

**The continuum of spreading depolarizations in acute cortical lesion development: examining Leão's legacy**

Journal:	<i>Journal of Cerebral Blood Flow and Metabolism</i>
Manuscript ID	Draft
Manuscript Type:	Review Article
Date Submitted by the Author:	n/a
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	and Experimental Neurology Woitzik, Johannes; Charité - Universitätsmedizin Berlin, Department of Neurosurgery; Dreier, Jens; Humboldt-Universität Berlin , Department of Neurology and Experimental Neurology
Keywords:	Spreading depression, Brain edema, Brain trauma, Cerebral Blood Flow, Electrophysiology, Focal Ischemia, Subarachnoid Hemorrhage, Vasospasm, Two photon microscopy, System biology, Stroke, Selective Neuronal Death, Neurovascular coupling, Neuroprotection, Neurocritical Care, Global Ischemia, Diffusion weighted MRI, cerebrovascular disease, Cardiac Arrest, Brain Ischemia

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February 24, 2016

Martin Lauritzen  
Editor-in-Chief  
*Journal of Cerebral Blood Flow and Metabolism*  
Copenhagen, Denmark

To the Editor:

We are pleased to submit our invited article "The continuum of spreading depolarizations in acute cortical lesion development: examining Leão's legacy" for publication as a Review Article in the special issue of the Journal of Cerebral Blood Flow and Metabolism devoted to Brain Injury Depolarizations.

Our manuscript contains 199 words in the Abstract, 7,985 words in the main text, and contains 7 figures and 2 tables. There are no conflicts of interest to disclose. This manuscript is new and is not being considered for publication elsewhere.

As corresponding author, I can be reached at the above address and phone, or at [jed.hartings@uc.edu](mailto:jed.hartings@uc.edu). We thank you in advance for consideration of our work and hope that you elect to publish it in this special issue.

Sincerely,

A handwritten signature in black ink that reads 'Jed Hartings'.

Jed A. Hartings, Ph.D.



## The continuum of spreading depolarizations in acute cortical lesion development: examining Leão's legacy

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**ABSTRACT**

A modern understanding of how cerebral cortical lesions develop after acute brain injury is based on Aristides Leão's historic discoveries of spreading depression and asphyxial/anoxic depolarization. Treated as separate entities for decades, we now appreciate that these events define a continuum of spreading mass depolarizations, a concept that is central to understanding their pathologic effects. Within minutes of acute severe ischemia, the onset of persistent depolarization triggers the breakdown of ion homeostasis and development of cytotoxic edema. These persistent changes are diagnosed as diffusion restriction in magnetic resonance imaging and define the ischemic core. In delayed lesion growth, transient spreading depolarizations arise spontaneously in the ischemic penumbra and induce further persistent depolarization and excitotoxic damage, progressively expanding the ischemic core. The causal role of these waves in lesion development has been proven by real-time monitoring of electrophysiology, blood flow, and cytotoxic edema. The spreading depolarization continuum further applies to other models of acute cortical lesions, suggesting that it is a universal principle of cortical lesion development. These pathophysiologic concepts establish a working hypothesis for translation to human disease, where complex patterns of depolarizations are observed in a variety of diseases and appear to mediate and signal ongoing secondary injury.

**Keywords:** spreading depression, brain trauma, cerebral blood flow, electrophysiology, focal ischemia, brain edema, subarachnoid hemorrhage, vasospasm, two photon microscopy, system biology, stroke, selective neuronal death, neurovascular coupling, neuroprotection, neurocritical care, global ischemia, diffusion weighted MRI, cerebrovascular disease, cardiac arrest, brain ischemia

## I. Introduction

The most important clinical conditions causing acute brain damage are cerebrovascular disease (ischemic and hemorrhagic stroke), head trauma, cardiac arrest, and asphyxiation-hypoxia. These neurologic emergencies affect people of all ages and backgrounds, and cardiac arrest and stroke in particular are the top two causes of mortality globally. In survivors, permanent brain damage often leads to severe disability and loss of independence. Thus, these conditions have been a major priority for public research funding and for pharmaceutical and medical device companies to improve prevention, diagnostics and treatment. In particular, the concept of neuroprotection emerged from advances in understanding the fundamental pathophysiology of these conditions, spawning hope that new therapeutic approaches could limit the extent of damage, and therefore, the burden of morbidity and mortality.<sup>1</sup>

This issue of *JCBFM* highlights a central mechanism in the development of acute brain injury that until recently has scarcely been recognized. The fundamental mechanism was discovered 70 years ago by the Brazilian physiologist, Aristides Leão, who described what is commonly known as “spreading depression” of cortical activity.<sup>2</sup> Beginning in the 1980’s, it was increasingly accepted that this mechanism was relevant to acute brain injuries, particularly those resulting from ischemic and hemorrhagic stroke, as well as trauma. Research on spreading depression and its variants gained further momentum when they were convincingly demonstrated in the human brain,<sup>3</sup> occurring with high incidence in each of these diseases.<sup>4</sup> The reader is referred to excellent reviews of spreading depression, covering topics of biophysics and cellular mechanisms,<sup>5,6</sup> vasculature and hemodynamics,<sup>7,8</sup> and relevance to human disease.<sup>4,8-10</sup>

While it is generally agreed among the small community of spreading depression researchers that these events have a causal role in the development of acute brain lesions, this

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3 concept has been slow to infiltrate the broader medical and research communities. This is partly  
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5 attributable to the recency of major research advances. However, we consider that this may also  
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7 be due to a historic anomaly, which is the erroneous conceptual separation of spreading  
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9 depression – often a benign, transient phenomenon – from what is known as anoxic  
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11 depolarization (Table 1). Anoxic depolarization of the cerebral cortex is a persistent consequence  
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13 of severe ischemia that defines the core of focal ischemia and is the foundation of the clinical  
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15 diagnosis of ischemic stroke by diffusion-weighted imaging. In reality, however, spreading  
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17 depression and anoxic depolarization represent two ends of a continuum of the same essential  
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19 phenomenon, and appreciation of this continuum is essential for understanding the causative role  
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21 of these propagating waves – spreading depolarizations – in the development and expansion of  
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23 acute brain lesions. Because the connection of these two extremes was first made by Leão,<sup>11</sup>  
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25 assertion of the continuum in a sense re-defines his legacy.  
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32 In this paper, we review the history and scientific rationale that establishes the spreading  
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34 depolarization continuum. On the basis of this understanding, we discuss the role of the initial  
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36 spreading depolarization in early damage from severe ischemia and then summarize the  
37  
38 conclusive evidence that subsequent, spontaneous waves of spreading depolarizations cause  
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40 secondary lesion growth in focal ischemia. We then review application of the same concepts to  
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42 other models of acute cortical lesions, including subarachnoid hemorrhage and traumatic brain  
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44 injury, and conclude with brief comments on clinical translation. Due to space limitations, we  
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46 focus on acute neuronal cell death in adult cerebral cortex; white matter, developmental changes,  
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48 specialized structures or cell types, subcortical or other gray matter, graded insults (e.g.,  
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50 concussion or hypoglycemia), and treatment implications are beyond the article's scope.  
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52 Collectively, the spreading depolarization continuum emerges as a conceptual and practical  
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3 framework that is essential to understanding the full spectrum of clinical neurologic conditions  
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5 resulting in acute brain damage.  
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## 8 9 10 **II. Foundations of the Spreading Depolarization Continuum**

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12 Mass depolarization is a fundamental response of cerebral cortical regions subjected to  
13 severe ischemia. This was first shown by Leão in 1947 when he described a negative “slow  
14 voltage variation” in cortical recordings that occurred 2-5 min after interruption of the cerebral  
15 circulation and persisted as long as the arteries were occluded.<sup>11</sup> He noted that this slow voltage  
16 variation – a negative shift in extracellular direct current (DC) potential – was similar in several  
17 respects to the slow voltage variation that accompanied the spreading depression of spontaneous  
18 cortical activity: in amplitude, in abrupt rate of development, and in the spreading, non-  
19 simultaneous onset across the cortex. He further observed that recovery of the negative DC shift  
20 of spreading depression in normally perfused cortex could be delayed indefinitely by sudden  
21 occlusion of the cerebral arteries. Thus, Leão speculated that in the spreading depression of  
22 activity, “some change of the same nature as one resulting from prolonged interruption of the  
23 circulation occurs in the cerebral cortex” (Figure 1).  
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41 In a striking manner, this single study established two poles of a continuum of a  
42 fundamental pathological response of the cerebral gray matter to injurious stimuli. In the  
43 following years, indirect evidence suggested that the nature of the response, in the case of  
44 spreading depression, was a profound depolarization of cortical neurons that blocked neuronal  
45 discharges, resulting in suppression of the electrocorticogram.<sup>12, 13</sup> With intracellular recordings,  
46 Collewijn and van Harreveld then confirmed that the DC potential shifts both of spreading  
47 depression and following cerebral circulatory arrest were indeed “of the same nature”,  
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3 corresponding in both cases to an abrupt loss of neuronal membrane potential to nearly 0 mV.<sup>14</sup>  
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5 They concluded that “these asphyxial changes are identical with the corresponding features of  
6  
7 (spreading depression)... and are caused by a common mechanism.”  
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11 Ironically, van Harreveld resisted this conclusion prior to his intracellular recordings<sup>15, 16</sup>  
12 since it had been shown that Leão’s spreading depression was not asphyxial, but on the contrary  
13 provoked a vasodilation and increase in blood oxygenation above the normal resting levels.<sup>17, 18</sup>  
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15 This is interesting as an illustration of the schism in thought that developed concerning spreading  
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17 depression on one hand and ‘asphyxial depolarization’ on the other, despite evidence for their  
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19 identity. In the decades after Leão’s publications, spreading depression was investigated  
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21 primarily as a phenomenon provoked in the otherwise healthy brain, in accordance with its  
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23 original description.<sup>2</sup> This was encouraged by early identification of its possible clinical  
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25 relevance in migraine aura. Karl Lashley’s mapping of the neurologic deficits during aura –  
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27 spreading scotomas in the visual field – predicted a neural correlate that closely matched the  
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29 speed of Leão’s spreading cortical depression.<sup>2, 19, 20</sup> Spreading depression was thus considered  
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31 clinically relevant only in migraine, a disease which, except in rare cases, does not cause  
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33 permanent neuronal damage.<sup>21, 22</sup> In agreement with the clinical condition, Nedergaard and  
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35 Hansen later demonstrated that even multiple repetitive waves of spreading depression do not  
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37 cause damage to the normally perfused brain.<sup>23</sup> Thus, contemporaneous studies on ischemic  
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39 brain injury and asphyxial (later called anoxic<sup>24</sup>) depolarization were conducted under a  
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41 framework quite distinct from those on spreading depression, and the two phenomena were  
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43 considered separate entities. Hansen notes in 1985, for instance, that “although the two  
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45 abnormalities resemble each other and may have common explanations, the common  
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47 denominator is not evident”.<sup>25</sup>  
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It was in this context that spreading depression-like events were first observed to occur spontaneously in animal models of focal cerebral ischemia,<sup>26-28</sup> a breakthrough discovery that now implicated spreading depression in the pathophysiology of acute brain injury. As evidence accumulated that these depolarization waves might augment injury in the context of an existing insult, new terminology emerged to differentiate them from spreading depression, a term with a benign connotation. These included peri-infarct, peri-focal, or peri-lesional depolarization, hypoxic spreading depression, ischemic depolarization, and spreading depression-like depolarization.<sup>5, 29, 30</sup> As a further matter, such waves were often evident only as DC shifts in electrically silent tissue, which precluded the possibility for the depolarization to induce spreading depression of the electrocorticogram. Thus, as the effects of these waves in injured brain were investigated, confused by proliferating terminology, a re-formulation of pathophysiologic concepts was needed.

The first hint of this new concept was provided by studies that, for the first time, demonstrated a true continuum between spreading depression and anoxic depolarization in focal cerebral ischemia. Recording from multiple electrodes across the cerebral cortex after middle cerebral artery occlusion (MCAO), these studies revealed persistent depolarization in the ischemic core, spreading depression in the periphery, and intermediate-duration depolarizations in the intervening penumbra.<sup>31-34</sup> Most notably, the first depolarization that erupts in the core propagates into the normal periphery as a single wave, but with gradually changing characteristics and durations (Figure 2A). In the ischemic core, this persistent depolarization is preceded by simultaneously developing suppression of the electrocorticogram (Figure 3A), identical to Leão's asphyxial depolarization in global ischemia (Figure 3B).<sup>11</sup> When the same wave spreads to normally perfused cortex with spontaneous electrical activity, however, it

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3 induces spreading depression of the electrocorticogram (Figure 3C). The continuity of such  
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5 singular waves may have been the “common denominator”<sup>25</sup> to finally unite prior descriptions of  
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7 spreading depression and persistent asphyxial depolarization.  
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10 While these concepts and their relation to lesion development are reviewed in detail  
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12 below, of relevance here is that the essential phenomenon of this *spreading depolarization* is  
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14 unchanged in its defining features as it transitions through the continuum from persistent  
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16 depolarization in severely ischemic tissue to spreading depression in normal brain,<sup>5</sup> including i)  
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18 degree of cellular depolarization and changes in principal ion concentrations (Figure 4), ii)  
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20 release of neurotransmitters, iii) structural changes of cytotoxic edema and dendritic beading, iv)  
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22 intrinsic optical signal, v) abrupt onset of extracellular DC shift, and vi) slow speed of spread (1-  
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24 8 mm/min). Thus, it has become clear that in the context of brain injury, Leão’s spreading  
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26 depression and asphyxial depolarization are two ends of the same spectrum, and are indeed “of  
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28 the same nature”.<sup>11</sup> The reader is referred to comprehensive reviews of differences that arise  
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30 along the spreading depolarization continuum, and how the continuum may explain diverse  
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32 clinical correlates.<sup>6, 9, 21, 35</sup> In particular, we note that there are some differences between  
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34 spreading depolarizations evoked by noxious stimuli in otherwise intact cortex, and those  
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36 triggered by ischemia. These include preceding tissue changes, such as gradual acidification and  
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38 rising  $[K^+]_e$  in ischemia, and thus the pharmacology of initiating mechanisms.<sup>5, 6, 36</sup> These  
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40 differences, including the nature of the precipitating insult, also exist on a continuum.<sup>9</sup>  
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48 Reflecting a new conceptualization of brain injury depolarizations, the term ‘spreading  
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50 depolarization’ (SD) is now used as a generic name for all variants of mass tissue depolarization  
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52 along the continuum originally described by Leão (Table 1). While the term he coined in his  
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54 1944 paper, spreading depression,<sup>2, 37</sup> has been widely used for decades, it was unknown at that  
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3 time that the underlying mechanism, reflected in the slow voltage variation, was mass  
4 neuronal/astrocytic depolarization. Nor could Leão have appreciated the extent to which he was  
5 correct in speculating that the slow voltage variation after cerebral circulatory arrest was the  
6 same essential phenomenon underlying spreading depression. In hindsight, therefore, we  
7 consider that Leão's contribution was discovery of the full continuum of SDs, not only the subset  
8 that induces spreading depression of the electrocorticogram. While a subtle shift in terminology  
9 may seem a disservice to Leão, we rather consider that it more fully honors his rightful legacy,  
10 while also installing a neutral and uniquely descriptive term that may prove durable in growth of  
11 the field.  
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### 27 **III. Spreading Depolarization Initiates Neuronal Injury in Global and Focal Cerebral** 28 **Ischemia** 29

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31 In the sudden onset of severe cerebral ischemia, as occurs in cardiac arrest, asphyxiation,  
32 or ischemic stroke, the first electrophysiologic consequence is the suppression of spontaneous  
33 activity, reflected in the 0.5-70 Hz band of the electrocorticogram, which develops  
34 simultaneously in widespread regions 10-20 sec after reduction of blood flow below the critical  
35 threshold of 15-23 ml/100g/min (Figures 2A and 3A,B).<sup>26, 38, 39</sup> Loss of synaptic activity results  
36 from neuronal hyperpolarization and adenosine-mediated suppression of vesicular transmitter  
37 release, which reduce energy consumption as a survival response to ischemia.<sup>40-43</sup> If blood flow  
38 is reduced below 5-10 ml/100g/min, the widespread depression of activity is followed, after a  
39 delay of 2-5 min, by mass tissue depolarization (Figures 2A and 3A,B).<sup>11, 14, 15, 44</sup> This  
40 depolarization, observed as a negative DC shift of extracellular potential, develops first focally  
41 and then spreads through contiguous cortex at 3.3 to 6.8 mm/min.<sup>45-50</sup> In some cases the apparent  
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3 propagation velocity of this initial SD developing in severely ischemic cortex may be greater  
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5 than SDs observed in normally perfused cortex. This occurs mainly because other SD foci  
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7 develop in adjacent ischemic regions before they are engulfed by the initial wave;<sup>50</sup> the full  
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9 extent of severely ischemic tissue is then rapidly depolarized by convergence of waves from  
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11 multiple focal origins.  
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15 In both global and focal ischemia, this initial depolarization is persistent in regions where  
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17 blood flow is below the 5-10 ml/100g/min threshold (Figure 2A). It has sometimes been  
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19 described as ‘terminal depolarization’,<sup>24</sup> which is accurate when blood flow is not restored, since  
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21 depolarization then persists until the tissue becomes infarcted (Table 1). As a generic term,  
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23 however, ‘terminal depolarization’ fails to recognize that, depending on time, the initial  
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25 persistent depolarization in severe ischemia is a reversible phenomenon.<sup>48, 51, 52</sup> This was first  
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27 demonstrated by Leão, who found that the negative DC shift could be reversed by restoring  
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29 perfusion after ischemia up to 12 min in duration.<sup>11</sup> There is no precise threshold duration  
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31 beyond which mass depolarization is irreversible, since recovery can occur in a graded or partial  
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33 manner. Importantly, reversal of the negative DC shift does not indicate that neurons will  
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35 survive.  
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41 As noted above, the initial persistent depolarization that erupts after severe ischemic  
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43 onset has historically been termed asphyxial or anoxic depolarization, with implications of  
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45 conditions and consequences that are distinct from SD.<sup>15, 24, 53</sup> However, specific definitions of  
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47 ‘anoxic depolarization’ prove to be elusive and are all misleading in some important respect,  
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49 which only further illustrates the nature of the SD continuum. First, hypoxia-anoxia during SD is  
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51 not unique to cases of proximal arterial occlusion, but occurs to some extent in normal tissue<sup>54-56</sup>  
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53 and more severely when SD induces the profound microvascular constriction known as  
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3 spreading ischemia, as discussed below.<sup>57-59</sup> Second, SDs can be triggered by decreased  
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5 perfusion not only after initial occlusive ischemia, but also by hypotensive transients in the  
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7 ischemic penumbra as a secondary injury process occurring hours after the initial ictus.<sup>60</sup> Third,  
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9 not all SDs induced by initial occlusive ischemia are persistent, and persistent depolarization is  
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11 not unique to this condition. For instance, SDs occurring within minutes of microembolization  
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13 are short and transient.<sup>61, 62</sup> On the other hand, spontaneous SDs occurring hours after more  
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15 severe focal ischemia can induce persistent depolarization in the inner penumbra, as reviewed  
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17 below.<sup>31, 63-66</sup> Finally, the term ‘anoxic’ is misleading since it is not only the lack of oxygen, but  
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19 also of glucose and blood flow that are essential in inducing and maintaining tissue  
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21 depolarization. Therefore, here we use the term ‘persistent depolarization’ to describe  
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23 indefinitely prolonged depolarizations under conditions of severe ischemia (Table 1). ‘Terminal  
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25 depolarization’ is used more specifically to describe persistent depolarization that endures until  
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27 tissue becomes infarcted.  
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34 As discussed in the following, it is the initial persistent depolarization that triggers a loss  
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36 of ion homeostasis and cytotoxic edema, and therefore marks the onset of structural injury and  
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38 countdown to irreversible damage, in both global ischemia and the core of focal ischemia.  
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40 Delayed lesion expansion in focal ischemia occurs in a similar fashion, triggered by secondary  
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42 spontaneous SDs that induce persistent/terminal depolarization and excitotoxic injury in the  
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44 penumbra. Definitions of the ischemic core and penumbra used here are provided in Figure 2 and  
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46 explained below.  
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4 *Spreading depolarization triggers the loss of ion homeostasis and release of neurotransmitters*  
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6 *after ischemic onset*  
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8 The sequence of pathophysiologic events following severe ischemia has been well  
9 characterized and generally involves the rise of  $[K^+]_e$ , depolarization of presynaptic terminals,  
10 excessive extracellular accumulation of neurotransmitters, activation of N-methyl-D-aspartate  
11 (NMDA) receptors, a general loss of ion homeostasis ( $Ca^{2+}$ ,  $K^+$ ,  $Na^+$ ,  $H^+$ ,  $Cl^-$ ,  $HCO_3^-$ ) with  
12 attendant rise of intracellular  $Ca^{2+}$ , and the onset of cytotoxic edema.<sup>67-69</sup> In the general literature,  
13 the central role of SD in these processes is rarely acknowledged. This again may be due to the  
14 historical view of asphyxial/anoxic depolarization as distinct from Leão's actively propagating  
15 phenomena. When the time course of the extreme ionic changes in ischemia were described,  
16 investigators noted the similarity to ionic changes during spreading depression (Figure 4).<sup>28, 36, 70-</sup>  
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73 However, since only SD in normal brain (i.e., spreading depression, "a pathological event without pathology"<sup>70</sup>) was considered, it was difficult to reconcile these transient changes without damage to the persistent ionic changes associated with anoxic-ischemic damage. Hence the concepts of the ischemic penumbra and neuroprotection developed initially, and persist largely today, without consideration of the SD continuum.<sup>26, 38, 39</sup> Marshall was an exception to this trend in describing asphyxial depolarization as asphyxial SD.<sup>74</sup>

Understanding now that persistent DC shifts (i.e. anoxic depolarization) are SDs, there is unequivocal evidence that SD is the network/systems process that triggers the major changes of the ischemic pathophysiologic cascade. In various *in vivo* models including hypoglycemia, respiratory paralysis, and ischemia,  $[K^+]_e$  first rises slowly over 2-4 min until it reaches 9-11 mM, a "ceiling level"<sup>75</sup> in the cortex which is only exceeded when SD occurs. Thus, the next phase is a sharp rise of  $[K^+]_e$  to a new equilibrium up to 75 mM, which coincides with the

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3 negative DC shift that signals the onset of persistent depolarization (Figure 4B).<sup>25, 44, 72</sup> In the  
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5 same simultaneous event, there is a sharp decline in extracellular  $\text{Ca}^{2+}$  ( $[\text{Ca}^{2+}]_e$ ) from 1.3 to  $<0.1$   
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7 mM,  $[\text{Cl}^-]_e$  from 150 to 95 mM, and  $[\text{Na}^+]_e$  from 150 to 60 mM.<sup>9, 25, 28, 73, 76-78</sup> These shifts  
8  
9 develop in seconds, while prior to depolarization there is little change in these ions. The same  
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11 results have been confirmed with imaging studies of global ischemia *in vivo* and oxygen-glucose  
12  
13 deprivation *in vitro*: after onset of hypoxia-ischemia, the steep rapid rise in intracellular  $\text{Ca}^{2+}$   
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15 occurs only as a consequence of persistent depolarization (Figure 5).<sup>48, 79-81</sup> By comparison, the  
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17 time course of glutamate changes in relation to the onset of persistent depolarization has been  
18  
19 more difficult to resolve *in vivo* since microdialysis techniques have limited resolution. However,  
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21 several studies suggested a close association, with glutamate increasing at the time of  
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23 depolarization or thereafter.<sup>82-84</sup> Recent use of amperometric techniques confirmed that a steep  
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25 rise in glutamate after arterial occlusion develops simultaneous with depolarization onset, and  
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27 does not precede it,<sup>85</sup> as for SD in normal cortex.<sup>86</sup>  
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34 The extreme ionic loading that occurs during the initial persistent depolarization wave  
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36 might be sufficient to kill neurons simply as a consequence of the increased metabolic demand  
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38 coupled with the lack of substrates to fuel ATP-dependent ion pumps. That is, even though many  
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40 of the mechanisms of excitotoxic injury are active at this end of the SD continuum, including  
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42 profound glutamate accumulation and  $\text{Ca}^{2+}$  loading, it appears NMDA-dependent  $\text{Ca}^{2+}$  pathways  
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44 may not be required for injury. In some global ischemia and *in vitro* models, for instance,  
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46 prevention of  $\text{Ca}^{2+}$  loading or antagonism of NMDA receptors fails to protect against the initial  
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48 structural injury induced by persistent depolarization.<sup>48, 50, 87</sup>  
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3 *Spreading depolarization causes cytotoxic edema and characteristic changes in imaging*  
4 *diagnostics of ischemic stroke*  
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8 Persistent depolarization following severe ischemia is also an important mechanism to  
9 recognize since it induces the structural changes, known as cytotoxic edema, that are the basis of  
10 clinical stroke diagnosis by diffusion-weighted imaging. Disruption of the physiological ion  
11 gradients during SD results in intracellular water accumulation and abrupt neuronal and  
12 astroglial swelling. Neuronal cytotoxic edema involves a plasma membrane reorganization that  
13 gives dendrites the appearance of beads on a string, termed dendritic beading (Figure 6A,B).  
14 Neuronal and astroglial swelling was first recognized as an abrupt increase in cortical tissue  
15 impedance that accompanied the ‘asphyxial depolarization’,<sup>88, 89</sup> a change also observed for SD  
16 in normal brain.<sup>74</sup> These results provided evidence of water and chloride uptake into apical  
17 dendrites,<sup>90, 91</sup> and dendritic swelling was visualized using electron microscopy.<sup>92</sup> Cytotoxic  
18 edema following persistent depolarization of severely ischemic cortex thus results in a 50-65%  
19 shrinkage of the extracellular volume, similar to that during SD in normal brain.<sup>52, 93-95</sup>  
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36 These structural changes can be visualized by magnetic resonance imaging (MRI) of  
37 restricted water diffusion in cerebral gray matter. Diffusion MRI techniques are a clinical  
38 standard in the diagnosis of ischemic stroke, since they have high sensitivity and specificity and  
39 characteristic changes are visible within minutes of stroke onset (Figure 6C,D).<sup>96</sup> The decrease in  
40 diffusion coefficient reflects restricted intracellular movement of water following edema onset  
41 and the development of beaded neurite morphology.<sup>97</sup> Importantly, the imaging changes occur  
42 abruptly and simultaneously (within the resolution of MRI sequences) with the negative DC shift  
43 of persistent depolarization.<sup>94, 98</sup> SD is sufficient to induce the imaging changes since diffusion  
44 restriction is observed as a propagating pattern even during SD in normally perfused cortex.<sup>99</sup>  
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3 Some have disputed whether diffusion restriction after stroke is a consequence of  
4 depolarization,<sup>100, 101</sup> but this question was conclusively resolved with two-photon real-time  
5 imaging of global ischemia in the mouse.<sup>48, 102</sup> About 2-3 min after ischemia onset, prior to any  
6 structural changes to cellular processes, persistent depolarization developed with its attendant  
7 spreading wave of increased intracellular  $\text{Ca}^{2+}$ . This was followed, ~6 seconds later, by abrupt  
8 beading of dendrites and astroglial swelling that progressed in a spreading pattern after the SD  
9 wavefront (Figure 6A,B). These structural changes persist as long as depolarization is maintained  
10 and mark the onset of permanent damage to synaptic circuitry.<sup>103</sup>

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12 While it has been assumed that neurons swell during SD because osmotically obligated  
13 water follows  $\text{Na}^+$ ,  $\text{Cl}^-$  and  $\text{Ca}^{2+}$  influx, pyramidal neurons do not express functional aquaporins  
14 and are largely water-impermeable under acute osmotic stress.<sup>104</sup> Recent evidence rather  
15 suggests that water is carried by select chloride-coupled neuronal cotransporters as a  
16 consequence of the altered electrochemical gradients during SD.<sup>105</sup>

### 17 *Persistent depolarization defines the core of severe focal ischemia*

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19 The work of Lindsay Symon and colleagues was the first to suggest a connection between  
20 persistent depolarization and permanent damage from severe focal ischemia. An early study  
21 suggested that damage from MCAO was confined to regions with the lowest post-occlusive  
22 blood flow (<10 ml/100g/min).<sup>106</sup> To investigate what changes occur at this threshold, they  
23 examined the effects of progressive ischemia on cortical physiology by combining MCAO with  
24 controlled steps of exsanguination (hypotension).<sup>26, 38</sup> They found that  $[\text{K}^+]_e$  levels changed  
25 steeply according to a flow threshold of 8-11 ml/100g/min, with  $[\text{K}^+]_e$  remaining <10 mM above  
26 this range, but rising *persistently* to a range of 30-90 mM below the threshold. Of note, sharp but  
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3 *transient* rises in  $[K^+]_e$  and decreases in  $[Ca^{2+}]_e$  were also observed at higher levels of flow.<sup>26, 28,</sup>  
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6 <sup>107</sup> A similarity to spreading depression was noted for the transients, but SD was not invoked to  
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8 explain the sharp, persistent increases, which were attributed generically to ion pump or  
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10 membrane failure. This membrane failure, marked by the persistent  $[K^+]_e$  increase, was proposed  
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12 as the sign of impending infarction. Subsequent work has confirmed that the  $[K^+]_e$  elevation (and  
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14  $[Ca^{2+}]_e$  depletion) in the core of focal ischemia is triggered and maintained by persistent  
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16 depolarization, as for global ischemia reviewed above.<sup>107, 108</sup>  
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20 The main legacy of this work was not the role of SD in focal ischemia, however, but  
21  
22 rather the classical concept of the ischemic penumbra (Figure 2A). The threshold for the steep  
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24 rise of  $[K^+]_e$  (persistent depolarization) and consequent structural damage was considerably  
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26 lower than that for functional failure (suppression of electrocorticogram), identified as ~20  
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28 ml/100g/min. This suggested the idea of a penumbra between the dual thresholds, where neurons  
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30 remain structurally intact but functionally inactive.<sup>38, 39, 109</sup> Thus, the inner boundary of the  
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32 ischemic penumbra was defined as the spatial limit of persistent depolarization, which defines  
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34 the ischemic core (Figure 2A).  
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#### 41 *Three determinants of neuronal damage: depolarization, impaired energy supply, and time*

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44 The threshold duration for persistent mass depolarization to cause permanent neuronal  
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46 injury has been termed the commitment point.<sup>35</sup> The commitment point is not a universal value  
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48 but depends strongly on the model and the level of residual perfusion, developmental stage and  
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50 cell populations, and also the marker of damage chosen.<sup>110-112</sup> Threshold durations have been  
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52 reported as short as 5 min for selective neuronal injury and as long as 60 min for pannecrosis.<sup>113,</sup>  
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56 <sup>114</sup> In another noteworthy study of global ischemia, it was shown that 15 min of persistent  
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3 depolarization, but not 10 min, is sufficient to cause massive neuronal necrosis.<sup>115</sup> Interestingly,  
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5 animals that did not develop SD, despite severe ischemia at 10% baseline blood flow for 20 min,  
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7 exhibited minimal or no histologic damage. Thus, depolarization appears to be a requirement for  
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9 severe acute neuronal damage to develop from ischemic insults of these durations.<sup>103, 115, 116</sup>  
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11 Critically, however, ischemia is also required. While persistent depolarization will eventually  
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13 cause infarction even in healthy brain,<sup>23</sup> 1 hr of persistent depolarization resulted in cortical  
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15 infarction only when accompanied by severe ischemia.<sup>117</sup> Thus, as discussed below, it appears  
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17 that there are three key determinants for lesion development: depolarization, impaired energy  
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19 supply, and a minimum duration of each.  
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25 It is noteworthy that the minimum durations for depolarizations to cause neuronal cell  
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27 death appear to be shorter than the maximum durations for reversibility of depolarization. In  
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29 focal ischemia, for instance, reversal of persistent depolarization in the ischemic core has been  
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31 observed even after 3 hr.<sup>63</sup> Importantly, this means that reversal of the negative DC shift (i.e. at  
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33 least partial repolarization) is not an indicator that tissue will survive, as the commitment point  
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35 for cell death may have already been crossed.<sup>63</sup>  
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#### 41 **IV. Lesion Expansion in the Focal Ischemic Penumbra**

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43 In comparison to global ischemia, focal ischemia presents a more complex scenario in  
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45 which a gradient of blood flow and impaired metabolism exists from the ischemic core, through  
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47 the classical penumbra, and into the normally perfused periphery. Following ischemic onset, SD  
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49 first develops in the core, where it is persistent and thus the only wave to affect this region.<sup>31, 32,</sup>  
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<sup>49</sup> The initial SD is not restricted to the core, however, but propagates outward in a radial pattern  
through ipsilateral cortex, traversing tissue with a gradient of increasing blood flow. In these

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3 regions, the wave transitions to a transient event with prolonged duration in the penumbra and  
4 short duration in non-ischemic cortex (Figures 2A,3). Different depolarization durations are  
5 determined by residual perfusion, and thus reflect the perfusion gradient.<sup>118</sup> With the passage of  
6 time, additional SDs arise due to energy supply-demand mismatch in the classical penumbra,<sup>62,</sup>  
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119 or even in more peripheral cortex with impaired metabolism but preserved excitability (Figure  
2B).<sup>60</sup> These waves can propagate in diverse patterns such as spreading radially or cycling  
through the penumbra around the ischemic core, sometimes repeatedly, following the path of  
greatest susceptibility.<sup>49, 63, 119, 120</sup>

#### *The causal role of secondary spreading depolarizations in ischemic lesion expansion*

The hypothesis<sup>109</sup> that these spontaneous, secondary SDs are a causal mechanism of secondary injury was suggested by an association between final infarct volume and the burden of transient SDs observed in the ischemic periphery/penumbra – burden being measured either in SD count or cumulative depolarization duration.<sup>29, 31, 60, 63</sup> Moreover, the time course of SD activity through 24 hr post-ischemia matches the kinetics of lesion growth: both exhibit an early (<2 hr) and delayed (8-18 hr) phase.<sup>121</sup> Therapeutic studies further supported this hypothesis, as interventions that reduced the frequency of spontaneous SDs also reduced infarct volumes. These included both physiologic (hyperoxia,<sup>122</sup> hypothermia,<sup>123</sup>) and pharmacologic (glutamate receptor antagonists<sup>121, 124-127</sup> and gap junction blocker<sup>128, 129</sup>) therapies. Nevertheless, a fair critique of this cumulative evidence is that these studies did not prove a causal role of SD in infarct growth, since neuroprotection from treatments may be achieved through other pathways that cannot be excluded, and SD may be an epiphenomenon of lesion growth, arising as a consequence but not contributing causally to the process.

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3 This pivotal question of causality (together with the non-injurious nature of SD in normal  
4 cortex) has perpetuated skepticism about the relevance of secondary SDs in acute brain injury.  
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6 There are now, however, several lines of evidence that directly address it (Table 2). The first is a  
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8 series of studies that were conducted independently in three different laboratories and,  
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10 remarkably, published in the same year.<sup>130-132</sup> Each study used the same general approach to  
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12 address causality: determine whether additional SDs, induced experimentally (in remote tissue)  
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14 and propagating into the ischemic focus, augment lesion growth compared to the ischemic insult  
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16 with sham SD elicitation. Considered together, the studies used two different methods to induce  
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18 SD ( $K^+$  microinjection, electrical stimulation), two models of focal ischemia (intraluminal thread  
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20 or photothrombosis with common carotid artery occlusion), and three methods to assess lesions  
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22 [diffusion-weighted MRI, H&E histology, and triphenyltetrazolium chloride (TTC)]. All studies  
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24 found that the experimental groups with a greater number of SDs had larger infarct volumes (25-  
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26 50% increases).  
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34 The study by Busch et al. also provided a second line of direct evidence.<sup>130</sup> A series of  
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36 diffusion-weighted MRIs (DWI) were obtained every 15 min after stroke in synchrony with  
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38 remote SD induction. They found that the persistent diffusion lesion grew in a stepwise fashion  
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40 through 2 hr post-stroke, in synchrony with the invasion of SD into the penumbra, thereby  
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42 directly confirming the effect of SD inferred from final lesion volumes. In particular, SDs caused  
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44 a transient increase in DWI signal intensity throughout the ipsilateral cortex, but a small rim  
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46 around the existing lesion did not recover, becoming indistinguishable from the lesion core.  
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48 Similar results were obtained in a separate study using NADH (nicotinamide adenine  
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50 dinucleotide) fluorescence imaging.<sup>63</sup> These experiments not only directly visualized a causal  
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52 role of SD in lesion growth, but also suggested how this is effected: by inducing terminal  
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3 depolarization in the inner penumbra, thus expanding the zone of the terminally depolarized core  
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6 (Figure 2B).

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8 This conclusion is supported by electrophysiological studies that have shown terminal  
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10 depolarizations develop in a delayed fashion as a consequence of the spread of spontaneous  
11  
12 SDs.<sup>31, 63, 65, 66, 85</sup> Surprisingly, these delayed terminal depolarizations are observed not only in  
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14 the classical penumbra, where metabolism is more severely impaired, but also in tissue with  
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16 preserved spontaneous activity.<sup>85</sup> That propagating SDs can induce terminal depolarization under  
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18 conditions of metabolic compromise is confirmed in single neurons<sup>133</sup> and in *in vivo* models of  
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20 partial global ischemia.<sup>46</sup> Together, these studies provide a third demonstration of SD's causal  
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22 effect, since it is the propagating wave that induces terminal depolarization, regardless of how or  
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24 where the SD was remotely initiated.  
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29 How can an invading SD cause terminal depolarization in tissue where baseline blood  
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31 flow was previously adequate to maintain membrane integrity? It is possible that the blood flow  
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33 requirement for restoration of membrane potentials is higher than that for maintenance.  
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35 However, it is also known that the SD wave itself induces complex perfusion changes that affect  
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37 the tissue's capacity to repolarize.<sup>7, 134, 135</sup> While SD in uninjured cortex evokes a spreading  
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39 hyperemia followed by oligemia,<sup>136</sup> SD in the penumbra elicits a blunted, absent or inverse  
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41 hemodynamic response, known as spreading ischemia,<sup>57</sup> due to impaired neurovascular coupling  
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43 and reduced perfusion pressure.<sup>49, 59, 137-140</sup> As in hyperemia, the perfusion decrease follows the  
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45 depolarization, and does not precede it.<sup>59</sup> This is also a graded phenomenon, with the perfusion  
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47 waveform changing shape as SD traverses the penumbra. The blood flow response is a transient  
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49 decrease followed by increase in the outer penumbra, a transient decrease only in a middle zone,  
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51 and a persistent decrease in the inner penumbra (Figure 2B).  
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As a fourth piece of direct evidence of SD's adverse effect, the persistent decreases in perfusion have a cumulative effect with each repetitive SD, progressively increasing the degree and spatial extent of ischemia in a stepwise manner.<sup>49, 60, 120, 137</sup> This effect of SD to exacerbate ischemia results in prolongation of depolarization by reducing energy supply and perhaps vascular clearance of  $[K^+]_e$ .<sup>7, 141</sup> In the most severe cases, it enables terminal depolarization (Figure 2B).<sup>46, 59, 139</sup> In other words, SD sows the conditions for its own destructive effect. The reader is referred to previous reviews on the vicious cycle of SD-induced  $[K^+]_e$  elevation leading to vasoconstriction and hence prolonged depolarization in nitric oxide-depleted tissue.<sup>8, 142</sup>

#### *Comparison of initial terminal and secondary spreading depolarizations*

As discussed, reasonable doubt of the causal role of secondary SDs in time-dependent growth of focal infarcts has been obviated by use of advanced real-time monitoring, including MRI, NADH fluorescence, two-photon and laser speckle imaging, in combination with electrophysiology (Table 2). The direct effect of SD as executioner of vulnerable tissue is observed, as spreading waves induce further terminal depolarization and expand the infarct core. Here, the effect of terminal depolarization to induce persistent loss of ion homeostasis and structural damage is very similar to that of the initial terminal depolarization in the ischemic core.<sup>66, 143</sup> There are, however, critical differences between initial and secondary SDs apart from the triggering mechanisms described above. The first is that the initial terminal depolarization in the ischemic core *follows* the critical reduction in blood flow from arterial occlusion, whereas delayed secondary SDs *induce* the perfusion decreases that prevent or delay recovery in the penumbra (Figure 2). Second, in the case of secondary SDs, neuronal damage can result not only from a single persistent depolarization, but also from the cumulative effects of multiple transient



SDs, particularly if they are prolonged or recur in high numbers.<sup>35, 63, 144, 145</sup> Dendrites, for instance, can undergo repeated rounds of beading and recovery with each transient SD until eventually beading becomes terminal.<sup>143, 146</sup> This progressive course toward permanent injury depends in part on the proximity of cells to capillaries and may develop in specific microdomains of more severe hypoxia/ischemia.<sup>56, 143</sup> During repetitive SDs, the failure of subsets of neurons to repolarize may underlie the shallow negative ultraslow potential (NUP) that is sometimes observed.<sup>10, 147</sup> Third, in comparison to the initial depolarization of ischemia, neuronal injury from secondary SDs may be more dependent on excess accumulation of extracellular glutamate and NMDA receptor-mediated  $\text{Ca}^{2+}$  loading<sup>148</sup>. In metabolically compromised but viable neurons, a single SD causes cell death by inducing an irrecoverable  $\text{Ca}^{2+}$  increase in apical dendrites that then spreads to the soma.<sup>133</sup> Excess glutamate release and NMDA receptor activation are required, since persistent  $\text{Ca}^{2+}$  loading and cell death are prevented in the same conditions by NMDA receptor antagonism. Moreover, bursts of glutamate release that occur *in vivo* during delayed penumbral lesion growth are observed only in synchrony with SDs, and not independently.<sup>64</sup> Thus, it appears that glutamate excitotoxicity occurs only in connection with SD and that excess glutamate signaling and SD are merely different facets of the same secondary injury process. The different role of NMDA receptors in secondary SDs as opposed to the initial persistent depolarization in the ischemic core has important treatment implications.<sup>6, 8</sup>

## V. Other Models of Cortical Lesion Development

Ischemic stroke is a model disease for the study of acute brain injury since the cause of cortical dysfunction is singular (vascular occlusion), the onset of pathology is extreme and

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3 abrupt, and animal models are well established. Furthermore, ischemia is an important  
4 component of other types of stroke as well as traumatic brain injury (TBI), which are more  
5 multifactorial, and the concepts of secondary injury and neuroprotection were conceived largely  
6 from the study of ischemic stroke. Accordingly, characterization of the SD continuum in  
7 ischemic stroke, as described above, has provided a conceptual framework for understanding  
8 how SD may be involved in acute neuronal damage from other causes.  
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### 20 *Subarachnoid hemorrhage*

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22 Aneurysmal subarachnoid hemorrhage (SAH) is a stroke sub-type in which cortical  
23 ischemic lesions are observed in two primary phases, early and delayed. Early lesions develop in  
24 laminar or band-like patterns of contiguous cortex or symmetrically in midline regions (Figure  
25 6D).<sup>149, 150</sup> Delayed lesions develop around 6-7 days after aneurysm rupture, often in conjunction  
26 with neurologic decline in a condition known as delayed cerebral ischemia (DCI). Cortical  
27 lesions are often found in regions covered with blood, such as a sulcus or fissure with a thick  
28 subarachnoid clot.<sup>151-154</sup> Furthermore, the likelihood of DCI correlates with the amount of  
29 subarachnoid blood<sup>155</sup> and the time-course of DCI development corresponds with that of  
30 hemolysis.<sup>156</sup>  
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43 These observations led to the idea that delayed lesions develop from the release of  
44 hemolysis products into the subarachnoid space. To model this, the rat cortex was superfused  
45 with artificial cerebrospinal fluid (ACSF) containing high concentrations of K<sup>+</sup> and free  
46 hemoglobin.<sup>57</sup> These conditions were sufficient to trigger SDs that recurred repeatedly over  
47 several hours. Importantly, the electrophysiologic changes developed independently, without  
48 prior decrease in blood flow. Rather, it was found that SDs induced a profound constriction in  
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3 the microvasculature, termed 'cortical spreading ischemia'. Repeated SDs thus induced and  
4 sustained ischemic conditions reaching ~10% basal cerebral blood flow for up to 2 hr. Despite  
5 return of baseline blood flow and tissue repolarization after washout with normal ACSF, band-  
6 like cortical infarcts were observed at sacrifice 24 hr later.<sup>157</sup> Therefore, cortical infarcts  
7 developed in this model through a pathophysiologic cascade similar to that in ischemic  
8 penumbra: SD induces ischemic conditions that preclude rapid recovery from depolarization.  
9 Also similar is that the commitment point for cell death was reached, despite subsequent  
10 recovery of perfusion. Importantly, spreading ischemia is an essential mechanism in this  
11 sequence, since lesion development could be blocked by application of a nitric oxide donor or  
12 the calcium channel antagonist, nimodipine, both of which at least partially reverted the  
13 spreading ischemia to spreading hyperemia.<sup>158, 159</sup>

### 31 *Endothelin model*

34 Another model of cortical neuronal necrosis investigates the effects of mild but prolonged  
35 focal cerebral ischemia. Endothelin is a peptide that promotes vasoconstriction and is implicated  
36 in a number of disease states. When 1  $\mu$ M endothelin-1 (ET-1) is topically applied to the rat  
37 cortex, CBF remains above the ischemic threshold ( $\sim 75 \pm 15\%$  of baseline), but several  
38 spontaneous SDs are observed in 50% of animals.<sup>160</sup> Interestingly, only the animals with SDs  
39 develop regions of selective neuronal death after 24 hr.<sup>144</sup> To determine whether SDs were a  
40 cause or consequence of neuronal injury, SDs were elicited experimentally in remote cortex and  
41 propagated into the ET-1 window in animals that did not develop SD spontaneously. In each  
42 case, necrosis developed similar to animals with spontaneous SDs, and neuronal death only  
43 occurred in regions exposed to ET-1. Similar to studies of severe focal ischemia<sup>130-132</sup>, these

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3 results support the causal role of SDs as a determinant of neuronal cell death even in mild  
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6 ischemia.

### 7 8 9 10 *Traumatic brain injury*

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12 Is the SD continuum observed in acute brain lesions developing from causes other than  
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14 cerebrovascular disease? TBI is the only other condition in which this question has been  
15  
16 addressed experimentally, albeit to a much lesser extent. The injury cascade following cerebral  
17  
18 contusion is very similar to that after ischemia, including an early rise in  $[K^+]_e$ , elevation of  
19  
20 extracellular glutamate, and neuronal injury resulting from excitotoxic  $Ca^{2+}$  overload.<sup>161-163</sup>  
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22 While an initial SD at the time of injury has been shown in multiple studies,<sup>164-167</sup> to date only  
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24 two studies have specifically examined early ionic changes in relation to histologically  
25  
26 confirmed lesions. Nilsson et al. found an increase in  $[K^+]_e$  (3.0 to 40 mM) and decrease in  
27  
28  $[Ca^{2+}]_e$  (1.1 to 0.1 mM) immediately after contusion that corresponded with a negative DC  
29  
30 shift.<sup>168</sup> These changes persisted for 5-15 min in the region where a lesion developed, but for  
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32 only a few minutes in adjacent tissue that survived. Similarly, Takahashi et al. found a permanent  
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34 increase in  $[K^+]_e$  (4.0 to 50 mM) in tissue with a histologic contusion after a high impact injury,  
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36 but only a transient increase in  $[K^+]_e$  (4.0 to 30 mM) in animals that did not develop a contusion  
37  
38 after low impact injury.<sup>161</sup> These results demonstrate that SD occurs at the moment of traumatic  
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40 impact, and that the SD is prolonged when a region of neuronal injury develops.

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43 After the initial impact, spontaneous secondary SDs have been observed for hours to days  
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45 in some models, but not others.<sup>167, 169-174</sup> Whether they occur and their impact on lesion  
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47 development seem to depend on injury severity,<sup>165, 174</sup> the extent of a metabolic penumbra, and  
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49 the presence of secondary insults (e.g., elevated intracranial pressure and hypotension). In the  
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3 controlled cortical impact model, von Baumgarten et al. found no effect of remotely elicited SDs  
4 on contusion volume, despite a 6-fold increase in the number of SDs in experimental vs. control  
5 animals.<sup>164</sup> Sword et al., by contrast, showed that remotely elicited SDs caused reversible but  
6 then terminal dendritic beading in the same model.<sup>146</sup> In fluid percussion injury, increases in  
7 intracranial pressure to levels observed in clinical severe TBI (30 mm Hg) were associated with  
8 continuous repetitive SDs, even in the contralateral hemisphere.<sup>165</sup>

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18 The anatomic pathology of clinical TBI consists not only of contusions, however, but  
19 may also include intracerebral hemorrhage, diffuse axonal injury, subdural or epidural  
20 hematoma, or any mix of these. Subdural hematoma in particular is one of the most common TBI  
21 lesions and is associated with high morbidity and mortality. To study its pathophysiology, Miller  
22 et al. developed a rat model in which 400  $\mu$ l of blood is injected into the subdural space.<sup>175</sup> This  
23 results in a large cortical lesion, comprising 15% of hemispheric volume, within 4 hr. The lesion  
24 is ischemic in nature, as indicated by H&E histopathology and measurement of blood flow, and  
25 is associated with cytotoxic edema and a large glutamate surge.<sup>176, 177</sup> Considering the  
26 similarities to focal ischemia, including neuroprotection by hypothermia and NMDA receptor  
27 antagonists,<sup>178, 179</sup> electrophysiology was performed to investigate the SD continuum (Figure 7).  
28 Preliminary results suggest that the infusion of subdural blood acutely induces terminal  
29 depolarization in cortex that evolves to infarction. In the infarct periphery, the initial SD is  
30 prolonged, lasting up to 15 min, and spontaneous SDs of shorter duration are subsequently  
31 observed throughout 4 hr of monitoring (JMH and JAH, unpublished). SDs are also observed in  
32 a swine model of intracerebral hemorrhage,<sup>180</sup> further suggesting that hemorrhagic lesions might  
33 explain the high incidence of SD in patients with severe TBI<sup>181, 182</sup> as compared with contusional  
34 rodent models. In general, the mixed pathology and heterogeneous nature of clinical TBI present  
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3 serious challenges for animal modeling, especially considering that the degree and volume of  
4 such injuries that are survivable in humans are orders of magnitude greater than those in rodents.  
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## 10 **VI. Pre-Conditioning: Is Spreading Depolarization Protective?**

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12 A substantial literature has accumulated showing that pre-conditioning the cortex with  
13 SDs has a neuroprotective effect against subsequent ischemia.<sup>183-187</sup> In such experiments, SD is  
14 typically provoked with KCl application to the cortex for 2-3 hr at an interval of 1-7 days prior to  
15 induction of global or focal ischemia. Compared to control hemispheres or animals, pre-  
16 conditioning results in significant reduction of the final cell death count or infarct size. The  
17 mechanism of neuroprotection likely involves upregulation of cytokines and growth factors,<sup>187-</sup>  
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189 down-regulation of metabolism,<sup>190</sup> and altered neurotransmission.<sup>191</sup>

The protective effects of SD pre-conditioning offer potential insight into mechanisms of  
ischemic tolerance, but have also been advanced as a reason for skepticism concerning the  
adverse effects of SDs that occur *after* brain injury. We note, however, that the pre-conditioning  
and post-injury concepts are entirely congruent. First, it is a general principle of physiology that  
sub-lethal challenges can build resistance and protection against subsequent threats. Thus,  
neuroprotective pre-conditioning is a phenomenon also observed with ischemia, hypoxia, and  
glutamate. Second, pre-conditioning is not applicable to an acutely developing brain injury since  
SDs are initiated by the injury and not before. An exception occurs in patients who are at risk for  
a 'second hit' within the time window (days) for pre-conditioning, as in the case of DCI after  
SAH. However, clinical data demonstrate the deleterious nature of both early and delayed SDs  
even in this condition.<sup>58, 192, 193</sup> Third, the effects of SD can be spatially disparate depending on  
local conditions of cortex, as explained above. A wave could confer a net benefit in normally

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3 perfused regions remote from an injury focus, but the same wave may trigger vasoconstriction  
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5 and persistent depolarization in the injury penumbra. Aside from pre-conditioning, remote  
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7 beneficial effects of SD may include induction of neurogenesis or facilitation of plasticity.<sup>194-196</sup>  
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10 Whether such effects influence functional recovery or counterbalance the harm of SD deserves  
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12 further study.  
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## 17 **VII. Conclusions**

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20 In view of evidence from these diverse animal models, it appears SD is a necessary and  
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22 ubiquitous mechanism for the development of pannecrotic and selective neuronal lesions of the  
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24 cerebral cortex. In every model studied to date, including global ischemia, focal ischemia,  
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26 products of hemolysis, endothelin, contusion, and hematoma, tissue fate is determined by the  
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28 occurrence of prolonged or persistent depolarization. The role of depolarizations is causal, as  
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30 shown by real-time monitoring/imaging of multiple physiologic variables during spontaneous  
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32 SDs, and also by experimental induction of exogenous SDs. Furthermore, observations from  
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34 these studies unite the historically separated phenomena of spreading depression and anoxic  
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36 depolarization into a singular class, as presaged by Leão and described as the SD continuum. The  
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38 continuum is most notably evidenced by the continuity of singular waves that transform from  
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40 persistent to progressively shorter SDs as they propagate peripherally from an initial ischemic  
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42 event, and the same full continuum of singular waves that arise spontaneously as a secondary  
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44 injury process.  
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51 Importantly, the SD continuum is not a semantic formulation, but rather is a  
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53 pathophysiologic concept that is essential for understanding how lesions develop in cerebral  
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55 cortex and some other gray matter structures. After ischemic onset, the occurrence of persistent  
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3 depolarization is the causal switch between mild ionic changes and the near-complete breakdown  
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5 of transmembrane gradients, coupled with the structural damage of cytotoxic edema. The study  
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7 of persistent depolarization in the ischemic core further informs the mechanism of secondary  
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9 ischemic lesion growth: spontaneous SDs propagate to the innermost penumbra where they  
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11 expand the core region of persistent depolarization that is fated for infarction. Finally, when it is  
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13 considered that the commitment point for cell death is reached after a finite duration, the lessons  
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15 of persistent/terminal depolarization also apply to transient depolarizations occurring in high  
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17 numbers or with prolonged durations. As illustrated in the hemolysis and endothelin models,  
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19 such repetitive SDs are a necessary and determining factor for neuronal lesion development. In  
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21 both the hemolysis model and in secondary ischemic lesion growth, SDs cause not only the ionic  
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23 and structural changes, but also the ischemic conditions (through spreading ischemia) necessary  
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25 for their lasting detrimental effects.  
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32 Results of ongoing translational research suggest that these pathophysiologic concepts are  
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34 also essential to understanding human disease. Continuous electrocorticographic (ECoG)  
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36 monitoring of more than 500 patients by the COSBID group has shown that SDs occur in a very  
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38 high proportion (55-90%) of patients with acute brain injury, and that they often occur in high  
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40 numbers for days to weeks after injury.<sup>182, 192, 197-199</sup> As in animals, SDs in patients are associated  
41  
42 with excitotoxic injury,<sup>200-202</sup> metabolic crisis,<sup>201, 203-206</sup> decreased blood flow,<sup>58, 207, 208</sup> new lesion  
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44 development,<sup>58, 192</sup> and poor outcomes<sup>182, 209</sup>. The framework of the SD continuum is necessary to  
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46 understand these events, since they often have characteristics that defy categorization in the  
47  
48 historic dichotomy of 'spreading depression' and 'anoxic depolarization'.<sup>10</sup> Furthermore, the  
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50 continuum provides a working hypothesis and informs interpretation of clinical neuromonitoring  
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52 data. For instance, the evidence from experimental stroke that each SD has detrimental effects  
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3 when it reaches the most metabolically compromised tissue, inducing decreased perfusion and  
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5 terminal depolarization, suggests that detection of any SD may be interpreted by the  
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7 neurointensivist as a warning sign that secondary injury is occurring somewhere in the brain,  
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9 even if the SD has a short duration with rapid recovery of spontaneous activity at the recording  
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11 site. This is analogous to detection of a tsunami by an ocean buoy, providing warning of  
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13 expected effects at distant shores. Experimental studies further demonstrate that SDs are an  
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15 essential link in the causal chain between triggering insult and neuronal cell death. Thus, it is a  
16  
17 fallacy to conclude that SDs in patients are an epiphenomenon based on their upstream triggers,  
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19 whether systemic insults such as hypoxia, hypotension, hypoglycemia, or pyrexia,<sup>60, 123, 210, 211</sup> or  
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21 more subtle changes in tissue microenvironment.<sup>212, 213</sup> Rather, identifying causes of secondary  
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23 SDs may lead to refinements in neurointensive care –whether medications, improved perfusion  
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25 and temperature management, or minimizing patient stimulation– that minimize the probability  
26  
27 of SDs and therefore limit damage and improve outcomes.  
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### 36 ACKNOWLEDGMENTS

37 Supported by the Mayfield Education and Research Foundation (JAH), Deutsche  
38  
39 Forschungsgemeinschaft (DFG DR 323/6-1 to JPD; DFG DR 323/5-1 to JPD, JW, OS, RG), the  
40  
41 Bundesministerium für Bildung und Forschung (Center for Stroke Research Berlin, 01 EO 0801;  
42  
43 BCCN 01GQ1001C B2 to JPD), Era-Net Neuron 01EW1212 (JPD), the Hungarian Scientific  
44  
45 Research Fund (Grant No. K111923 to EF), the Bolyai János Research Scholarship of the  
46  
47 Hungarian Academy of Sciences (BO/00327/14/5 to EF), U.S. Department of Defense (CDMRP  
48  
49 PR130373 to KCB), the National Institutes of Health (NS085413 to KCB; NS083858 to SAK),  
50  
51 and the Wellcome Trust (HICF 0510-080 to MGB).  
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**AUTHOR CONTRIBUTIONS**

JAH wrote the initial draft, conceived and prepared the figures and tables, and edited and approved the manuscript.

CWS, SAK, CA, JMH, and BF contributed to the initial draft, prepared figures, and edited and approved the manuscript.

DA, MGB, KCB, APC, MAD, CD, MF, EF, DF, RG, RH, ML, SM, AIO-F, FR, ESR, OWS, RS-P, ES, MS, AJS, AU, MBW, MKLW, OWW, and JW edited and approved the manuscript.

JPD contributed to conceptual design and initial draft of the paper and edited and approved the manuscript.

**DISCLOSURE/CONFLICT**

The authors have no conflicts of interest to disclose.

## TABLES

Table 1. Definitions of spreading depolarization and spreading depression

<b>Spreading depolarization</b>	A self-propagating wave of abruptly developing, near-complete breakdown of transmembrane ion gradients in neurons and astrocytes <i>en masse</i> that spreads through contiguous cerebral gray matter at a typical velocity of 2-5 mm/min (range: 1-9 mm/min) and is locally sustained for at least 15 sec. Depolarization can be recorded as a negative shift of the extracellular direct-current potential (frequency range <0.05 Hz).
<b><u>Modifiers</u></b>	Spreading depolarization can be characterized along the continuum with further descriptors. These typically apply to the wave only at the point of monitoring, as characteristics vary along the spatial path of propagation according to local baseline conditions, particularly in brain injury. The following adjectives are listed as suggestions and do not imply distinct classes or subtypes.
<i>Transient</i>	Indicates finite duration of depolarization with confirmed tissue repolarization. Durations may vary.
<i>Short-lasting</i>	Indicates a duration that is observed in intact, normally perfused brain, typically in the range of ~20 to 180 sec depending on species and recording method
<i>Prolonged or long-lasting</i>	Indicates a duration longer than that observed in intact, normally perfused brain and suggests some degree of dysfunction or metabolic compromise that delays repolarization
<i>Persistent</i>	Indicates that depolarization is indefinitely prolonged and tissue will not repolarize unless conditions change. Persistence refers to the depolarization duration of an individual wave in a local region of monitored tissue, and should not be interpreted to indicate multiple repetitive waves or to describe the spatial extent (or duration) of a wave's travel through adjacent tissues.
<i>Terminal</i>	The meaning is similar to persistent, but indicates specifically that repolarization did not occur or that depolarization persisted long enough for tissue infarction.
<i>Anoxic, asphyxial, or aglycemic</i>	Used historically to describe the initial spreading depolarization that occurs in tissue subjected to the corresponding insult. In such tissue, depolarization is persistent as long as the insult continues. For focal insults, the depolarization becomes transient, repolarizing without a change in conditions, when it spreads to tissue beyond the insult.
<b>Spreading depression</b>	A wave of depression in spontaneous activity of the electrocorticogram (>0.5 Hz) that propagates through contiguous cerebral gray matter at a typical velocity of 2-5 mm/min. Spreading depression is a consequence of spreading depolarization.
<b><u>Uses</u></b>	
<i>Historical/migraine</i>	Current use of the term in migraine research, and historical use throughout neuroscience, also implies the spreading depolarization of neurons/astrocytes (and corresponding negative shift of extracellular DC potential) that causes the depression of spontaneous activity. For purposes of historical review, spreading depression is used in this broad sense throughout the manuscript unless further specified. Spreading depression has also historically implied a short-lasting transient depolarization in normal brain with benign consequence. <sup>214</sup>
<i>Brain injury</i>	In the injured brain, spreading depolarization often occurs in tissue that is already electrically silent and the electrocorticogram (> 0.5 Hz) cannot be further depressed. Therefore, spreading

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3 depolarization is not always accompanied by spreading depression and the terms are not  
4 synonymous. The differentiation between spreading depolarizations with and without spreading  
5 depression has diagnostic and prognostic significance.<sup>10, 182, 197, 198, 209</sup> To allow this distinction, we  
6 recommend the term spreading depression be used only to describe the depression of spontaneous  
7 activity induced by spreading depolarization. Although spreading depression will always be  
8 accompanied by spreading depolarization, the converse is not true and the terms should not be used  
9 interchangeably when ambiguity may result. In contrast to historic use, spreading depression of the  
10 electrocorticogram in the injured brain may be prolonged or persistent.  
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**Table 2. Evidence for the role of spreading depolarizations in penumbral expansion of focal ischemic lesions**

*Correlational evidence*

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- 1 Greater burden of spontaneous SDs is associated with larger infarct volumes<sup>29, 31, 63</sup>
  - 2 Therapies that inhibit spontaneous SDs also reduce infarct volumes<sup>121-129</sup>
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*Proofs of causality*

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- 1 SDs that are elicited remote from lesion and propagate into penumbra increase infarct volumes<sup>60, 130-132, 144</sup>
  - 2 Ischemic core expansion is time-locked to SD entering penumbra, as visualized with serial DW-MRI and NADH fluorescence imaging<sup>63, 130</sup>
  - 3 Electrophysiologic recordings of delayed, terminal depolarization induced by propagating SD<sup>31, 63-66</sup>
  - 4 Blood flow recordings demonstrating persistent perfusion decreases induced by SD; corresponding expansion of ischemic zone<sup>46, 49, 59, 60, 120, 137, 139</sup>
  - 5 Demonstration of terminal dendritic beading of penumbral neurons induced by repetitive SDs<sup>143</sup>
- 

**Epiphenomenon** (noun): a secondary effect or byproduct that arises from a disease but does not causally influence it

SD=spreading depolarization; DW-MRI=diffusion-weighted magnetic resonance imaging; NADH=nicotinamide adenine dinucleotide

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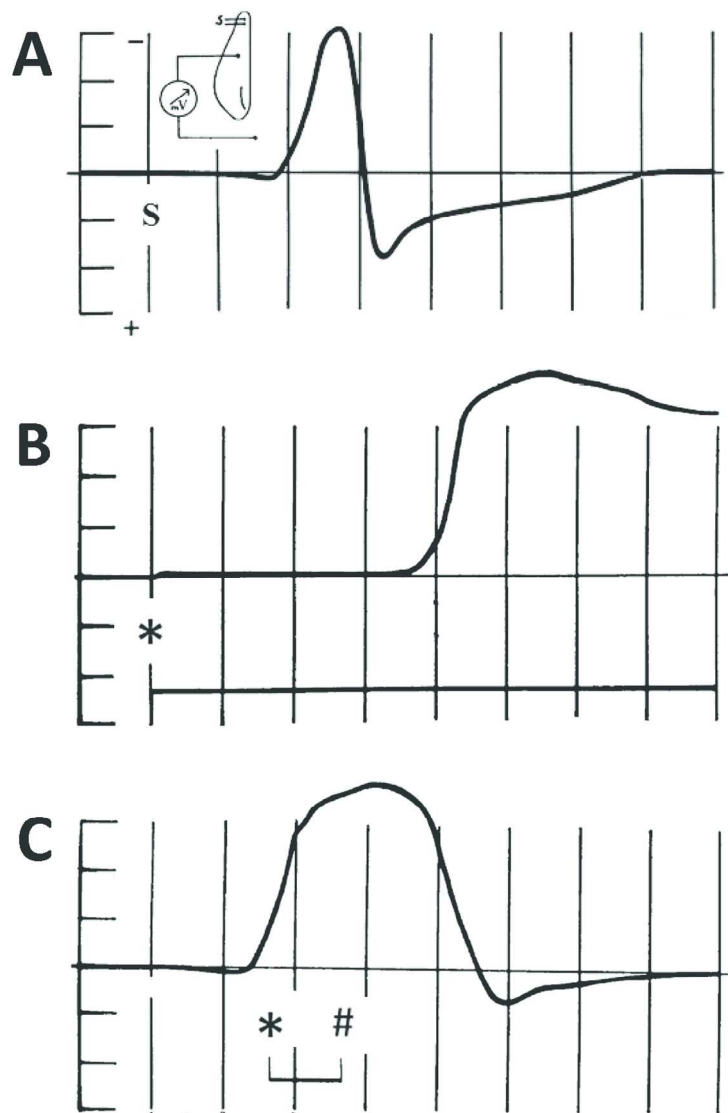


Figure 1. Direct-current potential shifts in spreading depression and after ischemia are of the same nature. In all traces, negative is up, amplitude divisions are 3 mV, and time divisions are 1 min. (A) The original tracing published by Leão showing the initially negative slow voltage variation that accompanies spreading depression.<sup>1</sup> Spreading depression was elicited by application of a tetanic current to the cortex at time marked 'S'. Inset shows recording and stimulation schematic. (B) In the same paper, he showed a similar but persistent negative slow voltage variation several minutes after clamping (denoted by \* in the trace) of the common carotid and basilar arteries. (C) The similar nature of these phenomena was further demonstrated by inducing spreading depression with electrical stimulation as in (A) and then clamping the arteries for 1 min (from \* to #) as soon as the slow voltage variation of spreading depression was observed. In this case, the negative slow voltage variation of spreading depression was prolonged. Subsequent studies showed that both spreading depression and the response to ischemia are spreading mass depolarizations that are dependent on energy supply for reversal. In the experiment shown in (C), we further find a prediction of how spreading depolarization causes infarct growth: if the depolarization itself, rather than an



investigator's hand, can worsen ischemia, the tissue should remain depolarized. Adapted from Leão (1947)<sup>1</sup>  
with permission.  
100x148mm (300 x 300 DPI)

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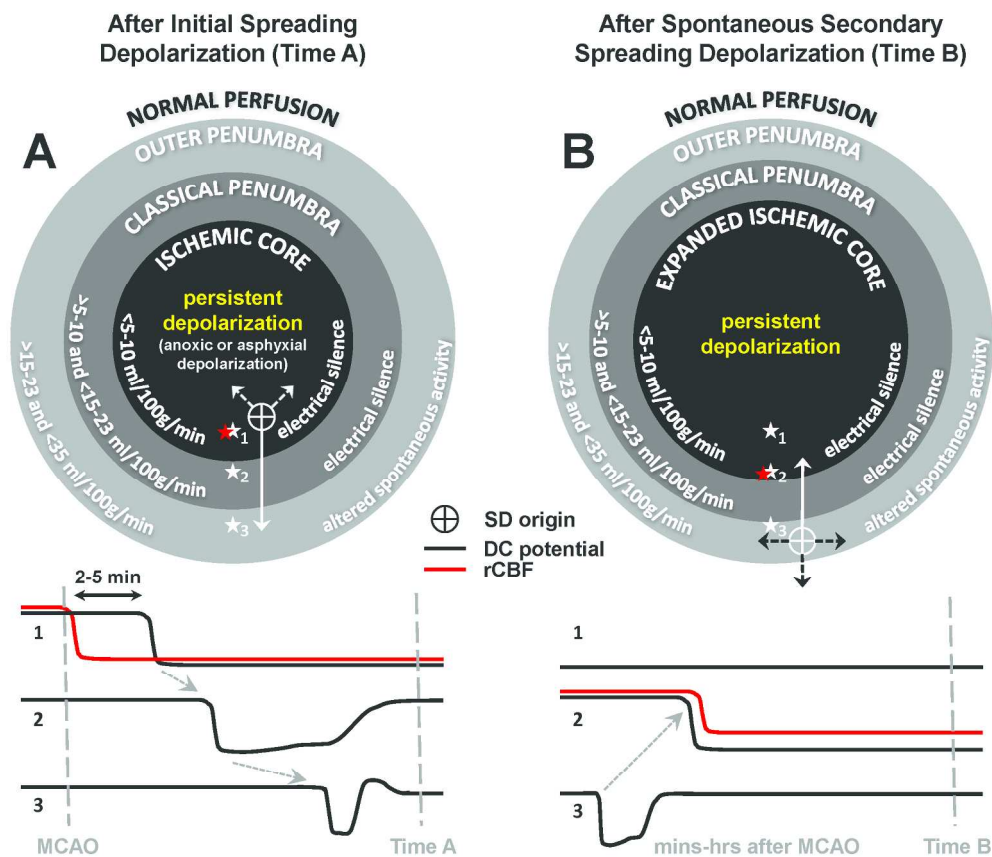


Figure 2. Persistent depolarization defines the ischemic core and causes initial and delayed secondary infarct development. Schematic diagrams illustrate various zones of focal ischemia, such as middle cerebral artery occlusion (MCAO), defined by electrophysiologic function and regional cerebral blood flow (rCBF). Direct current (DC) potential recordings (black) of spreading depolarizations and rCBF (red) are shown below for the three monitoring locations indicated by stars. The diagram in (A) depicts the status of ischemic zones after passage of the initial spreading depolarization (Time A). Within seconds of ischemic onset, a broad area with rCBF < 15-23 ml/100g/min simultaneously develops (in a non-spreading manner) complete suppression of the electrocorticogram (electrical silence). After 2-5 min, spreading depolarization then develops, as shown for instance by the cross-hairs, in a more central zone where rCBF is < 5-10 ml/100g/min. Here, the depolarization is persistent and will be terminal if perfusion is not restored. The zone of persistent depolarization and rCBF < 5-10 ml/100g/min is defined as the ischemic core, and the zone just beyond the core with electrical silence is the classical (inner) penumbra. The initial depolarization spreads through penumbra (arrows) and into normally perfused tissue as a transient wave with decreasing duration determined by local rCBF. (B) In the subsequent course of minutes, hours, or even days, spreading depolarizations arise repeatedly in penumbral hot zones as a result of local energy supply-demand mismatch. These hot zones may be located in the outer penumbra (oligemic tissue with preserved spontaneous activity) or in the inner penumbra. In the former case, as illustrated, the depolarization propagates not only outward, but also inward toward the ischemic core, inducing persistent depolarization and further perfusion decrease in the innermost penumbra (recording location 2). Thus, the ischemic core and region of developing infarction increase in a step-wise manner after the passage of each spreading depolarization (Time B). Note that spreading depolarizations that are prolonged or recur in high numbers can have the same cumulative effect as a single persistent depolarization, reaching the threshold duration of ionic derangement and metabolic impairment that commits neurons to cell death.

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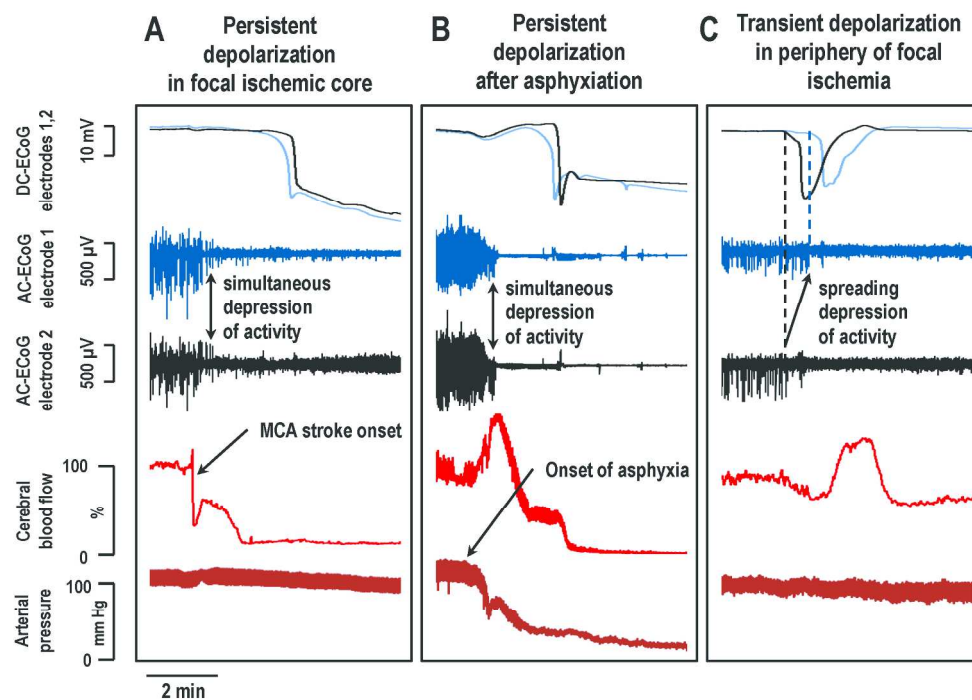


Figure 3. The spreading depolarization continuum unites anoxic (persistent) depolarization and spreading depression. (A) After middle cerebral artery occlusion, simultaneous suppression of the electrocorticogram (AC-ECoG, 0.5-70 Hz) develops within seconds in the ischemic core and classical penumbra. This is followed  $\sim$ 2 minutes later by persistent depolarization that develops in a spreading manner, as revealed by a negative DC shift of the cortical potential (DC-ECoG). (B) The same sequence of events occurs after asphyxiation, as shown in global ischemia by Leão.<sup>1</sup> (C) In focal ischemia, the initial spreading depolarization propagates into the normally perfused periphery, where it induces spreading depression of spontaneous AC-ECoG activity.<sup>2</sup> Here, asphyxiation was induced by reducing inhaled oxygen to 0% and focal ischemia was induced by injecting a blood clot near the origin of the middle cerebral artery.<sup>3</sup> ECoG was recorded from two glass micropipette electrodes separated by 2 mm and cerebral blood flow was monitored by laser Doppler flowmetry.

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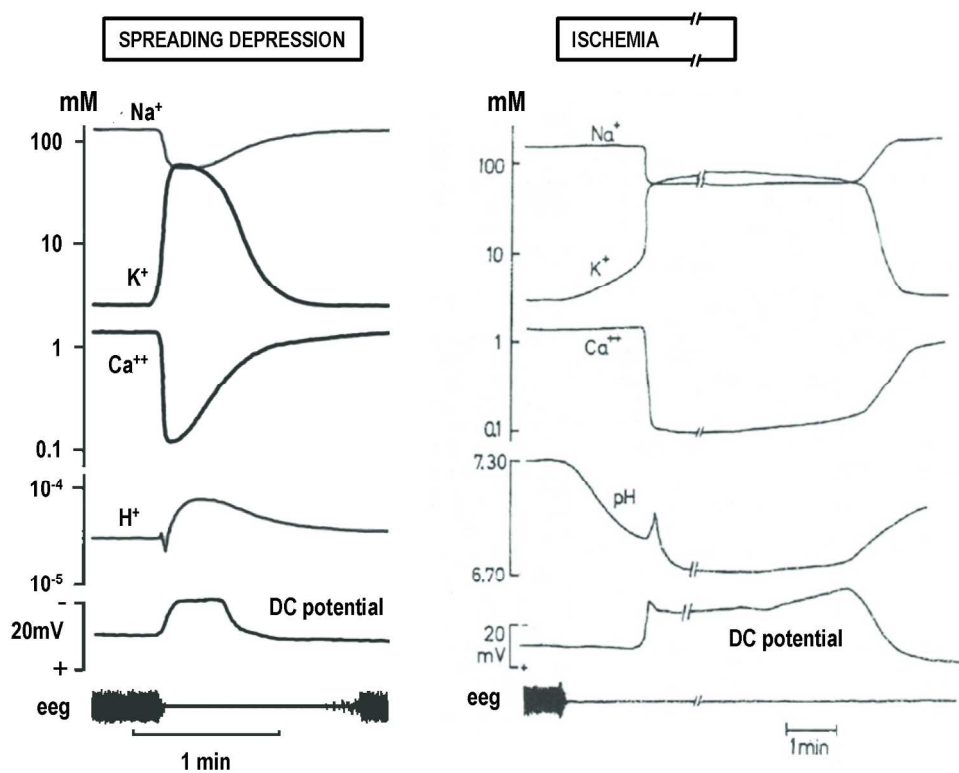


Figure 4. Changes in extracellular ion concentrations in spreading depression and asphyxial depolarization. Extracellular ion concentrations change to a similar degree and with similar time course during spreading depression (historical use, Table 1) in the normal brain and during the spreading depolarization that develops after asphyxiation. In the latter case, depolarization persists until cerebral perfusion is restored. A notable exception between the two cases is the gradual rise in  $[K^+]_o$  and decline of pH prior to the abrupt shifts during asphyxial depolarization. Adapted from Hansen and Lauritzen (1984).<sup>4</sup>  
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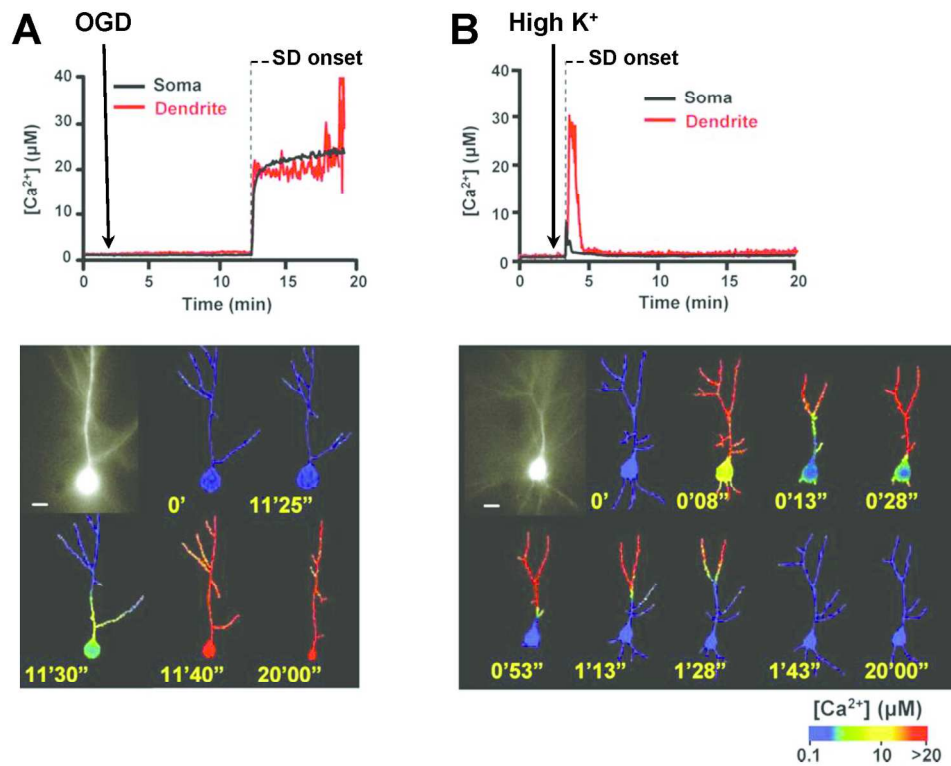


Figure 5. Spreading depolarizations evoked in hippocampal slices by oxygen-glucose deprivation and by high  $K^+$ . CA1 neurons were loaded with  $Ca^{2+}$  indicator fura-6F. The first image panel shows raw 380 nm fluorescence and subsequent panels are pseudocolor images that represent  $[Ca^{2+}]_i$ . (A) Following oxygen-glucose deprivation, there is no increase in  $Ca^{2+}$  in  $\sim 11.5$  min prior to onset of spreading depolarization (SD). After SD, there is a large irrecoverable  $Ca^{2+}$  increase ( $\sim 24 \mu M$ ) that originates in the soma and progresses toward apical dendrites, resulting in rapid neuronal injury. (B) SD evoked by high  $K^+$ , by contrast, produces a transient  $Ca^{2+}$  elevation in distal dendrites that propagates toward, but never fully involves the soma, and  $[Ca^{2+}]_i$  returns to basal levels in  $< 2$  min without neuronal injury. SD propagation rates are similar in the two conditions. Initiation and propagation of SD evoked by high  $K^+$ , but not by oxygen-glucose deprivation, is dependent on the intracellular  $Ca^{2+}$  influx and can be prevented by  $Ca^{2+}$  removal from the bath, illustrating a mechanistic difference that arises along the continuum. Reproduced from Dietz et al. (2008)5.

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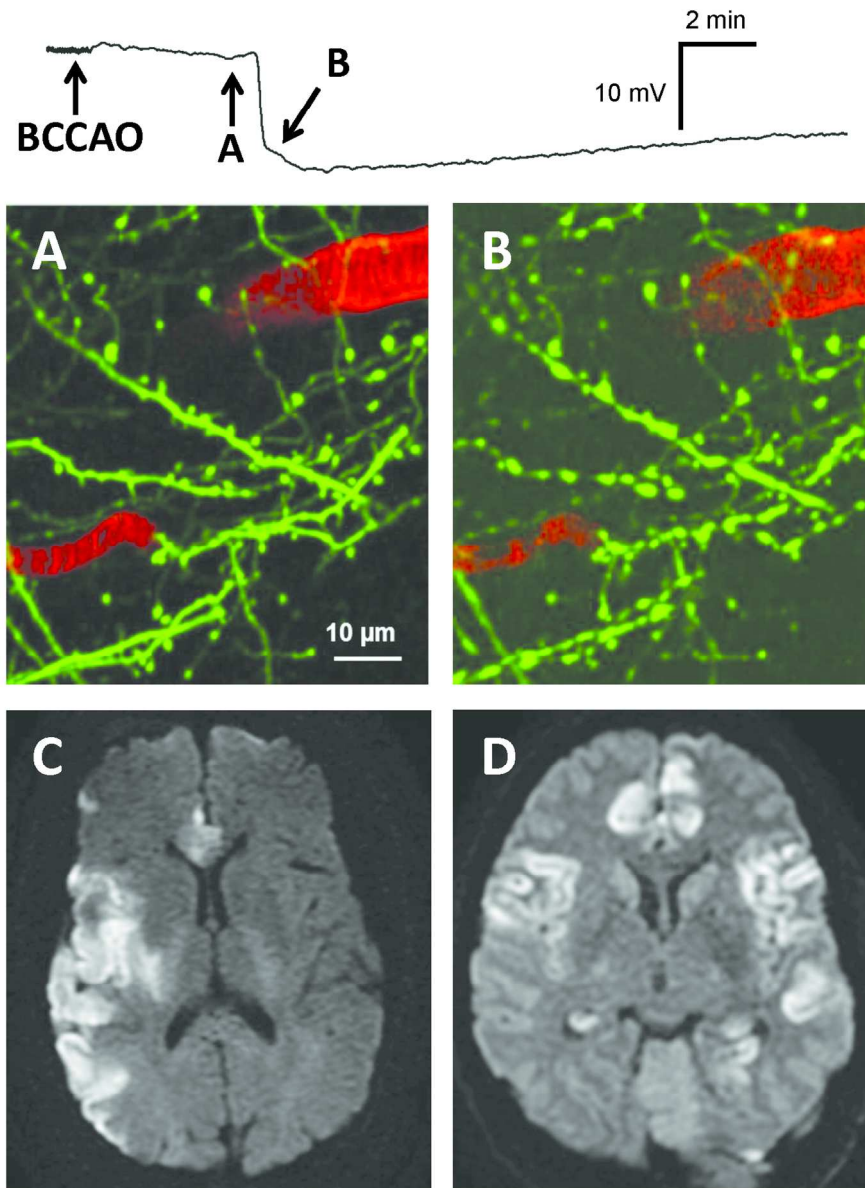


Figure 6. Cytotoxic edema consequent to persistent depolarization is the basis of diffusion lesions in clinical imaging of stroke. (A) and (B) show two-photon images of apical dendrites in superficial layers of mouse cerebral cortex following bilateral common carotid artery occlusion (BCCAO) at times shown in DC potential recording above. The recording shows the onset of spreading depolarization in the imaging field 4 min 30 sec after BCCAO. Normal dendritic morphology with spines, which are sites of excitatory synaptic transmission, is observed for the first minutes after BCCAO, but spines disappear and dendrites become beaded after the tissue depolarizes. Image in (B) is taken 26 sec after depolarization onset. (C) and (D) show diffusion-weighted MRI scans from patients with ischemic stroke and aneurysmal subarachnoid hemorrhage. The scan in (C) was taken within 48 hr of cardioembolic stroke in the right middle and anterior cerebral artery territory of a 41-year old woman with initial NIH stroke scale of 16. The scan in (D) was obtained 3 days following rupture of a basilar tip aneurysm in a 57-year old man who presented as Hunt-Hess grade 4 and modified Fisher grade 2. At this time, transcranial Doppler showed moderate-severe vasospasm in the right and mild-moderate vasospasm in the left middle cerebral arteries. Hyperintense

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regions reflect decreases in apparent diffusion coefficient caused by cytotoxic edema, dendritic beading and restricted intracellular diffusion of water. Two-photon imaging studies suggest that these changes reflect the occurrence of persistent tissue depolarization.

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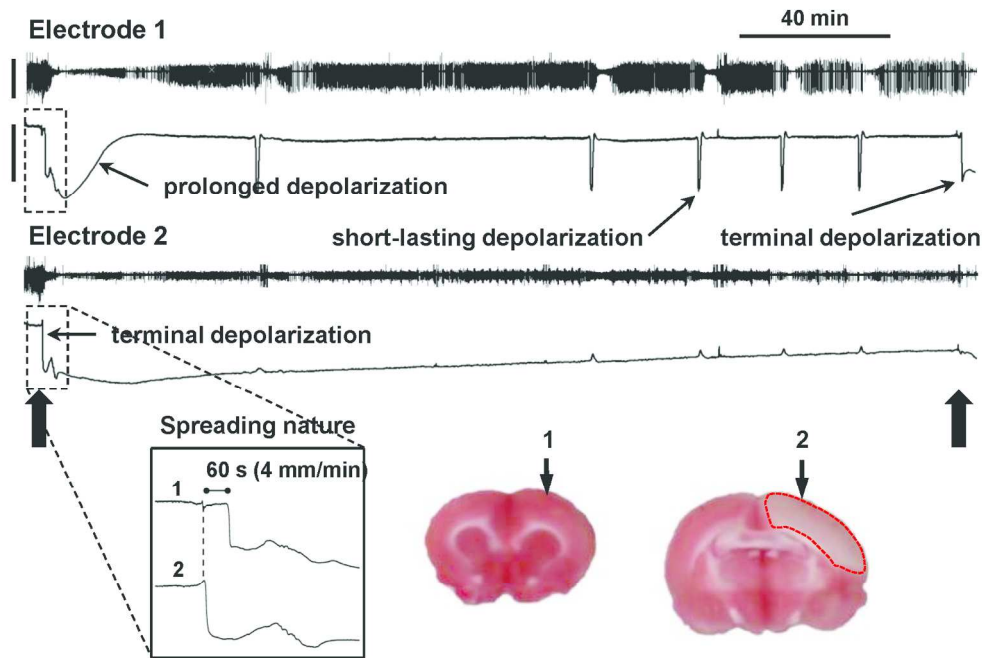


Figure 7. The continuum of spreading depolarizations in a rat model of acute subdural hematoma. Electrophysiologic recordings of spontaneous electrocorticographic activity (top traces; 0.5-50 Hz) and DC potential (bottom traces) from two micropipette electrodes in cerebral cortex. Slow infusion of 0.4 ml of arterial blood into the subdural space (large arrow, left) causes an immediate depolarization that begins at electrode 2 and spreads to electrode 1 at 4 mm/min. At electrode 2, the depolarization is persistent through 4 hr of monitoring and TTC-staining confirms tissue infarction at this location. The initial depolarization at electrode 1 is prolonged with recovery after 15 min, and spontaneous short-lasting depolarizations are subsequently observed. When the animal is killed by asphyxiation after 4 hr (large arrow, right), terminal depolarization is observed in viable cortex (electrode 1) but not in the infarct core. Scale bars are 1 mV and 20 mV for top and bottom traces, respectively, and apply to both electrodes.

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