CORTICAL SPREADING DEPOLARIZATIONS
Pathophysiology and clinical implications

Author: Arturo Sánchez Chillón

Director: Dr. José Luis Fernández-Torre
Head of Clinical Neurophysiology at:
- Marqués de Valdecilla University Hospital

Santander, June 2018
## INDEX

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Abstract</td>
<td>7</td>
</tr>
<tr>
<td>2. Introduction</td>
<td>8</td>
</tr>
<tr>
<td>3. Objectives</td>
<td>11</td>
</tr>
<tr>
<td>4. Discussion</td>
<td></td>
</tr>
<tr>
<td>A. Some brief and clarifying definitions: who is who in the spreading</td>
<td>12</td>
</tr>
<tr>
<td>depolarizations?</td>
<td></td>
</tr>
<tr>
<td>• Spreading depolarization (SD), negative DC shift &amp; spreading</td>
<td></td>
</tr>
<tr>
<td>depression</td>
<td></td>
</tr>
<tr>
<td>• Persistent spreading depression of activity &amp; non-spreading</td>
<td>13</td>
</tr>
<tr>
<td>depression</td>
<td></td>
</tr>
<tr>
<td>• Anoxic depolarization; spreading hyperemia, oligemia and ischemia</td>
<td>14</td>
</tr>
<tr>
<td>• Negative ultraslow potential (NUP) &amp; spreading convulsion</td>
<td>16</td>
</tr>
<tr>
<td>B. Molecular and cell biology: genesis and pathophysiologic processes</td>
<td></td>
</tr>
<tr>
<td>• Metabolism: igniters, ionic cataclysm, molecular pathways and</td>
<td>16</td>
</tr>
<tr>
<td>modifiers</td>
<td></td>
</tr>
<tr>
<td>• Astrocyte role</td>
<td>23</td>
</tr>
<tr>
<td>• Normal and inverse hemodynamic response</td>
<td>25</td>
</tr>
<tr>
<td>• Deleterious effects: cytotoxic edema and neuronal death</td>
<td>29</td>
</tr>
<tr>
<td>C. Movement patterns through time and space</td>
<td>31</td>
</tr>
<tr>
<td>D. Clinical correlation:</td>
<td></td>
</tr>
<tr>
<td>• Stroke</td>
<td>33</td>
</tr>
<tr>
<td>• Subarachnoid hemorrhage</td>
<td>36</td>
</tr>
<tr>
<td>• Intracerebral hemorrhage</td>
<td>37</td>
</tr>
<tr>
<td>• Cranioencephalic trauma</td>
<td>38</td>
</tr>
<tr>
<td>• Migraine with aura</td>
<td>40</td>
</tr>
<tr>
<td>• Epilepsy</td>
<td>43</td>
</tr>
<tr>
<td>• Other diseases related to CSD</td>
<td>44</td>
</tr>
<tr>
<td>• CSD in dying patients</td>
<td>44</td>
</tr>
<tr>
<td>• Detection and register methods: importance in neurocritical care</td>
<td>45</td>
</tr>
<tr>
<td>• Current and future therapeutic applications: pharmacology of CSD</td>
<td>47</td>
</tr>
<tr>
<td>5. Conclusions and striking messages</td>
<td>49</td>
</tr>
<tr>
<td>6. References</td>
<td>50</td>
</tr>
<tr>
<td>7. Acknowledgements</td>
<td>53</td>
</tr>
</tbody>
</table>
1. ABSTRACT

A cortical spreading depolarization consists on a sudden, transient, generally lessive and self-propagating wave of sustained depolarization in neurons and glia, which silences their function, due to a near-complete break-down of the transmembrane ionic gradients generated mainly by the Na⁺/K⁺-ATPase activity and ion-specific non-gated channels.

It self-propagates through the cerebral grey matter, without respecting functional or vascular divisions, causing a depression in spontaneous cerebral activity and inducing an inverse vasoconstrictive hemodynamic response. Genysis of the CDS consists on either mechanical or neurochemical stimulus.

CDS are observed in clinical frames where we find oxigen, glucose and other metabolites deficit such as craniocerebral trauma, subarachnoid hemorrhage, intracerebral hemorrhage, stroke (e.g. malignant hemispheric stroke) or migraine aura. It’s been also linked to epilepsy.

The gold-standard to register spreading depolarizations and associated electric events is the electrocorticography with subdural electrodes. The hallmark evidence of CDS in this exam is the negative direct current (DC) shift.

CDS are potentially reversible, but if the metabolic impairment persists too long they ignite the cascade to neuronal death. The more its number and longer, the worse the outcome. Specific-targeted drugs aiming to this process present as a great promise for stopping CSD-related consequences such as infarct expansion and cytotoxic edema.

Key words: depolarization, depression, vasoconstriction, infarct, aura
Back in the ancient ages, Roman Empire, in a time where the entertainment circled around new lands conquering and bacchanalia, physician of the first century Scribonius Largus, despite being maybe the first impulser of medical humanism, strongly believed that a deer’s liver dead by a weapon used to kill a gladiator had properties to cure epilepsy. This was soon followed by people jumping over the arena to drink the fresh blood of dead gladiators hoping that would cure their “sacred disease” (epilepsy). Until late XIX ce., this practice survived, specially in scandinavian countries and Germany, where crowds would queue to drink the blood of the recently decapitated.

In a period where mankind and medical knowledge were in the cradle, great part of diseases were associated to supernatural causes like gods or demons. We could easily think people in antiquity made no effort and were complete ignorants of the world that surrounded them. But the fact is that they tried, hard, like us, to understand what they saw according to the knowledge and tools they had then.

One of the first testimonies we have of strokes is found in the Psalms book in The Bible: “may my right be forgotten, may my tongue stick to my palate”, describing the symptoms of a hemiparesia. Even among the 37 miracles Christ, described in the Gospel, three were the healing of paralytic processes possibly due to a stroke.

In the pharaonic empire, egyptians used to extract the brain through the nose in mummification given that they believed it had no function. We find some of the first descriptions of ictal episodes in the Edwin-Smith papyrus. In the assyrian-babylonic culture, the Azu were the physicians tasked with healing thorough physical methods but the Ashipu were the physicians-priests committed to release the patients from demons that caused sacred-origin diseases, e.g. summoning god Ea against Pazuzu, lord of wind and bringer of storms, or Labarthu, the body possessor [1].

From the 500 a.C. and on a something changed in the thought thanks to the greek civilization, a drift from Mythos to Logos, where physicians started to rationally recognize nature as it is and use the observation (autopsy) and experience rather than religious beliefs. Alcmaeon of Croton, then Hippocrates and his school were the first ones to describe the brain and its superficial vasculature. Later on, Galen wouldablish the sudden paralysis as the most conspicuous symptom of apoplexy. In the middle of the heart of Islamic Medicine, Moshe Ben Maimon, also know as Maimonides, set the basis for stroke care in acute phases and rehabilitation [1]. Proud he would be of the issue that the present text verse about.

In Dante’s Divine Comedy (1307) we appreciate also a number of symbolic references to neurological diseases such as epilepsy, dystonia, amaurosis fugax or migraine aura [52]. Later, in 1549 Jason Pratensis published the first specialized book in Neurology, where he firstly related semiology, pathogenia and treatment. Nevertheless, tinctures of astrological and magical galenic ideas were present.

Of course during all history insane healing methods and practices never stopped. For example in XVII ce. people used to eat the molds (Usnea) stick to the unburied human skulls in order to treat epilepsy or menstruation problems.

Baroque. Sir Thomas Willis described the vascular circle that holds his name and also made a classification of ictus [1]. His work marked an inflection point that would lead to an age where neurological knowledge flourished leading us where we stand today.
This brief compilation of curious historical data, far from being a history treatise, pretends showing to the reader how some diseases we are going to discuss from the view-point of CDS, have always been present.

A question that has atributed the human spirit since the beginning of times is: where do we come from? Which is the origin of what we perceive with our senses? We are yet too far to understand the functioning and internal language of our brain but for now, we are approaching to solve one of the biggest challenges that cerebrovascular disease, migraine or cranoencephalic trauma present: cortical spreading depolarizations. Responsibilities for the most savage deleterious secondary effects of brain injury such as cytotoxic edema or infarct expansion. The next story tells how during last decades this new phenomena has been in constant elucidation:

In 1940, brazilian physiologist Aristides Leão [32] discovered by first time the spreading depression when he was running an electrocorticogram in a rabbit. He also did discovered and coined the therm non-spreading depression, the propagating slow potential change along with the normal neurovascular coupling response (hyperemic response) to CSD and a phenomenon that would be later called spreading convulsion, by van Harreveld and Stamm.

The dutch Dr. Antoine van Harreld was the first one to describe and record a large slow pontential change in the asphyxic spinal cord and linked it to mass depolarization [4]. He also found that cystotoxic edema is induced mainly by spreading depolarization and is related to a massive release of glutamate.

Dr. Bernice Grafstein hypothesized that potassium plays a key role in the self-propagation patter of CSD, as we shall see. Later on, other researches started to contribute their grain of sand. In the 70s, Dr. Sramka succeeded in recording potassium induced SD in caudate nucleus of humans and hippocampus. In the early 80s, Dr. Lauritzen, Olensen and Larsen give the first recording of a normal neurovascular response in patients with migraine aura.

An important link between CSD and epilepsy was made in the early 90s when Avoli et al. found spreading depolarizations in brain slices of patients with intractable epilepsy. Little bit latter, middle of 90s, we find the first electrophysiological evidence in patients with traumatic brain injury and later a connection was established between familial hemiplegic migraine type I and CADASIL to spreading depolarizations and the inverse neurovascular response was discovered.

In 2002, Dr. Anthony Strong and colleagues [50] are the first ones to develop a new methodological approach to register these events in brains undergoing through traumatic injury or intracerebral hemorrhage. After a meeting of the interested researchers in Copenhagen in 2003, the Co-Operative Studies on Brain Injury Depolarizations (COSBID) group was stablished with the primary aim of summoning the knowledge about this topic and provide a standarized method to register these depolarizations as well as to study its consequences in patients.
In Latin, the Spanish term *ictus* means “stroke” or “hit”. The origin of this Anglo-Saxon term comes from the quote “Stroke of God’s Hand”. Nowadays, the international COSBID group of physicians and researchers in basic and clinical sciences think that if we keep digging and investigating this issue, soon enough we’ll be able to counterattack with a great hit this pathologies improving the patient care and diminishing the secondary effects that worsen the outcome.

“And she to me: There is no greater sorrow
Than to be mindful of the happy time
In misery, and that thy Teacher knows.”

(Inferno, V, 121-123) - Dante Alighieri’s *Divine Comedy*

Though in this case, thy Doctor knows.
3. OBJECTIVES

The following work attempts to give a wide but precise vision of one of the most deleterious processes human central nervous system can suffer: the “brain tsunamis”, technically called cortical spreading depolarizations.

As I will analyze, understanding spreading depolarizations at its whole complexity will allow us one day to target specific molecules and receptors in human brain hence stopping secondary effects (e.g.: cytotoxic edema or infarct zone growing) of the CSD that cause so much morbidity and mortality specially among the acute injured patients and those undergoing through a subarachnoid hemorrhage or malignant hemispheric stroke. With more intense research migraine aura and further symptoms will also become susceptible to be effectively treated from the roots.

Being able to register spreading depolarizations at the bedside could provide us with a powerful prognosis tool in politraumatized patients with cranioencephalic trauma or stroke for example, allowing us to improve the patient care and expanding treatment options, given that spreading depolarizations increase in length and number when the outcome is worse.

Note: this issue is highly complex. In order to approach it efficiently I do first some definitions of the basic protagonists in CSD and the essentials without touching too many details about pathophysiology, electrophysiological recording or clinical implications, for they will be properly explained in the designated parts of the text.
4. DISCUSSION

4.A. CORTICAL SPREADING DEPOLARIZATIONS DEFINITIONS

**SPREADING DEPOLARIZATION:**

Any self-propagating wave in the cortical gray matter of the central nervous system. A pervasive abrupt break-down of transmembrane ion gradients that causes a continuous depolarization of neurons and its glia (indeed maybe all cells) above its depolarization threshold, which enables them to repolarize hence stopping them from properly functioning. We don’t find CSD in healthy brain tissue [2, 4, 9, 13].

**NEGATIVE DC SHIFT:**

Basically, the electrophysiological correlation of a spreading depolarization. This hallmark consists on a rapid negative change in the slow potential, recorded through electrocorticography (ECoG) using a DC (direct current) amplifier [4, 9]. We detect it in frequencies <0,05 Hz.

In the image on the right, we observe a polyphasic slow potential change at different neighboring ECoG electrodes (monopolar array). On the lower part, it’s illustrated the next concept we are reviewing, the spreading depression. A diminishing in the spontaneous brain activity in the AC (alternating current) ECoG (bandpass 0,5-45 Hz) [51].

**SPREADING DEPRESSION:**

Progressive silencing of the spontaneous electrical activity in the brain due to a blockade of the neuronal activity. Primary consequence of the spreading depolarization. But depression of activity, permanent status of the neuron with a membrane potential above threshold, is maintained by other factors as we’ll see, given that spreading depression outlasts spreading depolarization duration [13].

For the spreading depression to occur, indeed we should dispose previously an intact metabolism and energy supply. When a spreading depolarization crosses an inactive zone then we refer to an *isoelectric depolarization* and thereby can not induce further depression. The more its number recorded, the worse the outcome at 6 months [9].

A CSD supresses spontaneous and evoked synaptic activity for 5 to 15 minutes and normal functioning is restored in normal brain tissue. When we got metabolic impairment (hypoxia, ischemia, hypoglycemia, etc.) these spreading depolarizations and depressions will occur spontaneously and will take longer to repolarize, as we shall see in next chapters. This way we note that we got one kind of spreading depressions generated by a specific stimulus (e.g.: rise in K+ levels, release of glutamate, opening of voltage-gated Ca2+ channels, hypo-osmolality) and causes an impaired metabolism with depression; or those who come after an energy deficit (e.g.: stroke) [13, 31], as we’ll analyze later.
Spreading depression is the etiology of the migraine aura and migranous stroke symptoms [13]. As we shall see later with more detail, migraine aura is characterized by the following symptoms:

- **Visual**: shimmering, sparkling, blurred vision or scintillating scotoma.
- **Sensory**: unilateral paresthesias in one limb or side of the face that progresses down them, or even cheek or inside of the mouth, with upcoming feeling of numbness that can long for an hour.
- **Language**: temporary language troubles from mild word-finding difficulties to severe dysphasia with paraphasic problems.
- **Motor**: weakness in face or limbs symptoms [43].

This symptoms, and signs in migraine patients, progressively appear as the wave moves through the cortex from occipital to frontotemporal zones.

Spreading depression is not always the following event to a spreading depolarization. As a matter of fact, peri-infarct depolarizations (those who appear mainly in the penumbra zone of an ischemic brain tissue) can’t cause depression in an already compromised tissue [31, 56].

---

With this case we review the essential elements in this work: on the left part we appreciate spreading depolarizations that cause spreading depression. But on the right side the spreading depolarizations produced do not cause depression (isoelectric depolarizations).

It’s relevant to notice how the tissue in (a) is well supplied. We can deduce this for the next reason:

1. The negative DC shifts are short-lasting. This indicate neurons don’t lack for the ions and nutrients for repolarization.
2. Presence of spontaneous activity before CSD indicate rCBF (regional cerebral blood flow) >15-23 mL/100 g/min
3. Spontaneous activity is quickly restored.
4. $p_{1}O_{2}$ lies in the normal range
5. Cerebral perfusion pressure (CPP) remains steady before and after the CSD

During the following night (on the right), clusters of SD (at least three depolarizations within three or less consecutive hours of recording) [19] appeared with persistent spreading depression of activity. But in this case we appreciate how the negative DC shifts in the clusters are longer. This could indicate us energy compromise. We do also observe an increase in the $p_{1}O_{2}$ with each SD (a) but in (b) we see an absence of increase or even reduction in $p_{1}O_{2}$ with each SD.

Upper image [9]: a 53 year-old female with a 3rd Fisher grade aSAH (or World Neurosurgeons FS grade 5) due to an aneurysm rupture in the middle cerebral artery.

[Image of a diagram showing spreading depolarization and isoelectric depolarization]
PERSISTENT SPREADING DEPRESSION OF ACTIVITY:

As its name says, a constant state of depolarization (depressed AC-band or high-frequency electrical activity) where neurons won’t repolarize unless metabolic conditions drift to a better state. This way, we find it on the core of ischemic tissue and later it will trigger the ionic cataclysm in spreading depolarizations and associated cytotoxic edema [18]. Induced by one or a cluster of SD. The historical synonym for this term is anoxic, asphyxial or aglycemic depolarization.

NON-SPREADING DEPRESSION:

Simultaneous stop in the spontaneous electrical activity in neighbour electrodes under a severe energy compromise, such as hypoxia or ischemia, before SD appear. Given that it’s the general term used for the diagnosis of interrupted energy supply, at least one the two following criteria should be fulfilled to say we are the presence of a non-spreading depression [9]:

A. Global arrest of the circulation proved by arterial pressure measurement
B. Local $p_{O_2}$ falls to a critical level before nonspreading depolarization occurs.

An outstanding difference in non-spreading depression is that is associated with neuronal hyperpolarization instead of depolarization. Non-spreading depression diminishes as spreading depolarization moves to the outer zones of the ischemic zone where it’s capable to induce a depression given that the tissue is electrically active [9]. The changing properties of spreading depolarization and its consequences as it progresses form the ischemic core, through penumbra zones to the healthy tissue is what we call the continuum of spreading depolarization. The presence of this continuum is the reason of infarct territory expansion, as we’ll discuss with more detail in next chapters.

In the image, an ECoG recording of persistent spreading depression induced by clusters of spreading depolarizations.

Notice how spreading depolarizations expand the electrically inactive tissue: the first SD is isoelectric in electrode 6 but then depression in the rest; later, the third SD is depression only in electrode 2. Spreading depression causes the zone of electrically inactive tissue to expand, and also into healthy tissue (third SD in electrode 3 is isoelectric but the second one depression).
ANOXIC DEPOLARIZATION:

Those depolarizations originated in the ischemic core of a stroke, result of an ischemic injury. This is why the negative DC shift recorded at the center of an ischemic attack is long - due to an energy shortage that impairs repolarization- and shortens as it progresses to the outer zones of tissue (penumbra and then healthy tissue) [4, 9]. Even from the very first spreading depolarization we appreciate the continuum of different forms the spreading depolarization goes through given that the metabolic status of the tissue around the ischemic core changes.

This anoxic depolarization spreads against different gradients of O₂, glucose and perfusion into adequately supplied surrounding tissues. This is why, analyzing the length and characteristics of the negative DC shift is critical to evaluate energy status and risk of excitotoxicity in the recording place [9, 4]. A short shift for example will tell us there is enough ATP for repolarization.

SPREADING HYPEREMIA, OLIGEMIA AND ISCHEMIA:

These are basically three types of vascular response to an spreading depolarization. In any spreading depolarization we’ll find just one isolated type of response but all of them overlaid in time and space and form a continuum [38, 49] as we shall analyze later:

- **Hyperemia**: an increase in the regional cerebral blood flow. Constitutes the first phase of the normal response to a spreading depolarization. It nourishes the tissue due to an increased demand of energy and metabolites after a huge consumption of these.

- **Oligemia**: a long-lasting slight decrease in the focal cerebral blood flow after recovery from the spreading depolarization. Second phase of the normal response to a spreading depolarization.

- **Ischemia**: a vasoconstrictive, inverse response to the spreading depolarization. The lack of enough supply of blood prevents the neurons from repolarize and increases the releases of vasoconstrictors [17]. This is appreciated by a longer negative DC shift. This inverse response propagates with the wave of depolarization and so is referred as spreading.

In the image [9] on the left, we appreciate the normal response to CSD (short lasting negative DC shift in DC/AC ECoG electrode 3, slight depression in AC-ECoG electrode 3 and hyperemia detected in optode 3) and the inverse response (both longer-lasting shift and spreading depression in electrodes 5; pO₂ descends and so does flow in optode 5). Spreading ischemia is the result of a vicious circle.
result of the vasoconstriction produced by the SD. The narrowing of the vessels enhances the energy deficit hence increasing the membrane pumps failure and impaired repolarization. Spreading depolarization regenerate [2, 9, 41].

NEGATIVE ULTRASLOW POTENTIAL (NUP):

A long-lasting, shallow negativity of the DC potential with coexisting, superimposed spreading depolarizations. It indicate us incomplete recovery from spreading depolarization and developing of neuronal injury. In other words, not all neurons repolarized and some persist, capable of conduct a spreading depolarization [9, 4]. We can appreciate this phenomenon in upper figure of page 11 marked with (*) in DC/AC ECoG electrodes 5 and 6.

SPREADING CONVULSION:

Spreading depolarization who has epileptic field potentials (electrocorticographic correlate of epileptic seizure) on the tailing end of its DC shift [10].

Now that we have clarified the key elements in this affair, we ready to face it from the very molecular basis, going through the pathology, ending with the clinical correlates useful to us.

4.B. MOLECULAR AND CELL BIOLOGY
(GENYSIS AND PATHOPHYSIOLOGIC PROCESSES)

So the first question that naturally comes to my mind is: how is a spreading depolarization originated?

To answer this question I must add there are lots of theories about ionic disturbances and the mechanism of self-propagation. But, far from the times of Leão, we now have an intense knowledge about the pathophysiology of SD and we can stablish a solid scheme about how and why happens. To get started, some essential facts about the ignition of SD:

- Those stimuli that ignite the SD have been proofed they cause an increase in local $[K^+]_o$ (key initiating event) and the release of glutamate (and other neurotransmitters) owing to the presynaptic terminal depolarization and opening of voltage-gated $Ca^{2+}$ (CaV) channels.
- A net self-sustaining inward current across membrane is the main element that initiates the positive-feedback cycle making the depolarization self-regenerative. It leads to the opening of voltage gated and/or non-gated $K^+$ which completes depolarization if removal of $K^+$ from interstitial space do not pace with its release. This makes the vicious circle to happen.
- NMDA receptors are essential for both initiation and propagation of SD [41].
- Approx. 1 mm$^3$ is the minimum critical volume of tissue that needs to be depolarized to ignite SD [2].
IGNITION:

A spreading depolarization can be triggered when a strong enough stimulus rises the \([\text{K}^+]_o\) above the threshold of 12 mM in a critical minimum volume (1 mm³) of grey matter. This threshold can vary depending on the nervous system zone (for SD can happen also in corpus striatum, thalamus, cerebellum, brain stem, spinal cord, and retina) and its neuronal and excitatory density; and between species (either vertebrates like mammals, birds or amphibians; or invertebrates like cockroaches and cephalopods) [2, 41].

Some igniters of spreading depolarization, whether they are present in the body or applied experimentally, are:

- **Emerging or direct application of depolarizing substances:** concentrated KCl, NMDA agonists (glutamate, aspartate), Ca²⁺ or Na⁺ channel openers (e.g.: BAY-K-8644, aconitine)
- **Direct cathodic electrical stimulation on brain tissue**
- **Mechanical stimulation:** pin-prick, focused-ultrasound or laser
- **Na⁺/K⁺-ATPase blockers** (e.g.: ouabain, palytoxin)
- **Membrane-impermeable Cl⁻ substitutes**
- **Metabolic toxins:** cyanide, azide, 2,4-dinitrophenol, fluoride, fluoroacetate, iodoacetate
- **Hypoglicemia, ischemia or hypoxia**
- **Ictal epileptiform event (IEE)** [2, 41]

The igniter, whatever it is, causes a massive drop in the membrane resistance through the opening of non-selective large-conductance cation channels [2]. This stimulus induces an net self-sustaining inward current across the membrane to initiate the positive-feedback making gradual depolarization self-regenerative, conferring SD its all-or-none properties. This is due to opening of voltage-gated and/or \([\text{K}^+]_o\)-dependent channels. This current releases more K⁺ that induces more depolarization and further increase in potassium levels if reuptake mechanisms can’t keep up. The nature of this initiator channels remains incompletely understood [21, 41].

It’s clear that NMDA receptor, much more than AMDA-kainate receptors, play a key role in SD (in initiation, propagation or both, yet to be clarified). It’s been witness NMDA blockers stop SD of occuring even when stimulation is several times larger than the threshold [41].

If we compare the neurons to batteries, they store in their polarized membranes an important charge, contrasting with the highly regulated and brief currents comprising action potentials and synaptic transmission. So a spreading depolarization would like shorting out that battery, discharge the membrane potential in a narrow (2-3 mm) strip of brain tissue simultaneously. It can even generate heat in the tissue [2].

\[ a) \text{Stimuli produces rising of } [\text{K}^+]_o \text{ and initiating the net inward current due to } \text{K}^+ \text{-channels activation. This depolarizes the membrane increasing the } [\text{K}^+]_o \text{ which activates more the channels and on again. If glial reuptake mechanism can’t keep the pace with its release, complete depolarization happens.} \]

\[ b) \text{The glutamate released from cortical pyramid cell synapses and NMDA activation have a key role in positive-feedback cycle that ignites SD. Glial reuptake regulates the process.} \]
Cav channels also seem to play a crucial role for spreading depolarizations because they are blocked when we create a free-calcium medium or block Ca\(^{2+}\) channels with Cd\(^{2+}\) or Ni\(^{2+}\). I remark the special importance of Cav2.1 channels (P/Q type Ca\(^{2+}\)). Others like Cav2.2 (N-type Ca\(^{2+}\)) or Cav2.3 (R-type Ca\(^{2+}\)) could also play a less significant part. It’s been also studied the relevance of Nav channels with the use of tetrodotoxin, which blocks voltage-gated Na\(^{+}\) channels, and spreading depression induced by electric stimulation was prevented but not KCl crystals or high K\(^{+}\) dyalisis induced. Looks like spreading depressions K\(^{+}\) induced do not depend on glutamate release, action potential-dependent K\(^{+}\) efflux or activation of postsynaptic TTX-sensitive Na\(^{+}\) channels. To summarize the role of each channel in the ignition of a depolarization, we can appreciate the different degree of depolarization blockade depending which channel or ion was intervened (image above) [41].

Hypoxia and ischemic attacks are also igniters (anoxic depolarizations). The delay onset of depolarization is the time for ATP to fall down below necessary levels to sustain Na\(^{+}/K^{+}\)-ATPase and other membrane pumps and so initiating the inward current. Differently from other stimuli, in the phase preceding anoxic depolarization there is a brief hyperpolarization and then slow depolarization, along with NaCl and water influx, decrease in extracellular pH and gradual increase in [K\(^{+}\)]\(_{e}\), [Ca\(^{2+}\)] and [Zn\(^{2+}\)]. Mitochondria also depolarize (even without Ca\(^{2+}\)), which can be stopped by Zn\(^{2+}\) chelation. NMDARs, Cav channels and TTX-sensitivitive currents could be involved one way or the other in anoxic depolarization generation, and so [Ca\(^{2+}\)]-dependent and independent mechanisms of glutamate release are involved. Nav channels blockade posses the strongest inhibitory effect given that Na\(^{+}\) current have a relevant paper in it. Ouabain (Na\(^{-}/K^{-}\)-ATPase inhibitor) induced depolarization are similar in many ways to hypoxia or oxygen-glucose deprivation depolarizations. This drug has been used experimentally to determine the influence of different ions in generation of spreading depolarization [41].

The channels that participate in the initiation and spread of SD depend on the tissue conditions, mainly before the depolarization onset [12].
IONIC CATACLYSM AND MOLECULAR PATHWAYS:

Spreading depolarization occurs when neuronal cation outflux by the ATP-dependent Na⁺/K⁺-ATPase pumps locally fails to compensate for cation influx of sodium and calcium [4].

A SD is a complex phenomenon where we appreciate various phases that could be roughly divided this way:

- **Early phase**: lasts for a few seconds. It begins with the opening of channels in apical dendrites (which are completely depolarized) of pyramidal cells, while soma partially.
- **Main phase**: lasts for 15-20 seconds. Entire somatodendritic membrane depolarized.
- **Late phase**: just a narrow band of apical proximal dendrites remains depolarized while the soma is partially repolarized.

The rapid change in interstitial direct current potential (V_o) during early phase goes with increase [K⁺]e to 30-60 mM, rapid decrease in [Na⁺]e and [Cl⁻]e to 50-70 mM and in [Ca²⁺]e to 0.2-2.8 mM. Also a short increase and then sustained decrease of pH_e during depolarization [13, 41, 42].

Spreading depolarization are ignited in neurons primarily and astrocytes follow: [Ca²⁺]i rises first in neurons. Also CSD-associated neuronal [Ca²⁺]i wave is unaffected by supression of [Ca²⁺]i. Due to the influx of ions, the extracellular space shrinks more than 50% [33]. Also, we appreciate loss and beading of dendritic spines, which lasts about 8-10 min., time for the depression to recover [41].

In this image we appreciate the different ionic currents and elevation of glutamate (also aspartate, GABA, acetylcholine, glycine, of taurine increase), basic components of the depolarization spectrum in SD. The increasing in [K⁺]e has been proposed as the element that causes the spreading pattern in the depolarization and self-perpetuates the wave [13, 41].
The core of a spreading depolarization consists basically on the Na⁺/K⁺-ATPase failure.
In normal state, cations influx produced by intracellular negative-charged proteins is counteracted by the sodium-potassium pump (double Gibbs-Donnan equilibrium). In SD the sodium and calcium pumps do not provide sufficient outward current to balance the inward currents (pink and purple channels). In other words, the influx of calcium and sodium is greater than the potassium outflux generating intracellular hyperosmolality with subsequent edema and distortion of dendritic spines [13].

The ionic changes increase further release of excitatory transmitters. The key element that promotes self-propagation of a spreading depolarization is still on debate. There are several theories, divided in two main groups depending on the central element:

- Intercellular diffusion and opening of gap junctions in either neurons or glial cells.
- Diffusion of a humoral agent like K⁺ or glutamate.

There are arguments and studies which either prove or invalidate this positions. The role of gap junctions is challenged by the fact that SD propagation is not inhibited by carbenoxolone (gap-junction blocker). The flooding of K⁺ and glutamate increase their extracellular levels and induce further increase of [K⁺]ₑ that activate the positive-feedback circle we previously described. The propagating wave of K⁺ goes with an almost simultaneous wave of [K⁺]ₑ-dependent synaptic glutamate release at the depression wavefront, essential for activation of NMDARs and propagation. Cav2.1 channel-dependent synaptic glutamate release are also involved in the propagation, specially in SD induced by local K⁺ stimulation [41].

**METABOLIC DISRUPTION AND MOLECULAR PATHWAYS:**

The fact that spreading depolarizations are called brain tsunamis is not random for they cause a complete caos in the tissue they cross. We can infer then that it’s in part energy-dependent and stimulates O₂ and glucose consumption. Therefore, restoration of homeostasis is hard in severely compromised gray matter and this will worsen its viability [2].

So ATP consumption is increased during a spreading depolarization for restoration of homeostasis after the massive release of neurotransmitters and ion imbalance. These changes match with the onset of the DC shift. The cerebral metabolic rate of glucose (CMRGlu) peaks at repolarization and returns to normal in 15 min. Clusters of SD can double or triple it in broad areas of the cortex. In the end, tissue’s glucose decreases by 30-60% and lactate increases by more than twofold at or within a minute of peak depolarization. Glucose returns to normal after 5 minutes [2].
ATP, which is paradoxically also increased during depolarization waves, has been proposed to have a neuroprotective role against ischemia due to the activation of neuronal receptor P2Y [47].

The spreading depolarizations will rise the O$_2$ demand and the oxidative phosphorylation. Rises in cerebral metabolic rate of O$_2$ (CMRO$_2$) will be controlled by the ATP turnover, which depends of the energy used by the Na$^+$/K$^+$-ATPase to restablish ionic gradients. This rise supports then the increased Na$^+$/K$^+$-ATPase activity. One of the reasons for CMRO$_2$ to increase could be the huge rise in cytosolic Ca$^2+$ after SD depolarizes neuronal mitochondria and may trigger the increasing in CMRO$_2$ via the Ca$^2+$ uniporter in the inner mitochondrial membrane. But there are also data suggesting that during the depolarization (i.e., preceding the hyperemia) there may be a transient impediment to mitochondrial O$_2$ utilization. CMRO$_2$ rise function could be decreasing the size of the intermediate pools glucose must go through before being used in metabolism to restore glycogen storage for example [2].

**GLYCOGEN ROLE IN SPREADING DEPOLARIZATION [27]:**

Lack of sleep is a risk factor for spreading depolarizations

A single spreading depolarization can decrease tissue glycogen by up to 50 %, which can be detected within 2 min. and last for 10-90 min. This falling down is even larger than in moderate hypoxic conditions, seizures or days of fasting.

Phosphorilase is the enzyme tasked with breaking down the glycogen. Its activity is increased with higher cAMP levels, peaks shortly after DC shift recovery and lasts more than 10 min. The adenyl cyclase, highly expressed in astrocytes, increases the cAMP levels in response to the alkaliniza/on (↑HCO$_3$) of astrocytes during SD due to increased activity of Na/HCO$_3$ cotransporter. This enhances glycogen break-down, glycolisis and release of lactate to extracellular space. This whole process is triggered by situations like extra interstitial K$^+$ or aglycemic episodes [33].

Indeed, glycogen storage and its use is more relevant in astrocytes for they maintain glutamate and K$^+$ in proper levels around excitatory synapses. Sleep deprivation, an important migraine trigger, can induce the expression of PTG (protein targeting glycogen) -that increases activity of glycogen synthase- in astrocytes impairing glycogen break-down. When glycogen can’t match synaptic energy demand, the extracellular K$^+$ increases activating pannexin-1 channels (also activated by glutamate, ATP or high [Ca$^{2+}$] and the downstream inflammatory pathway with it (migraine headache, through stimulation of meningeal trigimenal afferents). It also lowers the S threshold. Specific hormonal and genetic factors can influence this making some migraineurs more susceptible to sleep pattern changes by predisposing to synaptic conditions that activate large pore channels (e.g.: Panx-1/P2X7) and subsequent inflammation (migraine without aura) or lowering the threshold for CSD (migraine with aura).

In order to prove this, researchers reduced the glycogen-derived energy supply to synapses with 1,4-dideoxy-1,4-imino-D-arabinitol (DAB, inhibitor of glycogen phosphorilase) and an antisense oligodeoxynucleotide to impair the function of MCT2 (transport lactate, energy metabolite, from astrocytes to neurons). They also provoked extended wakefulness in order to decrease glycogen deposits. They found out reduced glycogen mobilization triggers caspase-1 cleavage and HMGB1 release from neurons even in the absence of CSD, activating inflammatory cascade to Panx-1 complex.
In the image above [27] we observe basically the fluorescent correlate of the previous facts we have described:

- In the first row of images it’s shown how propidium iodide, which normally doesn’t label cells, enters in neurons thanks to the Panx-1 channels opening induced by DAB. In A & C PI enters and not in B (no DAB). This influx could be blocked by carbenoxolone (D).

- In the second row we see how DAB activate the caspase-1 (E,G)

- In the third row we appreciate how HMGB1 [normally confined to neuronal nucleus (arrows in I) or non-neuronal cells (arrow heads)] translocates to cytoplasm under the influence of DAB [H & J (perinuclear staining)] or it’s released massively (*). Notice how HMGB1 was not released from non-neuronal cells and remained nuclear (arrowheads in H & J).

- Last two rows show a diminishing fluorescence due to massive release of HMGB1 after DAB (K, L) from dentate gyrus (DG) granular neurons. HMGB1 levels in CSF also increased (O).

Speaking of nucleus, at some point a group of researchers found interesting to investigate if spreading depolarizations involved only membrane alterations and some organeles in neurons or if it also affected the nuclear structures. They discovered indeed a SD can induce epigenetic chromatin modifications, through measuring levels of methylated histone H3K4 & H3K9 and histone methyl-transferases MLL & SET7 [39].
Another issue on everyone’s lips in medical community is radical reactive oxygen species. While trying to find how spreading depolarizations can have a neuroprotective role against subsequent ischemia events as we said about ATP, researchers discovered that SD induction causes an increase in H$_2$O$_2$, increase in SOD (superoxide dismutase) activity and so a decrease in O$_2^-$ suggesting a neuroprotective role of this enzyme [52].

It’s been discussed that one of the main reasons CSD could have a protective function against ischemia is the induction of genes who codify proteins with neuroprotective function such as iNOS (nitric oxide synthase), HIF-1α (hypoxia-inducible factor) and LDH-A (lactate dehydrogenase). iNOS -expressed in situations like stroke, trauma, infection, etc.- can be induced by HIF-1, whose subunit α degradates under normal conditions but dimerizes with subunit β in hypoxic conditions. CSD increases HIF-1 activation which induces the expression of iNOS and LDH-A [51].

A link between CGRP (calcitonin gene-related peptide) and CSD has also been stablished, suggesting that CGRP -vasoactive neuropeptide- is released during spreading depolarizations inducing more vasodilatation than the produced by SD itself, and playing a role in migraine given that antagonists of CGRP receptors produce anti-migraine effects on the trigeminovascular system [54].

**MODIFIERS:**

A large number of physiological (e.g.: pH, temperature, fasting, hormones,…), pharmacological (Ca$^{2+}$ channel blockers, K$^+$ channel openers, nitric oxide synthase inhibitors) and genetic [e.g.: CACNA1A gene encodes the pore-forming α1 subunit of neuronal Ca$_{V}$2.1 (P/Q type) calcium channels; or loss of function mutations in the ATP1A2 gene that encodes the A1A2 subunit of the astrocytic sodium pump -these can lower the threshold for SD and are related to increased glutamatergic neurotransmission--; also NOTCH3 receptor gene mutation, described in CADASIL, explains why a link has been stablished between SD and these subcortical vasculopathy] modulators have been described [13].

_Further molecular mediators and modifiers shall be studied in the chapter that verses about the hemodynamic response to SD given that most of them are in intimate relation with it. Other specific metabolic pathways will be discussed with each specific pathology they are related to._

**ASTROCYTE ROLE:**

Astrocytes, protoplasmic in the case of the grey matter, provide structural isolation of neurons and their synapses and also tasked with ionic (K$^+$) sequestration, trophic support, and look after growth and signaling functions in neurons. Astrocytes are of capital importance in brain energy utilization. Excess of glutamate for example is retaken along with a Na$^+$ cation through a cotransporter. This Na$^+$ is returned to the synaptic cleft through α2 Na$^+$/K$^+$/ATPase (almost exclusive is astrocytes), which uses ATP, obtained from enhanced glycolisis. This process also increases glucose uptake from capillaries and rises the lactate production which is used as energy source in synapsis. Glutamate is converted to glutamine and excreted again to the interstitial space where in this form can be taken up by the presynaptic neuron [43].

_Astrocyte reactivity is a hallmark in many neurological diseases and targeting it has been discussed in order to treat SD-related diseases [18]._
It’s easy to infer then that an impaired astrocytic function can interfere with the K⁺ flux.

As we referred before, CSD causes parenquimal inflammation via Pannexin-1 megachannel opening as well as caspase-1 activation followed by HMGB1 release from neurons and nuclear factor κB activation in astrocytes. CSD has been also linked to meningeal inflammation which is associated with activation of macrophages and mast cells along with a rise in cytokine levels [18].

It’s been proved spreading depolarizations under ischemic situations (upper image - hallmarks of alterations ischemic SD) increases the number and volume of astrocytes (reactive astrocytosis - lower image) [18, 12]. Astrocytes facilitate recovery from SD. The fact that neurons lead and astrocytes follow in a SD is supported by the facts that calcium rises first in neurons and the SD-associated calcium wave wasn’t affected by the block of astrocytic calcium wave through internal calcium store depletion [12].

CSD enhances the expression of GFAP (glial fibrillar acyd protein), vimentin (both of them typical in reactive astrocytosis), S100B (a calcium-binding protein) and NDGR2. Moreover, expression of immuno modulatory enzyme IDO is enhanced and also toll like receptors (TLR3 & 4) whose activation generates pro-inflammatory cytokines such as IL-6 or and TNF-α [18].

The synaptic guardian role of astrocytes has been demonstrated in studies with genetically modified animals with familial advanced sleep phase syndrome (FASPS) and migraine, a genetic disease of migraine with uncomplicated aura. A mutation in the gene encoding casein kinase 1δ (CKIδ)- T44A was introduced generating hypophosphorylation of connexin43 (Cx43), thereby reducing gap junctional communication. This lead to a lower K⁺ threshold for SD ignition. This proved the key role of gap-junctions in potassium buffering and hence in SD [12].
NORMAL AND INVERSE HEMODYNAMIC RESPONSE:

This is the cornerstone in the spreading depolarizations issue given that hemodynamic responses are one of the most studied topics and maybe one with the most viable therapeutic implications. But before digging into I must say it’s one of the most controversial zones in spreading depolarizations for there are lots of theories about its exact pathophysiology but there are some facts we can get clear and here they are.

The hemodynamic response to spreading depolarization will depend not only in the metabolic conditions but the pathology related to the SD too. We can say there are four temporal phases in the normal hemodynamic response. The tricky thing is that these are not linear but sometimes they are overlapped and we do not always appreciate the same pattern for it depends on a variety of factors like whether it’s recorded on animals or humans for example [2, 3]:

✦ **Component II**: hyperemia peak under physiological conditions that dominates the vasomotor response. Due to the increased metabolic demands. Starts after 15-20 s. the depolarization onset and peaks after full repolarization, normally 1-2 min. after DC shift onset. CBF can increase 30-250%. Lasts up to 3 min. and leads to a prolonged post-SD oligemia that keeps CBF 10-40% below baseline an hour or longer.

✦ **Component I**: an initial hypoperfusion coincident with the DC shift. This transient vasoconstriction decreases CBF (cerebral blood flow) by 5-30% for 5-30 s. and can either precede the hyperemia (II) as an early dip in CBF or overlaps with the rising hyperemic slope giving the response a notched or jittery upstroke (upper image)

✦ **Component III**: a second smaller (10-50%) but longer-lasting hyperemia (or dilation) peaking 3-5 min. after the peak hyperemia, forming a characteristic hump (upper and lower image). A mild hyperemia (1-2 h. after) and a much delayed oligemia (3 day later) can appear although the latter could be triggered by clusters of SD.

✦ **Component IV**: decline in CBF of 60% up to an hour consisting on a post-SD oligemia.

The heterogeneity of this response could be due to species or more probably differences among brain regions and arterial territories. It’s been suggested different vascular compartments and arterial territories (e.g.: arteries, penetrating arterioles, capillaries, veins) respond in diverse ways to SD [33]. During the surgical preparation, SDs could be generated iatrogenically and their recording would differ from those SDs in naive tissue. Other systemic physiological parameters such as blood pressure or PO$_2$ could alter the vasomotor response to SD as well (*) [2].
But in the injured brain, CSDs come with initial CBF reduction (severe microvascular spasm), instead of increase, coupled to the depolarization phase (spreading ischemia), which persists as long as the tissue remains depolarized. When this inverse response appears, the risk of tissue damage is higher [9, 31, 38].

This spreading ischemia results from local microvascular dysfunction:

- **Left image:** normally, at capillary level, when brain needs increase, capillaries dilate in order to diminish what’s called capillary time transit heterogeneity (CTH). This is occurs naturally and consists on different rates of erythrocyte velocities among capillaries. In spreading depolarization this CTH is increased and oxygen in tissue descends. The capillary disfunction (pericyte dysfunction, changes in blood viscosity or capillary wall morphology, or external capillary compression) impairs the oxygenation of tissue [38]. In B we appreciate the histological changes produces: massive swelling of peryvascular astrocytic feet (AE) and flattening or compression of the capillary lumen (L, *red arrows*). Notice also the endothelial protrusion (*middle image, arrow*). We also see how ischemia induce pericyte constrictive action through horseradish peroxidase (HRP)-filled capillaries in (iii).

- **In this image we got a patient suffering an aneurysmal subarachnoid hemorrhage.** We observe a single spreading depolarization that propagates at a rate of 1.9 mm/min. Notice the normal hyperemic response in optodes 6 & 4 and the transient spreading depression in electrodes 6 & 4. But in the lower recordings we appreciate the inverse hemodynamic response to SD in optodes 5 and 3 and very important to highlight the prolonged negative cortical DC shift (electrodes 5 & 3), electrophysiological feature of inverse hemodynamic response [31].
It’s been also proposed that the hyperemic response to SD doesn’t happen to supply the mismatch between energy needs and supply but due to release of diffusible vasoactive mediators from the cells of neurovascular unit (astrocyte end feet, perivascular nerves, pericytes, smooth muscle cells & endothelium). Astrocyte mechanisms in vascular regulation acquire a more relevant role in CSD than neurons. The astrocytic increase in Ca²⁺ activates stimulates the arachidonic acid production by phospholipase A₂, which can then be turned into dilator epoxyeicosatrienoic acids (EETs) by epoxygenase CYP42C11, constrictor 20-HETE by CYP4A in vascular smooth muscle or dilator prostaglandin E₂ by cyclooxygenase-1, to relay the vasomotor signal to the vessel wall. Pyramidal neurons and interneurons do also have a role in vasoregulation (image below) [2].

Vasomotor tone also depends on the rich inervation provided by peryvascular nerves originated from superior cervical ganglion (sympathetics containing norepinephrine and neuropeptide Y), sphenopalatine, otic and internal carotid ganglia (parasympathetics containing acetylcholine, VIP and NOS) and trigeminal ganglion (holding inside CGRP, substance P, neurokinin A, and pituitary adenil-cyclase activating polypeptide). Sympathetic constricts and parasympathetics vasodilates [2].

Diagram showing the elements conforming the neurovascular unit:
Gap junctions (e.g.: connexin 43) directly couple endothelial and smooth muscle cells to allow electrotoneic and chemical spread of vasomotor signals along the vessel length as a mechanism to induce adaptive changes upstream to the activated segment.
SD induces a furious storm of practically every vasoactive mediator or modulator that has been discovered:

- **Ions**: as we commented previously, [K]e concentration can either provoke vasodilation at levels <20 mM and constricts at levels >20 mM.

- **N.O.**: main vasodilation mediator of CBF response to SD, synthetized overall by NOS, whose activity is increased by glutamate. It acts as a permissive factor in cortex and cerebellum (e.g.: blocking effects of NOS inhibition are reversed by NO donors-image on the left-).

- **Arachidonic acid metabolites**: SD triggers rapid arachidonic acid production through increased phospholypase A2 activity. A single SD increase up to 30-90% perivascular prostaglandins E2, I2 and F2α levels and a cluster of SDs up to 50-300% which also increase thromboxane B2 production. This arachidonic acid, released from astrocytes, is converted to its main vasoconstrictor mediator 20-HETE by cytochrome P-450 (CYP4A) peaking within 20 min. and lasting up to 2 h., approximately the duration of post-SD oligemia.

- **Peptide neurotransmitters**: release of CGRP, potent endothelium-independent vasodilator, from perivascular trigeminal sensory nerves, has been detected with high [K]e. Endothelin, a potent vasoconstrictor released from endothelium with discussed role in SD, acts over smooth muscle, endothelium, neurons and glia.

- **Small molecule neurotransmitters**: acetylcholine (muscarinic receptors), serotonin (5-HT2A/C receptors), ATP (A1 receptors) or glutamate (NMDA receptors) through activation of NOS synthase, have vasodilator effects.

- **Free radicals**: they are produced by glial cells during SD. Few studies have tried scavenger antioxidants (e.g.: tirilizad, superoxide dismutase, oxypurinol) without success.

- The formation of a **mitochondrial permeability transition pore** (mPTP) due to increase in Ca2+ and fatty acids in SD impairs mitochondrial function and induces apoptosis [2].

The upper image shows the disappearance of pial arteries (*) and propagating paleness during spreading ischemia.

In the right image, the two patterns of vascular response. When tissue is damaged, the inverse response occurs and the spreading ischemia can be witnessed by a prolonged slow potential change and depression due to insufficiency of pumps in repolarizing the neurons.
DELETERIOUS EFFECTS: CYTOTOXIC EDEMA AND NEURONAL DEATH

Spreading depolarization ignites a vicious cycle that worsen the prognosis and creates, if prolonged, tissue damage. Let’s take the example of one of the pathologies with highest prevalence of CSD: subarachnoid hemorrhage, where a blood clot covers a zone of brain tissue altering the microenvironment (increasing K⁺ and decreasing NO) rising up the vasoconstrictor effect, deactivating the vasodilator one. This ignites the spreading ischemia which propagates along with the front of depolarization [13].

The deficit of oxidative substrate supply while energy demands increases, creates a deficit in ATP that will impair the Na⁺/K⁺-ATPase function which increases extracellular glutamate (promoting excitotoxicity) and preventing neurons from repolarizing. Hence increasing the vasoconstrictors production and again the depolarization. This excitotoxic process increases the [Ca²⁺]ᵢ, enhancing the cellular death signals [13, 30].

The intracellular calcium overload will trigger mitochondrial outer membrane depolarization (MOMP) and subsequent cell death. Water influx following sodium and chloride intracellularly causes swelling of cells and edema (“cytotoxic”). As commented before, free radicals are also produced during spreading depolarization and they trigger a vicious cycle in mytochondria, through electron chain inhibition, that leads to superoxide excess and also to activation of mPT -contributes to neuronal swelling- and induction of MOMP [30].

In the left image [19], A & B show a photon images from apical dendrites of mouse cerebral cortex after bilateral common carotid artery occlusion (BCCAO). For the first minutes (A) spine morphology is normal but 26 s. after depolarization onset (4 min. 30 s. after BCCAO) dendritic spines disrupt and become beaded (B). C: 48 h. after cardioembolic stroke in right middle and anterior cerebral arteries; and D: taken 3 days following rupture of of basilar typ aneurism. Hyperintense signals represent decreases in apparent diffusion coefficient caused by cytotoxic edema, dendritic beading and restricted intracellular diffusion of water.
The SD-induced apoptosis of neurons will eventually occur, specially in the penumbra zone of an ischemic attack. The cornerstone of this process is the activation of aspartate-specific cysteine proteases constitutively expressed in brain and are activated by either intrinsic (↑[Ca$^{2+}$], DNA damage, ROS or glutamate) or extrinsic (activation of surface cell death receptors such as TNF-R, CD95/Fas, DR/5 triggered by TNF, FasL and Apo2I/TRA1L respectively) stimulus [30].

The resultant mitochondrial damage generated leads to the caspase activation -specially caspase-3, activated in early ischemia and particulary in the peri-infarct region- that target DNA repair enzymes such as PARP, cytoskeletal proteins, presenilin, huntingtin and caspase-activated deoxyribonuclease (ICAD). The leakage of cytochrome-c from inside mitochondria plays as well a key role for it activates the apoptosome complex, which contains cytosolic protein Apaf-1 and procaspase-9, leading to a sequential activation of downstream caspases. This cytochrome release is also regulated by the integrity of the mitochondrial outer membrane, partially regulated by Bcl-2 family of proteins [30].

SD also exacerbates the inflammatory reaction that will worsen the progression of the disease. Early after ischemia, pro-inflammatory genes (e.g.: NF-kB, IRF-1, PPARs, etc) along with cytokines (TNFα, IL-1β, IL-6, etc) induce expression of adhesion molecules (e.g.: ICAM-1, VCAMs, integrins, etc) that will attract leukocytes and platelets. It’s been proposed iNOS as well contributes to the damage caused by post-ischemic inflammation [30].
This whole process, along with the ion disbalance we described, promotes the cytotoxic edema. The persistent depolarization of the tissue in the ischemic core will cause this edema with structural changes (e.g.: dendrites beading), that are the basis of clinical stroke diagnosis by diffusion-weighted imaging with magnetic resonance. The cytotoxic edema results in a shrinkage of 50-65% of the extracellular volume [19].

4.C. MOVEMENT PATTERNS THROUGH TIME AND SPACE

SD movements through the cortex and other structures are not completely elucidated yet for gyrencephalic brains in evolved species are different gray and white matter conformation, glial cells proportion or sulci and deep fisures distribution that can affect SD propagation [45].

It’s important to notice this, given that veins for example affect SD propagation while arteries react to it but do not consistently modify its propagation. Hypoperfused or dead tissue can act as anatomical barriers too -like necrotic tissue in the heart after an infarct-. But also functional blockades do also exist like tissue in temporary refractory periods [44].

Several patterns of wave propagation have been described and classified depending on their timing. First we got the initiation patterns [45]:

✦ **Radial wave**: closed circular wave that spreads out radially in all directions, originated in a single point (like a water drop fall). The wave-front can break at some point and be divided in two or more semiplanar waves.

✦ **Irregular wave (broken radial wave)**: an initial radial wave which doesn’t remain close and an asymmetric expansion and discontinuous wavefront evolves into a semi-planar wave.

✦ **Irregular wave with cycling**: they break in the very beginning and surround the stimulation zone with ring-shaped form. Opens ends of the wave are attached to the inner and outer borders of this domains and they tend to evolve into more semiplanar waves.

And then we got the propagation (evolving) patterns:

✦ **Semiplanar wave**: solitary wave with a flat-rounded front and two open ends.

✦ **Spiral wave**: it rotates either around a dynamic functional block, the “spiral core” [e.g.: hypoperfused tissue or infarct tissue (⊙)]; or around an anatomical blockade.

✦ **Reverberating wave**: a semi-planar wave circles around a functional or anatomical block in a closed-loop pathway. This pattern produces periods and during these, more semi-planar waves that move in other regions can be produced.

✦ **Collision**: when two fronts collide they block each other given the refractoriness of the excitable medium. If two
collide in lateral extremes, the two waves fuse and continue together through non-exicted tissue in a new direction.

The sulci, fissures (deeper in human brain) and pial vessels are limit and modify the propagation direction. But it happens though that the same vessel that first stops a SD, later it allow another to go through. So their blocking effect is variable (image on the right). Specifically, the curves and irregularities of a gyrencephalic brain, like the human one, makes it more susceptible to re-entrance patterns waves such as spiral or reverberating waves [45]. The spreading through contiguity in the grey matter is independent of action potentials and physiological synaptic transmission and therefore, of long-range axonal connections [41].

As we talked about before, a massive increase in [K+] enough to depolarize neighbouring cells is the critical factor for spreading (reaction-difussion model for SD propagation) [2], so changes in the microenvironment can alter the propagation pattern.

Several studies have tried to establish the exact velocity of a spreading depolarization but it’s variable depending on the tissue characteristics. It’s been accepted SD move at a rate of 2-9 mm/min. SD usually shows deviations from concentricity in both lyss- and gyrencephalic brains. Apparently, it also tropism for superficial rather than deep layers of the cortex. Cytoarchitectonic peculiarities do affect propagation in the horizontal plane. The exact mechanism regarding this anisotropy of SD remain enigmatic [44, 45].

Movements of multiple SD record with intrinsic optic signal (IOS). A) The SD moves from the stimulation site to different gyrus and didn’t respect sulcus between two gyri. A hypovolemic phase was followed by a hyperemic phase and a recovery phase. B) Collapsing wave when underlying tissue did not fully recovered from last SD [45].
Now that we have reviewed the foundations of what spreading depolarizations are, we’re analyzing the clinical implications and how we could apply it to the patient care in a near future.

**STROKE**

When an ischemic attack is produced, a non-spreading depression, simultaneously in the whole area of oxygen depletion, is produced and later what’s called anoxic/asphyxial depolarization in the very center of the injury.

The first SD erupts in the ischemic center ~2-5 min. after (in one or multiple foci) the occlusion of the vessel or ischemia zone establishment. If the tissue is reperfused in time, this initial depolarization is totally reversible. If the depolarization outlasts the commitment point, cellular death increases and is reflected with a negative ultraslow potential. After the first SD has been produced and concentrically invades the penumbra and surrounding tissue, subsequent SDs are generated at the rim of the permanently depolarized core: peri-infarct depolarizations [12, 50].

In this image (12) we observe a diagram of different parameters in both ischemic model and migraine model. A) Notice how the SD runs into the normal surrounding tissue. B) In the embolic model, the zone of persistent depression of activity extends into the normally perfused cortex. In both models spreading depression activity will only be short-lasting far away from the trigger. C) Notice how the only in embolic model there’s ischemic zone. If embolus or K⁺ stimulus would resolve, cells would repolarize without damage.
The infarct size expansion is tightly related to the number of PIDs. PIDs are then responsible to integrate more healthy tissue into the ischemic core. In the image on the left we appreciate a patient suffering from malignant stroke whose EEG recordings worsened in the next days after the event. Notice how in the first day we see punctual suppression episodes in electrodes III & IV and 5 days later suppression is prolonged from I-IV. Slow potential changes in the absence of EEG background activity are signatures of PIDs [31].

Peri-infarct depolarizations are very present in the infarcts and malignant hemispheric strokes. Upper image: They usually start at the edge of the ischemic core (due to partial neuronal depolarization, glucose and oxygen availability or brain temperature, etc) and spread clockwise around the edge of the core as anticlockwise spread is prevented by the slow recovery from previous event. The net effect of this is vasoconstriction nearest the core (blue arrow part) and vasoconstriction further the core (red arrow part) which will be less sustained or severe allowing repolarizing and future SD. This way the original penumbra is included in the ischemic core and grows [31].

During the developing of new infarcts, a pattern of midly prolonged SD is observed rather than terminal depolarizations. So any kind of SD can lead to cell death, not just terminal ones [12].

The SD that are ignited in the center of attack can not produce depression given that it’s an isoelectric tissue [49]. This way, when SD propagates outside the core, against different metabolic gradients, the SD changes its characteristics and electrophysiological record because non-spreading depression weaker and it can depress the tissue. This is what it’s call the spreading depolarization continuum, between anoxic depolarization in focal ischemia and spreading depression [19].

Persistent depolarization defines the core causing initial and secondary infarct development. A) Within seconds of ischemic onset, a broad area (<15-23 mL/100 g/min) simultaneously develops complete suppression (non-spreading way) with electrical silence. After 2-5 min. a SD develops in a central zone and if circulation is not restored it becomes permanent. This initial SD spreads into penumbra (arrows) and healthy tissue. B) In the following minutes, hours or days PIDs are generated in hot spots in penumbra zones as a result of energy supply-demand mismatch, either inner or outer (oligemic tissue with preserved spontaneous activity). These new PIDs spread not only outwards but inwards towards ischemic core inducing more perfusion deficit, expanding in a stepwise-manner the core with each SD.
PIDs surge specially triggered by episodic drops in metabolic supply (e.g.: hypotensive or hypoxic transients) or increases in metabolic demand (e.g.: functional activation) that cause supply-demand mismatches in PIDs hot zones. This is why hyperglycemia or hyperoxia can suppress PIDs [2].

We refered before to SD using therms like persistent or short-lasting. It made more sense analyzing this classification in relation with the continuum [19]:

- **Transient SD**: finite duration of depolarization with repolarization of the tissue.
  - Short-lasting: indicates a duration that is observed in intact, normally perfused brain, typically in the range of 20-180 s. depending on species and recording method.
  - Prolonged or long-lasting: indicates some degree of dysfunction or metabolic compromise.

- **Persistent**: depolarization will be indefinite unless conditions change so tissue can repolarize.

- **Terminal**: similar to persistent, but it implies that depolarization has been present long enough to cause tissue infarction.

In the image on the left [2] patterns of CBF are analyzed according to the ischemic zone. It’s relevant noticing how the vascular response turns more vasoconstrictive the deeper we go towards the core. In midly hypoperfused regions response is usually biphasic with a slight initial hypoperfusion (component I) followed by peak hyperemia (comp. II). In more severely ischemic regions, the hypoperfusion turns more severe and hyperemia fades away. Hypoperfusion depth depends mainly on PIDs duration. Near to the core, hyperfusion becomes permanent if depolarization fails to recover.

Some evidence that target PIDs as responsible for penumbral expansion could be [19]:

**CORRELATIONAL EVIDENCE:**
- Greater burden of spontaneous SDs is associated with larger infarct volumes and SD inhibitory therapies reduce infarct size.

**PROOFS OF CASUALTY:**
- SD that are elicited remote from lesion and propagate into penumbra increase infarct sizes.
- Ischemic core expansion is time-locked to SD entering penumbra.
- Blood flow recordings showing repeated perfusion decreases by SD (ischemic zone expansion).
- Demonstration of terminal dendritic beading of penumbra neurons induced by repeated SDs.
- Electrophysiological recordings of delayed, terminal depolarization induced by propagating SD.
Subarachnoid hemorrhage comes with a high morbidity and mortality, not only due to the blood loss that implies, but also to the neurological deficit produced by vasospasm and cortical ischemic lesions after the aneurysm rupture (delayed cerebral ischemia -DCI-) that usually arise between days 5-14 afterwards. However, DCI accounts for only 13% mortality but initial injury (acute cerebral ischemia -ACI-) implies 86% of deaths. Therefore, we distinguish:

![Early lesions: developed in laminar or band-like patterns of contiguous cortex or symmetrically in midline regions (lower image on page 26, D). They usually are patchy, bilateral and multifocal, often present in ACA territory. Presence of these <48 h. determines a worse neurological status and long-term outcome [19, 21].](image)

SDs may also be involved in the generation of early cortical infarction in the period of ACI [47]. ACI and cortical ischemic lesions have been hypothesized to result from increased intracranial pressure and intracranial circulatory arrest the time of the aneurysm rupture, ultra-early vasospasm or local vessel thrombosis [21].
Delayed lesions: develop around 6-7 days after aneurysm rupture, often in conjunction with neurological decline (delayed cerebral ischemia) [18]. DCI occurs in 25% of the patients that survived the hemorrhage [31].

Cortical lesions are often found in in the region covered with blood. It’s been proposed that delayed lesions are produced by spreading depolarizations ignited by the hemolysis products of blood in subarachnoid space. Experiments have been conducted in both rats and swine through infusion of CSF with high [K+] or adding blood (that acts as a NO scavenger) in subarachnoid space respectively, finding that these conditions were sufficient to trigger SDs, which recurred over several hours. The topical application of hemoglobin makes the monophasic peak hyperemia turn into a biphasic response with initial hypoperfusion followed by diminished hyperemia. When extra K+ is added, vascular response turns into a severe monophasic hypoperfusion (80% CBF decrease). Therefore, the erythrocytes-breaking products impair the neurovascular coupling and induce spreading ischemia [2, 21].

The repeated SDs thus induced and sustained ischemic conditions reaching approx. 10% basal CBF for up to 2 h. [19].

INTRACEREBRAL HEMORRHAGE

Spontaneous intracerebral hemorrhage (ICH) represents 15% to 20% of strokes. Its relative importance derives from the high mortality associated (35-60% at 6 months, the highest within stroke spectrum) and the severity of the permanent sequelae in survivors: only 20% are independent at 6 months compared with 60% after ischemic stroke. Its mortality and mortality is related, not only to the place and hematoma’s size (<30 cm³ or 30-60 cm³ usually have a good prognosis and >60 cm³ a bad one), but to the SDs (>60% of patients) that are produced after the process and contribute to the perihematoma edema formation [23, 31, 43].

Advancing age, hypertension, vascular formations, cerebral amyloid angiopathy or anticoagulant and fibrinolytic agents are among the risk factors. And the most common location is putaminal (35%) [43]. Aggravation of brain injury after ICH is linked to rebleeding, mass effect and brain edema, which develops in a stepwise manner: transient initial peri-hematoma edema is associated with transient reduction in blood flow in the very same area, and after 24-48 h. there is a disruption of the BBB along with a thrombin-dependent edema. Later, the erythrocyte lysis unleashes inflammatory reaction in the parenchima and SDs [31].

Serial CT images: a) admission, b) hematoma evacuated, c) 10 days after, d) four weeks after. Patient 1 hasn’t perihematoma edema progression and 2 does. Midline shift increased 6-8 mm in patient 2. Panel E (patient 1) shows the placement of subdural electrode (†) and intraparenchymal ICP probe (⊲) [23].
In this image [32]↑, the duration (in min.) of recovery time from cortical silence induced by SDs in different diseases. Depression's duration is an indicator for the ability of the tissue to recover after CSD and thereby the perfusion and metabolic status at the point of measurement. The group above 8 min. (—) reflect a reduced blood flow response indicating penumbral conditions. Notice how ICH group of patients showed fast recovery, possibly because a widespread penumbra seems to be a rare feature in it.

Here, a recording of different types of SDs: a) clusters of isoelectric SDs in a patient with significant perihematomal progression 24 h. after evacuation; b) isolated SD that begins in channel D and spreads to channel A; c) 5h. ECoG showing clusters of steteotypated spreading depressions starting on channel D and spreading to A.

Hence, there is a strong association between SDs and perihematomal progression [23].

CRANIOENCEPHALIC TRAUMA

Traumatic brain injury (TBI) to human brain can be classified in two major groups [30]:

- Closed. Further subclassified depending of the velocity of the force transmission process:
  - Static
  - Dynamic: more common mechanism to cause TBI. Depending of the loading:
    ➤ Impulsive
    ➤ Impact

- Penetrating

The damage generated by TBI can be classified in primary (direct effects of mechanical energy in the brain tissue that can be focal (epidural, subdural, or intracerebral hematomas) and/or diffuse (diffuse axonal injuries - DAI-) or secondary, which need latency of hours to days to occur and include events with consequences such as ischaemic and/or hypoxic brain injury or brain swelling [30].

In the post-concussion syndrome, transient global amnesia could be due to cortical spreading depression [37]
It’s been found after a blow to the head, there is an increase in interstitial glutamate concentrations. This leakage of glutamate excess either provides from the direct damage to neurons and other parenchymal cells or loss through newly formed micropores in cell membranes; by BBB damage and extravasation of glutamate; or by posttraumatic impairment of GLUT-1 transporters, which, under physiological circumstances, efficiently clear great part of synaptic glutamate. The increasing NMDA/AMPA receptor activation induces \([Ca^{2+}]_i\) overload with subsequent activation of enzymes dependent on this ion that will ignite inflammatory cascade to apoptotic pathways [30].

After TBI there also elevated risk of secondary cerebral ischemia mainly due to two mechanisms: direct trauma to cerebral arteries, and vasospams induced by leakage from damaged BBB and extravasated blood from injured vessels. Also the increasing demands of glucose after TBI to compensate ionic disbalances creates a lactate acidosis in regions lacking of oxygen. The cerebrovascular uncoupling leads to ischemic injury in areas already damaged by trauma [30].

Other factors that worsens the prognosis as well is the cytotoxic edema we previously described, result from a water influx into astrocytes and neurons due to a brutal ionic disbalance. Isolated posttraumatic cytotoxic edema is not enough to explain the massive brain edema common in post TBI patients. Other mediators and factors (image on the left), apart from mytochondrial damage and ionic disbalance that contribute to cytotoxic edema, include aquaporins, matrix metalloproteinases, enzymes and proinflammatory, vasoactive agents that increase BBB permeability (e.g.: bradykinins and neuropeptides like CGRP) [19, 30].

SDs are present in 50-70 % of TBI patients. A study was conducted in TBI patients requiring craniotomy that also received implanted subdural electrodes for 7 days after surgery. Patients showed worse outcomes linked to CSD and an increase in the probability for SD to occur with low mean arterial pressures, low cerebral perfussion pressures and increasing body temperature [29].

In summary, SDs have been shown to worsen tissue perfusion in injured human brain [2].
Migraine aura (1/3 migraineurs) consists of transient focal neurological symptoms (visual, sensory, language, motor) that tend to last for 5 min. to an hour and may occur before or during the headache phase. The most common is **visual** aura and consists of either positive symptoms (shimmering, sparkling, flashes of light) or negative symptoms (blurred vision or its loss) in both eyes. Scintillating scotoma is the classical hallmark and starts as a small shimmering or blurred spot lateral to the visual fixation point. Then it expands assuming a curve or sickle shape with a zigzagging or serrated border (fortification spectra), sometimes multicolored or sparkling in appearance. When **sensory** aura accompanies it usually does within minutes after visual one and consists of unilateral parasthesias in one side of the face or a limb that progresses down the limb or face with numbness. **Language** aura (transient language problems like dysphasia) is much less common and **motor** aura involves unilateral weakness of a limb or one side of the face [18, 43].

The aura results from a cortical spreading depolarization causing spreading oligemia that travels from the occipital lobe (dorsal) to ventral usually stopping at the central sulcus.

Aura symptoms description may differ from patient to patient. The order of appearance of some symptom may differ between individuals [40]. Again, we remark the heterogeneity of CSD.

As we explained earlier, there are modifying genetical factors that increase susceptibility to CSD. Misense mutations in mice in the gene encoding the pore forming α1 subunit of voltage gated P/Q Ca\(^{2+}\) channel, responsible for the rare autosomal dominant for of MwA familial hemiplegic migraine type 1 (FHM1), induce a gain of function for it decreases the threshold and slower voltages activate the channel. Mice carrying this mutation also showed greater velocity of CSD. In FMH2, mutations in the α2-subunit of the Na+/K+-ATPase, an isoform exclusive in adult astrocytes. Its impairment prevents proper glutamate clearance from synaptic cleft which increases excitatory neurotransmission [6].
CSD is a powerful igniter for the migraine headache. Products released because of CSD (K⁺, NO, arachidonic acid, H⁺), facilitated by MMP activation and mild disruption of BBB, discharge a network of trigeminal axons, promotes conduction to and discharge of second-order neurons and cause local neurogenic inflammation and mast cell degranulation within dura mater. The trigemino-vascular (small-caliber pseudounipolar sensory neurons arising from trigeminal ganglion and upper cervical dorsal roots that project to innervate pial vessels, dura mater, large cerebral vessels and venous sinuses) activation plays a key role in migrainous headache sphere (image below) [15, 41].

In addition to peripheral activation following aura, there are central modulators (image above) playing a substantial role either enhancing or inhibiting responses in the trigeminal nucleus caudalis. So let’s see: nociceptive receptors in the head send signals via primary sensory afferent neurons through the trigeminal nerve and upper cervical roots (trigemino-cervical complex) to synapse in TNC, which projects to thalamus and then to cortex. It also synapses with periaqueductal gray substance with further projections to thalamus and hypothalamus and then to cortex. The latter regulates back the TNC via descending projections to the hypothalamus, and directly from the PAG through rostroventromedial medulla (RVM). The insula projects corticofugal fibers contralaterally to facilitate response in laminae I-II in within the spinal trigeminal nucleus, whereas primary somatosensory cortex inhibits laminae III-IV. Because these cortical regions are somatotopically disposed, it’s been proposed corticofugal projections may contribute to top-down regulation by fine tuning the topographic localization of headache pain [41, 43].

CSD changes may trigger activations of meningeal endings and trigemino-vascular system, causing the headache phase. The latter can occur through MMP activation that increases vascular permeability releasing nociceptive molecules from mast cells (e.g.: cytokines). The migraine pain phase is due to peripheral and central sensitization of the trigeminal system as well as to the CGRP (vasodilator) [6] release both centrally and peripherally. Periaqueductal gray (PAG) - its anomalies leads to hyperexcitability in trigeminal nociceptive pathways - locus coeruleus (LC) or nucleus of raphe magnus (NRM) - whom impairment leads to vasomotor instability- are key parts of the brainstem possibly affected in MwA that would explain the headache spectrum [6, 43].

**TRIGEMINOVASCULAR ACTIVATION BY CSD [41]**

The CSD triggers the opening of Panx-1 channels followed by the release of proinflammatory mediators such as HMGB1 or IL-1β. We see also nuclear translocation of astrocytic NF-κB and enhanced expression of iNOS and COX-2 along with sustained cytokine and prostaglandin production from glial cells. These cross the glia limitans (formed by the astrocyte feet) to reach the sensory axons. Following discharge of pial and dural trigeminal axons, impulses are carried centrally (orthodromically; arrows from vessel to trigeminal nucleus caudalis -TNC- and trigeminal ganglion cells).

Then antidromic (towards the middle meningeal artery) impulses promote CSD-induced neurogenic inflammatory response and mast cell degranulation within meninges plus reflex dilation of the middle meningeal artery via brainstem connections.
CSD can inhibit the activity of neurons in the ipsilateral caudate nucleus down to 20%. Reduced activity in TNC may be assumed to contribute to pain as well as changes in cognition and behavior in patients suffering from MwA [48].

Cranial parasympathetic outflow stems from a reflex connection from the TNC to the superior salivatory nucleus (SSN). Efferents from the SSN (via facial nerve) connect with sphenopalatine ganglion, which innervates intracranial vessels (vasodilation), as well as the nasal and lacrimal glands [43].

**Several factors appear to influence in the ignition of CSD related to migraine** *(image above)* [15]. Microembolism or head trauma can evoke CSD and lead to migraine aura and headache. CSD susceptibility in enhanced or decreased by diverse factors either endogen (e.g.: monogenic and polygenic determinants and sex hormones) or exogenous (weather, fasting, sleep deprivation, drugs, etc.). Only this very last item has been proved to affect CSD susceptibility [15]. Serotonergic system, known for modulate the excitability of cortical neurons (5HT1A, 5HT2A & 5HT3A receptor activation), contributes to the SD propagation [7].

Migraine is associated as well with increased risk of stroke, especially in younger women suffering from MwA. Some cardiac or pulmonary defects (e.g.: patent foramen ovale or pulmonary arteriovenous formation) with right-to-left shunts that increase the risk of cryptogenic stroke are associated with higher incidence of migraine. It’s been shown that cerebral microembolism can initiate CSD without causing lasting tissue injury [15].

FHM patients, given that their threshold for CSD is lower, may display a particular tendency to respond with CSD events to a mild head trauma or ICH, and deteriorate much more severely in such incidents than in the general population [31].

*In this last image [31] we review the presence of CSD along the pathologies we have analyzed:*

**Various EEG measures reflect the severity of the CSD observed in the human brain, including the DC shift duration (1), the duration of depression of high-frequency spontaneous activity (2), the extent of recovery of HF-EEG after depression (3) and the periodicity of recurrent CSD events (4). The bottom panel shows the prevalence of each type in different diseases.**
Learning to distinguish an ictal epileptiform event (IEE) - the pathophysiological correlate of convulsive and non-convulsive epileptic seizures - from a spreading depolarization comes in handy. The DC shift of the SD is several times larger than the negative shift of an IEE. The latter causes rhythmic discharges whereas the SD induces a depression of spontaneous activity as we have reviewed. An IEE can spread at a much faster rate than SD, even at 90 mm/min. [10]

They both rise in response to an energy-supply mismatch, found in severe cerebral injuries that increase neuronal excitability. Anyway, SD are more common [9].

But due to the hyperexcitability context they exist in, a link between them has been made: the spreading convolution. An hybrid phenomenon between IEE and SD, characterized by epileptiform field potentials (iEFP) on the tailing end of the DC shift instead of the usual spreading depression. A reduction of inhibitory tone seems to play a crucial role in spreading convolution genesis [9, 10].

The relation between epileptic activities and SD is complex. Spreading depolarizations can be ignited in a susceptible area by a single discharge of an epileptic focus termed spike-triggered spreading depolarization. And repeated spreading depolarizations may enhance epileptic activities by selective suppression of GABAergic function [10].

iEFP can precede SD in acute epilepsy and were also demonstrated in the front of SD in brain-injured patients. iEFP and SD usually occur in an alternating fashion and, after recovery from SD, iEFP are still blocked for periods up to 10 min. [10].

It’s been found that the development of late post-hemorrhagic seizures is related to a higher peak number of SD in the ECoG on the subacute phase after aSAH. Targeting SD in the early course of the disease could potentially interrupt this cycle and lower the incidence of post-hemorrhagic seizures.

Here, cooccurrence of iEFP & SD at the vicinity of a right frontal intracerebral hematoma [10].
OTHER DISEASES RELATED TO CSD

MwA is usually the first manifestation of cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) caused by NOTCH3 mutations genes mutations expressed predominantly in vascular smooth muscles. In laboratory, mice with a R90C mutation in NOTCH3 or knock-out mutation showed enhanced CSDs. This fact support the importance of the trigeminovascular unit in the development of migraine aura. Lastly, relation between SD and other vasculopathies like RVLC (retinal vasculopathy with cerebral leukodystrophy) and COL4A1 mutations has raised the possibility of primary vascular determinant of SD susceptibility or induction [2, 6].

Spreading ischemia has been proposed as a model for migrainous infarction, or in the pathophysiology of MELAS syndrome (mitochondrial encephalopathy, lactic acidosis and stroke) [2].

CSD IN DYING PATIENTS [11]

Researchers found interesting to study the presence of spreading depolarizations in terminal patients (monitoring as well arterial pressure, \( p_{O_2} \), with a Do Not Resuscitate-Comfort Care order after devastating brain injuries. Massive irreversible injury of most vulnerable cells (e.g.: neocortical pyramids of layers III, IV & V, hippocampal CA1 pyramid cells, striatal cells and cerebellar Purkinje cells) happens within <10 min. after circulation ceases in situation such as cardiac arrest.

Then, the anoxic/asphyxial depolarization arrives as we described talking about CSD in stroke. It initiates a plethora of toxic changes, including intracellular \( Ca^{2+} \) and \( Na^+ \) loading, rise in extracellular glutamate and cytotoxic edema as we commented. This cellular injury develops after the SD ignition, not during the non-spreading depression that occurs after circulatory arrest.

Deeper knowledge about neurophysiological processes in the final stage of life would not inform not only research on treatment strategies after cardiac arrest but also the debate on organ donation after circulatory where death is declared between 2-10 min after cardiocirculatory function cessation.

Circulatory arrest is followed by non-spreading depression and then terminal SD.

**Note how terminal depolarization arises in electrode 4 after arterial pressure reached its minimum.**

**Similar to a normal SD, terminal SD recorded with depth electrodes shows an initial and a late negative DC component. A) CT of a patient with a traumatic subarachnoid hemorrhage and skull vault fractures due to train struck. B) The late component, the NUP is similar to the DC shifts of prolonged SDs, but specifically refers to a negative potential component generated by progressive recruitment of neurons into cells death in the wake of SDs.**
In this study they found out in patients with acute brain injury, persistent states of electrical silence in cortex are in many cases induced by SD, because the initial depression occurs in a spreading pattern. SDs can repeatedly invade silent tissue can not induce further depression (isoelectric).

In patients undergoing global cerebral ischemia non-spreading depression occurred, which typically preceded the terminal SD by several tens of seconds. It’s been suggested the underlying mechanism of non-spreading depression is based on a neuronal or glial oxygen sensor that shuts down neuronal activity once it senses a step decline in pO2. Here non-spreading depression occurred during a step decline in pO2. The rest of SDs, like focal ischemia, happened accord with the characteristics we have described in this work, inducing depression in healthy tissue.

Terminal SD (anoxic-triggered SD) is fully reversible without any signs of cellular damage, if the oxidative substrate supply is reestablished before the so called commitment point (time when neurons star dying under persistent depolarization).

In summary, non-spreading depression and terminal SD have been found in patients with acutely injured brain undergoing global cerebral ischemia in the dying process. A non-invasive clinical method to diagnose the onset of terminal SD, as well as its reversal upon restoration of the circulation, may have practical value for recovery prognosis and as indication/endpoint point for resuscitation efforts. Hence, human brain death should require at least the developing of terminal SDs in cerebral gray matter but nowadays there is no evidence that confirms the point when terminal SD turns irreversible.

**DETECTION AND REGISTER METHODS: IMPORTANCE IN NEUROCRITICAL CARE**

The gold-standard to monitor SDs is the electrocorticography with linear subdural platinum electrodes placed operatively through burr holes or craniotomy [4, 9].

Now that we have seen the whole complex of what a spreading depolarization is and implies, I shall end this work with the two most critical parts: how to apply it in the patient care.

Depolarizations are a sensitive measure of relative ischemia, vulnerable brain regions and ongoing secondary injury, and may serve as a clinical guide for personalized, mechanistically targeted therapy [22].
The SDs create a large negative shift of the baseline, or direct-current (<0.05 Hz), potential of cerebral cortex in the electrocorticogram. Because shift reverses with the repolarization, DC recordings measure the length of the depolarization and in this way, the cortex metabolic status and the potential for local depolarization-induced injury [22].

SD also causes a depression of the spontaneous activity in the 0.5–70 Hz band of the electroencephalogram, termed spreading depression, which, through mechanism yet to discover, can last much longer than the SD itself. SD don’t create depression when the tissue is already electrically silent (isoelectric SD). They indicate vulnerable but viable tissue [22].

Several clinical studies have proved that the presence of SDs is linked to a worse outcome in disease progression. In TBI, isoelectric depolarizations were associated with increased risk of poor 6-month outcome. In aSAH, temporal clusters of depolarizations were associated with delayed ischemic neurologic deficits, including new infarcts. As we’ve discussed previously, they also associated to perihematomal lesion expansion. The ups and downs, the waxing and waning of neurological deficits commonly observed in neurointensive care patients could be due to SDs [22].

Appart from the intracranial pressure (ICP), cerebral perfusion pressure (CPP), oxygen availability (pO2) or scalp electroencephalography, it would come in handy to develop a system or device that allow to register precisely SDs so the clinicians are provided with a powerful diagnostic weapon that allow them to detect and prevent the secondary damage due to SDs we have described. This ideal measure for disturbed brain energy metabolism should be: i) available at bedside in order to diagnose early

Every full band ECoG [9] contains intel on both negative DC shift that identifies SD and the SD-induced depression of activity. We can observe SDs in different ranges depending of how much filter we use, ranging from full-band to band-pass filtering with AC-ECoG that allow us to appreciate the minimum details.

These are the wave-widths and durations for each signal that serve as footprint of a SD [9].

Clusters of SDs [22] in a 19-year-old patient that suffered a fall and went under bilateral decompressive craniectomy with right frontal contusion evacuation. SDs began to occur in clusters at intervals of 40–70 min. the day after surgery. Even when patient was extubated and monitorization retired 37 hours post-surgery SD still occurred. a) ECoG from final hours before strip removal showing recurrent SDs from 4 to 2. b) High resolution of full-band recording of a SD denoted by (*). c) Intraoperative photo with the electrode strip placement and ICP probe as well. d) Postsurgical CT shows right frontal hemorrhagic contusions. e) T2 FLAIR MRI at 4 months after trauma.
and prevent damage; ii) high sensitivity and specificity; iii) be non or minimally invasive; iv) procedurally simple to implement and durable; vi) responds rapidly to treatment and reflect treatments efficacy in real-time [9, 14]. Scalp EEG alone is not enough to provide reliable data on SDs [9, 20].

For further technical aspects about guidelines to properly register SDs, electrode placement, surgical preparation or processing of the data obtained, I refer the reader to a group of clinical guides and papers [4, 9, 14, 19].

There also other ways to observe the SDs like intrinsic optic signaling (IOS), based on illuminating the cortex surface with different wave-lengths of light between 500-650 nm. [page 29]. Fast voltage-sensitive dye imaging techniques can be used as well (image).

CURRENT AND FUTURE THERAPEUTIC APPLICATIONS: PHARMACOLOGY OF CSD

It’s obvious to think that if we can target the molecular mechanisms underlying SDs, we could treat the deleterious effects these have over brain under pathological circumstances, hence reducing morbidity and mortality greatly. Many drugs have been tried, focusing on the wide range of excitotoxic mechanism under the SDs and processes related. Great part of the neurophysiological aspects in CSD have been discovered through inhibition of excitotoxicity-related channels or membrane ion channels for example. And given the existant amount of knowledge about the CSD-related, there is an equal amount of drugs which have been tried to stop SDs or its clinical frames. So we can do nothing but enumerate some of the most relevant, specially applied in migraine treatment.

The symptomatic drugs used to treat migraine haven’t proved to affect CSD given that they do not inhibit the aura symptoms. Some among those used in the preventive treatment (tricyclic antidepressants, β-blockers, Ca²⁺ channel blockers and antiepileptics) have been proved effective treating both MwA and MwoA. Currently, drugs like dydroergotamine (DHE), acetysalicylic acid, lignocaine, metoprolol, clonazepam and valproate at single dose prior to CSD induction, were able to inhibit the CSD, reduce the rate of propagation and modify the amplitude of cortical blood flow increase. CSD on the other hand has been succesfully blocked by NMDA-R blocker MK-108 or halothane [5, 6, 16].

Nowadays the administration of specifics prophylactic drugs (topiramate, valproate, propanolol, amitriptyline or methysergide) dose-dependtly proved to inhibit CSD frequency and increase the cathodal stimulation threshold. Although acute administration of these drugs was ineffective. Lamotrigine, a potent Na⁺ channel blocker, as well has been proved to affect MwA but not in MwoA. Chronic treatment with them appears to have a marked suppressive effect on CSD [16].
There tons of other novel drugs currently under research. Tonabersat is a unique molecule with gap-junction inhibitory activity that has been suggested to be useful in both treatment and prophylaxis of migraine. However, results are troubling. Amiloride has also been studied as intractable MwA drug due to its trigeminal inhibition through via acid-sensing ion channel (ASIC) mechanism. ASIC is a family of voltage-independent cation channels that are activated by pH. Its activation lead to membrane depolarization, neuronal excitation and calcium influx through voltage-gated Ca\(^{2+}\) and NMDA-R. Specific ASIC1a inhibitor psalmotoxin has also been researched [5, 6]. ASIC3 for example regulates the CGRP release in astrocytes. CGRP is one of the most relevant trigeminal pain mediators. We can infer then that inhibition of CGRP (e.g.: olcegepant) constitutes a therapeutic target to combat CSD effects [5, 6].

Kynurenic acid, an endogenous derivate from of tryptophan metabolism, acts as a NMDA-antagonist and so it’s also been studied to increase its effects through the systemic administration of its precursor to treat CSD. Ketamine is another NMDA-R antagonist which showed CSD inhibition in early experiments. It’s been proposed as a putative treatment option for severe and prolonged aura. Analgesics and sedative drugs have also proved to diminish [24, 5]. Panx-1 channels inhibitors (e.g.: carbenoxolone) were also used to treat SD susceptibility but the result showed disappoining. It blocked the inflammatory cascade but had no effects on SD susceptibility [24].

Even cannabis has been empirically used for centuries as both symptomatic and prophylactic treatment for different types of headaches, including migraine. Recent studies suggest that indeed it has a dose-dependent suppression of CSD amplitude, duration and propagation velocity after delta9-tetrahydrocannabinol (THC) administration [6].

Insulin-like growth factor 1 (IGF-1) breaks the vicious cycle where SDs generates oxidative stress in cortex, meninges and trigeminal ganglion, that in return, enhances the SD causing by causing metabolic deterioration. So it could be used to decrease SD susceptibility. Magnesium levels have been described to increase possibilities for an SD event so keeping them in proper measure is also a therapy [16].

Endothelin-1 (a main responsible for vasoconstrictive SD mechanisms) receptors antagonists could be also used to treat CSD-related ischemia [29].

Other techniques such as transcranial magnetic stimulation or transcranial direct current stimulation have been described for suppressing or modulate SD effects. Vagus nerve stimulation as well has been described and used as a treatment for migraine symptoms [5].
5. CONCLUSIONS AND STRIKING MESSAGES

There are several centers around the globe investigating spreading depolarizations. The issues I have explained in this work should be among neurologists basic concerns given that successful treatment of the secondary effects these have, could imply a change in neurological therapies equivalent to the discovery of penicillin for infectious diseases.

The understanding of spreading depolarization complex should become very important in the neurological knowledge. Here we are dealing with the neurophysiological basis of infarct growth, perihematomatol edema progression and neuronal death associated to cranioencephalic trauma. So finding new ways to target and stop this process is critical, in order to reduce the morbidity and mortality greatly.

- **KEY MESSAGES:**

⭐ A spreading depolarization is a pervasive self-propagating wave that can potentially occur in any zone of the gray matter in the central nervous system, specially in the cortex, but always in non-healthy brain tissue.

⭐ It consists on the greatest transmembrane ionic gradient disbalance, in both in neurons and glia, seen in the brain which is the basis for the two most outstanding deleterious effects: cytotoxic edema and neuronal death.

⭐ It can be ignited by several factors. But ischemic, mechanical or a rise in extracellular K⁺ are specially relevant for it’s also the possible mechanism under the self-propagating properties.

⭐ It’s present in stroke, intracerebral or subarachnoid hemorrhage, migraine with aura, cranioencephalic trauma and epilepsy. The higher its number and the longer they are, the worse is the patient’s outcome.

⭐ It induces, but not always -when tissue is already silent-, a spreading depression of the spontaneous neuronal activity, responsible for the deficitary symptoms in migraine with aura or stroke.

⭐ Under physiological circumstances it induces an spreading hyperemia followed by a sustained oligemia. But under pathological circumstances it will create a spreading ischemia that will last as long as the mismatch between energy demand and supply is not satisfied.

⭐ A SD is a shape-shifter that changes its characteristics depending on the metabolic status of the tissue. After an ischemic injury we appreciate this along its way through different zones of nutrients supply and we call it the spreading continuum.

⭐ In the electrocorticography (gold-standar to register SDs), its hallmark is the negative DC shift and the negative ultraslow potential of ischemic injury is the largest electrophysiological signal at the human brain surface.
6. REFERENCES

4. Brain Tsunami’s Website (https://www.charite-academy.de)


32. Leão. Spreading depression of the activity in the cerebral cortex. Harvard Medical School. 1944


38. Østergaard, Dreier. Neurovascular coupling during cortical spreading depolarization and depression. 2015. Stroke; 1392-1401


40. Petrusic, Zidverc-Trajkovic. Cortical spreading depression: origins and paths as inferred from the sequence of events during migraine aura. Functional Neurology. 2014; 29 (3) 207-212


47. Schock, Munyao. Cortical spreading depression releases ATP into the extracellular space and purinergic receptor activation contributes to the induction of ischemic tolerance. Brain Research. 2007; 1168: 129-138


7. ACKNOWLEDGMENTS

I’d specially like to thank Dr. José Luis Fernandez-Torre for his help, not just in the elaboration of this job, but for his guidance along my medical studies in this university and further.

“If you follow the classical pattern, you are understanding the routine, the tradition, the shadow... You are not understanding yourself.”

Bruce Lee
END OF THE DOCUMENT