# MOLECULAR MECHANISMS IN PATHOPHYSIOLOGY OF MIGRAINE AURA

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**Abstract**

Cortical spreading depression (CSD), the underlying mechanism behind migraine aura, occurs when neurons become depolarized and lose function, while releasing signaling molecules that propagate this condition. Spreading depression occurs when abnormal flow of ions across the cell membranes of a neural cell depolarizes the cell and activates detrimental signaling pathways that cause the same disruptive ion flow to occur in nearby neural or glial cells. After depolarization, cells affected by CSD are not able to return to their normal state and remain nonfunctional for some time. This lack of function causes symptoms of migraine aura. Calcium and glutamate signaling may be primary drivers of the CSD; inhibition of either tends to prevent CSD, whereas their activation promotes it. Cerebral blood flow and oxygen consumption are considered measures of cell function during CSD and further demonstrate the significance of CSD on a larger scale.

This is a review of current research about these elements of migraine aura.

## Introduction

According to a survey of symptoms, approximately 11% of U.S. citizens over 12 years old had migraines within a one year time period, with women being more likely than men to have the condition (Lipton, et al., 2007). Additionally, in the 2015 Global Burden of Disease study, migraine was credited with being the 6$^{th}$ leading cause of days spent disabled (Steiner, Stovner, & Vos, 2016). Considering their prevalence, migraines are remarkably misunderstood. These headaches have been studied for decades, but despite all the information collected, the underlying mechanisms and causes are quite mysterious. Migraine aura is one of the better-characterized elements of migraine; cortical spreading depression is the widely accepted mechanism behind aura and many of its components are well-described. Cortical spreading depression can be most simply defined as a massive depolarization of one nerve cell that results in the depolarization of nearby cells, propagating in a wave-like manner.

Glutamate is likely a driving factor of migraine pathophysiology, especially in cortical spreading depression, the leading proposed mechanism for aura in migraines. This mechanism involves propagation of signals between neurons as driven by glutamate receptors *N*-methyl-*D*-aspartate (NMDA) and non-NMDA receptors (and their related pathways. Movement of calcium and potassium ions is closely tied to function of these receptors and likely plays a role in susceptibility to cortical spreading depression through glutamate signaling. Calcium channels are particularly significant in CSD; gain-

of-function mutations in calcium channels cause a severe form of migraine and inhibition of certain calcium channels in mouse models can prevent CSD from occurring.

Cerebral blood flow to regions of the brain that are affected by CSD and oxygen consumption in the same areas indicate the level of activity in these cells. The general pattern is that blood flow increases as the cell begins to depolarize, then drops off once they becomes depolarized. This is likely because the cells must generate more ATP than usual as they attempt to maintain normal ion balances, but once they have passed the threshold, they are essentially non-functional and are using little or no energy as only minimal cellular function is ongoing.

All of these elements are important to consider when attempting to understand the pathophysiology of migraine aura. Though this is not an exhaustive list of components of aura, or even CSD, each represents a key point of the condition that must be studied to form a complete picture. Understanding the condition is important for developing new drugs to help the millions of migraineurs world wide.

## Medical Definition of Migraine and Epidemiology

The International Headache Society is an organization of headache specialists from academic and medical backgrounds in neurology with the goal of furthering knowledge about headache disorders through research and education. One of their main functions is to create and maintain the International Classification of Headache Disorders, a document containing the information needed to identify and diagnose different types of headache disorders. Migraines fall into two main categories: migraine with aura and migraine without aura. Migraine without aura is described as “Recurrent headache disorder manifesting in attacks lasting 4-72 hours. Common characteristics of the head ache are unilateral location, pulsating quality, moderate or severe intensity, aggravation by routine physical activity and association with nausea and/or photophobia and phonophobia” (International Headache Society, 2013). Migraine with aura includes these criteria for the migraine itself, but also includes aura, a set of nervous system malfunctions that include sensory, auditory, visual, speech/language, motor, brainstem, or retinal symptoms. This typically precede the pain phase of migraine, but can persist through, or even beyond, it  (International Headache Society, 2013). Triggers vary widely but often consist of events or substances that make migraineurs more susceptible ot migraine onset. Most are neither necessary nor sufficient to cause migraine; often multiple triggers must be present before a migraine or migraine aura begins. Instigators of migraine attack vary between patients, but include hormonal

changes, stress, lack of sleep, going to long without eating, certain foods/drinks, smells, weather changes, physical activity, and others (Spierings, Donoghue, Mian, & Wober, 2014).

Millions of people suffer from migraines, but the underlying mechanisms for this type of headache are still being identified and specified. Many biochemical markers and changes have been found and some of the biological pathways and relationships have been described; however, the relationships between these markers and pathways are not completely understood. There is strong evidence that migraines have a genetic component; most migraineurs have a close relative with migraines and some forms of migraine are associated with a known genetic mutation. Genome wide association studies (GWAS) have been carried out within populations of migraine sufferers, where they found single nucleotide polymorphisms (SNPs) within genes linked to migraine, including vascular and metabolic mutations, metalloproteinases, pain sensitivity, synapse function, and glutamate-related neurotransmission (Ferrari, Klever, Terwindt, Ayata, & van den Maagdenberg, 2015). Twin studies provide further support for a genetic basis of migraine, as monozygotic twins are 1.5 to 2 times more likely to both have migraines than dizygotic twins (de Vries, Frants, Ferrari, & van den Maagdenberg, 2009) Some of these genetic links have been uncovered and pursued, but for most types of migraine, researchers have not been able to identify a particular mutation

responsible for the condition. The genetic mutations that have been identified have been used to provide insight into how migraine works on a larger scope.

## Familial Hemiplegic Migraine

Familial hemiplegic migraine (FHM) is an extreme form of migraine with aura (MA), primarily differentiated from general MA by the presence of motor weakness during the aura phase (International Headache Society, 2013). FHM is a monogenic disease with three main types distinguished by mutation type. FHM type 1 (FHM1) is characterized by mutations to the $\alpha$1 subunit of neuronal $Ca_V$ 2.1 (P/Q-type) voltage-gated calcium channels, which are also associated with cerebellar ataxia and epilepsy during aura (de Vries, Boukje, Frants, Rune R., Ferrari, Michel D., van den Maagdenberg, Arn M.J.M., 2009). FHM type 2 (FHM2) mutations affect the $\alpha$2 subunit of sodium–potassium pumps and are associated with cerebellar problems, childhood convulsions, epilepsy, and permanent mental retardation, and non-hemiplegic migraines. FHM type 3 (FHM3) mutations are in the $\alpha$1 subunit of neuronal $Na_V$ 1.1 voltage-gated sodium channels, which have previously been identified as epilepsy mutations (de Vries, Boukje, Frants, Rune R., Ferrari, Michel D., van den Maagdenberg, Arn M.J.M., 2009).

## FHM as a model for migraine with or without aura

Though the genetic mutations that cause FHM are specific to that disease, the similarities between FHM-migraines and other types of migraines allow studies of these mutations to apply more broadly across migraine types. Each of the identified mutations affects the function of a particular ion-transport protein, indicating that these proteins and their function must have some kind of link to migraine and migraine aura. Identifying non-functional genes and their products provides insight into how the pathway involving the gene works. Knowledge of these mutations pushed researchers toward more detailed examination of the role that ion transport malfunctions play, especially when considering the connections between ion concentration, cell function, and signaling pathways. These functions can be studied in protein isolates and cell or tissue isolates.

Additionally, the monogenic nature of FHM has allowed the disease to be studied in relative detail, which combined with the severe and predictable symptoms makes it a useful condition for extending studies using model organisms. FHM mutations make it almost guaranteed that research techniques designed to provoke migraine symptoms will do so, allowing researchers to measure various metrics of the migraine headache and/or aura. Techniques for preventing migraine can also be tested in these systems.

## Cortical spreading depression

Modern research has provided significant support for cortical spreading depression (CSD) as the mechanism behind migraine aura. This phenomenon is explained as a “slowly propagating wave of rapid, near-complete depolarization of brain cells that lasts for about 1 minute and silences brain electrical activity for several minutes… It is characterized by the collapse of ion homeostasis, profound disruption of transmembrane ionic gradients and the release of neurotransmitters and other molecules from cellular compartments” (Pietrobon & Moskowitz, 2015). In their resting state, neurons maintain a potential difference between their cytoplasm and the extracellular space. Carefully controlled efflux and influx of ions creates a steep concentration gradient and a charge difference that ions can follow when needed. CSD occurs when this balance is disrupted. It begins with some perturbation of a neural cell that causes it to depolarize via imbalanced ion flux, triggering certain pathways such that some neurotransmitter or other signaling molecule is released, causing unusual ion flux and depolarization of the next neuron, which causes the process to repeat and propagate throughout a section of the brain.

Normal neurotransmission follows a similar pattern. Change in ion concentration and resultant depolarization is a common way for neurons to release normal signaling molecules. The key difference between CSD and normal neurotransmission, however, is that cells during normal neurotransmission are able to quickly re-polarize after the signal is propagated, whereas cells during CSD are unable to re-establish correct

voltage differences between the inside and outside of the cell. This causes the affected neurons to be non-functional for a period of time following the passing of a CSD wave and could lead to aura symptoms. Although this inability to repolarize can happen through various mechanisms, the general result that leads to spreading depression is a group of nearby cells that are either especially susceptible to depolarization or are unable to repolarize normally. This could come from ion channel mutations at the synapse that lead to problematic ion movements or from other types of mutations that cause abnormal levels of neurotransmitter to be present at the synapse (Khennouf, Gesslein, Lind, van den Maagdenberg, & Lauritzen, 2016).

CSD occurs in three phases called early, main, and late. Early is characterized by extreme depolarization of apical dendrites and soma, rapid influx of potassium ions (10 times normal levels), efflux of chlorine ions (2-3 times lower) and calcium ions (about 2 times lower). The main phase includes complete depolarization of soma and dendrites, and increased extracellular pH as a result of alterations to ion flow. Late phase includes return polarization, normal ion concentrations for most cells, and normal pH. During the early and main phases, neurons swell and interstitial space decreases (Pietrobon & Moskowitz, 2015) This propagates in a wave-like pattern, where ion flux in one neuron induces the same changes in the next neuron.  Astrocytes tend to follow these steps as well, although this is thought to be a response to changes in extracellular ion concentrations from neurons and therefore, not a cause of CSD. Vascular function

linked to these cells is also affected; local hypoperfusion prevents adequate blood flow from reaching the neurons and further inhibits their ability to repolarize.

Though CSD itself has been characterized in quite some detail, the source of the original depolarized cell in the brains of migraineurs is not certain. In the lab, CSD is triggered using chemical, mechanical, or electrical techniques that manually disrupt the polarization of one cell or a region of cells such that they will cause the same in adjacent cells. It is postulated that similar events in the human brain occur to cause CSD, but none have enough support to be considered a primary cause.

Acknowledging CSD as the main mechanism behind migraine aura gives researchers the opportunity to study aura by narrowing their focus to one of the many elements of CSD. Research into CSD components, such as ion channels and cellular metabolism, has allowed scientists to put together a more accurate picture of migraine aura pathophysiology and may pave the way for better therapeutics.

## Calcium channel mutations and their role in CSD

Twenty-one different calcium channel mutations have been identified in FHM1 patients. They are found in the gene CACNA1A gene that encodes the $\alpha_1$ subunit of the channel which forms its pore (Pietrobon, Cav2.1 channelopathies, 2010). These mutations are gain-of-function and only affect $Ca_V2.1$ (P/Q-type) channels, which are

specific to “presynaptic terminals and somatodendritic membranes throughout the mammalian brain and spinal cord” including a number of variants found in particular neuron types (Pietrobon, Cav2.1 channelopathies, 2010). FHM1 mutations are known to reduce the threshold for CSD and increase its propagation speed (Tottene, Urbani, & Pietrobon, 2011). It is thought that these mutations’ role in FHM1 is that increased influx of calcium ions aids in the extreme depolarization of neural cells that is needed to cause and propagate CSD. Additionally, calcium levels within the cell are closely related to certain signaling pathways that help spread CSD and can lead to vascular reactions of migraine aura. Their significance is further highlighted by the fact that these channels are found in all of the brain strucutrures implicated in migraine pathology and are major regulators of the signaling pathways of several key neurotransmitters that have also been connected to migraine and aura.

These mutations are quite rare, making it unlikely that they are responsible for migraines or migraine aura in most migraineurs, but knowing that gain-of-function mutations lead to such a severe phenotype gives insight into the role of calcium flux in other migraine conditions. Increased movement of calcium into the cell may be part of the reason why neurons in non-FHM migraine sufferers are susceptible to CSD, a hypothesis upon which additional research can be based. It must also be considered, however that though calcium channels are a useful focus for research about migraine in general, these mutations are also associated with other, more severe, conditions that

differ greatly from general MA including progrssive damage to brain tissue or certain types of ataxia (Pietrobon, Cav2.1 channelopathies, 2010). This may indicate that these mutations have a larger role than simply making neurons more susceptible to depolarization and could mean that research designed to elucidate the role of these channels in FHM1 may not always apply to understanding migraine in a more general sense.

The significance of calcium in CSD is shown in several studies. One uses a mouse model for FHM1 with a $Ca_v2.1$ calcium channel mutation, R192Q, which was found to increase $Ca^{2+}$ influx at pre-synaptic terminals and increases glutamate release in pyramidal cells without affecting inhibitory neurotransmission at interneurons (Khennouf, Gesslein, Lind, van den Maagdenberg, & Lauritzen, 2016). When CSD was triggered by researchers, R192Q mutants showed a faster CSD propagation and had higher maximum intracellular $Ca^{2+}$ levels when compared to the wild type (WT) overall and for each phase of calcium concentration change. The first phase reaches its maximum quickly and the calcium concentration at that maximum is much greater than the other phase before returning to low calcium levels. The second increases more slowly, reaching a smaller maximum concentration (Khennouf, Gesslein, Lind, van den Maagdenberg, & Lauritzen, 2016). Calcium participates in a number of cellular pathways, particularly those associated with release or uptake of certain neurotransmitters, which have various downstream effects including propagating the

spreading depression, altering ion uptake of surrounding cells, changing cell metabolism, release of signals that alter blood flow to the region, and others. Of these, glutamate signaling is thought to be one of the most significant in CSD propagation.

## Calcium and glutamate signaling

The relationship between glutamate and calcium is a positive feedback loop, meaning that more calcium influx means more glutamate release and vice versa (Gasparini, Smith, & Griffiths, 2016). In an FHM1 mouse model, the amount of calcium ion influx was shown to positively correlate with glutamate release in cortical pyramidal cells (Tottene, Urbani, & Pietrobon, 2011). In CSD, when initia2l depolarization of the neuron occurs, calcium ions flow into the cell at much higher levels than normal, which leads to higher-than-normal rates glutamate release at neural synapses. This in turn causes the neuron receiving the signal to take up more calcium than normal and become depolarized, propagating CSD (Khennouf, Gesslein, Lind, van den Maagdenberg, & Lauritzen, 2016).

When extracellular glutamate concentrations are very high, two problems arise. First, glutamate molecules in the intercellular space constantly stimulate calcium uptake. This means that affected neurons are stuck in a depolarized state, effectively maintaining continuous signaling within and between neurons (Gasparini, Smith, & Griffiths, 2016). This inability to recover from depolarization is one of the elements of

CSD that leads to aura symptoms; non-functioning neurons in certain areas of the brain are responsible for visual, auditory, and other symptoms. It also sets the disease condition apart from normal neuronal signal propagation, which relies on rapid repolarization of neurons so they may continue to function after releasing their neurotransmitters.

The second issue with abnormally high rates of glutamate signaling is that it will likely lead to higher than normal glutamate concentrations in the extracellular space, particularly when paired with mutations that reduce normal glutamate uptake, a condition found in some migraineurs. In high concentrations, glutamate is toxic to neurons. This means that in addition to the problems with downstream signaling, the most direct impact of high glutamate levels on brain tissue is excitotoxicity (Gasparini, Smith, & Griffiths, 2016). This may play a role in some migraine symptoms, but the relationship is not well understood and excitotoxicity itself is not thought to directly affect CSD.

In slices of cerebral cortex from an FHM1 mouse model, the causative role of glutamate signaling in CSD was demonstrated when researchers found that adding a glutamate-release inhibitor to knock-in FHM1 mouse pyramidal cells restored glutamate to wild-type levels, and CSD rate and threshold were completely rescued (Tottene, Urbani, & Pietrobon, 2011). Additionally, experiments that inhibited the NDMARs and different types of calcium channels, prevented researchers from eliciting CSD in the

tissue (Tottene, Urbani, & Pietrobon, 2011). Since the calcium channels tested in this experiment help control the calcium levels needed in the glutamate release pathway, these results provide strong evidence that glutamate and calcium signaling proteins is key to instigating and propagating CSD. Further support for the significance of this pathway over others involving calcium flux it that when other types of voltage-gated calcium channels were blocked, which did not cause major changes in CSD initiation or propagation. This indicates that the P/Q-type channels are indeed the channels driving CSD in FHM1 cortical tissue, likely because of their role in the glutamate signaling pathway (Tottene, Urbani, & Pietrobon, 2011)

## Normal NMDAR pathways

The N-methyl-D-aspartate receptor (NMDA receptor or NDMDAR) is a receptor found in neurons that plays a key role in calcium and glutamate signaling. The specific mechanism by which calcium and glutamate signal is through the activation of this receptor. NMDARs have a very high affinity for glutamate, but require simultaneous binding of glycine in order to open. When both of these molecules bind; the membrane begins to depolarize as calcium cations begin to enter the cell through the channel. As voltage within the cell changes, this increases the likelihood that an inhibitory, magnesium ion is forced to leave the NMDAR, where it normally resides when the receptor is not active, opening the channel. This allows greater movement of $Ca^{2+}$ and

$Na^+$ into the neuron while $K^+$ moves out of the cell, eventually reaching the voltage threshold and causing the cell to become depolarized (Zito & Scheuss, 2009).

The depolarization that allows release of $Mg^{2+}$ comes from influx of glutamate through the non-NMDA receptors, especially $\alpha$-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors. These receptors have a lower affinity for glutamate, but they respond more quickly to glutamate influx and also return to their resting state more quickly. When glutamate is released into the synaptic cleft, it is taken up into the post-synaptic neuron by AMPA and other non-NDMA receptors. AMPA is also permeable to large cations whose influx begins to depolarize the membrane. As depolarization occurs and intracellular glutamate levels rise, the glutamate begins to bind NMDA in higher numbers and the inhibitory $Mg^{2+}$ molecules begin to leave the NMDARs. This ultimately results in an active conformation of NMDA that will allow the ion movement earlier mentioned.

The molecule exchange that occurs serves two purposes; propagation of the Excitatory Postsynaptic Potential (EPSP) and downstream signaling of the entering cations. Propagation essentially moves glutamate signaling effects between neurons, often in an excitatory pathway. Movement of $Na^+$ into the cell and $K^+$ out of cells is important for maintaining the concentration gradients needed for normal neuron resting potential and ability to respond to stimuli through action potentials. Cations that are

brought into the neuron by glutamate signaling, notably $Ca^{2+}$, also cause their own signaling cascades within the cell (Lynne Oland, 2016) (Tottene, Urbani, & Pietrobon, 2011).

**Glutamate processes and their relationship with migraine aura**

There is strong evidence linking glutamate pathway malfunction to migraines, particularly as it relates to CSD. Initial evidence for glutamate abnormalities came from studies that found abnormal glutamate concentrations in bodily fluids of migraine sufferers and a variety of problems within glutamate-regulation processes (Gasparini, Smith, & Griffiths, 2016). This showed that glutamate release was higher in migraine sufferers and indicated that it and its related pathways may be important to consider when trying to understand migraine.

Glutamate is one neurotransmitter that has been linked to CSD from several different angles. Its relationship with calcium signaling and the effects of that pathway have already been explained in previous section, but there are several other reasons why it is likely to play a major role in CSD. One study found that when a mutant $Ca_v2.1$ calcium channel implicated in FHM1 patients was expressed in mice glutamate release increased in the cortex and that researchers could “induce, maintain, and propagate CSD” more easily than in wild-type mice (de Vries, Frants, Ferrari, & van den Maagdenberg, 2009). This is supported by another study that indicated that an FHM2

mutation in a $Na^+/K^+$ ATPase would lead to “reduced glial uptake of $K^+$ and glutamate from the synaptic cleft,” which leads to higher concentrations of glutamate at the synaptic cleft (de Vries, Frants, Ferrari, & van den Maagdenberg, 2009). Both of these mutations connect higher glutamate levels to known mutations in FHM and increased sensitivity to CSD. Another way to examine glutamate signaling in CSD is to focus on the interactions between glutamate and glutamate receptors. Some studies found that “CSD is strongly dependent on the interaction between glutamate and presynaptic and postsynaptic glutamate *N*-methyl-*D*-aspartate” (Khennouf, Gesslein, Lind, van den Maagdenberg, & Lauritzen, 2016). This method supports evidence found when inhibition of NMDARs was able to prevent CSD from occurring in an FHM1 mouse model.

Further support for glutamate’s significance in migraine comes from studies that identified glutamate-pathway mutations in migraine sufferers. Several glutamate receptor mutations were found to be present in higher levels in a population of migraine sufferers than in the normal population. GRIA1 and GRIA3 are AMPAR subunits were implicated in one study. The identified GRIA3 mutation is found in the promotor region of the gene and leads to decreased gene expression through reduced promotor activity. Cells with this mutation had reduced expression of AMPA (Gasparini, Smith, & Griffiths, 2016). These particular mutations along with GRIA2 and GRIA4 have been studied since then, but results have proven inconclusive between studies. Several genome-wide

association studies (GWAS) of migraine sufferers found single nucleotide polymorphisms (SNPs) in genes related to glutamatergic neurotransmission (Ferrari, Klever, Terwindt, Ayata, & van den Maagdenberg, 2015). Unfortunately, these mutations and their role in migraine are much more challenging to study than the FHM-associated ones. Rather than being sufficient for CSD, as some of the calcium-channel knock-outs were, these SNPs are more likely to have a less direct role in patients' susceptibility to CSD. More research into these and other proteins in glutamate-signaling pathways is needed to develop a better understanding of their significance.

## Cerebral Blood Flow During Aura

Several studies over the course of the last few decades have demonstrated that the flow of blood through affected portions of the brain during migraine aura is different from flow during non-migraine phases and between migraineurs and non-migraineurs, though each has its own take on the specific relationship between cerebral blood flow and migraine pathophysiology (Fabjan, Zaletel, & Zvan, 2015; Denuelle, Fabre, Payoux, Chollet, & Geraud, 2008). Most of the current evidence supports a model in which some perturbation of the normal levels of certain ions and signaling molecules causes cellular changes that result in an increase of blood flow to these neurons (Fabjan, Zaletel, & Zvan, 2015; Huneau, Benali, & Chabriat, 2015). Because many experiments that are uncovering the relationship between CBF and migraine merely demonstrate a

concurrence of CBF and migraine stages, more causative explanations require interpretation of this data. One strong support for linking increased blood flow to increased cellular metabolism is neurovascular coupling, the model that describes how neuronal activation releases signaling molecules that induce changes in blood vessel dilation/constriction and consequently induces CBF changes. For certain regions of the brain, this hypothesis is accepted to demonstrate that higher neuronal activity causes blood vessel dilation and increased blood flow in order to provide metabolites needed for cellular function (Huneau, Benali, & Chabriat, 2015). When considering the link between CSD and increased metabolism as specified in its section of this paper, it is reasonable to accept that monitoring CBF as an indicator of this metabolism change can provide significant insight into each of these components.

One study quantifies the observed changes in CBF during migraine, specifying that the hypoperfusion during migraine was found “bilaterally in the occipital cortex and the posterior temporal and parietal cortex” and that the rate of this blood flow change was 10.34% lower than was measured in the patients during a non-headache phase. This study also noted that the decrease lasted into the headache phase and increased to 12.32% lower than normal, even after treatment with a popular migraine drug called sumatriptan (Denuelle, Fabre, Payoux, Chollet, & Geraud, 2008).

In migraine with aura, a slightly different pattern emerges. A study measuring CBF, oxygen, and vascular response in a mouse model of FHM-1 found that there is an

initial increase in CBF at the onset of CSD, then a reduction that lasts beyond the end of CSD and into the pain phase (Khennouf, Gesslein, Lind, van den Maagdenberg, & Lauritzen, 2016). In considering the hypothesis that CBF increase is a result of neurons attempting to prevent the massive depolarization of CSD, this initial increase may be an indicator of high activity as neurons use ATP to push ions across their cell membranes to counteract depolarization. Once depolarization occurs, the neurons are no longer functioning, leading to much lower metabolic activity, which requires significantly below average blood flow to maintain.

## CSD Effects on Neuronal Metabolism

Changes in blood flow to areas affected during migraine have been observed for decades, but the relevance and causes for these patterns are only beginning to be identified. During the course of an episode of migraine with aura, the cell goes through several stages of energy consumption. In their resting state, neurons must maintain a particular balance of ions, requiring movement of ions through transmembrane channels or pumps, some of which require ATP to function. When their homeostasis is disrupted, the neurons must move ions at a higher rate as they attempt to re-create the normal ion balance rather than maintain it. This requires more ATP, which comes from higher rates of cellular respiration. In CSD, neurons experience the disruption and attempt to return to normal, but are unable to do so and instead remain depolarized and essentially non-

functional. When they have lost most of their function, neurons are using much less ATP than normal and cellular respiration occurs at a lower rate. Since cellular respiration requires oxygen, the rate of cellular respiration can be measured by tracking blood oxygen concentration. The amount of blood flow can also be a sign of cellular metabolism rates because as rates increase and more oxygen is needed, blood flow tends to also increase. This is especially true in neurons because of neurovascular coupling, the relationship between neurons and glial cells and blood vessels within the brain. The accepted definition of this process is that as neuronal activity increases, vasodilating molecules are released to nearby blood vessels, increasing their diameter and rate of blood flow (Girouard & Iadecola, 2006). Researchers have measured cerebral blood flow (CBF) and blood oxygen levels during the course of migraine in order to demonstrate metabolic changes within the cell during the episode.

One study focused on observing patients who experienced migraine with aura, monitoring the blood flow and oxygen usage in affected regions of the brain using angiography in large veins thought to contain significant portions of blood flowing out of the brain. The researchers measured changes in $O_2$ and $CO_2$ levels in eight patients over the course of 75 minutes, during which four of the patients developed aura symptoms. They found that cerebral blood flow to certain regions of the hemisphere where migraine occurred increased or stayed constant before decreasing by 20-30% after about 20 minutes, that oxygen extraction increased overall, and that a calculated

value for cerebral metabolic rate for oxygen ($CMRO_2$) decreased. $CMRO_2$ is a metric that takes into consideration oxygen-saturated and unsaturated hemoglobin levels, cerebral blood flow, and oxygen extraction. The four patients who did not develop migraine with aura showed no such changes (Friberg, Olesen, Lassen, Olsen, & Karle, 1994. At onset of aura, increased blood flow and oxygen consumption correlate with the idea that the vascular response supports increased cellular activity, while the decreasing rates are likely a response to reduced function after CSD occurs.

Another paper provided support for this idea by tracking blood flow and oxygen levels throughout a CSD wave in a mouse model of FHM1 which is commonly used for *in vivo* migraine research. They found that cerebral blood flow (CBF) increased 40% above normal levels shortly after the onset of CSD, then decreased to 20% below the normal levels. Blood oxygen pressure decreased from 40 mmHg to 0 mmHg after CSD onset, then increased very gradually, but did not return to resting levels within the window of observation (Khennouf, Gesslein, Lind, van den Maagdenberg, & Lauritzen, 2016). This study also tracked levels of calcium ions and cell potentials as known markers of CSD and found that the largest influx of calcium ions and initial depolarization occurred at the same time as those CBF- and oxygen-level increases (Khennouf, Gesslein, Lind, van den Maagdenberg, & Lauritzen, 2016). Because calcium ion influx occurs at the beginning of CSD, this is a more direct demonstration of the relationship between CSD and blood flow and oxygen levels. It indicates that when the

neuron depolarizes as calcium ion influx occurs and CSD begins, its rate of cellular respiration increases, causing increased blood flow to that region and removing large quantities of oxygen from the blood. The observed decrease in blood flow is linked to the end of CSD when metabolism decreases below normal rates.

## Conclusion

Cortical spreading depression is the best explanation for how migraine aura occurs. Within this event, calcium and glutamate signaling are important for understanding the exact conditions of neurons that are about to undergo or are currently experiencing CSD. They are important regulatory elements of CSD that could be manipulated in efforts to aid migraine sufferers. Cerebral blood flow and oxygen consumption are similarly important, though their significance appears to lie more in understanding migraine aura than in preventing or stopping it. It may also be significant in uncovering the impact of migraine or aura on an individual's health overall; blood flow changes in the brain could be related to changes in other parts of the body or could implicate a wider cardiovascular dysfunction.

Modern migraine research has uncovered many important elements of the disease using a variety of approaches across different fields of biology. It will take extensive research throughout these fields to truly

Genetic techniques have proved to be extremely useful in uncovering the mechanisms behind CSD and migraine. Using the FHM mutations, researchers could gain insight into the roles of calcium channels, sodium-potassium pumps, and potassium channels in migraine pathogenesis and create useful model organisms. Portions of research about FHM can be applied to migraine in a broader sense. Unspecific genetic analysis like GWAS is also useful for finding mutations that are common amongst migraineurs. Those mutations are probably not severe enough to cause migraine on their own, but may be part of a larger combination of mutations and can implicate which processes may be important to study. Simply finding the mutations is the first step in identifying what they do. Once mutations are identified, biochemists, cell biologists, physiologists, and neurobiologists can use that information to study what the mutations actually do.

After describing the processes involved in a disease condition, researchers can begin developing treatments that are specific to the disease, focusing on fixing exactly which parts of the body are malfunctioning. This means that targeted therapies more likely to work and less likely to cause side effects throughout the body, making them highly desirable. At least one therapy specific to migraines is being developed and several possible therapies have been identified, but there are currently no widely accessible therapeutics designed to target the migraine pathways mentioned in this paper. The pathways in this paper may be good targets for therapy, but most would be

very challenging; inhibiting ion channels to compensate for increased function may cause more harm than good. Compensating for neuronal metabolism changes or loss of cell function may also not work especially well.

This being said, all of the research cited in this paper was very useful in describing how migraine and aura work, which could always lead to new information and better therapeutics in the future. As genetic and molecular techniques improve and as the breadth of knowledge about this subject increases, existing treatments can be improved and new treatments can be developed to alleviate the suffering of people with migraines.

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