Electrical prodromals of spreading depression void Grafstein’s potassium hypothesis

To the Editor: Anthony Strong recently essayed (Strong 2005) on Grafstein’s classic manuscript (Grafstein 1956) that launched the potassium hypothesis for the propagation of spreading depression (SD). Strong maintains that her conclusions are still valid today. However, our team and others have refuted it some time ago. The potassium hypothesis is no longer valid and a number of results have accumulated that question an essential role for potassium at any time on SD. Because Grafstein’s is still a very influential manuscript, an updated appraisal may be appropriate, so as not to mislead present and future workers in the field.

Since Leão’s initial description (Leão 1944), a confusion too often found in the literature is to assume that the mechanism of SD movement can be grasped by looking into its main phase. However, it has long been established that SD is a complex chain of tightly bound events (Marshall 1959) with different thresholds, which may share or not share their macroscopic, cellular, and subcellular mechanisms. The advancing front and the main phase can be dissociated in several ways (see e.g., Herreras and Somjen 1993a). Also, many authors reported “patches” of depression that remained motionless in the elicitation locus (e.g., Largo et al. 1997). In Grafstein’s hypothesis, intense neuron firing, potassium elevation, and excitation of nearby neurons constitute the crucial cycle of events for SD movement and also accounted for the subsequent major neuron depolarization. Its long-standing success must be credited to the early finding of the associated interstitial potassium flood, which conditioned the interpretation of subsequent findings, and to the simplicity of these biophysical relations: an excitatory extracellular moraine fed by the neurons themselves (the same principle underlies the glutamate hypothesis; see Van Harreveld 1959). A number of results, scarcely mentioned in the literature, undermine the potassium hypothesis. We enlist here only a few: 1) tetrodotoxin blockade of neuron firing causes no change to SD, and thus neuron firing is not required (Sugaya et al. 1975). 2) Voltage clamping does not avoid SD-related membrane conductance (Czéh et al. 1993); initial excitation is thus not a requisite (see Somjen et al. 1991). 3) SDs may change into spreading convulsions moving at the same speed (Van Harreveld and Stamm 1953), which differentiates spreading and inactivating mechanisms. 4) Potassium and DC voltage signals follow a similar temporal course but opposite changes in magnitude (Herreras and Somjen 1993b). 5) Our latest most surprising finding shows that neurons undergo longitudinal gradients of depolarization, which are explained by the zonal dendritic opening of a large ion conductance, new equilibrium potentials, and axial currents (Canals et al. 2004), not potassium levels.

The best refutation of Grafstein’s hypothesis comes from our lab (Herreras et al. 1994). We found in the hippocampal CA1 a synchronization of the initial firing among nearby neurons that presented as a high-frequency burst of population spikes ahead of the DC negativity. Even more striking is the presence of an earlier subthreshold pacemaker field oscillation seconds ahead of the potassium flood and negative potential. All this activity takes place milliseconds away from the depolarizing front and is resistant to synaptic transmission blockade. These and other electrical peculiarities led us to propose direct neuron-to-neuron communication, possibly by gap junctions, to bring cells into synchronic operation and would also offer a transcellular pathway for SD propagation. The essential view of a reaction–diffusion process still holds, but available results, including Grafstein’s, are compatible with potassium being a mere coadjuvant on the chain reaction.

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Oscar Herreras
Departamento Investigación-Histología
Hospital Ramón y Cajal
Madrid, Spain
E-mail: oscar.herreras@hrc.es

REPLY

To the Editor: In a letter to the Editor Oscar Herreras criticizes the Editorial Focus Essay by Anthony Strong (2005) concerning the classic paper by Grafstein (1956), “Mechanism of spreading cortical depression.” Specifically, Herreras objects to the statement that Grafstein’s potassium theory of spreading depression (SD) is still valid today. Actually, he has a point.

Of the several arguments marshaled by Herreras one stands out. If one records interstitial K+ concentration with an ion-selective microelectrode in normally perfused and oxygenated cerebral gray matter, an oncoming wave of propagating SD is usually not preceded by an increase in [K+]o. Rather, [K+]o increases nearly simultaneously and at the same relative rate as does the shift of extracellular voltage (for sources see Somjen...
2001, 2004). Only when the energy supply is inadequate, or active transport of ions is blocked, or the tissue is cooled, do we see \([K^+]_o\), increasing ahead of the large-amplitude, accelerating negative extracellular sustained potential (SP), and the depolarization of neurons that causes the SP shift. The absence of a prodromal increase in \([K^+]_o\) seems to preclude the idea that diffusion of \(K^+\) ions in interstitial space is the agent mediating the propagation of SD.

The numerous other data mentioned by Herreras are all important, but none refutes the \(K^+\) theory. For example, the fact that tetrodotoxin (TTX) does not block SD carries little weight in this respect because \(K^+\) evidently does flow out of neurons during SD in the presence of TTX by mechanisms other than action potentials. The impulse showers at the onset of SD were already described by Grafstein (see Fig. 2 in Strong 2005). Herreras emphasizes that synchronized subthreshold high-frequency oscillations and sometimes also impulse showers precede the increase in \([K^+]_o\). This does indeed suggest a propagating wave of electrical interaction among neurons, although similar synchronized activity can occur without subsequent SD. When SD does follow the synchronized activity, there has to be some additional mediating or triggering event.

Still, even if we accept that normoxic SD does not propagate by means of the diffusion of \(K^+\) ions, Grafstein’s assertion linking the release of excess \(K^+\) from excited neurons to SD generation attests of inspired insight. In 1956 Grafstein could not measure \(K^+\) in tissue; she based her hypothesis on the careful analysis of other data. Then, a few years later, the massive release of \(K^+\) was confirmed by the Czech team (Krivánek and Bureš 1960; Vyskocil et al. 1972).

To appreciate the role of \(K^+\), one must consider separately the mechanism of SD propagation from the mechanism of its generation. Marshall (1959) already emphasized that SD need not propagate. “Stationary spreading depression” might sound like a contradiction in terms, but this is a problem of semantics, not of principles. There can be little doubt that the redistribution of \(K^+\) is an essential component in the positive feedback chain that produces SD, even if it is not responsible for its initiation and its spread. A substantial amount of experimental data and computer simulations support this role of \(K^+\).

The ignition of SD requires first a slowly inactivating inward membrane current (Kager et al. 2002). The main charge carrier of this current is Na\(^+\), with Ca\(^{2+}\) usually but not necessarily playing an important ancillary role. Under ordinary conditions, the inward current is carried through several simultaneously activated ion channels. Blocking any one of them can delay but not prevent SD (Müller and Somjen 1998). Some but not all the Na\(^+\) is accompanied by Cl\(^-\). Entry of Na\(^+\) without an anion would force \(K^+\) out of the cells in any event, but the ensuing depolarization and concomitant Ca\(^{2+}\) entry open various \(K^+\) channels, greatly favoring its exit. The accelerating tidal release of \(K^+\) causes additional depolarization, resulting in the nearly total neuron depolarization. The release of \(K^+\) is an essential factor giving SD its all-or-none characteristic.

With respect to the mechanism of SD propagation, there are at least four competing hypotheses extant (for review see Somjen 2001, 2004). One of them has been suggested by Herreras (for references see his letter to the Editor). It is based on the concept of a wave of the opening of previously closed gap junctions among neurons. Computer simulations by Shapiro (2001) make his scheme plausible.

Grafstein’s experiments were conducted on cat neocortex, whereas Herreras and coinvestigators work in hippocampal formation of rats. Differences in cytoarchitecture and in the distribution of ion channel types modify the process, but the essential features of the biophysical mechanism generating SD appear to be identical in the two regions and in all species.

In conclusion, the outflow of \(K^+\) is an essential element in the SD process but it is not starting or spreading it. It was Grafstein (1956) who first suggested a critical role for \(K^+\).

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George Somjen
Departments of Cell Biology and Neurobiology
Duke University Medical Center
Durham, North Carolina
E-mail: g.somjen@cellbio.duke.edu

Anthony Strong
Departments of Cell Biology and Neurobiology
Department of Clinical Neuroscience
King’s College
London, United Kingdom
E-mail: anthony.strong@kcl.ac.uk