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Electromagnetic interventions as a therapeutic approach to spreading depression

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ELECTROMAGNETIC INTERVENTIONS AS A THERAPEUTIC APPROACH TO SPREADING DEPRESSION

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VAMSEE REDDY

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Approved by

First Reader

Richard Jarrett Rushmore III, Ph.D.
Assistant Professor of Anatomy and Neurobiology

Second Reader

Gwynneth D. Offner, Ph.D.
Director M.S. Medical Sciences Program,
Associate Professor of Medicine

DEDICATION

I dedicate this work to Milton H. Saier Jr., who has been my friend and mentor.

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ABSTRACT

Spreading depression (SD) is a slow propagating wave of depolarization that can spread throughout the cortex in the event of brain injury or any general energy failure of the brain. Massive cellular depolarization causes enormous ionic and water shifts and silences synaptic transmission in the affected tissue. Large amounts of energy are required to restore ionic gradients and are not always met. When these energetic demands are not met, brain tissue damage can occur. The exact mechanism behind initiation and propagation of SD are unknown, but a general model is known. It may be possible to prevent or delay the onset of SD using non-invasive electromagnetic techniques. Transcranial magnetic stimulation (TMS), electrical stimulation (ES), and transcranial direct coupled stimulation (tDCS) could be used to decrease neuronal excitability in different ways. In theory, any technique that can reduce cortical excitability could suppress SD initiation or propagation.

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LIST OF ABBREVIATIONS

ACF	artificial cerebrospinal fluid
ASD	anoxic spreading depression
CBF	cerebral blood flow
CSD	cortical spreading depression
CYC	D-cycloserine
DMO	dextromethorphane
ECF	extracellular fluid
EEG	electroencephalogram
FAc	fluoroacetate
FC	fluorocitrate
LTD	long-term depression
LTP	long-term potentiation
MT	motor threshold
NMDA	N-methyl-D-aspartate
rTMS	repetitive transcranial magnetic stimulation
SD	spreading depression
SSRI	Selective Serotonin Reuptake Inhibitor
tDCS	transcranial direct current stimulation
TEA	tetraethylammonium

TMA^+	trimethylaluminum
TMS	transcranial magnetic stimulation
TTX	tetrodotoxin

INTRODUCTION

What is Spreading Depression?

Spreading depression (SD) was first observed in the cerebral cortex of rabbits. In 1944, Aristides Leao noticed that small mechanical or electrical stimuli in the rabbit cortex produced a wave of electroencephalogram (EEG) silence that slowly spread through the cortex (1-5mm/min) in a radial manner (Leao, 1944). The suppression of EEG activity is the result of a preceding wave of depolarization in the grey matter of the central nervous system. Cortical depolarization begins at the site of the mechanical/electrical stimulation, and propagates like a wave in a radial manner throughout the tissue. This period of prolonged depolarization occurs in neurons and glia, and precludes synaptic transmission. The cessation of synaptic transmission results in EEG silence. The term "spreading depression" is thus misleading because the cellular substrate of this process takes on a higher intracellular potential (excited state) than at rest. The term 'depression' describes the prolonged suppression of cortical activity lasting tens of minutes after the insult.

SD has been experimentally demonstrated in a variety of species including certain mammals, reptiles, cephalopods, birds and locusts (Yu et al., 2012). The condition shared by these species that are conducive to SD is the presence of a continuous cortical grey matter since interruption of gray matter continuity prevents SD propagation(Fujita et al., 2016). While SD is conducted through continuous grey matter, it is also impeded in the proximity of large cerebral arteries. This impedance suggests some soluble protagonists as a driving factor that triggers depolarization (Fujita et al., 2016). Spreading

depolarization propagates at a reduced velocity near regions of high astrocyte density, which suggests that astrocytes may play a role in buffering these soluble driving factors (Peters, Schipke, Hashimoto, & Kettenmann, 2003).

SD also has clinical relevance in human neurological conditions. SD has been observed in ischemic and hemorrhagic stroke, brain trauma, and transient global amnesia (Dohmen et al., 2008; Fabricius et al., 2006; Hartings et al., 2009). Recent evidence indicates that SD may also be responsible for the aura experienced during a migraine (Hadjikhani et al., 2001). In light of its clinical prevalence, SD has become an important diagnostic and therapeutic target in neurovascular disease. SD is an attractive therapeutic target because it is known to worsen the outcome of brain injury through its precipitating metabolic and vascular changes (Pinard et al., 2002). Depolarization of the cellular substrate induces massive ionic and water shifts that flood neighboring cells with excitatory neurotransmitters and ions, which perpetuate a cycle of depolarization. Large amounts of energy are required to restore ionic homeostasis after SD. Such energetic demands are greater than of brain seizures, and are especially problematic in the context of brain injury (Dreier et al., 2013).

General Mechanism of Spreading Depression

A sufficiently strong electrical or mechanical stimulus can evoke SD. The rodent cortex has a critical volume of roughly $1mm^3$ that must be depolarized to initiate SD (Matsuura & Bures, 1971). The depolarized neurons release large amounts of potassium, which overwhelms the brain's potassium clearing mechanisms, allowing the extracellular

potassium concentration ($[K^+]_o$) to reach a critical threshold (Grafstein, 1956; Hansen & Zeuthen, 1981). Unmyelinated cells in the central nervous system experience a sudden drop in membrane resistance as large-conductance non-selective cation channels open in cells exposed to high potassium concentrations (Cenk Ayata & Lauritzen, 2015). Intracellular and extracellular ions flow down their concentration gradient (Table 1). K^+ efflux increases $[K^+]_o$ from 3mM to 30-50mM in an all-or-none manner (Brinley et al., 1960). Extracellular Na^+ flows into the cell through the same cation pores, followed by chloride via separate chloride channels (Freygang Jr & Landau, 1955). Extracellular water follows Na^+ and Cl^- into the neuron, causing the cell to swell. The massive movement of water shrinks the extracellular space by more than 50% and further concentrates extracellular solutes, including K^+. The accentuated $[K^+]_o$ causes nearby neurons to depolarize and perpetuates the cycle of spreading depolarization seen in SD (Windmüller et al., 2005). Although neuronal depolarization does not occur through the typical action-potential manner, voltage-gated calcium channels in axonal terminals still trigger the release of neurotransmitters during the depolarization phase of the SD (Cenk Ayata & Lauritzen, 2015). While K^+ is likely the primary driver of SD, a combination of ions, and excitatory neurotransmitters play a role in SD propagation. The precise mechanism by which SD proceeds is unknown, but there are several theories in the literature, which are not mutually exclusive. These theories offer insight to the mechanism of SD and may hold the key to novel therapeutic interventions that can improve the clinical outcomes of SD events.

SPECIFIC AIMS AND GOALS

Overall Objective

The objective of the present review is to explore the potential application of electromagnetic (EM) based interventions in the treatment of SD. A non-invasive protocol that can prevent or reduce the spread of SD may prove very useful in clinical settings. In order to understand and predict how various EM interventions could affect SD, a detailed mechanistic model of SD must be first illustrated.

Specific Objectives

1. Literature review of neurophysiological features of spreading depression
    a. Understand certain aspects of initiation & propagation mechanisms
2. Literature review of electromagnetic (EM) stimulation protocols
    a. Transcranial magnetic stimulation (TMS)
    b. Transcranial direct-coupled stimulation (tDCS)
3. Discuss the feasibility of using EM protocols to treat SD

Neurophysiological Features of Spreading Depression

Extracellular Potential shifts

The most prominent and observable feature of SD is a change in the extracellular potential. Neurons are like capacitors that hold a charge and are fully discharged when they are involved in SD. Massive ionic shifts occur when neurons are depolarized, causing their intracellular potential to increase while the extracellular potential declines. The extracellular potential shifts are on the order of millivolts, and can reach up to -30mV in magnitude (Hansen & Olsen, 1980). The large depolarization of neurons is analogous to shorting out a circuit. The depolarization of neurons during SD can be distinguished from the features of a typical action potential. Action potentials are short events (~1-2ms) that produce extracellular potential shifts on the order of microvolts and can propagate over a meter in one second (myelinated axons propagate as much as 40m/s). SD depolarization (spreading depolarization) on the other hand, produces extracellular shifts on the order of millivolts and travels only a few millimeters per minute (Ayata & Lauritzen, 2015). The extracellular potential shift in SD can be measured using ultrafiltered direct-coupled (DC) amplification. Due to this shift's slow onset and decay, typical high-pass AC amplifiers are not capable of detecting this phenomenon (Ayata & Lauritzen, 2015). For these reasons, this electrophysiological event has been coined as the DC shift (Figure 1).

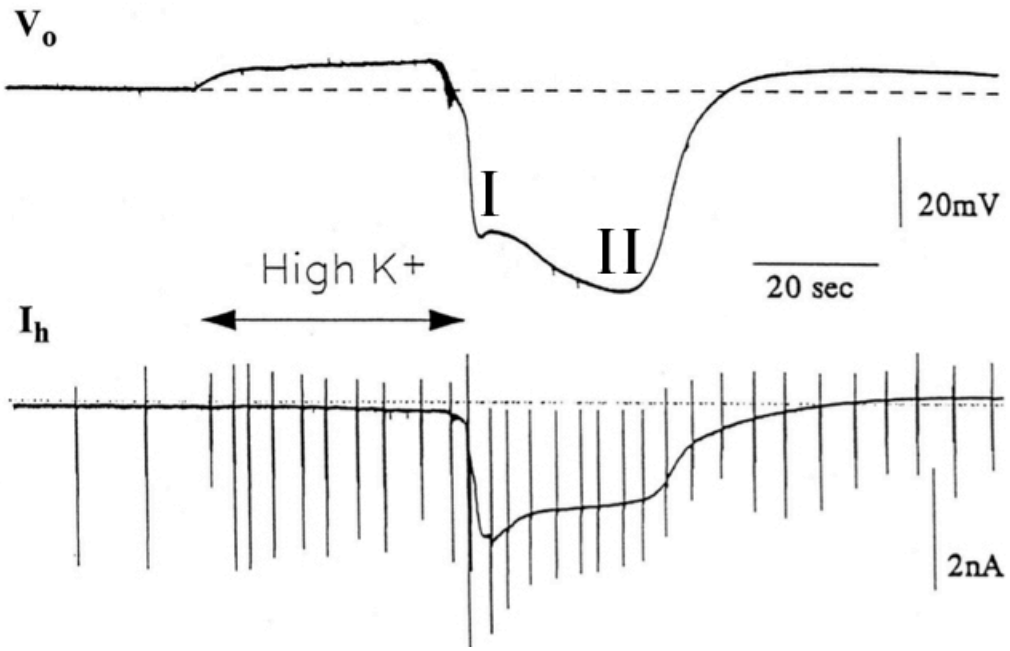

Figure 1. Extracellular voltage recording (DC-shift) and whole-cell current reading during SD of a pyramidal neuron from CA1 of hippocampus slice. Top graph is the extracellular potential near the neuron. Bottom graph is the holding current needed to maintain a holding potential of -71mV. The vertical lines represent voltage ramp voltage probes that were used to test voltage-dependent responses. KCl was applied in the time-frame denoted by the double headed arrows. This region does not represent phase 0, but that is where it would appear if recorded. Phase 1 & 2 are indicated. A second minimum rather than a plateau characterizes phase 2. Figure modified from (Czéh et al., , 1993)

Electrophysiological recordings have revealed a burst of high frequency spikes immediately before the onset of the DC shift. This spike in activity is characteristic of increased spontaneous neuronal activity (Grafstein, 1956). The onset of SD, characterized by the arrival of the DC shift, has two phases: I) a rapid negative voltage peak, followed by II) a less negative plateau (hump) or decline to a more negative peak (second maximum). Sometimes a positive inflection is seen before both phases (referred to as

phase 0), but it is usually not observed in SD recordings (Czéh et al., 1992, 1993). Depending on the types of currents driving the spreading depolarization process, the second phase can present itself as either a notch/hump or as a second minimum (Herreras & Somjen, 1993). Although there are multiple ways to initiate SD, the DC shift is an all-or-nothing event. These electrophysiological properties are fairly uniform once propagation of SD has occurred with some subtle variations in its second phase (Somjen, 2001).

Ion Flux During Spreading Depression

The use of ion-selective microelectrodes has made it possible to measure specific ion flux during SD. Extracellular K^+ increases and is the largest ion flux seen during SD. A precipitous drop in extracellular Cl^-, Na^+, and Ca^{2+} parallels the K^+ efflux (table 1), and results in a net decrease in extracellular potential. The ion movements that occur during phase I of the DC shift are the most profound and is the major current that drives SD as a whole. No specific channel has been identified, but circumstantial evidence strongly suggests some large conductance non-selective cation pore is responsible for the initial ion flux (Ayata & Lauritzen, 2015; Martins-Ferreira et al., 2000; Somjen, 2001). The timing of the individual ion fluxes offers some insight into the mechanisms driving SD. The efflux of K^+ and the influx of Na^+, Cl^-, and Ca^{2+} have been temporally correlated with the emergence of phase I of the DC-shift (Hansen & Zeuthen, 1981). It appears that NMDA channels are largely

responsible for generating phase II of the DC-shift. Phase II of the DC-shift is dampened during microdialysis with the NMDA antagonist, (±)-3-(2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP) and does not affect phase I. The second phase reappears further away from the source of the CPP dialysis (Herreras & Somjen, 1993), indicating that phase II of the DC-shift is largely driven by NMDA mediated currents. While extracellular Ca^{2+} concentration begins to decline during phase I, a more abrupt drop also occurs at the emergence of phase II of the DC-shift (Hansen & Zeuthen, 1981). NMDA channels are non-selective cation conductors and are probably responsible for the increased Ca^{2+} permeability. Neither phase II nor I is affected by tetrodotoxin (TTX), which indicates that voltage-gated Na^{+} channels are not directly involved in SD propagation, but voltage-gated Na^{+} channels may still be recruited as a secondary response to depolarization (Tobiasz & Nicholson, 1982).

Table 1. Extracellular ion concentrations before and after spreading depression*.

Ion	Extracellular Conc. Before	Extracellular Conc. After
K+	3 mM	30-60 mM
Ca2+	1.2 mM	0.2 mM
Cl-	120 mM	50-70 mM
Na+	150 mM	50-70 mM

*Measurements obtained from the lissencephalic cerebellar molecular layer of the catfish, Corydoras aneus. SD was induced via micropipette injection of 1M KCl. Liquid ion exchangers with ion-selective electrodes were used to measure extracellular concentrations 50uM beneath the cerebellar molecular layer under normothermic conditions (adapted from: Kraig & Nicholson, 1978; Somjen, 2001).

Sodium currents

Sodium currents are essential for the initiation and/or propagation of SD. Substitution of Na^{+} with choline or trimethylaluminum (TMA^{+}) attenuates or abolishes

SD in isolated retinal tissue in a concentration dependent manner (Martins-Ferreira, De Oliveira Castro, Struchiner, & Rodrigues, 1974). When voltage-gated Na^+ channels are blocked using TTX, SD is only postponed or attenuated but not abolished (Sugaya, Takato, & Noda, 1975; Tobiasz & Nicholson, 1982). However, when SD is induced under anoxic conditions, TTX acts as a stronger inhibitor and can even abolish SD (Aitken, Jing, Young, & Somjen, 1991). These results indicate that there are several routes of Na^+ influx during SD and provide some insight into other currents driving the process. Both forms of depolarization (normoxic & anoxic) approach intracellular potentials of 0mV without becoming positive (Müller & Somjen, 2000), and whole-cell currents reverse at negative potentials (Czéh et al., 1993). These observations suggest the presence of a mixed ionic current that could be explained by the existence of large nonselective cation conductance as previously mentioned. It has been suggested that a combination of individual cation channels (Na^+, K^+, Ca^{2+}) could also produce a current with a reversal potential near 0mV (Somjen, 2001). NMDA mediated channels seem like a reasonable explanation for the mixed current, given that they are non-selective cation channels with reversal potentials near 0mv (Langomoen & Hablitz, 1981). However, it is unlikely that NMDA channels are the primary channels driving the DC-shift because NMDA currents appear to be temporally correlated with phase II, but not phase I of the DC-shift. The observation that TTX is a stronger inhibitor of anoxic SD (ASD) than SD has some interesting implications. ASD occurs in tissue that is more acidic, and NMDA channels are strongly inhibited by protons (Traynelis & Cull-Candy, 1990). Voltage-gated Na^+ channels seem to play a secondary role in SD, and are particularly noticeable

only when NMDA currents are attenuated.

Calcium Currents

Ca^{2+} appears to play a critical role in the initiation of SD and a lesser role in its propagation. Extracellular Ca^{2+} levels begin to decline at the onset of the DC-shift and experience a sharper decline after the K^+ flux has passed. The initial decline in Ca^{2+} is thought to be due to the activation of the postulated non-specific cation pores responsible for the onset of the DC-shift. Several other channels may be collectively responsible for the second sharper decline at phase II of the DC-shift. These channels include NMDA channels, voltage gated P-channels, and voltage gated Q-channels (Kunkler & Kraig, 2004). The P/Q channels are largely localized on presynaptic terminals and are essential for the release of neurotransmitters. KCl-mediated induction of SD is inhibited in hippocampus slices when non-selective Ca^{2+} blockers (Co^{2+} or Ni^{2+}) are applied to the tissue (Jing, Aitken, & Somjen, 1993). These observations suggest that Ca^{2+} mediated release of glutamate is necessary for the induction of SD. These findings are further supported by experiments done with transgenic mice, which contained R192Q mutants of the human P/Q gene (Tottene et al., 2009; van den Maagdenberg et al., 2004). These mice demonstrated increased Ca^{2+} currents and a reduced K^+ threshold for focally induced SD. These observations indicate that both extracellular K^+ and excitatory neurotransmitters likely play a role in the initiation of SD.

The role of Ca^{2+} in SD is drawn into question when SD is evoked under different experimental conditions. Removal of extracellular Ca^{2+} from cortical slices completely

abolishes KCl-mediated induction of SD. When cortical slices are immersed in a KCl bath, the inhibition of Ca^{2+} influx does not prevent SD from propagating (Basarsky et al., 1998). These results suggest that Ca^{2+} is only required for the initiation of SD and not necessarily needed to sustain its propagation. When cortical slices are immersed in a KCl bath, the typical initiation process is circumvented and SD propagation occurs. The currents driving initiation and propagation are not necessarily the same. These findings suggest that Ca^{2+} / Ca^{2+}-mediated currents fall out as SD evolves from the site of initiation. While extracellular Ca^{2+} may not be important for propagation, it is still possible that intracellular Ca^{2+} stores (i.e. endoplasmic reticulum or mitochondria) could play a role.

pH Shifts During Spreading Depression

A transient period of alkalinity followed by a period of sustained acidity can be temporally correlated with the emergence of the DC shift. The brief alkaline phase lasts only a few seconds and may be partially due to the activation of the non-specific cation channel at the onset of the DC shift (Kraig et al., 1983; Tong & Chesler, 1999). Several other mechanisms may also be responsible for the alkaline shift. Smith & Chesler (1999) have shown that electrically-evoked alkalization is partially dependent on Ca^{2+}-dependent H^+ uptake by glutamate-activated AMPA receptors. Other experiments have revealed that electrically evoked alkalization is also dependent on HCO^{3-} excursion at $GABA_A$ channels (Kaila, 1994). A combination of factors is likely to be responsible for the

increase in pH at the onset of SD and is generally less clear than the mechanism driving the acidic shift.

A sustained period of extracellular acidity follows the alkaline shift, and decays shortly after the DC shift subsides. This pH shift is believed to be a result of proton efflux as a result of increased cellular metabolism. A multitude of cellular mechanisms are quickly employed to restore ionic homeostasis at the onset of SD, and generate large amounts of lactate and CO_2 during the metabolic response that ensues (Scheller, Kolb, & Tegtmeier, 1992). Lactic acid and CO_2 are believed to be the primary source of protons during the acid-shift (Cruz, Adachi, & Dienel, 1999). It has been observed that astrocytes experience a period of intracellular alkalization that temporally coincides with the extracellular acid-shift. The negative extracellular potential that is acquired from ionic fluxes depolarizes astrocytes and activates Na^{+}/HCO^{3-} co-transporters which help restore ionic homeostasis while simultaneously alkalizing astrocytes (Chesler & Kraig, 1987). Astrocytes play a secondary role in SD, and are recruited after neuronal depolarization. These specific roles will be explored in later sections, and are important for understanding some of the precipitating metabolic and vascular changes that are observed in SD.

The changes in interstitial pH have the ability to attenuate and facilitate SD. The SD wave travels slower and over shorter distances under acidic conditions and travels faster and further under alkaline conditions (Tong & Chesler, 2000). It is believed that protons are able affect SD by modulating NMDA channels (Tong & Chesler, 1999). NMDA channels activities are 50% inhibited at physiological pH (Traynelis & Cull-

Candy, 1990). Under acidic conditions, NMDA channels are likely to be further inhibited and contribute less to the propagating SD wave.

Potassium Currents

The role of K^+ in SD has been generally accepted as the primary driving force behind SD initiation and propagation ever since Grafstein's initial hypothesis. The general model that has been accepted describes K^+ accumulating in interstitial spaces, which depolarizes neurons and releases more K^+, forming a self-perpetuating cycle. The various molecular pathways that K^+ travels are not clear, but there is evidence for several models that need not be mutually exclusive. Blocking voltage-gated K^+ channels with tetraethylammonium (TEA) reduces the amount of K^+ released during SD and lowers the amplitude of the DC-shift in a dose-dependent manner in chick retina (Ramos, 1975). High doses of TEA do not abolish SD completely in rat hippocampus slices, which suggest that voltage-gated K^+ channels are only partially responsible for the efflux of K^+ during SD (Jing et al., 1994). An increase in $[K^+]_o$ is observed at the onset of the DC-shift. One would expect $[K^+]_o$ levels to rise ahead of the DC-shift if K^+ is indeed the driving force behind propagation. Some experimental SD models, but not all, indicate that this prodromal rise in $[K^+]_o$ does not occur, and only presents coincidently with the onset of the DC-shift (Herreras & Somjen, 1993; Lehmenkühler, 1990). To align these two different findings, it has been suggested that K^+ can spread to distant neural tissue via intercellular gap junctions of glial cells (Reid et al., 1988). Glial cells are highly permeable to K^+ and act as an ionic sink to keep $[K^+]_o$ low, which has the effect of preventing SD initiation (Gardner-Medwin, 1981). Once SD is initiated however, the

same glial cells may be capable of delivering K^+ to more distant interstitial spaces surrounding neurons. K^+ can circumvent most interstitial space and does not passively diffuse and accumulate in the extracellular fluid (ECF) in this model. Instead, K^+ uptake mechanisms of glial cells displace distant intracellular glial K^+ stores via the gap junction network. The displacement of K^+ to the extracellular space can generate a high local $[K^+]_o$ that is capable of triggering depolarization of nearby neurons. When observing the DC-shift a small positive inflection (phase 0) can be recorded under certain experimental circumstances. The phase 0 of the DC shift is usually not observed, but it is possible that the sudden displacement of glial K^+ is responsible for the positive inflection. The glial network model is supported by observations that drugs that block gap junctions (heptanol, octanol, and halothane) abolish SD propagation in the isolated retina and hippocampus (Nedergaard et al., 1995). More recent studies have attributed the effects of heptanol and octanol to the closure of gap junctions between neurons and not glial cells, because selective glial poisons (fluorocitrate [FC] and fluoroacetate [FAc]) failed to abolish SD (Somjen, 2001). Somjen's modified gap-junction theory states that K^+ spreads only between neurons via intercellular gap-junctions. These findings do not definitively preclude the possibility of glial cell involvement in K^+ spread. The majority of astrocyte populations in the mature brain express large amounts of passive inwardly rectifying K^+ channels, which play a role in determining the $[K^+]_o$ threshold for SD initiation (Price et al., 2002). Even in the presence of an astrocyte-specific metabolic poison, the astrocyte network may still be capable of broadcasting K^+ through gap junctions and passive K^+ channels. It is possible that intercellular gap junctions between both glial cells and

neurons are involved in the spread of K^+ during SD. Lastly, astrocytes play an active role in preventing SD and is explained in detail in later sections. Metabolically poisoning astrocytes may have facilitated SD for reasons unrelated to gap-junction networks. Despite these criticisms of Somjen's modified gap-junction theory, there is one phenomenon that seems to strongly support his proposed gap-junction linked neuron model. An SD wave in urethane-anesthetized rats is preceded by population spikes of EEG activity in cells distant from the focal region (Herreras & Somjen, 1993). This burst of activity is different from seizure-discharge seen during the negative DC-shift. This particular burst of EEG activity occurs while $[K^+]_o$ is still normal, is synchronized, and occurs over long distances (Somjen, Aitken, Giacchino, & McNamara, 1985). The cells that discharge during this phase are too far from the focal site to be explained by ephaptic coupling. Rather, it may be that the presence of these population spikes can be explained by the opening of neuronal gap junctions that were previously closed (Herreras et al., 1994; Herreras & Somjen, 1993). Lastly, It is well known that acidosis has the effect of closing gap junctions (Spray et al., 1981). SD is attenuated under acidic conditions, and it has been proposed that gap junctions are responsible for mediating these effects (Somjen, 2001). This piece of evidence still has some flaws because it is also known that protons, even at physiological pH, inhibit NMDA channels. NMDA channels represent an important pathway for K^+ flux during SD and may also be responsible for acid-dependent attenuation of SD.

Not all SD models lack the prodromal $[K^+]_o$ rise, which suggests that K^+ delivery via the gap junction network is not the only route of K^+ propagation. Diffusion and

accumulation through interstitial spaces is likely the more common mechanism of K^+ propagation. The current available evidence seems to favor Somjen's modified gap-junction theory over Reid's original glial network model (Reid et al., 1988). However, the possibility of K^+ dispersal via glial networks has not been entirely precluded. It is unclear exactly how to produce SD that appears to prefer propagation via the gap junction networks. It is possible that it is dependent on the astrocyte cell density near the sight of initiation, as these cells play a role in maintaining $[K^+]_o$. No experiments have been conducted to verify this theory, but they would prove helpful to understanding the different modes of SD propagation. In addition, experiments correlating the appearance of phase 0 of the DC-shift and disappearance of the prodromal $[K^+]_o$ rise could strengthen the gap junction model if such a correlation is found.

Cortical Silencing

One of the major features of SD is an hour long period of cortical silence that occurs after the wave of depolarization. While most neurotransmitter vesicles are released during depolarization, it is not the depletion of these vesicles that causes the extended period of cortical silence. The rate at which neurotransmitters are synthesized and re-packaged is too high to explain this phenomenon (Rizzoli & Betz, 2005). There are two separate but closely related mechanisms that are believed to contribute to the period of cortical silence. The first and most important mechanism is due to an overall reduction in action potential firing, due to an increased ratio of inhibitory/excitatory tone which begins to exert its effect minutes after the wave of depolarization (Sawant-Pokam et al., 2016). The second mechanism is due to a decreased synaptic release probability due to

changes to the vesicular release mechanism at the post-synaptic terminal. This second mechanism does also contribute to an overall reduced action potential frequency, but is independent from the first pathway and begins to exert its effects 30 minutes after the depolarizing wave (Sawant-Pokam et al., 2016). The reduction in release probability is speculated to be due to Hebbian plasticity changes by long-term-depression (LTD) induction. LTD can be induced by postsynaptic depolarization of NMDA channels, and exerts its effects over the observed time-scale (30 mins). The reduction in the rate of action potential firing is due to a shift toward inhibitory tone over excitatory tone. When individual postsynaptic potentials (PSPs) of inhibitory & excitatory cells were observed (after SD induction), the frequency and amplitude of both cells' potentials increased (Sawant-Pokam et al., 2016). The inhibitory cells demonstrate higher amplitudes and more temporally consistent spontaneous PSPs (SPSPs) and thus exert a stronger effect than excitatory cells. The net effect of the cells' postsynaptic potentiation is inhibitory. The fact that both cell types experience a form of postsynaptic potentiation is interesting because classical plasticity models exert opposite effects on excitatory and inhibitory neurons (Malenka & Bear, 2004 cf Sawant-Pokam et al., 2016). Consequently, the observed postsynaptic potentiation of both cell types is unlikely due to classical long term potentiation (LTP) mechanisms. Non-classical LTP mechanisms could explain this phenomenon. However, brain-derived neurotrophic factor (BDNF) is known to be released after SD and can promote postsynaptic potentiation within minutes of high frequency or weak synaptic stimulation (Kawahara et al., 1997 cf Sawant-Pokam et al., 2016). It is believed that BDNF-induced postsynaptic potentiation affects both inhibitory

and excitatory neurons during SD, resulting in a net inhibitory effect. There is another possible explanation for the increased inhibitory tone, which is not mutually exclusive of the BDNF-LTP model. Increased cation permeabilities are observed during SD, and are responsible for the DC-shift. These cation permeabilities may persist and elevate the resting membrane potential closer to the action potential threshold for both excitatory and inhibitory neurons. The elevated membrane potential would not necessarily explain the increased PSP amplitudes observed, but it could help explain the increased frequency of SPSPs. It could be that SD affects inhibitory neurons more than excitatory neurons, resulting in an overall increase in inhibitory tone.

Vascular Changes during Spreading Depression

The hemodynamic response to SD is complex, and is composed of multiple opposing vasomotor responses. There is a great deal of heterogeneity in the vascular response, which is partially determined by experimental conditions, organism type, age, and a range of physiological parameters (i.e. pO_2, pCO_2, blood glucose, etc.). The vascular changes that occur with SD affect cerebral blood flow (CBF) and can be partitioned into four phases: I) initial vasoconstriction, II) hyperemia (increased blood flow), III) late hyperemia, and IV) sustained oligemia (decreased CBF).

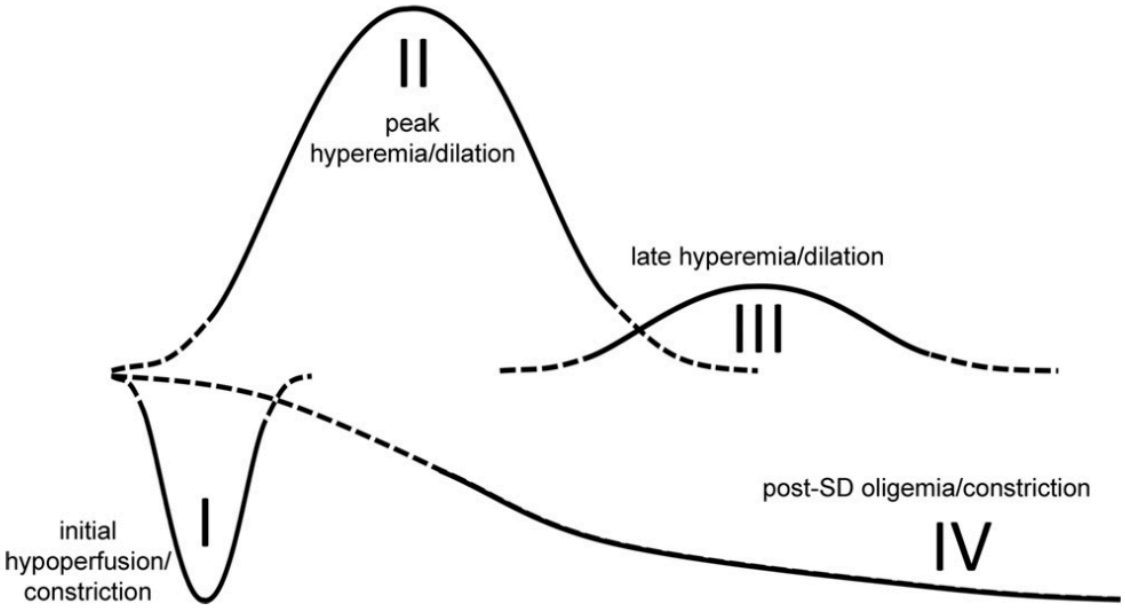

Figure 2. Components of the cerebral blood flow response during SD. There are four distinct vasomotor responses that shape the overall CBF response to SD. The initial hypoperfusion (phase I) is usually not seen in SD. In cases of spreading ischemia however, phase I is exaggerated and phase II is attenuated. Phase IV is a therapeutic target of interest because it generates a period of oligemia that can last over an hour. Figure modified from (Ayata, 2013).

Table 2. Summary of perturbations to SD vasomotor response.*.

Physiological State	**Vasomotor Response**
Hypotension	Phase I ↑; Phase II ↓
Hypoxia	Phase I ↑; Phase II ↓
Hypercapnia	Phase II ↓
Hyperglycemia	Phase I ↑; Phase IV↑

*Phases 1-4 are canonical vasomotor responses seen during SD. They are augmented or attenuated in different proportions depending on the physiological state of the organism. The malleable nature of each phase constitutes a complex and heterogeneous vascular response in SD models. Contents adapted from Ayata & Lauritzen, 2015)

A period of increased CBF and blood volume (phase II) is the most consistent vascular response across species and experimental conditions after SD induction. Phase II hyperemia begins between 15-20 seconds after the onset of the DC-shift, and peaks after re-polarization of cortical neurons have occurred (1-2 minutes after the DC-shift) (T. Obrenovitch, Chen, & Farkas, 2009). This phase is characterized by dilation of plial arteries (by 25-120%) and an increase in CBF between 30-250% (Lauritzen, 1987). A period of initial vasoconstriction (phase I) can either precede hyperemia or be superimposed on it. When superimposed, this phase I can be difficult to notice and may appear as a notched or jittery upstroke of CBF. The onset of phase I vasoconstriction is coincident with the onset of the DC-shift (Unekawa et al., 2013). It is likely that the ion fluxes that trigger the DC-shift are the causal factor for the initial vasoconstriction observed during this phase. A second period of hyperemia occurs roughly 3-5 minutes after the phase II, but is less pronounced and more sustained; CBF increases by 10-15% and lasts between 4-8 minutes (Farkas et al., 2008; Obrenovitch et al., 2009). The final phase is the post-SD oligemia. This event lasts for over an hour after the initial insult and is only partially responsive to vasodilators. CBF is reduced by 10-40% below baseline and is likely due to a combination of vasoconstriction factors and physical swelling of astrocyte end-feet (Bullock et al., 1991; Sehba & Friedrich, 2013).

The vasomotor response in naïve vs. subsequent SD evocations is different. Subsequent SD events display a diminished or abolished initial vasoconstriction response and an exaggerated late hyperemia response (Chang et al., 2010). The oligemia phase is only slightly increased in subsequent SDs. It is likely that much of the vasomotor

heterogeneity reported in the literature is a result of experimental preparations. SD can be accidently induced during sample preparation while handling the tissue. SD can also be induced mechanically while inserting electrodes into the cortical samples, causing unintended variation in the vascular response (Tomida et al., , 1989). A wide range of physiological factors can also affect the vasomotor effect and are listed in table 2. Under certain pathophysiological conditions (such as subarachnoid hemorrhage), these vasomotor responses can shift to produce a phenomenon called "spreading ischemia". Spreading ischemia is characterized by an exaggerated period of initial vasoconstriction and less pronounced periods of hyperemia (Dreier, 2011). Spreading ischemia can be induced experimentally using nitrous oxide (NO) scavengers. It is not yet understood how different pathophysiological conditions encourage the shift from hyperemia to spreading ischemia, but it is suspected that hemoglobin may have a role in scavenging NO from endothelial cells (Dreier et al., 1998). Spreading ischemia produces a wave of vasoconstriction that is closely coupled with the spreading wave of depolarization, and can worsen the outcome of the initial insult by restricting blood flow to the brain, causing metabolic mismatches. Therapeutic interventions that can relieve phases I or IV of the vasomotor response are clinically significant and are current topics of investigation (Dreier, 2011). An approach to attenuating phase IV is a greater priority because it occurs in most pathophysiological conditions that produce SD and lasts for hours.

Metabolic Support Offered by Astrocytes

Recovering from SD is an energy intensive process and is characterized by a drop in brain glucose, increase in lactate, and an increase in O_2 consumption (Cruz et al., 1999;

Csiba et al., 1985). The drop in glucose is coincident with repolarization, which is observed when $[K^+]_o$ begins returning to normal levels (Feuerstein et al., 2010). Astrocyte glycogen stores are rapidly depleted during SD and is likely largely responsible for the increase in lactate and sustained acidosis observed after the DC-shift (Bures, 1956; Seidel et al., 2016). Oxidative metabolic processes in astrocytes likely play an important protective role of neurons during SD. When astrocyte glycogen stores are experimentally increased, the onset of SD is delayed in cortical brain slices (Seidel & Shuttleworth, 2011). The oxidative processes in astrocytes protect neurons in three ways: 1) they provide lactate to neurons, 2) they permit active K^+ uptake and allow repolarization, and 3) allow uptake of glutamate to avoid excitotoxicity. Both astrocytes and neurons remove glutamate from the ECF through secondary active transporters. Astrocytes provide lactate to neurons through a lactate shuttle, which help neurons to restore the ionic milieu and remove glutamate and other excitatory amino acids from the ECF (Cruz et al., 1999). Mature astrocytes highly express the alpha-2 isoform of Na^+/K^+-ATPase, which plays a significant role in reducing $[K^+]_o$ levels and repolarizing surrounding neurons (Cameron, Klein, Shyjan, Rakic, & Levenson, 1994). Astrocytes also help remove excitatory amino acids from the ECF using secondary active transport mechanisms. It is very important that neurons are able to repolarize in a timely manner. When neurons remain depolarized for too long, cell-death can occur. Neurons have their own glycogen store, which is sometimes exhausted during the repolarization process. Lian & Stringer (2004b) determined that SD alone is not sufficient to cause damage to brain tissue. When SD was initiated with and without FC/FAc (selective inhibitors of

astrocyte citric acid cycle), tissue damage was only observed when FC/FAc was applied. These findings suggest that astrocytes preserve tissue viability through their oxidative capacity which neurons alone can't meet. Further studies revealed that FC/FAc focal injection or dilation near the sight of SD initiation can actually increase the velocity of SD propagation and reduce the electrical threshold needed to initiate the process (Lian & Stringer, 2004a). In some cases FC/FAc application alone was able to initiate SD (Canals et al., 2008). These findings imply that failure of astrocyte metabolism might be sufficient to trigger SD. It is now understood that astrocyte oxidative processes play an active role in preventing SD and are not just recruited once SD has already occurred.

Astrocyte Calcium Waves in Spreading Depression

Astrocytes exhibit spreading waves of Ca^{2+} through their gap-junction network. These Ca^{2+} waves share several similarities with SD, and it has been speculated as to whether or not these Ca^{2+} waves are a causal factor for SD. Astrocyte Ca^{2+} waves spread at a rate of 15-50 μm/second, which is similar to the rate of SD propagation. Astrocyte Ca^{2+} waves are evoked during SD and can be elicited by electrical or mechanical stimulation (Cornell-Bell et al., 1990; Martins-Ferreira et al., 2000). There is strong evidence that these Ca^{2+} waves are not the causal factor of SD and are probably an epiphenomenon. The exact effect that astrocytic Ca^{2+} waves produce is unclear, but attempts to answer this question have shed light on some interesting features of SD as a whole. High resolution imaging studies have revealed that KCl induced SDs exhibit elevated neuronal Ca^{2+} levels before the onset of astrocytic Ca^{2+} waves (Chuquet et al.,

2007). These findings suggest that astrocytic Ca^{2+} waves are not a causal factor in the neuronal depolarization in SD. Additional experiments have been performed that supports this conclusion. The gap-junction inhibitor, carbenoxolone, only slows down the propagation of the Ca^{2+} wave but does not affect the rate of SD propagation (Peters et al., 2003). Imaging studies have revealed that the Ca^{2+} wave travels further than the SD wave in most cases (Peters et al., 2003). Additionally, NMDA antagonists are able to abolish SD while still permitting a reduced astrocytic Ca^{2+} wave (Peters et al., 2003). Brain slices immersed in Ca^{2+} free environments are still able to produce SD (Somjen, 2001). Martins-Ferreira & Ribeiro (1995) observed some interesting results while studying astrocytic Ca^{2+} waves using the gap-junction blockers, octanol & heptanol. They observed that SD velocity increased under low concentrations of gap-junction blockers and was abolished under high concentrations. These experiments are very similar to Nedergaard's experiments, which provided support for the glial gap-junction hypothesis for K^+ dispersal. Unlike Nedergaard, Martins-Ferreira & Ribeiro (1995) demonstrated the drugs' effects were actually dose-dependent. The rationale offered was that octanol & heptanol are selective to astrocytic hemichannels. At higher concentrations, these gap-junction blockers start to occlude neuronal gap-junctions. This explanation supports Somjen's model of K^+ dispersal, which states that K^+ is distributed through gap-junctions between neurons and not astrocytes. When only astrocyte hemichannels are occluded, K^+ experiences a smaller effective volume to concentrate and is capable of projecting a critial $[K^+]_o$ to distant neurons at a faster rate. Lastly, transgenic mice that are lacking the astrocyte-specific connexin43 gene have been shown to produce SD with an increased

propagation velocity (Theis et al., 2003). SD velocity is increased for the same reasons, given that the K^+ effective volume is reduced without the intracellular astrocyte network. While these experiments were performed to demonstrate the non-causal role of the astrocyte Ca^{2+} wave, their rationale can be extended to support Somjen's modified K^+ dispersal theory.

Astrocytes Facilitate NMDA Currents

The NMDA receptor is a glutamate receptor ion channel. The acronym stands for its selective agonist, N-methyl-D-aspartate. The NMDA channel is unique because it requires two events to activate its channel. A ligand must bind to its receptor site (usually glutamate) and depolarization must also occur. An Mg^{2+} cation (sometimes Zn^{2+}) is associated with the channel on the extracellular side at rest. The Mg^{2+} cation is associated strongly with the channel enough to block all ion flow from occurring (Nowak et al., 1984). A depolarizing stimulus must expel the Mg^{2+} blockade in order to activate the channel. Because two events are needed to activate the NMDA receptor, it is considered a "coincidence" detector. The NMDA channel is a non-selective cation pore that conducts Ca^{2+}, K^+, and Na^+. The receptor binding interaction is unique, because it requires both an agonist and a coagonist. Glycine and D-serine are the two coagonists known to modulate NMDA currents between synapses (Johnson & Ascher, 1987; Panatier et al., 2006). Typically ambient glycine concentrations are sufficient to permit NMDA activation. Previous studies have shown that glycine can even act as a partial agonist and can activate NMDA channels to some degree without glutamate binding (given Mg^{2+} block

removal) (Hood et al., 1989). D-serine binds to the same site as glycine, but no studies have been conducted to see if it too is a partial agonist. Astrocytes were once considered the sole producer of D-serine due to their selective expression of the serine racemate enzyme. It is now understood that neurons are also a source of D-serine (Wolosker et al., 1999). Astrocyte Ca^{2+} waves are known to instigate the release of D-Serine (Mothet et al., 2005); because astrocyte waves follow neuronal depolarization, it is possible that both cell types facilitate NMDA activation during SD.

Anoxic Spreading Depression

Severe hypoxia or any general energy failure can precipitate a form of spreading depression that is called anoxic spreading depression (ASD). Spreading depolarization under these circumstances is especially important because their precipitating energetic demands are not readily met. If neurons remain depolarized for too long, brain tissue damage can occur. The reduced energy supply under anoxic conditions leaves cortical tissue more vulnerable to cell-death than under normoxic conditions. The increased basal $[K^+]_o$ may be induced by energy depletion as in ischemic penumbra, tissue damage as in brain trauma, or potassium release from erythrocytolysis as seen in subarachnoid hemorrhage (Astrup et al., 1981; Ayata & Lauritzen, 2015).

One long-standing question has been if ASD and SD proceed by the same mechanisms. Somjen (2001) performed experiments to determine how these forms of depolarization compared. A gas-liquid interface chamber was used to deprive and provide O_2 to cortical slices. When ASD was induced by O_2 deprivation, followed by O_2

restoration after the DC-shift, the ionic gradient recovered within the same time course as SD. Somjen also observed that hypoxia induced depolarization propagated at the same velocity as under normoxic conditions. These findings suggest that both forms of depolarization probably propagate by a similar mechanism. There are some important differences between ASD and SD however. For example, synaptic transmission is depressed several minutes before the onset of the DC-shift in ASD. In SD, synaptic transmission is depressed at the onset of the DC-shift. This observation has been explained by reduced Ca2+ entry due to O_2 deprivation which prevents the release of neurotransmitters (Young & Somjen, 1992). ASD and SD have different pharmacological features. It has been observed that NMDA receptor antagonists can stop the spread SD where basal $[K^+]_o$ levels are normal (Krüger, Heinemann, & Luhmann, 1999). NMDA receptor antagonism does not have the same inhibitory effect during ASD, where basal $[K^+]_o$ is pathologically high. While NMDA receptor antagonism does not stop the spread of ASD, it does limit its spread into les hypoxic areas (Aitken et al., 1991). As mentioned earlier, TTX is more effective at abolishing ASD than SD. It appears that the depolarizing current is less reliant on the NMDA pathway and consequently more dependent on voltage-gated Na^+ channels. Hypoxic tissue is often accompanied by increased tissue acidity. The increased acid concentration is likely to inhibit the NMDA channels. Because the NMDA channels are partially deactivated, the presence of an NMDA antagonist probably does relatively little to affect the overall depolarizing ionic currents. Anoxic conditions may do more than just shunt ionic currents away from NMDA channels. These anoxic conditions may also activate various other membrane

permeabilities that conduct depolarizing ion currents. This theory is justified because it is known that membrane permeability does change to some extent during ASD. For example, TMA+, which is normally impermeable to cell membranes, becomes permeable to neurons during ASD (Scheller et al., 1992). The exact nature of these membrane changes are unknown, but it stands to reason that they may conduct depolarizing ionic currents in place of the NMDA channels.

There are many pathways that might contribute to K^+ efflux during spreading depolarization. These sources are broadly grouped into two categories: 1) K^+ currents occurring during phase 1 of DC-shift and 2) K^+ currents during phase 2 of DC-shift. Hypoxic tissue has higher than usual basal $[K^+]_o$ and requires less K^+ efflux to continue propagation in ASD. For this reason, only K^+ from the first phase of the DC-shift is needed to propagate the depolarization wave. NMDA channels contribute to the second phase of the DC-shift. When these channels are antagonized, phase 2 disappears but ASD continues to propagate. One would expect the velocity of the ASD wave to be higher than the SD wave since K^+ threshold is more readily met. One explanation is that the acidosis that occurs in hypoxic tissue causes the closure of gap-junctions between neurons. According to Somjen's K^+ dispersal theory, if gap-junction networks between neurons are occluded then propagation velocity will be reduced. There may be several other opposing forces on ASD velocity, but its resultant velocity is reminiscent of SD.

The Role of Glutamate

Glutamate levels rise many times higher during ASD than SD, yet the depolarizing waves travels at the same velocity Fabricius et al., 1993). Glutamate has long been considered a driving factor of SD propagation, so one would expect ASD waves to travel faster than under normoxic conditions. Researchers have observed another seemingly contradictory finding demonstrating that SD may only depend on K^+ and not glutamate in order to propagate. Micropipettes were inserted into live cortical tissue and perfused with artificial cerebrospinal fluid (ACF). The ACF perfusion was able to abolish SD propagation when focally induced (Obrenovitch & Zilkha, 1995). This was presumably due to the ACF buffering depolarizing solutes in the ECF. Obrenovitch's group exploited this experimental setup to determine if additional K^+ and/or glutamate (delivered via ACF perfusion) can restore the inhibited propagation of SD. These results showed that only K^+ perfusion was able to restore propagation, while gultamate perfusion alone was unable to restore it. These findings contradict the observations that NMDA receptor antagonism stops the propagation of SD. If glutamate is not essential for propagation, then NMDA antagonism should have no effect on its spread. These findings illustrate a paradox where NMDA receptor activity is needed for SD propagation, but glutamate is not. One possible explanation that was proposed by Obrenovitch is that K^+ induced depolarization of neurons expels the extracellular Mg^{2+} block within the NMDA channel. The NMDA receptor is a coincidence detector and requires receptor activation and displacement of the Mg^{2+} block. Glutamate might only play a permissive role in NMDA activation, but the rate-limiting step is determined by K^+ efflux from phase 1 of

the DC-shift. If glutamate only plays a permissive role in NMDA activation, this could help explain why ASD and SD experience similar propagation velocities despite their large differences in glutamate concentrations.

Electromagnetic Based Interventions

Transcranial Magnetic Stimulation

Transcranial magnetic stimulation (TMS) is an effective method to diagnose and treat many neurological disorders. The technique involves inducing one or many pulses of current through an electromagnetic coil (primary coil) that is centered over a region of the brain. A magnetic field is generated perpendicular to the plane of the coil and induces a second current within some electrically conductive substrate near the device. The substrate in this procedure is brain tissue, and the magnetic fields are able penetrate the skull making this procedure entirely non-invasive. In theory, the current induced should move in the opposite direction as the primary coil. In reality, the direction of the induced current follows a path determined largely by the heterogeneous orientations of the neurons encompassed by the magnetic fields (Chervyakov et al., 2015). TMS is a useful diagnostic tool because different stimulation protocols can elicit a response from a specific population of cells. For example, a single pulse above the motor center in a human can elicit an observable contraction of the person's extremities. The threshold required to elicit such a response reveals useful diagnostic information when studying how a subject's neuronal excitability may have been altered (Barker et al., 1985). A list of common diagnostic TMS protocols are tabulated in Table 3 and are discussed in the present research.

Table 3. **Common diagnostic TMS protocols.***.

Measure	Abbreviation	Protocol	Neurons Stimulated
Motor threshold	MT	Single pulse	Corticospinal tract (CST) neurons and associated interneurons.
Input/Output curves	I/O curve	Single pulse with multiple intensities	CST & intracortical neurons over a wider surface area than MT.
Short interval intracortical inhibition	SICI	Paired pulse, subthreshold, then suprathreshold pulse	$GABA_A$ interneurons
Intracortical facilitation	ICF	Paired pulse, subthreshold, then suprathreshold pulse	Glutamatergic interneurons

*Different measurements can be made to assess the excitability of brain tissue. These protocols are often used to study the effects of other stimulation protocols, such as transcranial direct current stimulation (TDCs). Table modified from (Shin et al., 2015)

The TMS procedure has been developed into a therapeutic paradigm to target a handful of neurological disorders. When TMS is applied repeatedly under high/low frequencies with various intensities, long lasting neurophysiological changes can be conferred. Repetitive TMS (rTMS) treatments have shown improvement in pathologies like depression, Parkinson's disease, dystonia, migraines, pain syndromes and many more (Chervyakov et al., 2015; Matsumoto & Ugawa, 2010). Changes in neuronal excitability can be conferred using rTMS and can last up to an hour or even weeks. Short-term modulations in neuronal excitability are partially due to changes in ionic compositions while long-term changes are the result of altered gene expression (Kuwabara et al., 2002). For example, the therapeutic effect of rTMS on Parkinson's disease is believed to be due to an increase in endogenous dopamine production in certain parts of the brain, presumably due to alterations in gene expression (Cho & Strafella, 2009). The precise

mechanisms by which magnetic fields alter neuronal excitability are largely unknown, but most researchers agree that it is closely related to the phenomenon of long-term potentiation (LTP) and long-term depression (LTD) (Hoogendam et al., 2010).

LTP enhances synaptic strength and can be achieved in situations where a presynaptic terminal is stimulated followed by the postsynaptic terminal in the span of a few milliseconds. It is presumed that high-frequency (>5 Hz) rTMS can stimulate synaptic pairs in the correct manner to strengthen their communication (Bi & Poo, 1998). On the other hand, synaptic strength can be decreased in LTD when a postsynapse is stimulated followed by its presynapse within milliseconds. Like a series of knocks, low-frequency (>1 Hz) TMS stimulation is believed to produce LTD by activating the synaptic pair in the right fashion (Bi & Poo, 1998). The mechanism of LTP/LTD induction appears to be mediated by the activation of NMDA channels on the postsynaptic surface (Cooke & Bliss, 2006). Depolarization of the postsynaptic element displaces the NMDA Mg^{2+} block while glutamate coincidently stimulates the influx of Ca^{2+} through the channel. The synaptic plasticity pathways appear to be in part determined by the frequency/rate of Ca^{2+} influx, but the mechanism further downstream is largely unknown.

Transcranial Direct Current Stimulation (tDCS)

Like TMS, transcranial direct current stimulation (tDCS) has been demonstrated to modulate neuronal excitability. The procedure is painless and well tolerated, and is an

attractive approach to modulating behavior in clinical and experimental conditions. The procedure has also been used to treat stroke, pain conditions, epilepsy, and other neurological pathologies (Stagg & Nitsche, 2011). The procedure involves running a current through the skull in order to target a particular region of the brain. The current strength, duration, and polarity (anodal or cathodal) determine what type of effect will be produced in the brain tissue. A single session of tDCS has only been capable of conferring behavioral changes that last on the order of minutes (Reis et al., 2009). Repeated and spaced interval tDCS sessions have been able to extend these behavioral effects to the order of weeks (Reis et al., 2009). For long lasting behavioral changes to occur, functional changes must be occurring in the cortex. The only known mechanisms by which these changes can occur in the brain are through modulation of cortical synaptic plasticity via LTP or LTD (Stagg & Nitsche, 2011). The knock-series activation of synaptic pairs via NMDA-channels is only one way of modulating synaptic plasticity. There may be a handful of other mechanisms that are specific to the region of the brain. For example, LTD is dependent on NMDA channels in rat slices of the agranular cortex but independent of these channels in the sensorimotor cortex of live rats (Castro-Alamancos et al., 1995; Froc & Racine, 2004). A class of soluble factors called neuromodulators (i.e. serotonin, dopamine, histamine, etc.) in the neocortex facilitates LTP. Neuromodulators are a group of poorly defined molecules that either attenuate or augment the response of a neurotransmitter. Neuromodulators can affect long-term synaptic changes depending on the distribution of receptors and the concentration of modulators and neurotransmitters at the site of action (Barchas et al., 1978). The receptor

distribution is heterogeneous throughout the brain, and even throughout the cortex alone. For these reasons, the mechanism and capacity for conferring long-term synaptic changes through tDCS cannot be generalized from one brain region to another, and are studied on a case-by-case basis (Malenka & Bear, 2004). These same arguments can be extended to any form of electromagnetic stimulation.

Spaced interval tDCS and rTMS have the potential to confer long lasting changes to cortical excitability by modulating synaptic plasticity through LTP and LTD-like mechanisms. When tDCS is performed in a single session (without spaced intervals) the effects on cortical excitability are short-lived but might still be closely related to LTP/LTD mechanisms, varying only in intensity (Stagg & Nitsche, 2011). Early studies on animals have demonstrated that weak polarizing currents can either make a population of neurons more excitable or depress their activity while a current was applied. The modulatory effect conferred on the neuron population is short-lived but reaches its peak a few minutes after the current is removed (Priori et al., 1998). The effects produced during stimulation and after stimulation are considered separately and are likely generated through different mechanisms.

Effects During tDCS

Anodal currents raise the intracellular potential of cells within the cortex. Typically, more spontaneous activity is observed in the region receiving anodal

stimulation. Cathodal stimulation on the other hand, tends to depress cortical activity by hyperpolarizing certain cell populations. Interestingly, TMS experiments have indicated that motor threshold (MT) measurements (Table 3) are unaffected by either cathodal or anodal tDCS. These observations indicate that anodal/cathodal currents do not readily affect pyramidal neurons. Cathodal currents have been shown to modulate input/output (I/O) curves (table 3), which is a diffuse measurement of interneuron excitability (Nitsche et al., 2005). These findings suggest that tDCS modulates predominately interneuron cell populations. The effects of the tDCS are highly dependent on duration and intensity of current, and different cell populations respond differently to various parameters. Other experiments have suggested that tDCS affects nonpyramidal tract neurons at a lower charge density than pyramidal neurons. Pyramidal neurons appear to be activated at charge densities above 0.008 $\mu C/cm^2$ (Purpura & McMurtry, 1965). These findings are important because they suggest that tDCS can affect both pyramidal and non-pyramidal neurons in humans with the correct parameters. Because the experiments by Purpura & McMurtry utilize direct current stimulation rather than tDCS, their parameters cannot be extended to tDCS without experimental confirmation first. Although these techniques are similar, there exist some important differences that must be considered.

The effects of anodal/cathodal currents are not consistent throughout the brain. The orientation of neurons relative to the electric field can change how the cell responds to the current. Anodal current depolarizes cell membranes, but some neurons that are deeper in the cortical layers are actually inactivated at the same time. Cathodal stimulation favors hyperpolarization, but deeper neurons are actually depolarized for the

same reason (Purpura & McMurtry, 1965). The orientations of neurons are not homogenous and it is difficult to produce a consistent response throughout the cortex with a single tDCS regimen.

Effects After tDCS

The modulatory effects of tDCS persist after the current is removed and proceeds to reach its maximum effect a few minutes after cessation. The after-effects of anodal stimulation are dependent on the initial membrane depolarization produced by the anodal current. When voltage gated Ca^{2+} and Na^{+} channels are blocked, the after-effects of anodal tDCS are abolished (Nitsche et al., 2003). The excitatory after-effects are the result of several complex pathways that are poorly understood. It is known that glutamatergic synapses are involved and require the activation of NMDA receptors. Administration of dextromethorphane (DMO), an NDMA antagonist, abolishes the after-effects of anodal stimulation (Liebetanz et al., 2002). The NMDA receptor agonist, D-cycloserine (CYC), can only extend the duration of the anodal after-effects but not the magnitude (Nitsche et al., 2004). Catecholamines have also been implicated as a potential causal factor of these after-effects. Amphetamine has been observed to augment the excitatory after-effects, but only in the absence of DMO. In addition, beta-adrenergic antagonists have also been shown to reduce the after-effects of anodal stimulation. These observations suggest that the catecholamine pathway involves NMDA activation downstream, but the precise mechanism is unknown (Nitsche et al., 2004). Cholinergic tone appears to oppose the after-effects of anodal simulation. Increasing the cholinergic tone by administering rivistigmine abolishes the anodal after-effects (Kuo et al., 2007).

Serotonin appears to be the most powerful modulator of the anodal after-effects. The application of an SSRI increases both the magnitude and duration of the after-effects, unlike NMDA agonists that only extends the duration (Nitsche et al., 2009).

The after-effects of cathodal stimulation are inhibitory and are the result of various forms of modulation. Like anodal stimulation, the after-effects of cathodal stimulation are also dependent on glutamatergic synapses. The antagonism of NMDA channels with DMO abolished the after-effects of cathodal stimulation but its activation with CYC did not produce any changes Nitsche et al., 2004, 2005). It appears that NMDA channels play a fairly promiscuous role and are implicated in long-term and short-term plasticity. The observation that the NMDA agonist CYC did not enhance the after-effects of cathodal seems counter-intuitive. One explanation that has been offered is that cathodal hyperpolarization can modify the CYC binding site, making it unable to produce its effect. Unlike anodal after-effects, amphetamine does not modulate the after-effects of cathodal stimulation (Nitsche et al., 2004). This observation is surprising because catecholamine modulation appeared to be dependent on NMDA receptors downstream in the anodal after-effect model. Lastly, Serotonin appears to be the strongest modulator of cathodal after-effects. The administration of a SSRI has been shown to reverse the inhibitory effect of cathodal currents and facilitate excitation instead (Nitsche et al., 2009).

The precise mechanisms behind anodal/cathodal after-effects are still largely unknown. The involvement of NMDA channels in both modes of modulations is reminiscent of their role in LTP/LDP. Although the after-effects of tDCS produce

shorter-lived phenomenon than LTP/LTD it is likely that these mechanisms are closely related and are often referred to as LTP/LTD-like effects.

Experimental Effects of Electromagnetic Stimulation on Spreading Depression

The methods of TMS and tDCS offer different approaches to modulating cortical excitability. In theory, any approach that can reduce cortical excitation can be wielded to suppress or prevent SD. Because these electromagnetic based procedures are entirely non-invasive and well tolerated, they may be ideal for treating patients who are prone to SD, such as in the case of traumatic brain injury. SD can worsen the outcome of brain injuries through precipitating metabolic and vascular changes. A non-invasive procedure to delay or prevent the onset of SD would be of tremendous value.

There has only been one published study that explores the effects of tDCS alone on SD propagation in vivo. In the experiments by Liebetanz et al., (2006), live rats were treated with 20 minutes of either anodal or cathodal stimulation at 200 μA. These rats were anesthetized during the entire experiment. KCl was applied directly to the brain (through a drilled hole in the skull) to initiate SD and the velocity of propagation was recorded. Three episodes of SD were evoked within 20-minute intervals, the first one being five minutes after tDCS stimulation. It was found that anodal stimulation increased SD propagation velocity by 0.3mm/min above baseline. The increased velocity decayed and restored to baseline after the third SD evocation, 40 minutes after the first. The after-effect of anodal stimulation produced the expected response. The duration of the after-effects is not reliable however. As mentioned previously, naïve SD is known to have

different properties than subsequent SD events. It takes at least 60 minutes between SD evocations to restore naïve-like vascular responses. The increased propagation rate can be explained by the after-effects of sustained membrane depolarization of neurons. LTP-like mechanisms temporarily improve synaptic strength, which facilitate an increased rate of SD propagation. Because SD does not proceed through synaptic transmission, it is not entirely clear how LTP facilitates SD conduction. A multitude of factors are likely to be responsible, such as neurotransmitter concentrations, neuromodulators, and altered ionic compositions. Although increased neuronal excitability and augmented SD conduction are not therapeutically useful effects, the after-effects of anodal stimulation might be able to shed light on some of the mechanistic theories surrounding SD. One explanation is that K^+ dispersal through gap-junction networks is improved. According to Somjen's modified K+ dispersal theory, gap-junction networks between neurons conduct and project K+ to distant cells and help propagate SD non-synaptically. It is possible that neuronal gap-junction networks are transiently elaborated during LTP and LTP-like after-effects of anodal tDCS. Increased gap-junction expression between neurons can reduce the resistance that intracellular K^+ experiences and facilitates its spread. It is known that morphological neuronal changes can occur and disappear in LTP over the course of minutes, and has been well documented in dendritic spines (McEachern & Shaw, 1996). It has also been established that blocking neuronal gap-junctions (i.e. acid or pharmacological agents) can modulate SD velocity. This theory is supported by LTP studies in mice hippocampus slices. Mice with the neuronal gap-junction gene (connexin36) knocked out experience reduced long-term learning (Wang & Belousov,

2011). LTP is considered a cellular model to learning, and these findings suggest that gap-junctions play an important role in LTP. It is possible that increased neuronal gap-junction expression is one of the functional changes that facilitate LTP and thus facilitate SD propagation. This theory does have a weak point, because it is not known to what extent anodal after-effects are similar to LTP. If future experiments were performed to assess changes in neuronal gap-junction expression after anodal tDCS, those results could prove useful in light of this theory.

The accelerating effects of tDCS on SD velocity can also be explained by NMDA receptor dynamics. Anodal stimulation depolarizes cell membranes and dislodges the Mg^{2+} block. There are several possible ways that Mg^{2+} dislodgement can confer after-effects for minutes after the tDCS stimulation is removed. The concentration of excitatory amino acids and other neuromodulators certainly increase during anodal stimulation. Neuromodulators include partial agonists and coagonists for the NMDA receptor. It is unlikely that glutamate agonizes the NMDA receptor after anodal stimulation. Glutamate is tightly regulated and uptake mechanisms work to quickly remove these amino acids from the ECF to avoid excitotoxicity (Aiba & Shuttleworth, 2012). Glutamate concentrations would not persist for tens of minutes after the initial stimulus. Astrocytes are known to release coagonists (and possibly partial agonists) of NMDA channels, and could play a role in forging an extracellular milieu that is conducive to NMDA activation. These astrocyte-derived factors (glycine & D-serine) might persist for longer periods of time than glutamate. The presence of these ligands may confer coincident NMDA activation when anodal current displaces the Mg^{2+}. The

ionic flux that occurs through the NMDA channel may be persistent enough to modify the ECF sufficiently to accelerate SD conduction for minutes after the current is removed. Even if a population of NMDA channels experience Mg^{2+} block removal but remain inactive (no ligand binding), this molecular state is still conducive to SD propagation. Excitatory amino acids like glutamate are no longer just permissive to NMDA-mediated propagation, but become a driving factor in the scope of these molecular states. Mg^{2+} ions don't necessarily revert back to blocking the channel immediately after anodal current is removed. An increased presence of cations (such as K^{+}) can displace and delay the Mg^{2+} block from associating with the NMDA channel, allowing a population of NMDA channels to remain in this state for some time after the anodal current is removed.

The experiments by Liebetanz successfully demonstrated the effects of anodal tDCS on SD propagation. The research group's observations of cathodal stimulation however are not as clear and produced some unexpected results. The velocity of SD propagation remained the same after cathodal current was applied. This was unexpected, since LTD-like after-effects of cathodal currents reduce the excitability of neurons and should attenuate SD propagation. One explanation offered by the group is that the rats were anesthetized. The polarity specific tDCS effects that have been observed in the past were from awake subjects (Liebetanz et al., 2006). In order to obtain electrical epicortical readings of SD, the rats must be anaesthetized. The anesthetic decreases the cortical excitability beyond what the hyperpolarizing cathodal current could. The bottom-out effect of the anesthetic could make it impossible to observe the cathodal effects in Liebetanz's experimental design.

The work done by Liebetanz was thereafter extended by Fregni et al., (2007) who explored the effects of tCDS on SD from a different angle. The experiments performed by Fregni's team demonstrated the effect of tCDS treatment (20 minutes at 200 μA) followed by 20 minutes of low frequency repetitive electrical stimulation (ES) at 1 Hz. Repetitive ES is known to mimic the effects rTMS, but circumvents some practical inconveniences encountered when using rTMS with rats. The objective was to observe how tDCS pre-treatment could modify the effects of repetitive ES on SD propagation.

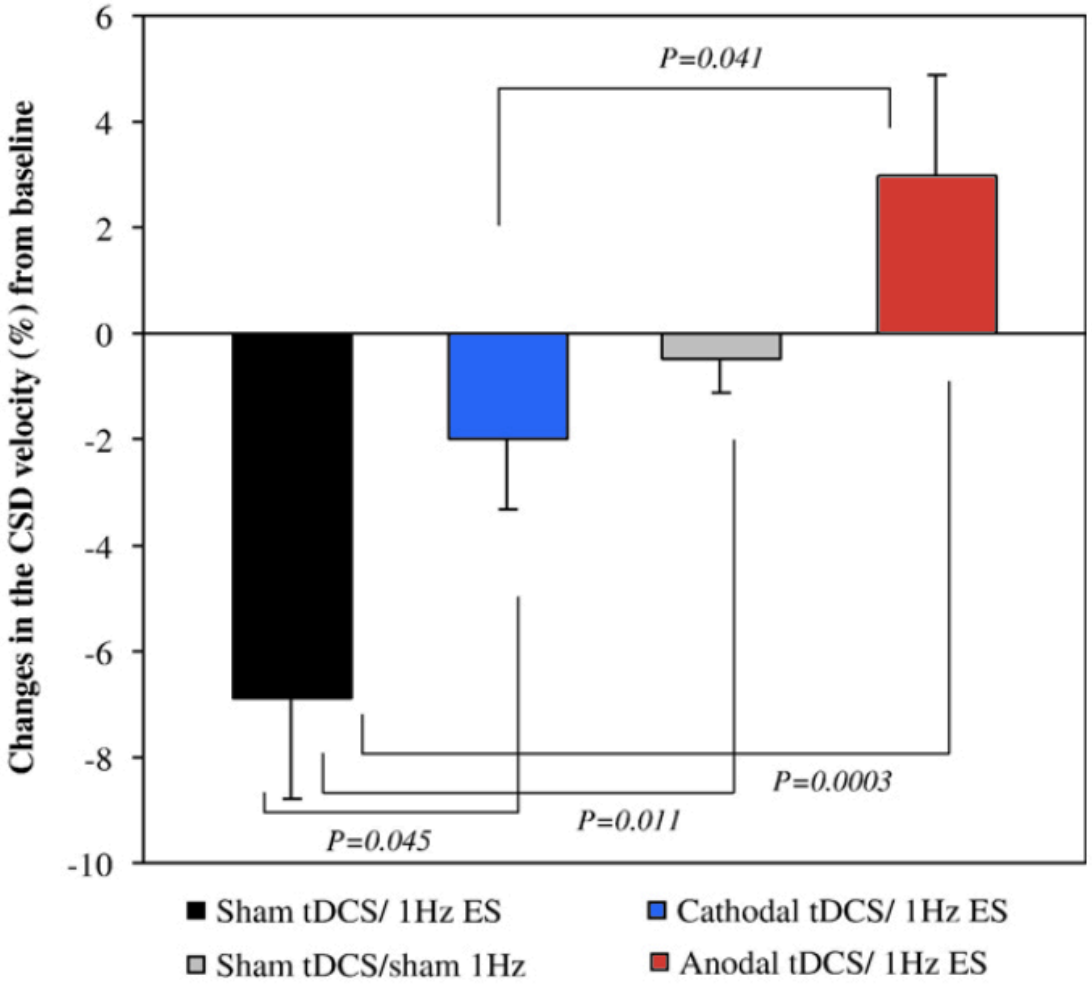

Figure 3. Changes in cortical spreading depression (CSD) velocity after tDCS/ES. Anaesthetized rats were pre-treated with 20 minutes of anodal/cathodal tCDS at 200 μA. Rats were then treated with 20 minutes of 1Hz ES. SD velocity was recorded with sham controls. Figure modified from (Fregni et al., 2007).

The results indicated by Figure 3 offer insight into the therapeutic potential of electromagnetic based SD interventions. The black bar indicates that the greatest depression in SD velocity was accomplished using repetitive ES alone. Because repetitive ES mimics rTMS, mechanistic arguments pertaining to rTMS can be extended to explain Fregni's results. The reduction in SD velocity can be explained by LTD induction. Low frequency rTMS induces current within targeted neuron populations. When a presynapse is activated followed by its postsynapse (within milliseconds), LTD is produced. A prolonged decrease in synaptic strength is the result of the stimulation protocol but no measurements were taken to observe how long these effects lasted. One would expect the effects to be longer lasting than Liebetanz's tDCS protocol, since repetitive protocols seem to confer the longest synaptic changes. The rats used in this experiment were also anaesthetized during SD recordings. In Liebetanz's cathodal tDCS experiments, the absence of any effect was attributed to a "bottom-out" effect produced by the anesthetic reducing cortical activity as a whole. The fact that Fregni's LTD inducing ES protocol decreased SD propagation in anaesthetized rats by such a large magnitude (~7%) implies that basal cortical activity is not the only factor that is modulating SD velocity in LTD/LTP. These observations support the aforementioned theory that functional molecular changes that facilitate LTP/LTD (i.e gap junction expression) also facilitate/attenuate SD propagation.

Fregni's results show that tDCS can modify the LTD conferred upon the cortex by repetitive ES. Cathodal stimulation appears to have restored some of the attenuated SD velocity, but still remained under baseline (Figure 3). Unlike Liebetanz's experiments, which demonstrate no cathodal effect, Fregni's results show that cathodal current may only exert an observable effect when LTD is in effect. The explanation proposed by Fregni's group is that the cathodal current hyperpolarized postsynaptic terminals, which might diminish the effect of LTD. It is known that slight postsynaptic depolarization (tDCS) combined with low frequency stimulation (ES) is a pre-requisite for LTD inducing protocols (Malenka & Bear, 2004). Another explanation is that deeper cortical layers experience membrane depolarization due to their orientation relative to the electric field induced during tDCS cathodal stimulation. Membrane depolarization can lead to LTP-like after effects, as previously discussed. The anodal current had the effect of completely abolishing the ES induced LTD and further increasing SD velocity past its baseline. Postsynaptic depolarization combined with low frequency stimulation has an LTP inducing effect (Malenka & Bear, 2004).

Therapeutic Potential of Electromagnetic based Interventions

tDCS's after-effects, rTMS, & ES are weak candidates for SD interventions

The experiments performed by Liebetanz and Fregini demonstrate the effects of tDCS after-effects and rTMS/ES on SD propagation velocity. Both sets of experiments reveal some important mechanistic underpinnings of SD. However, these experiments cannot be extrapolated to determine if tDCS after-effects and/or rTMS/ES can be used to prevent SD from occurring in the first place. These experiments are limited because only propagation velocity was measured after SD was experimentally initiated using K+ focal injection. The mechanism of initiation and propagation are not necessarily the same and thus cannot be generalized. In theory, any paradigm that can induce LTD has some therapeutic potential. A reduction in spontaneous activity could reduce the accumulation of extracellular K^+, and thus prevent SD initiation. Inducing LTD using rTMS/ES on a cortical region involved in traumatic brain injury could be one clinical approach to SD. The use of tDCS's after-effects in a similar capacity is not as promising given the available literature, but still has potential. It is unclear if the after-effects of cathodal stimulation actually attenuate or augment the velocity of SD propagation. It would be enormously insightful if experiments were performed that analyzed the effect of spaced interval tDCS on SD velocity. Spaced interval tDCS appears to confer long lasting changes that are probably attributable to classical LTD/LTP pathways. It is unclear if the brief after-effects of a single tDCS session proceed by the same (albeit less intense) mechanism of classical LTD/LTP. It would be useful to see experiments using connexin-

36 knockout mice that undergo single session tDCS. If anodal currents were unable to confer augmented SD propagation velocities in the mice models, then that would further support Somjen's modified gap-junction theory and its involvement in LTP. Overall, any LTD inducing paradigm has some theoretical potential to prevent SD initiation, but is a weak candidate as a therapeutic intervention.

tDCS hyperpolarization is a strong candidate for SD intervention

The after-effects of tDCS are not the only side of the coin that has clinical value. The effects during tDCS stimulation have their own unique properties that have been treated separately from its after-effects. No experiments have been performed to study the effects of tDCS hyperpolarizing currents on SD initiation. Mild sustained cathodal tDCS could be an option for patients who recently experienced traumatic brain injury or are prone to SD for other reasons. Cathodal currents can hyperpolarize pyramidal and non-pyramidal cells in the outer layers of the cortex. In theory, mild hyperpolarization should postpone or prevent the onset of SD if sustained long enough. A significant portion of SD's depolarizing currents are conducted through NMDA channels. NMDA antagonists have been demonstrated to abolish SD entirely under normoxic conditions. A hyperpolarizing current can prevent the NMDA channel's Mg^{2+} block from being expelled, thus mimicking the effect of an NMDA antagonist in a completely non-invasive way. Cathodal tDCS is known to produce some depolarization in cells deep in the cortex. This is probably due to the inhomogeneous orientation of neurons relative to the electric field. These populations of neurons might thwart the attempt to suppress SD by depolarizing and triggering SD deeper in the cortex. The use of an NMDA antagonists in

combination with tDCS may serve to protect the deeper regions of the cortex. Cathodal tDCS may also work to prevent ASD from initiating. NMDA antagonism does not block ASD, but it does prevent it from spreading to less hypoxic regions. Other cation permeabilities appear to drive ASD, but the identity of these channels/pores are unknown. A sustained cathodal current will provide an electric field that could prevent cations (Na^+, K^+, Ca^{2+}) from flowing down their concentration gradient through NMDA & non-NMDA pathways. If the hyperpolarizing current is sustained long enough, extracellular K^+ removal mechanisms (i.e. astrocytes) can reduce $[K^+]_o$ so that its concentration is below threshold, thus suppressing ASD/SD entirely.

Summary and Concluding Remarks

A general mechanism of SD exists, but the specific details behind initiation and propagation are still debated. Despite the incomplete picture, there are enough hints distributed throughout the literature to build a model with some predictive power. The mechanistic arguments made in the present review can be used to explain and predict the effects of rTMS, ES, & tDCS on SD. It has been experimentally demonstrated that ES/rTMS can reduce the velocity of SD propagation. It is unclear if these techniques can prevent SD from happening entirely. The LTP/LTD-like after-effects of tDCS have not been demonstrated to modulate SD in a therapeutically useful way. It is unclear if cathodal after-effects can suppress SD. It is clear that anodal after-effects greatly facilitate SD. Evidence suggests that LTP/LTP-like effects may include increased neuronal gap-junction expression which facilitates SD propagation, further supporting Somjen's theory. Two unexplored aspects of tDCS on SD are repetitive spaced protocols and cathodal effects during stimulation. Repetitive spaced cathodal stimulation can confer LTD-like effects that persist for weeks and might be an alternative to ES/rTMS protocols. The effects of cathodal after-effects have been studied with SD, but the effects during stimulation have not. Cathodal currents combined with an NMDA antagonist could be a novel technique to prevent SD and ASD from occurring, and is the most promising approach in the scope of this review. While there is still much to be discovered about SD, these findings illustrate an experimental trajectory that could develop electromagnetic therapies in the future to treat a wide range of conditions that involve SD.

REFERENCES

Aiba, I., & Shuttleworth, C. W. (2012). Sustained NMDA receptor activation by spreading depolarizations can initiate excitotoxic injury in metabolically compromised neurons. The Journal of Physiology, 590(22), 5877–5893.

Aitken, P. G., Jing, J., Young, J., & Somjen, G. G. (1991). Ion channel involvement in hypoxia-induced spreading depression in hippocampal slices. Brain Research, 541(1), 7–11.

Astrup, J., Siesjö, B. K., & Symon, L. (1981). Thresholds in cerebral ischemia - the ischemic penumbra. Stroke, 12(6), 723–5.

Ayata, C. (2013). Spreading Depression and Neurovascular Coupling. Stroke, 44(6, Supplement 1), S87–S89.

Ayata, C., & Lauritzen, M. (2015). Spreading Depression, Spreading Depolarizations, and the Cerebral Vasculature. Physiological Reviews, 95(3), 953–993.

Barchas, J., Akil, H., Elliott, G., Holman, R., & Watson, S. (1978). Behavioral neurochemistry: neuroregulators and behavioral states. Science, 200(4344).

Barker, A. T., Jalinous, R., & Freeston, I. L. (1985). Non-invasive magnetic stimulation of human motor cortex. Lancet (London, England), 1(8437), 1106–7.

Basarsky, T. A., Duffy, S. N., Andrew, R. D., & MacVicar, B. A. (1998). Imaging spreading depression and associated intracellular calcium waves in brain slices. The Journal of Neuroscience : The Official Journal of the Society for Neuroscience, 18(18), 7189–99.

Bi, G. Q., & Poo, M. M. (1998). Synaptic modifications in cultured hippocampal neurons: dependence on spike timing, synaptic strength, and postsynaptic cell type. The Journal of Neuroscience : The Official Journal of the Society for Neuroscience, 18(24), 10464–72.

Brinley, F. J., Kandel, E. R., & Marshall, W. H. (1960). Potassium outflux from rabbit cortex during spreading depression. Journal of Neurophysiology, 23, 246–56.

Bullock, R., Maxwell, W. L., Graham, D. I., Teasdale, G. M., & Adams, J. H. (1991). Glial swelling following human cerebral contusion: an ultrastructural study. Journal of Neurology, Neurosurgery, and Psychiatry, 54(5), 427–34.

Bures, J. (1956). Some metabolic aspects of Leao's spreading depression. Journal of Neurochemistry, 1(2), 153–8.

Cameron, R., Klein, L., Shyjan, A. W., Rakic, P., & Levenson, R. (1994). Neurons and astroglia express distinct subsets of Na,K-ATPase alpha and beta subunits. Brain Research. Molecular Brain Research, 21(3–4), 333–43.

Canals, S., Larrosa, B., Pintor, J., Mena, M. A., & Herreras, O. (2008). Metabolic challenge to glia activates an adenosine-mediated safety mechanism that promotes neuronal survival by delaying the onset of spreading depression waves. Journal of Cerebral Blood Flow and Metabolism : Official Journal of the International Society of Cerebral Blood Flow and Metabolism, 28(11), 1835–44.

Castro-Alamancos, M. A., Donoghue, J. P., & Connors, B. W. (1995). Different forms of synaptic plasticity in somatosensory and motor areas of the neocortex. The Journal of Neuroscience : The Official Journal of the Society for Neuroscience, 15(7 Pt 2), 5324–33.

Chang, J. C., Shook, L. L., Biag, J., Nguyen, E. N., Toga, A. W., Charles, A. C., & Brennan, K. C. (2010). Biphasic direct current shift, haemoglobin desaturation and neurovascular uncoupling in cortical spreading depression. Brain, 133(4), 996–1012.

Chervyakov, A. V., Chernyavsky, A. Y., Sinitsyn, D. O., & Piradov, M. A. (2015). Possible Mechanisms Underlying the Therapeutic Effects of Transcranial Magnetic Stimulation. Frontiers in Human Neuroscience, 9, 303.

Chesler, M., & Kraig, R. P. (1987). Intracellular pH of astrocytes increases rapidly with cortical stimulation. American Journal of Physiology - Regulatory, Integrative and Comparative Physiology, 253(4).

Cho, S. S., & Strafella, A. P. (2009). rTMS of the left dorsolateral prefrontal cortex modulates dopamine release in the ipsilateral anterior cingulate cortex and orbitofrontal cortex. PloS One, 4(8), e6725.

Chuquet, J., Hollender, L., & Nimchinsky, E. A. (2007). High-resolution in vivo imaging of the neurovascular unit during spreading depression. The Journal of Neuroscience : The Official Journal of the Society for Neuroscience, 27(15), 4036–44.

Cooke, S. F., & Bliss, T. V. P. (2006). Plasticity in the human central nervous system. Brain, 129(7), 1659–1673.

Cornell-Bell, A. H., Finkbeiner, S. M., Cooper, M. S., & Smith, S. J. (1990). Glutamate induces calcium waves in cultured astrocytes: long-range glial signaling. Science (New York, N.Y.), 247(4941), 470–3.

Cruz, N. F., Adachi, K., & Dienel, G. A. (1999). Rapid efflux of lactate from cerebral cortex during K+ -induced spreading cortical depression. Journal of Cerebral Blood Flow and Metabolism : Official Journal of the International Society of Cerebral Blood Flow and Metabolism, 19(4), 380–92.

Csiba, L., Paschen, W., & Mies, G. (1985). Regional changes in tissue pH and glucose content during cortical spreading depression in rat brain. Brain Research, 336(1), 167–70.

Czéh, G., Aitken, P. G., & Somjen, G. G. (1992). Whole-cell membrane current and membrane resistance during hypoxic spreading depression. Neuroreport, 3(2), 197–200.

Czéh, G., Aitken, P. G., & Somjen, G. G. (1993). Membrane currents in CA1 pyramidal cells during spreading depression (SD) and SD-like hypoxic depolarization. Brain Research, 632(1–2), 195–208.

Dohmen, C., Sakowitz, O. W., Fabricius, M., Bosche, B., Reithmeier, T., Ernestus, R.-I., … Graf, R. (2008). Spreading depolarizations occur in human ischemic stroke with high incidence. Annals of Neurology, 63(6), 720–728.

Dreier, J. P. (2011). The role of spreading depression, spreading depolarization and spreading ischemia in neurological disease. Nature Medicine, 17(4), 439–447.

Dreier, J. P., Isele, T., Reiffurth, C., Offenhauser, N., Kirov, S. A., Dahlem, M. A., & Herreras, O. (2013). Is spreading depolarization characterized by an abrupt, massive release of gibbs free energy from the human brain cortex? The Neuroscientist : A Review Journal Bringing Neurobiology, Neurology and Psychiatry, 19(1), 25–42.

Dreier, J. P., K??rner, K., Ebert, N., G??rner, A., Rubin, I., Back, T., … Dirnagl, U. (1998). Nitric Oxide Scavenging by Hemoglobin or Nitric Oxide Synthase Inhibition by N-Nitro-L-Arginine Induces Cortical Spreading Ischemia When K+ Is Increased in the Subarachnoid Space. Journal of Cerebral Blood Flow & Metabolism, 18(9), 978–990.

Fabricius, M., Fuhr, S., Bhatia, R., Boutelle, M., Hashemi, P., Strong, A. J., & Lauritzen, M. (2006). Cortical spreading depression and peri-infarct depolarization in

acutely injured human cerebral cortex. Brain, 129(3), 778–790.

Fabricius, M., Jensen, L. H., & Lauritzen, M. (1993). Microdialysis of interstitial amino acids during spreading depression and anoxic depolarization in rat neocortex. Brain Research, 612(1), 61–69.

Farkas, E., Pratt, R., Sengpiel, F., & Obrenovitch, T. P. (2008). Direct, live imaging of cortical spreading depression and anoxic depolarisation using a fluorescent, voltage-sensitive dye. Journal of Cerebral Blood Flow & Metabolism, 28(2), 251–262.

Feuerstein, D., Manning, A., Hashemi, P., Bhatia, R., Fabricius, M., Tolias, C., … Boutelle, M. G. (2010). Dynamic metabolic response to multiple spreading depolarizations in patients with acute brain injury: an online microdialysis study. Journal of Cerebral Blood Flow and Metabolism : Official Journal of the International Society of Cerebral Blood Flow and Metabolism, 30(7), 1343–55.

Fregni, F., Liebetanz, D., Monte-Silva, K. K., Oliveira, M. B., Santos, A. A., Nitsche, M. A., … Guedes, R. C. A. (2007). Effects of transcranial direct current stimulation coupled with repetitive electrical stimulation on cortical spreading depression. Experimental Neurology (Vol. 204).

Freygang Jr, W. H. , & Landau, W. M. (1955). Some relations between resistivity and electrical activity in the cerebral cortex of the cat. Journal of Cellular and Comparative Physiology, 45(3), 377–392.

Froc, D. J., & Racine, R. J. (2004). N-methyl-d-aspartate receptor-independent long-term depression and depotentiation in the sensorimotor cortex of the freely moving rat. Neuroscience, 129(2), 273–281.

Fujita, S., Mizoguchi, N., Aoki, R., Cui, Y., Koshikawa, N., & Kobayashi, M. (2016). Cytoarchitecture-Dependent Decrease in Propagation Velocity of Cortical Spreading Depression in the Rat Insular Cortex Revealed by Optical Imaging. Cerebral Cortex (New York, N.Y. : 1991), 26(4), 1580–9.

Gardner-Medwin, A. R. (1981). Possible roles of vertebrate neuroglia in potassium dynamics, spreading depression and migraine. Journal of Experimental Biology, 95(1).

Grafstein, B. (1956). Mechanism of spreading cortical depression. Journal of Neurophysiology, 19(2), 154–71.

Hablitz, J. J., & Heinemann, U. (1989). Alterations in the microenvironment during

spreading depression associated with epileptiform activity in the immature neocortex. Developmental Brain Research, 46(2), 243–252.

Hadjikhani, N., Sanchez del Rio, M., Wu, O., Schwartz, D., Bakker, D., Fischl, B., … Moskowitz, M. A. (2001). Mechanisms of migraine aura revealed by functional MRI in human visual cortex. Proceedings of the National Academy of Sciences, 98(8), 4687–4692.

Hansen, A. J., & Olsen, C. E. (1980). Brain extracellular space during spreading depression and ischemia. Acta Physiologica Scandinavica, 108(4), 355–365.

Hansen, A. J., & Zeuthen, T. (1981). Extracellular ion concentrations during spreading depression and ischemia in the rat brain cortex. Acta Physiologica Scandinavica, 113(4), 437–45.

Hartings, J., Strong, A. J., Fabricius, M., Manning, A., Bhatia, R., Dreier, J., … Bullock, R. (2009). Spreading Depolarizations and Late Secondary Insults after Traumatic Brain Injury. Journal of Neurotrauma, 110306202455053.

Herreras, O., Largo, C., Ibarz, J., Somjen, G., & Martin del Rio, R. (1994). Role of neuronal synchronizing mechanisms in the propagation of spreading depression in the in vivo hippocampus. Journal of Neuroscience, 14(11).

Herreras, O., & Somjen, G. G. (1993). Analysis of potential shifts associated with recurrent spreading depression and prolonged unstable spreading depression induced by microdialysis of elevated K+ in hippocampus of anesthetized rats. Brain Research, 610(2), 283–94.

Hood, W. F., Compton, R. P., & Monahan, J. B. (1989). D-cycloserine: a ligand for the N-methyl-D-aspartate coupled glycine receptor has partial agonist characteristics. Neuroscience Letters, 98(1), 91–5.

Hoogendam, J. M., Ramakers, G. M. J., & Di Lazzaro, V. (2010). Physiology of repetitive transcranial magnetic stimulation of the human brain. Brain Stimulation, 3(2), 95–118.

Jing, J., Aitken, P. G., & Somjen, G. G. (1993). Role of calcium channels in spreading depression in rat hippocampal slices. Brain Research, 604(1–2), 251–9.

Jing, J., Aitken, P. G., & Somjen, G. G. (1994). Interstitial volume changes during spreading depression (SD) and SD-like hypoxic depolarization in hippocampal tissue slices. Journal of Neurophysiology, 71(6), 2548–51.

Johnson, J. W., & Ascher, P. (1987). Glycine potentiates the NMDA response in cultured mouse brain neurons. Nature, 325(6104), 529–531.

Kaila, K. (1994). Ionic basis of GABAA receptor channel function in the nervous system. Progress in Neurobiology, 42(4), 489–537.

Kawahara, N., Croll, S. D., Wiegand, S. J., & Klatzo, I. (1997). Cortical spreading depression induces long-term alterations of BDNF levels in cortex and hippocampus distinct from lesion effects: implications for ischemic tolerance. Neuroscience Research, 29(1), 37–47.

Kraig, R. P., Ferreira-Filho, C. R., & Nicholson, C. (1983). Alkaline and acid transients in cerebellar microenvironment. Journal of Neurophysiology, 49(3), 831–50.

Kraig, R. P., & Nicholson, C. (1978). Extracellular ionic variations during spreading depression. Neuroscience, 3(11), 1045–59.

Krüger, H., Heinemann, U., & Luhmann, H. J. (1999). Effects of ionotropic glutamate receptor blockade and 5-HT1A receptor activation on spreading depression in rat neocortical slices. Neuroreport, 10(12), 2651–6.

Kunkler, P. E., & Kraig, R. P. (2004). P/Q Ca2+ channel blockade stops spreading depression and related pyramidal neuronal Ca2+ rise in hippocampal organ culture. Hippocampus, 14(3), 356–67.

Kuo, M.-F., Grosch, J., Fregni, F., Paulus, W., & Nitsche, M. A. (2007). Focusing Effect of Acetylcholine on Neuroplasticity in the Human Motor Cortex. Journal of Neuroscience, 27(52), 14442–14447.

Kuwabara, S., Cappelen-Smith, C., Lin, C. S.-Y., Mogyoros, I., & Burke, D. (2002). Effects of voluntary activity on the excitability of motor axons in the peroneal nerve. Muscle & Nerve, 25(2), 176–84.

Langomoen, I. A., & Hablitz, J. J. (1981). Reversal potential for glutamate responses in hippocampal pyramidal cells. Neuroscience Letters, 23(1), 61–5.

Lauritzen, M. (1987). Regional cerebral blood flow during cortical spreading depression in rat brain: increased reactive hyperperfusion in low-flow states. Acta Neurologica Scandinavica, 75(1), 1–8.

Lehmenkühler, A. (1990). Spreading depression - Reaktionen an der Hirnrinde: Störungen des extrazellulären Mikromilieus. Klinische Neurophysiologie, 21(1), 1–6.

Leo, A. A. P. (1944). SPREADING DEPRESSION OF ACTIVITY IN THE CEREBRAL CORTEX. Journal of Neurophysiology, 7(6).

Lian, X.-Y., & Stringer, J. L. (2004a). Astrocytes contribute to regulation of extracellular calcium and potassium in the rat cerebral cortex during spreading depression. Brain Research, 1012(1–2), 177–84.

Lian, X.-Y., & Stringer, J. L. (2004b). Energy failure in astrocytes increases the vulnerability of neurons to spreading depression. The European Journal of Neuroscience, 19(9), 2446–54.

Liebetanz, D., Fregni, F., Monte-Silva, K. K., Oliveira, M. B., Amâncio-dos-Santos, A., Nitsche, M. A., & Guedes, R. C. A. (2006). After-effects of transcranial direct current stimulation (tDCS) on cortical spreading depression. Neuroscience Letters, 398(1–2), 85–90.

Liebetanz, D., Nitsche, M. A., Tergau, F., & Paulus, W. (2002). Pharmacological approach to the mechanisms of transcranial DC-stimulation-induced after-effects of human motor cortex excitability. Brain : A Journal of Neurology, 125(Pt 10), 2238–47.

Malenka, R. C., & Bear, M. F. (2004). LTP and LTD. Neuron, 44(1), 5–21.

Martins-Ferreira, H., De Oliveira Castro, G., Struchiner, C. J., & Rodrigues, P. S. (1974). Circling spreading depression in isolated chick retina. Journal of Neurophysiology, 37(4), 773–84.

Martins-Ferreira, H., Nedergaard, M., & Nicholson, C. (2000). Perspectives on spreading depression. Brain Research. Brain Research Reviews, 32(1), 215–34.

Martins-Ferreira, H., & Ribeiro, L. J. (1995). Biphasic effects of gap junctional uncoupling agents on the propagation of retinal spreading depression. Brazilian Journal of Medical and Biological Research = Revista Brasileira de Pesquisas Medicas E Biologicas, 28(9), 991–4.

Matsumoto, H., & Ugawa, Y. (2010). [Transcranial magnetic stimulation (TMS) in clinical neurology]. Rinsho Shinkeigaku = Clinical Neurology, 50(11), 803–7.

Matsuura, T., & Bures, J. (1971). The minimum volume of depolarized neural tissue required for triggering cortical spreading depression in rat. Experimental Brain Research, 12(3), 238–49.

McEachern, J. C., & Shaw, C. A. (1996). An alternative to the LTP orthodoxy: a plasticity-pathology continuum model. Brain Research. Brain Research Reviews, 22(1), 51–92.

Mothet, J.-P., Pollegioni, L., Ouanounou, G., Martineau, M., Fossier, P., & Baux, G. (2005). Glutamate receptor activation triggers a calcium-dependent and SNARE protein-dependent release of the gliotransmitter D-serine. Proceedings of the National Academy of Sciences of the United States of America, 102(15), 5606–11.

Müller, M., & Somjen, G. G. (2000). Na+ and K+ Concentrations, Extra- and Intracellular Voltages, and the Effect of TTX in Hypoxic Rat Hippocampal Slices. Journal of Neurophysiology, 83(2).

Nedergaard, M., Cooper, A. J. L., & Goldman, S. A. (1995). Gap junctions are required for the propagation of spreading depression. Journal of Neurobiology, 28(4), 433–444.

Nitsche, M. A., Fricke, K., Henschke, U., Schlitterlau, A., Liebetanz, D., Lang, N., … Paulus, W. (2003). Pharmacological Modulation of Cortical Excitability Shifts Induced by Transcranial Direct Current Stimulation in Humans. The Journal of Physiology, 553(1), 293–301.

Nitsche, M. A., Grundey, J., Liebetanz, D., Lang, N., Tergau, F., & Paulus, W. (2004). Catecholaminergic Consolidation of Motor Cortical Neuroplasticity in Humans. Cerebral Cortex, 14(11), 1240–1245.

Nitsche, M. A., Jaussi, W., Liebetanz, D., Lang, N., Tergau, F., & Paulus, W. (2004). Consolidation of Human Motor Cortical Neuroplasticity by D-Cycloserine. Neuropsychopharmacology, 29(8), 1573–1578.

Nitsche, M. A., Kuo, M.-F., Karrasch, R., Wächter, B., Liebetanz, D., & Paulus, W. (2009). Serotonin Affects Transcranial Direct Current–Induced Neuroplasticity in Humans. Biological Psychiatry, 66(5), 503–508.

Nitsche, M. A., Seeber, A., Frommann, K., Klein, C. C., Rochford, C., Nitsche, M. S., … Tergau, F. (2005). Modulating parameters of excitability during and after transcranial direct current stimulation of the human motor cortex. The Journal of Physiology, 568(Pt 1), 291–303.

Nowak, L., Bregestovski, P., Ascher, P., Herbet, A., & Prochiantz, A. (n.d.). Magnesium gates glutamate-activated channels in mouse central neurones. Nature, 307(5950), 462–5.

Obrenovitch, T., Chen, S., & Farkas, E. (2009). Simultaneous, live imaging of cortical spreading depression and associated cerebral blood flow changes, by combining voltage-sensitive dye and laser speckle. Neuroimage.

Obrenovitch, T. P., & Zilkha, E. (1995). High extracellular potassium, and not extracellular glutamate, is required for the propagation of spreading depression. Journal of Neurophysiology, 73(5), 2107–14.

Panatier, A., Theodosis, D. T., Mothet, J.-P., Touquet, B., Pollegioni, L., Poulain, D. A., & Oliet, S. H. R. (2006). Glia-Derived d-Serine Controls NMDA Receptor Activity and Synaptic Memory. Cell, 125(4), 775–784.

Peters, O., Schipke, C. G., Hashimoto, Y., & Kettenmann, H. (2003). Different mechanisms promote astrocyte Ca2+ waves and spreading depression in the mouse neocortex. The Journal of Neuroscience : The Official Journal of the Society for Neuroscience, 23(30), 9888–96.

Pinard, E., Nallet, H., MacKenzie, E. T., Seylaz, J., & Roussel, S. (2002). Penumbral microcirculatory changes associated with peri-infarct depolarizations in the rat. Stroke, 33(2), 606–12.

Price, D. L., Ludwig, J. W., Mi, H., Schwarz, T. L., & Ellisman, M. H. (2002). Distribution of rSlo Ca2+-activated K+ channels in rat astrocyte perivascular endfeet. Brain Research, 956(2), 183–93.

Priori, A., Berardelli, A., Rona, S., Accornero, N., & Manfredi, M. (1998). Polarization of the human motor cortex through the scalp. Neuroreport, 9(10), 2257–60.

Purpura, D. P., & McMurtry, J. G. (1965). INTRACELLULAR ACTIVITIES AND EVOKED POTENTIAL CHANGES DURING POLARIZATION OF MOTOR CORTEX. Journal of Neurophysiology, 28, 166–85.

Ramos, J. G. (1975). Ionic movements in the isolated chicken retina during spreading depression. Acta Physiologica Latino Americana, 25(2), 112–9.

Reid, K. H., Marrannes, R., & Wauquier, A. (1988). Spreading depression and central nervous system pharmacology. Journal of Pharmacological Methods, 19(1), 1–21.

Reis, J., Schambra, H. M., Cohen, L. G., Buch, E. R., Fritsch, B., Zarahn, E., … Krakauer, J. W. (2009). Noninvasive cortical stimulation enhances motor skill acquisition over multiple days through an effect on consolidation. Proceedings of the

National Academy of Sciences of the United States of America, 106(5), 1590–5.

Rizzoli, S. O., & Betz, W. J. (2005). Synaptic vesicle pools. Nature Reviews Neuroscience, 6(1), 57–69.

Sawant-Pokam, P. M., Suryavanshi, P., Mendez, J. M., Dudek, F. E., & Brennan, K. C. (2016). Mechanisms of Neuronal Silencing After Cortical Spreading Depression. Cerebral Cortex, 81((Pt 1)), bhv328.

Scheller, D., Kolb, J., & Tegtmeier, F. (1992). Lactate and pH change in close correlation in the extracellular space of the rat brain during cortical spreading depression. Neuroscience Letters, 135(1), 83–6.

Scheller, D., Kolb, J., Tegtmeier, F., & Lehmenkühler, A. (1992). Extracellular changes of inorganic phosphate are different during spreading depression and global cerebral ischemia of rats. Neuroscience Letters, 141(2), 269–72.

Sehba, F. A., & Friedrich, V. (2013). Cerebral Microvasculature Is an Early Target of Subarachnoid Hemorrhage. In Cerebral Vasospasm: Neurovascular Events After Subarachnoid Hemorrhage (Vol. 115, pp. 199–205). Vienna: Springer Vienna.

Seidel, J. L., Escartin, C., Ayata, C., Bonvento, G., & Shuttleworth, C. W. (2016). Multifaceted roles for astrocytes in spreading depolarization: A target for limiting spreading depolarization in acute brain injury? Glia, 64(1), 5–20.

Seidel, J. L., & Shuttleworth, C. W. (2011). Contribution of astrocyte glycogen stores to progression of spreading depression and related events in hippocampal slices. Neuroscience, 192, 295–303.

Shin, Y.-I., Foerster, Á., & Nitsche, M. A. (2015). Transcranial direct current stimulation (tDCS) – Application in neuropsychology. Neuropsychologia, 69, 154–175.

Smith, S. E., & Chesler, M. (1999). Effect of Divalent Cations on AMPA-Evoked Extracellular Alkaline Shifts in Rat Hippocampal Slices. Journal of Neurophysiology, 82(4).

Somjen, G. G. (2001). Mechanisms of spreading depression and hypoxic spreading depression-like depolarization. Physiological Reviews, 81(3), 1065–96.

Somjen, G. G., Aitken, P. G., Giacchino, J. L., & McNamara, J. O. (1985). Sustained potential shifts and paroxysmal discharges in hippocampal formation. Journal of Neurophysiology, 53(4).

Spray, D. C., Harris, A. L., & Bennett, M. V. (1981). Gap junctional conductance is a simple and sensitive function of intracellular pH. Science (New York, N.Y.), 211(4483), 712–5.

Stagg, C. J., & Nitsche, M. A. (2011). Physiological basis of transcranial direct current stimulation. The Neuroscientist : A Review Journal Bringing Neurobiology, Neurology and Psychiatry, 17(1), 37–53.

Sugaya, E., Takato, M., & Noda, Y. (1975). Neuronal and glial activity during spreading depression in cerebral cortex of cat. Journal of Neurophysiology, 38(4), 822–41.

Theis, M., Jauch, R., Zhuo, L., Speidel, D., Wallraff, A., Döring, B., … Willecke, K. (2003). Accelerated hippocampal spreading depression and enhanced locomotory activity in mice with astrocyte-directed inactivation of connexin43. The Journal of Neuroscience : The Official Journal of the Society for Neuroscience, 23(3), 766–76.

Tobiasz, C., & Nicholson, C. (1982). Tetrodotoxin resistant propagation and extracellular sodium changes during spreading depression in rat cerebellum. Brain Research, 241(2), 329–33.

Tomida, S., Wagner, H. G., Klatzo, I., & Nowak, T. S. (1989). Effect of Acute Electrode Placement on Regional CBF in the Gerbil: A Comparison of Blood Flow Measured by Hydrogen Clearance, [3H]Nicotine, and [14C]Iodoantipyrine Techniques. Journal of Cerebral Blood Flow & Metabolism, 9(1), 79–86.

Tong, C. K., & Chesler, M. (1999). Endogenous pH shifts facilitate spreading depression by effect on NMDA receptors. Journal of Neurophysiology, 81(4), 1988–91.

Tong, C. K., & Chesler, M. (2000). Modulation of spreading depression by changes in extracellular pH. Journal of Neurophysiology, 84(5), 2449–57.

Tottene, A., Conti, R., Fabbro, A., Vecchia, D., Shapovalova, M., Santello, M., … Pietrobon, D. (2009). Enhanced excitatory transmission at cortical synapses as the basis for facilitated spreading depression in Ca(v)2.1 knockin migraine mice. Neuron, 61(5), 762–73.

Traynelis, S. F., & Cull-Candy, S. G. (1990). Proton inhibition of N-methyl-D-aspartate receptors in cerebellar neurons. Nature, 345(6273), 347–350.

Unekawa, M., Tomita, Y., & Toriumi, H. (2013). Potassium ‐ induced cortical spreading depression bilaterally suppresses the electroencephalogram but only ipsilaterally affects red blood cell velocity in. Journal of.

van den Maagdenberg, A. M. J. M., Pietrobon, D., Pizzorusso, T., Kaja, S., Broos, L. A. M., Cesetti, T., … Ferrari, M. D. (2004). A Cacna1a knockin migraine mouse model with increased susceptibility to cortical spreading depression. Neuron, 41(5), 701–10.

Wang, Y., & Belousov, A. B. (2011). Deletion of neuronal gap junction protein connexin 36 impairs hippocampal LTP. Neuroscience Letters, 502(1), 30–2.

Windmüller, O., Lindauer, U., Foddis, M., Einhäupl, K. M., Dirnagl, U., Heinemann, U., & Dreier, J. P. (2005). Ion changes in spreading ischaemia induce rat middle cerebral artery constriction in the absence of NO. Brain : A Journal of Neurology, 128(Pt 9), 2042–51.

Wolosker, H., Blackshaw, S., & Snyder, S. H. (1999). Serine racemase: a glial enzyme synthesizing D-serine to regulate glutamate-N-methyl-D-aspartate neurotransmission. Proceedings of the National Academy of Sciences of the United States of America, 96(23), 13409–14.

Young, J. N., & Somjen, G. G. (1992). Suppression of presynaptic calcium currents by hypoxia in hippocampal tissue slices. Brain Research, 573(1), 70–6.

Yu, Y., Santos, L. M., Mattiace, L. A., Costa, M. L., Ferreira, L. C., Benabou, K., … Rozental, R. (2012). Reentrant spiral waves of spreading depression cause macular degeneration in hypoglycemic chicken retina. Proceedings of the National Academy of Sciences of the United States of America, 109(7), 2585–9.

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