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REVIEW

ECS Dynamism and Its Influence on Neuronal Excitability and Seizures

Robert Colbourn[1,2] · **Aditi Naik**[1,2] · **Sabina Hrabetova**[1]

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Abstract
Seizure activity is governed by changes in normal neuronal physiology that lead to a state of neuronal hyperexcitability and synchrony. There is a growing body of research and evidence suggesting that alterations in the volume fraction (α) of the brain's extracellular space (ECS) have the ability to prolong or even initiate seizures. These ictogenic effects likely occur due to the ECS volume being critically important in determining both the concentration of neuroactive substances contained within it, such as ions and neurotransmitters, and the effect of electric field-mediated interactions between neurons. Changes in the size of the ECS likely both precede a seizure, assisting in its initiation, and occur during a seizure, assisting in its maintenance. Different cellular ion and water transporters and channels are essential mediators in determining neuronal excitability and synchrony and can do so through alterations in ECS volume and/or through non-ECS volume related mechanisms. This review will parse out the relationships between how the ECS volume changes during normal physiology and seizures, how those changes might alter neuronal physiology to promote seizures, and what ion and water transporters and channels are important in linking ECS volume changes and seizures.

Keywords Extracellular space · Diffusion · Seizure · Volume fraction · Glia

Introduction

Epilepsies are chronic disorders that affect over 50 million people worldwide [1], with approximately 30% of those cases being untreatable using modern anti-epileptic drugs [2]. This discrepancy is likely due to seizures having a variety of causes, with different cases not necessarily having the same pathophysiology behind it. For a seizure to initiate, a region of the brain requires the local neuronal circuits to shift into a hyperexcitable and synchronous state that begins a pathological pattern of network oscillation [3]. However, the exact biological mechanisms that can tip a system into that state are varied, ranging from a non-specific insult or injury to a portion of tissue, such as a traumatic brain injury, or as a consequence of a specific molecular change, such as that induced by a drug such as pilocarpine [4, 5]. Current antiepileptic drugs are almost entirely focused on directly targeting ion channels, neurotransmitter receptors, or vesicular proteins involved directly in synaptic neurotransmission [6], which does not cover the wide range of mechanisms that may promote seizures on a case-by case basis, synaptic or otherwise.

One prominent non-synaptic mechanism that represents a potential target of manipulation is the brain's extracellular space (ECS). The ECS is a network of interconnected gaps between cells that substances diffuse through to reach different destinations in a local region of tissue (Fig. 1a). Contained within it are substances such as ions and neurotransmitters that help determine neuronal firing patterns at a given point in time. It is also involved in various mechanisms of chemical neurotransmission, such as those of synaptic and volume transmission (Fig. 1b, c). The ECS exhibits a high degree of dynamism across physiological and pathophysiological states, which can affect the balance of these substances across the extracellular and intracellular compartments in the brain. This review will focus on how the ECS can change, the mechanisms these changes affect, and how these mechanisms can ultimately lead to seizures.

✉ Sabina Hrabetova
sabina.hrabetova@downstate.edu

[1] Department of Cell Biology, SUNY Downstate Medical Center, 450 Clarkson Avenue, Brooklyn, NY 11203, USA

[2] Neural and Behavioral Science Graduate Program, SUNY Downstate Medical Center, 450 Clarkson Avenue, Brooklyn, NY 11203, USA

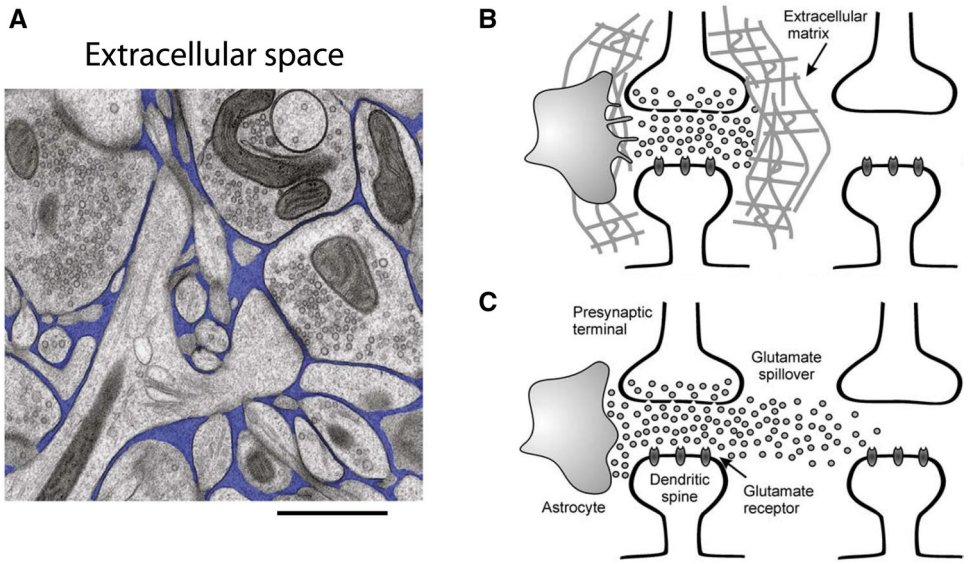

Fig. 1 The ECS and Neurotransmission. **a** Electron micrograph of the brain's ECS highlighted in blue. Tissue was preserved using cryofixation, preserving the size of the ECS. Scale bar 1 µm. Photograph courtesy of Korogod et al. [7]. **b** A schematic depicting synaptic transmission between neurons. A truly closed synapse is wrapped by astrocytes and ECM, which keeps the glutamate isolated to a particular pre- and post-synaptic neuronal system. **c** A schematic depicting volume transmission between neurons. Astrocytes and the ECM can cover the synapse only partially, allowing for the spillover of neurotransmitter into the ECS. The neurotransmitter can then reach distant synapses and neurons to affect them. The distance neurotransmitter can travel is highly dependent on the configuration and parameters of the ECS. Images **b** and **c** reproduced with permission from Syková [8]. (Color figure online)

Overview of the ECS and ECS Dynamics

The brain's ECS has been traditionally viewed to simply be a passive, structural component of the brain. However, this review will discuss the growing body of evidence that shows that the ECS plays an active role in neuronal physiology and in the initiation and propagation of seizures. First, we will look at the composition of the ECS, how the ECS changes, and how those changes relate to neuronal firing.

Composition and Function of ECS

The brain ECS has two components: the interstitial fluid that fills the spaces and is the medium for transport, and the matrix that gives structural support to the neighboring cells.

The main components of the interstitial fluid are water, physiologically important ions (e.g. sodium, potassium, calcium, chloride), neurotransmitters, nutrients, metabolites, gases, extracellular vesicles, macromolecules, and proteins secreted by cells [9–11]. This fluid forms the microenvironment of the cells and occupies the entire ECS, resulting in the formation of continuous channels throughout the brain that are in constant interaction with cellular and vascular components [12]. Importantly, the composition of this fluid affects the functionality of neurons, while the amount of this fluid in the ECS will affect the proportion of brain volume that the ECS occupies.

The main constituents of the brain's extracellular matrix (ECM) are hyaluronan (a glycosaminoglycan), lecticans (a large family of proteoglycans), tenascins (large multimeric glycoproteins) and small link proteins [13]. Hyaluronan is a large molecule that forms the framework for other matrix molecules to attach on, with or without the link proteins. Hyaluronan is also able to be hydrated by the water present in the interstitial fluid [14]. Other components, such as the proteoglycans heparin sulfate and chondroitin sulfate, provide the ECM with negatively charged domains that have the potential to interact with ECS cations, such as Ca^{2+} [15, 16]. Although the composition of the matrix is more or less similar everywhere in the brain, the concentration of the matrix and its constituents can vary in different regions. This leads to changes in the structural and functional properties of the matrix in different brain areas, such as around synapses in the form of perineuronal nets [17]. Study of the three-dimensional arrangement of these molecules led to several models that likely represent how ECM components combine to form the completed matrix. One such model is the Hyaluronan, Lectican, and Tenascin-R (HLT) model, which envisions a complex that places lecticans as scaffolding links between hyaluronan

and tenascin-R, with these complexes being directly anchored into cellular membranes [17–19].

A lot of the ECM's importance lies in its involvement in brain development and cellular function: it helps guide neurite outgrowth [20], provides important neural cell-adhesion sites [21], interacts with various cell-surface signaling proteins [22], and is involved in the formation of perineuronal nets [23]. However, the matrix also helps determine a lot of the impact that the ECS has on normal brain physiology. One of the main functions of the matrix is structural: it provides a framework that connects different components across spaces and provides rigidity and structure to the ECS. Its presence also imposes hindrance to the diffusion of molecules due to charge-based interactions with diffusing molecules and an imposed viscous drag by the components of the matrix itself [10]. Therefore, the brain's ECM provides many components of the structure of the brain and helps determine how molecules can interact with a variety of cellular elements within it.

How the ECS Is Described and Measured

One of the main ways that the brain's ECS is described is in terms of the ratio of ECS volume to total tissue volume. This measurement is termed α in the literature and is defined mathematically as:

$$\alpha = \frac{V_{ECS}}{V_{total}}$$

The ECS volume fraction has been studied using many different techniques. Because the interstitial spaces are below the resolution of light microscopy, visualization of them was attempted using electron microscopy. These experiments initially led to the estimate of 0.05 for α [24]. The advent of freeze substitution preparation of electron microscopy samples, however, revealed that those estimates were faulty due to water re-distribution of the preparations, and this new technique led to the estimate of 0.15–0.2 for α [25]. The disadvantage with this technique is that it cannot be directly applied to acute, living brain tissue samples, hindering study of the ECS in physiological or pathophysiological settings. Therefore, there are many other techniques that were developed that could be used in slices and/or whole brains in living animals, ranging from the measurement of diffusing radiotracers as one of the oldest techniques [26], to modern techniques such as the utilization of super-resolution microscopy to directly visualize interstitial space [27]. The real-time iontophoresis (RTI) technique specifically can be credited with providing most of the measurements of α taken across animal models and many physiological and pathological states of the brain. Today, it is well accepted that the ECS has an α of approximately 0.2, with physiological values for α ranging between 0.15 and 0.3 in the brain, depending on several factors such as the age of the organism and the region being measured. For a review of the history of measuring techniques for ECS volume fraction, see [10].

For the purposes of this review, it is important to understand techniques of measuring ECS volume using double-barreled ion-selective microelectrodes (ISMs). These ISMs are capable of recording voltages whose magnitude directly corresponds to the local concentration of an individual ion around its tip [28–30]. In order to utilize these tools to measure ECS volume, they must be made to sense an ion that can serve as a suitable probe of the ECS. To qualify as a suitable probe, the ion must be small, exogenous, and unable to enter cells freely during the time course of the recording. Tetramethylammonium (TMA^+) is one such ion that will stay largely confined to the extracellular space [31], due to its hydrophilic nature and lack of endogenous transporters into cells, and ISMs can be made to sense its concentration [32]. While there are other ions that have similar characteristics, TMA^+ is the most commonly used probe of the ECS in techniques that rely on ISMs to obtain measurements of ECS volume.

All of these properties of TMA^+ allow it to be used in several techniques to measure α. The main technique that uses them, RTI [32], is still one of the main techniques used that can provide an accurate measurement of α in a small region of brain tissue. Historically, this allowed for extensive characterization of α across many brain regions and animal preparations [10]. The technique relies on the sensing of TMA^+ by an ISM that diffuses away from a point source, provided by a fabricated iontophoresis electrode containing a solution of TMA-Cl (Fig. 2a, b). The ISM and iontophoresis electrode are placed deep in brain tissue at a fixed distance away from each other. The iontophoresis electrode has a current passed through it for a set period that forces the release of the TMA^+ from its tip, creating a point-source of ions diffusing away from it. From the resulting concentration curve measured by the ISM, a value for α can be derived that represents the ECS volume fraction for that local region of tissue [33, 34]. A second, but similar technique can be employed if the experimenter is only interested in measuring fast changes in α, such as those induced when cells are suddenly osmotically stressed (Fig. 2c). To employ this technique, an ISM is placed into a brain slice that is being perfused by artificial cerebrospinal fluid containing a baseline concentration of TMA-Cl. The local concentration of TMA^+ is recorded by the ISM both before and after the volume change takes place. The magnitude of that change is directly proportional to the volume of the ECS, allowing for the calculation of a % volume change between the two states [35, 36]. This technique, referred to in this review as relative volume monitoring (RVM), is incapable of measuring α directly, but has been used to obtain relative changes

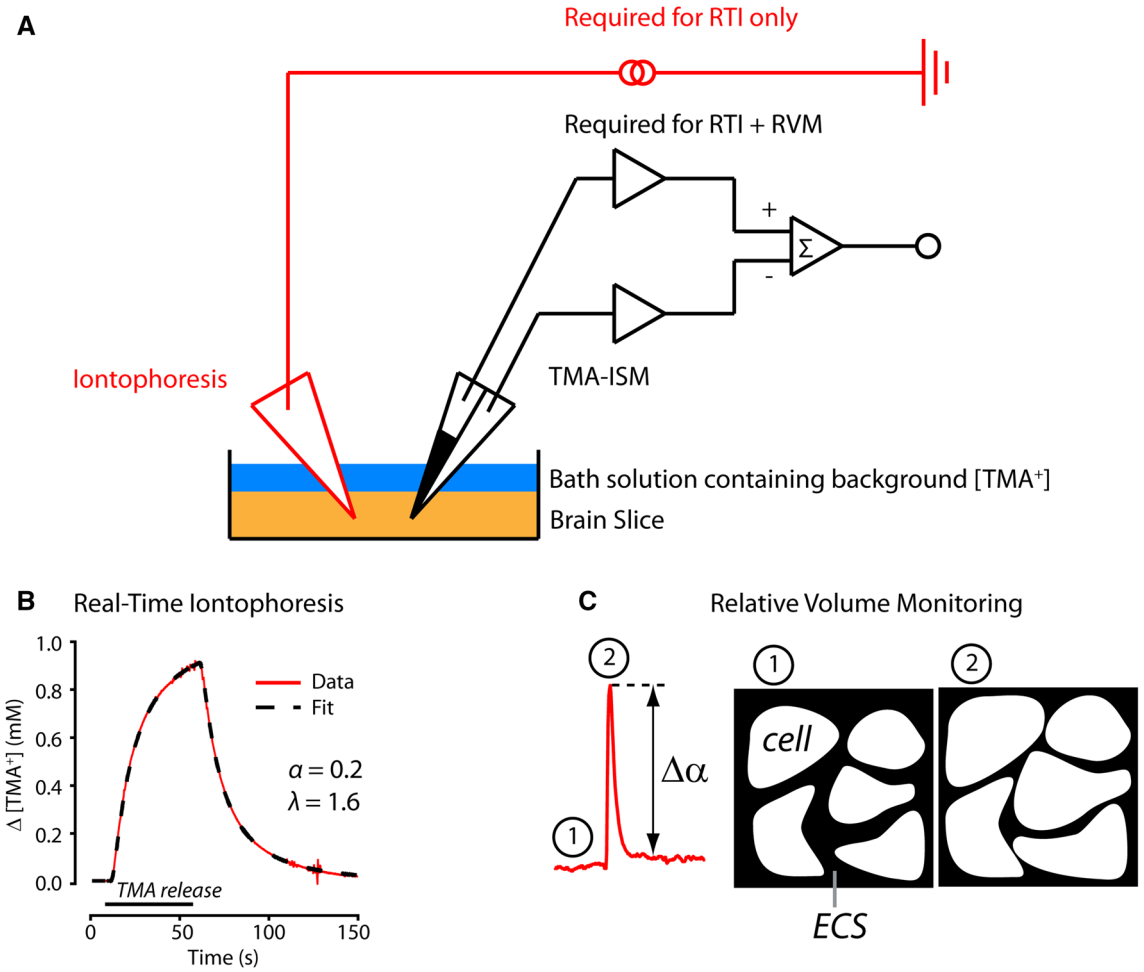

Fig. 2 TMA-ISM based methods for measuring ECS volume. **a** A diagram outlining the setup of RTI and RVM. For both techniques, a TMA-ISM must be placed deep into a brain slice that is being perfused with a baseline concentration of TMA-Cl. The two signals measured by the ISM will be subtracted, and the resulting signal will represent a change in the concentration of TMA^+ measured at the tip of the electrode. For RTI to be performed, an iontophoresis electrode is typically placed 100 µm away from the ISM and is set up to release a point-source of TMA^+ from the tip (red). **b** The resulting signal of a measurement taken using RTI. TMA^+ was released from the iontophoresis electrode for a period of 50 s. The resulting rise, then fall of $[TMA^+]$ was recorded. This curve allows for the quantification of both α and λ. Image courtesy of Hrabetova et al. [37]. **c** A TMA^+ signal that represents a transient ECS volume change is shown on the left. During state 1, the ECS is at a particular baseline volume. During state 2, the $[TMA^+]$ is higher, which represents a smaller ECS than during state 1. A relative change in α can be calculated between those two states. (Color figure online)

of α during different physiological states, as discussed in later sections.

ECS volume fraction has profound effects on the structure and organization of the brain. Aside from affecting local concentrations of substances when a sudden change occurs, it also can affect the rate of diffusion of substances throughout the ECS. The tortuosity for molecules diffusing through the ECS, labelled as the parameter λ in the literature, is defined mathematically as:

$$\lambda = \left(\frac{D_{free}}{D^*}\right)^{1/2}$$

where D_{free} is the diffusion coefficient of the molecule in an unhindered medium and D^* is the diffusion coefficient of the molecule in the hindered medium (i.e. the brain's ECS) [10]. The relationship between α and λ is complicated by the fact that a change in α does not always necessitate a change in λ [38]. Therefore, describing the geometry of the ECS and how its structure affects the molecules in it requires both parameters. Experimentally, λ can be obtained through RTI along with α, as well as through several other techniques. For review of these techniques, see [28, 34].

ECS Changes

The ECS is a dynamic compartment that changes its composition as well as its volume. These changes can occur over varying time scales and some of them, such as those during pathological processes, are likely irreversible. Significant changes in the brain ECS volume occur during development and aging. As the brain develops, the extracellular volume fraction is known to decrease. It was reported that 2–3 day old rats had an ECS volume fraction of approximately 0.36 in layer III of neocortex, with similarly large values in other regions of cortex and white matter, whereas adult rats had an approximate volume fraction of 0.2 in layer III, with similar values elsewhere [39]. This decrease is most likely attributed to neurogenesis and gliogenesis during development. Interestingly, there is no change observed in the tortuosity in the neocortex during postnatal development in rats, showing that while young animals may have a more dilute environment of neuroactive molecules, ions, and neurotransmitters due to a large volume fraction, the diffusion of those substances is likely the same during development [39]. A similar course of changes of ECS parameters seems to occur in elderly animals. RTI studies revealed that the ECS volume fraction in multiple brain regions dropped between adult and aged animals, with the largest drop existing in the somatosensory cortex and CA1 hippocampus ($\alpha = 0.13$–0.16 in the aged mice, $\alpha = 0.17$–0.20 in the adult mice) [40, 41]. However, there was no apparent change in tortuosity between the adult and aged rats. These aging data suggest that the ECS changes on a slow time course throughout several stages of an animal's life, with α dropping during development, then once again during advanced age.

The ECS can also change quite rapidly. These changes are commonly reversible and are associated with physiological functions of the body. One example was found in the spinal cord, where repetitive stimulation of peripheral nerves revealed that the ECS volume in L4-L5 reduced by 20–45% several minutes after stimulation [42]. Another example is the change associated with the sleep-wake cycle. It has been shown that during sleep, the ECS volume is measured as $\alpha = 0.23$, whereas in awake animals this volume becomes smaller ($\alpha = 0.14$) [43]. There is also a change observed in the extracellular concentrations of various ions between states, such as an increase in extracellular potassium, and a decrease in extracellular calcium and magnesium during the awake state [44]. However, the RTI measurements made during the sleep-wake cycle show no change in the tortuosity of the ECS. Later studies have looked into how the ECS can be investigated in an awake state and attempted to determine why the ECS parameters change during wakefulness. Through these studies, a new method of investigating the awake brain was developed by applying β-adrenergic agonists to a brain slice, which created an awake-like state [45]. In this awake-like state, it was observed that the reduced ECS volume fraction can directly be attributed to the volume of astrocytes, which were found to be significantly larger [45]. The ECS can also change rapidly during an osmotic challenge. A systematic study of osmotic challenges performed on brain slices showed that RTI measurements were affected by both hypo- and hyper-tonic changes in osmolarity after a 15-min application period of the various solutions. This study showed that α decreased and λ increased as tonicity was lowered, to values of 0.12 and 1.86, respectively, in a 150 mOsm medium. As osmolarity increased, α continuously increased to a maximum of 0.42 in a 500 mOsm medium, whereas λ did not increase in any hypertonic challenge [38]. All of these data show that the ECS is capable of changing in a variety of conditions on a short time scale.

Brain ECS Dynamics and Neuronal Firing

Extracellular space is present in all the tissues of the body. However, the brain's ECS is special in that it plays a direct role in neuronal physiology, largely through ion balance between cellular compartments. If we break down neuronal communication into its individual steps, we have two important processes: action potential generation, and chemical transmission of the action potential. First, action potential generation involves the opening and closing of ion channels, resulting in a flow of ions between the intracellular and extracellular environment. This movement is largely dependent on the electrochemical gradients belonging to each species of ion [46]. The ECS can affect these concentrations transiently, through changing its own volume [47]. Thus, volume changes of the ECS can affect the excitability of the individual neurons by affecting the baseline concentration of ions adjacent to neurons [48, 49]. Second, chemical transmission involves the release of neurotransmitters into the synaptic cleft. The molecules must diffuse through the space to reach their receptors but can also spillover out of the cleft and into the perisynaptic area. The diffusion through this area can get affected by various changes in the ECS [50]. Action potential generation and chemical transmission together comprise one form of communication between neuronal cells, and it is called "wiring transmission" or "synaptic transmission" as these neurons are wired together through synaptic interactions (Fig. 1b) [8]. However, when the synaptic neurotransmitters escape the synaptic cleft, they can diffuse over distances and affect other cells as well. This leads to hetero-synaptic modulation. Some neurotransmitters and neuropeptides are not released into the cleft, but are released from extrasynaptic sites, either as paracrine-like, or endocrine-like secretions. These molecules also diffuse through the ECS in order to reach their targets and affect other neurons or neuronal networks in the vicinity. This alternate form of communication is called "volume

transmission" and is largely dependent on diffusion through the ECS (Fig. 1c) [51]. Thus, both kinds of intercellular communication can be affected by ECS, and any changes occurring in ECS. This relationship results in a powerful control that the ECS can exert on neuronal excitability and inter-neuronal crosstalk.

Volume changes of the ECS have been heavily investigated as a mechanism that can affect neuronal excitability in various ways. The ECS can change in volume due to net movement of the ionic extracellular fluid into or out of the cells, thus altering their distribution in intracellular and extracellular space. In some cases, these volume changes can also occur because of changes in the composition of the matrix. For instance, when one of the genes required for the production of hyaluronan synthase enzyme, *Has3*, is knocked out, there is an overall reduction in the amount of hyaluronan seen in the brain. There is also a corresponding reduction in volume of the extracellular space, which is believed to occur due to a loss of the hydrated hyaluronan in the ECS [52]. Volume changes can affect neuronal excitability in three different ways. First, because of the changes in volume, the components of the extracellular fluid, such as the ions, will change their concentrations transiently. This will have a direct effect on the resting membrane potential and, as a result, may make neurons closer or further away from resting potential at a given point in time [53]. Second, a change in volume will alter the way that electric fields generated by neurons overlap with each other. These so-called ephaptic interactions will be strengthened by a shrinkage of the ECS due to neurons shifting closer together and weakened by an expansion of the ECS due to neurons drifting apart [54]. Third, changes in volume of the ECS can affect volume transmission, which can then affect intercellular cross-talk [51].

Reciprocally, the local ECS volume surrounding a neuron is also directly altered through the time course of neuronal firing. To understand this relationship, it is important to consider the ionic shifts that occur during the firing of a neuron. As the cell fires, there is an exchange of ions, with potassium flowing out of the cells, and sodium flowing in. For the cell to be ready to fire again, all these changes need to be reversed. The glial cells help in this recovery by taking up K^+ from the ECS. As the ions move across the membranes of these cells, there is also an associated movement of water molecules through membrane proteins. For glial cells, the net movement of ions is mostly inward, with a corresponding inward flow of water. Thus, the volume of glial cells increases, resulting in a decrease in brain ECS volume [36]. It is important to note that while one might expect water to flow back into neurons in a similar manner as glia, experiments done in a developmentally immature rat optic nerve without glial cells showed that this phenomenon cannot occur without glia [55]. This paradigm had been used to explain the phenomenon of activity-induced shrinkage, which has been shown to occur across many investigations [36, 56–58].

One of the most recent investigations performed by Larsen and MacAulay [58] shows how these volume changes occur in living slices following local neuronal stimulation. Measurements of both extracellular ion concentrations and volume changes using several ISMs were made in the CA1 region of rat hippocampus. They first observed that after electrically stimulating the cells via the Schaffer collateral pathway, field potentials were generated, along with changes in extracellular ion concentrations and ECS volume changes. During the period of stimulation, the combined effect of all the action potentials led to an increase in extracellular $[K^+]$ of about 2–10 mM and a relative decrease in the extracellular volume of 2–14%. After the stimulation was stopped, both ion concentration and ECS volume returned to baseline along similar time courses. Since astrocytes are mostly involved in the movement of potassium ions, it would be logical to expect that the K^+ flowing into the astrocytes are directly bringing water in and forcing the volume changes. However, blockade of Na^+/K^+-ATPase, NKCC1, KCCs, and Kir 4.1, the main K^+ transporters on astrocytes and neurons, were not required for the activity-induced shrinkage. Instead, it was shown that NBCe1, a pH-regulating transporter, and MCT, a lactate transporter, are required for the activity-induced shrinkage. Furthermore, their activity is stimulated through astrocytic-depolarization induced by the increase in ECS $[K^+]$. Therefore, this body of work modifies the previously held paradigm that K^+ directly transports into the glia and drags water along with it. Instead, K^+ clearance induces the activity of multiple other transporters responsible for pH regulation that, in turn, drag water into the glia [58, 59].

In summary, there is a large body of evidence linking ECS volume fraction and neuronal firing under physiological conditions. The following section will explore whether this relationship can extend to the pathological state of excessive neuronal firing—a seizure.

Seizures and the ECS

ECS as a Possible Mechanism for Seizure Activity

There is a large body of work surrounding the dynamics of the ECS during seizures. Seizures are defined by their state of synchronized and frequent neuronal activity, which causes large scale changes in the concentrations of ions in the intracellular and extracellular compartments. The first studies on the changes in ionic composition of the extracellular space were made possible by the advent of ion-selective microelectrodes (ISMs), which allowed for the real-time measurement of various endogenous extracellular ions, including sodium,

potassium, chloride and calcium [28–30, 60]. Through using these measurements, it became clear that there is a sequence of ionic shifts between the intracellular and extracellular compartments that occurs following the propagation of epileptiform activity: $[K^+]_{ECS}$ increases from 4 to 12 mM, $[Cl^-]_{ECS}$ increases from 145 to 152 mM, $[Na^+]_{ECS}$ decreases from 145 to 139 mM, and $[Ca^{2+}]_{ECS}$ decreases from 2 mM to 100 µM [61]. Soon after their advent, ISMs have also been used to cleverly estimate the size of the ECS by taking advantage of the real-time measurement of concentration they provide. ISMs can be fabricated to measure the concentration of TMA^+, and various methods were developed that allowed for an estimation of ECS volume based on changes in TMA concentration under different conditions: RTI and RVM (see "How the ECS Is Described and Measured" section). One of the first studies into ECS volume and seizure activity investigated changes in volume during repetitive stimulation of the sensorimotor cortex. By using a TMA-selective ISM, it was observed that there was a 30% volume decrease during the induced activity [47]. This change has been thought to be largely driven by the $[K^+]$ changes during seizures, which shift large amounts of K^+ from neurons, to the ECS, and into glia. This creates an osmotic gradient that would drive water from the ECS into the glia, and the subsequent cellular swelling could account for this large ECS volume shrinkage [36].

Since this study, there have been many other investigations into ECS dynamics during seizures utilizing a variety of models and methods. Investigations into the absolute ECS volume using the real-time iontophoresis method showed that during pilocarpine-induced status epilepticus, there is a gradual shift in α that starts at 0.19 from before pilocarpine application and reaches a minimum of 0.13 80–100 min after the pilocarpine application, with no change in tortuosity from baseline values [62]. While RTI is capable of giving absolute values of ECS volume in a given region of tissue, other techniques have been used to provide measurements of ECS parameters under seizure conditions. Because tissue electrical resistance is indirectly proportional to ECS volume, tissue resistance measurements have been used to demonstrate ECS volume shrinkage, and it has indeed been shown that tissue resistance increased to 108% during the first 15 s of potassium-induced seizures in rat hippocampal slices, with the resistance continuing to grow to a maximum of 122% [63]. More recently, super-resolution shadow imaging (SUSHI) allowed for direct visualization and measurement of ECS width during seizure induced by picrotoxin in a hippocampal mouse slice and found that the median ECS width shifted from 0.26 to 0.15 µm, which serves as another confirmation that the ECS shrinks significantly during seizures [27].

The significance of the relationship between ECS size and seizures has to do with how changes in α can actually influence the likelihood of seizure propagation. As discussed previously, seizures require several main conditions to occur in a region of brain tissue: a group of neurons must be hyperexcitable, fire synchronously, and participate in an oscillating network. In order to get this specific firing pattern to occur, influences that promote neuronal firing must affect a large group of neurons at the same cross section in time. A large-scale shrinkage of the ECS, such as a sudden change in ECS osmolarity, has the capability of changing the environment around many neurons from one that allows normal physiological activity to one that promotes a hypersynchronous oscillating network. The factors that ECS volume can affect that would promote such a network mostly fall under changes in neurotransmitter or ion concentration and the alteration of ephaptic interactions. For instance, ECS shrinkage can simultaneously concentrate ambient glutamate and K^+ and cause the electric fields of neighboring neurons to overlap more. All three of these factors would promote both more neuronal excitation and, if the shrinkage occurs in a large area at a single point in time, would promote a synchronous discharge. This insult could then push the system to oscillate, thereby producing a seizure. Many documented mechanisms that can promote this state are ultimately controlled and influenced by the ECS: electrolyte disturbances in the ECS that result in increased neuronal firing, such as elevated K^+ [64, 65]; increases in signaling of excitatory neurotransmitters, such as glutamate [66, 67]; decreases in signaling of inhibitory neurotransmitters, such as GABA in the adult population [68]; or increases in nonsynaptic/ephaptic interactions between neurons [69].

Various regions of the brain have different baseline ECS volumes, and there have been some studies that suggest this has an impact on the prevalence of seizures in those regions. The hippocampus is frequently involved in many epilepsy syndromes, with temporal lobe epilepsy being the most common form of complex partial seizures [70]. Its heterogeneous structure also allows for some varied and unique ECS characteristics. RTI in the CA1 and CA3 regions of the hippocampus have revealed that the stratum pyramidale regions have an α of 0.12 and 0.18, respectively [71]. This indicates the severe restriction in the ECS size of the CA1 region in comparison to other regions such as the cortex, where α ranges from 0.17 to 0.25 [10]. This restricted size of the ECS in the CA1 region has been suggested to be a key reason for the common implication of the hippocampus in epilepsies, due to the impact a small ECS can have on neurotransmitters, ions, and ephaptic interactions.

To demonstrate the overall control that the ECS has on ictogenesis, multiple studies have utilized experiments that directly affect the state of the ions contained in the ECS and observed the consequences on neuronal activity. A common way to affect the ECS is through osmotic stress, which results in the swelling or shrinking of neurons and glia [38,

72]. This results in widespread consequences that affect the excitability and synchrony of neurons through many mechanisms. Hypoosmotic stress, which results in a lower α dependent on the severity of the stress, has been shown numerous times to result in hyperexcitability, and at times, enhance ongoing epileptiform activity [73–76]. Additionally, the reverse, which resulted in a larger α, demonstrated a suppression of epileptiform activity. Recent studies have identified specific neuronal consequences that occur during the onset of induced osmotic edema. For instance, an NMDA-receptor dependent slow inward current has been observed to occur 1 min following the onset of hypoosmolar stress. This phenomenon occurred during the time course of astrocytic swelling during the stress and correlated with its intensity. Furthermore, because it was determined that these slow inward currents were generated in the absence of action potentials or miniature spontaneous vesicular release, it is believed that the glutamate source must be non-synaptic in origin, making diffusion through the ECS necessary for binding to its target receptors [76]. Earlier studies have also found non-synaptic based effects of hypoosmolar stress. Whether CA1 neurons are orthodromically or antidromically stimulated during hypoosmolar stress, there was an increase in population-spike amplitude that was dependent on the magnitude of the hypoosmolar stress, indicating that the effect is simply tied to the increase in electrical resistance imposed on the current by swelling cells. This directly supported the idea that ephaptic interactions helped generate neuronal synchrony and was affected by the state of the ECS [73, 74]. Other studies have also been able to show that even addition of K^+ into the ECS of the hippocampus has been able to cause the propagation of spontaneous seizures [71]. The experiments showed that manipulations of the ECS had a direct effect on the propagation of epileptiform activity.

Affecting the ECS through manipulations of the extracellular matrix has also revealed another way that this space can influence neuronal activity. Hyaluronan, a main constituent of the extracellular matrix, is synthesized by three main enzymes: HAS1, HAS2, and HAS3 [77]. Through histology, it was determined that the CA1 region of a Has3KO mouse had a significantly diminished concentration of hyaluronan compared to WT controls [52]. When the ECS parameters were measured using the RTI method in tandem with multilayer analysis [78] in the stratum pyramidale of the CA1 region, α was significantly reduced in the Has3KO compared to WT controls ($\alpha = 0.07$ in Has3KO whereas $\alpha = 0.12$ in WT). This discrepancy was attributed to the hydration capacity of hyaluronan, which allows it to expand and push other cells away from the cell that it was synthesized from, effectively creating a larger ECS [79]. Interestingly, in CA1 hippocampal slices, recordings of spontaneous field potentials were also made using extracellular electrodes, indicating enhanced excitability of the region. Furthermore, the Has3KO animal was also shown to exhibit spontaneous ictal seizures on EEG. Put together, these phenotypic consequences of the *Has3* gene deletion indicate that the reduced ECS volume may have directly led to an epileptic phenotype in these animals.

Taken together, the findings in this section thus far show that there are many possible ways the ECS volume can change and, in doing so, lead to a phenotype predisposed to seizures. To understand this further, it is important to explore the various ways that the brain can be impacted to promote ECS volume changes that could then lead to seizures and ultimately epilepsy.

Mechanisms

The composition of interstitial fluid is of prime importance in the determination of how excitable a region of brain tissue is. Because excitability of a neuron is determined in part by proximity of its resting voltage to its action potential threshold, the ionic concentrations in the extracellular and intracellular compartments directly affect the excitable state of the neuron. Taking K^+ as an example, an increase in $[K^+]$ in the extracellular compartment of a region of tissue induces an increase in the resting potential of a neuron and, in turn, will cause it to be closer to threshold. A change in the concentration of an ion such as $[K^+]$ can be caused in two main ways: a dysregulation in the flux of K^+ into or out of the ECS, causing an increase in the absolute number of ions present in the compartment, or a change in the volume of the ECS, causing the total number of K^+ ions to exist in a smaller space. An unfortunate complication is that these two mechanisms are frequently tied together, as ECS volume frequently changes due to ionic fluxes between the extra- and intracellular compartments. Therefore, in order to understand the mechanistic role that ECS volume plays in excitability and seizures, it is important to look at mechanisms that regulate ionic flux through the ECS.

Membrane channels and transporters are the main ways that ions are allowed to enter and exit cells, making them key components that affect ionic flux through the ECS. Some are responsible for actively maintaining ionic gradients that are necessary for normal neuronal activity (i.e. Na^+/K^+-ATPase), some take advantage of the energy provided by ionic gradients to tightly regulate important neuromodulators (i.e. GLT-1), and others assist in volume regulation by allowing for water to shift between the intra- and extracellular space (i.e. AQP4). Because dysfunctions in all of these applications can be responsible for the generation of neuronal hyperexcitability, channels and transporters of all of these types have historically been investigated as potentially being important in ictogenesis.

Na^+/K^+-ATPase

Most ionic gradients in the brain begin with an initial input of energy into the Na^+/K^+-ATPase, that then generates a relatively high ECS $[Na^+]$ and a low intracellular $[K^+]$. It accomplishes this by binding and hydrolyzing ATP (providing the initial activation energy), then pumping 3 Na^+ ions out of the cell and 2 K^+ ions into the cell. The 3/2 ratio of Na^+ pumped out and K^+ pumped in per cycle is primarily responsible for creating the ionic gradients that, in concert with ion channels, result in the negative resting membrane potential of neurons [80, 81]. Because the normal function of this pump is to create a hyperpolarizing neuronal environment, dysfunctions of the pump have long been implicated as a potential cause for neuronal hyperexcitability. Multiple experiments using Na^+/K^+-ATPase blockers, such as ouabain, have been shown to extend or even initiate seizure activity across multiple animal models [82]. Hippocampal tissue taken from patients suffering from epilepsy have also shown decreased Na^+/K^+-ATPase activity, indicating a potential causative role for its dysfunction in the disease [83].

There are many indications that Na^+/K^+-ATPase dysfunction can promote excitability of neurons through impairment of K^+ clearance. As discussed before, excess K^+ in the ECS promotes neuronal firing through shifting of the Nernst potential in a depolarizing manner. ECS $[K^+]$ measurements using ISMs demonstrate that a brain slice under the effects of oubain exhibits about 75% of the clearance rate of K^+ compared to controls [59]. Glial Na^+/K^+-ATPases, as opposed to their neuronal counterparts, have been primarily believed to be responsible for the clearance of excess K^+ in the ECS. The Na^+/K^+-ATPase is composed of two subunits, with the neuronal and glial populations of this protein having different subunit isoforms in its composition [84]. This structural heterogeneity allows for a difference in the kinetics of the two populations: the glia have a Na^+/K^+-ATPase subpopulation that has a lower affinity for K^+ (i.e. higher K_m) than others. This lower affinity, combined with a higher total glial Na^+/K^+-ATPase V_{max}, allows the glial Na^+/K^+-ATPase to clear K^+ effectively at a higher range of concentrations. The glia can therefore respond to the transient increase in $[K^+]_{ECS}$ that is evoked during neuronal firing, allowing the glia to act as K^+ sinks [85, 86]. Furthermore, a recent study has shown correlations between increased developmental age, increased expression of various isoforms of the Na^+/K^+-ATPase, and faster removal of the K^+ in the ECS added after neuronal stimulus [87]. These data, taken together, show an important link between the activity of the Na^+/K^+-ATPase and control of $[K^+]$ in the ECS.

The K^+ accumulation in the ECS that occurs as a result of Na^+/K^+-ATPase dysfunction can also be worsened by the high energy consumption that occurs during seizure as a result of repeated neuronal firing. These proteins require approximately 50% of the energy available to the brain under physiological conditions, making the lower energy availability that occurs during seizures a potential impediment for normal Na^+/K^+-ATPase function [88]. Therefore, dysfunction Na^+/K^+-ATPase can be a direct causative factor of a seizure [89] or be a prolonging factor of a seizure when dysfunction arises sometime after ictogenesis.

NKCC1 and KCC2

The NKCC1/KCC2 proteins are membrane bound cotransporters that work to regulate the concentrations of Na^+, K^+, and Cl^- based on their respective concentration gradients. NKCC1 brings Na^+, K^+, and 2 Cl^- ions into or out of cells, whereas KCC2 brings 1 K^+ and 1 Cl^- into or out of cells, depending on the electrochemical gradients that exist for each population of ion. They work very differently from the Na^+/K^+-ATPase, because their activity is reactive to deviations from ionic equilibrium, whereas the Na^+/K^+-ATPase works to create the imbalance of ions across cell membranes necessary for normal neuronal activity [90]. This function is especially important when considering the fluxes of Na^+, K^+, and Cl^- that result from neuronal activity, which cause large changes in the concentrations of these ions in the ECS that, if not restored to their original baseline, influence the overall excitability of the neuronal environment.

NKCC1 and KCC2 are critically important in determining the action of GABA receptors on neurons. The ionotropic $GABA_a$ receptor, when opened, transports $[Cl^-]$ down its electrochemical gradient; the direction of this gradient determines whether this action has a depolarizing (i.e. the gradient is pointed intracellularly) or hyperpolarizing (i.e. the gradient is pointed extracellularly) effect on neurons. NKCC1 brings its substrates into the neuron, making it responsible for neuronal Cl^- loading and therefore pushing GABA to a more depolarizing function, whereas KCC2 extrudes Cl^- and therefore has the opposite effect [68]. During infancy NKCC1 activity is relatively high compared to KCC2, making GABA activation result in neuronal depolarization. STE20/SPS1-related proline–alanine-rich protein kinase (SPAK) phosphorylates both NKCC1 and KCC2, which results in downregulation and upregulation of activity, respectively. This results in Cl^- extrusion to become more dominant during brain development, allowing for the functional switch of GABA to become hyperpolarizing [91].

When dysregulated control of these transporters results in a switch to depolarizing GABA, ictogenesis is promoted in multiple ways. In vitro studies that attempt to downregulate KCC2 function using pharmacological blockers or functional mutations of KCC2 resulted in the promotion of status epilepticus-like events in tissue [92]. Furthermore, the usage of NKCC1 blockers, such as bumetanide, have

reported some anti-seizure effect in in vivo animal models across multiple studies [93–95]; however, other studies that show no anticonvulsant effect of bumetanide [96, 97] suggest the drug may not exert the same effect across all models. Diminished KCC2 function and increased NKCC1 function have also been identified in different epilepsy syndromes, including acquired epilepsy resulting from gliomas [98]. These studies highlight the importance of Cl^- concentration balance in neurons in order to maintain normal, hyperpolarizing GABA.

However, there are reasons to suspect that the importance of NKCC1/KCC2 has to do with regulating ECS volume in addition to their effects on GABA. Multiple seizure models that involve non-GABA-dependent mechanisms (and even non-synaptic mechanisms) have shown to be affected by the application of furosemide, a potent NKCC1/KCC2 blocker [99]. This meant that while the GABA-related control exerted by NKCC1/KCC2 is likely important in some models, there is still likely a common mechanism across the other models that also affects neuronal excitability that is non-synaptic in origin; ECS volume regulation fits this criterion. Intrinsic optical signaling (IOS) was used to measure relative volume changes of the ECS during activity-induced shrinkage and observe the effects that NKCC1/KCC2 inhibition has on the magnitude of shrinkage. Both furosemide and Cl^- replacement with gluconate resulted in significant decrease of activity induced shrinkage of the ECS, indicating that these transporters may play a role in the phenomenon [100]. This may have been through indirect changes in K^+ clearance from the ECS or directly from inhibition of water transport through NKCC1, which is able to transport 500 molecules of water per cycle of transport [101]. However, later studies have shown that not only is there negligible expression of NKCC1 on astrocytes in vivo [102], but that blockade of NKCC1 using bumetanide does not alter the rate of stimulus-induced K^+ clearance [59]. Furthermore, TMA-ISM studies measuring ECS volume shrinkage during neuronal stimulation and furosemide application have not replicated the effect seen in the IOS studies [58]. Another study has also contended that the anti-epileptic effect may be partly due to its ability to block other transporters at the high concentration needed to suppress epileptiform activity [103]. Despite this discrepancy, there still has not been a consistent explanation for the effects that furosemide has on non-synaptic seizure models, making NKCC1/KCC2's role in ECS volume regulation and seizures not entirely elucidated.

Kir4.1 and AQP4

The astrocyte-specific Kir4.1 and AQP4 channels represent the main passive mechanisms that are involved in regulation of ECS $[K^+]$ and volume. They allow for the passage of K^+ or water down their concentration gradients into or out of cells. These two proteins are predominantly coexpressed at astrocytic endfeet facing capillaries, thus providing an exit path for both water and K^+ to the outside of the brain. Kir 4.1 is also expressed at perisynaptic glial membranes [104]. The action of this protein here allows the glia to participate in a K^+ clearing mechanism called spatial buffering. The mechanism is different from other K^+ clearance mechanisms in that it allows the glia to redistribute K^+ from an extracellular region that experiences a transient increase in $[K^+]$ concentration (such as after a neuronal depolarization) to a distant extracellular region that did not have any transient increase occur. This process has been hypothesized to be functionally coupled to AQP4, which would allow for water to follow and balance the ionic shift caused by K^+ spatial buffering [105]. Evidence for this coupling was provided by studies on mice with impaired or knocked-out AQP4, which revealed impaired K^+ clearance in these models [106]. However, some published data has shown that the K^+-dependent current through Kir4.1 was unaffected in isolated AQP4-null glial cells. In the same study, inhibition of Kir4.1 did not alter AQP4 water permeability [107]. Similar findings have been reported in retinal Muller cells [108]. These studies do suggest that there may be some functional independence of these two transporters under some conditions.

The role that these transporters play in seizures and neuronal excitability has been controversial in the literature. Their ability to regulate water and K^+ dynamics make them seem mechanistically important in these settings. Looking at studies that investigated the relative expression of Kir4.1 and AQP4 supports this hypothesis. Kir4.1 has been shown to be significantly downregulated in tissue taken from patients with mesial temporal lobe epilepsy [109, 110]. Multiple loss-of-function mutations in KCNJ10, which codes for Kir4.1, have been identified in groups of patients with temporal lobe epilepsy [111, 112]. AQP4 also showed downregulation in temporal lobe epilepsy patients, specifically around the perivascular astrocytic endfeet [113]. These changes are believed to be responsible for impaired K^+ regulation and therefore contribute to seizure susceptibility in patients with temporal lobe epilepsy [114]. Supporting this are AQP4-null mice, which have been shown to exhibit increased seizure duration [106]. However, several studies go against the idea that Kir4.1 and AQP4 are involved as causative factors in ictogenesis. First, while AQP4-null mice show slower K^+ clearance, this may be blamed by the baseline enlargement of the brain's ECS in these knockouts [115]. Secondly, spatial buffering may not be a dominant mechanism for K^+ clearance in a large, synchronous network of firing neurons, especially when compared to NKCC1 and the Na^+/K^+-ATPase. Inhibition of Kir4.1 using $BaCl_2$ during high-frequency stimulation of the CA1 region of the hippocampus revealed no reduction in K^+ clearance or ECS volume shrinkage compared to control stimulation [116].

Clearance of K^+ was only affected when $[K^+]$ was increased through local iontophoretic release. This implies that spatial buffering is only relevant in a highly localized region of elevated $[K^+]$, likely because a glial network needs to be in contact with a region of lower $[K^+]$ to dump the excess ions. This situation, however, is unlikely to be the case when tissue is engaged in synchronous neuronal activity, where K^+ is flooding a large region of ECS simultaneously.

Na^+/HCO_3^- Symport (NBCe1)

The Na^+/HCO_3^- symport (NBCe1) has come under some scrutiny regarding its role in osmotic and pH regulation of the ECS, and how this relates to neuronal excitation. The transporter is present on astrocytes and normally functions as a modulator of extracellular pH by transporting Na^+ and HCO_3^- in a 1:2 ratio [117]. At baseline, this process is directed towards the ECS, but under conditions of higher extracellular $[HCO_3^-]$, lower intracellular pH, or depolarization, the current is reversed into the astrocyte [118]. The net absorption of 3 ions caused per cycle of transport of this protein indicates that its activity may result in osmotic imbalance and cellular swelling. Evidence was obtained for this in a study that looked at activity-induced shrinkage of the ECS during application of 4,4′-diisothiocyano-2,2′-stilbenedisulfonic acid (DIDS), a non-specific blocker of the Na^+/HCO_3^- transporter. Application of DIDS resulted in a 25% reduction of activity-induced ECS shrinkage, an increase in post-stimulus alkalization, and no change in $[K^+]$ increase [58]. The effects of the increase in post-stimulus alkalization is important to consider when evaluating the impact this drug may have on neuronal excitability. It has been demonstrated that alkalization of the ECS results in an increase in neuronal excitability due to downstream effects on synaptic transmission [119]. Taken together, this suggests that blockade of the Na^+/HCO_3^- transporter has mixed effects on neuronal excitability: reduction in ECS volume shrinkage is inhibitory, whereas increase in alkalization is excitatory.

The relationship between DIDS' effect on activity-induced ECS shrinkage and its effect on seizure activity provides evidence for ECS volume playing a large role in seizure propagation. DIDS application has previously been shown to have a seizure attenuating effect when applied in vivo to rats undergoing lithium-pilocarpine induced status epilepticus [120]. If the antiepileptic effect of DIDS is due to a change in the local neuronal circuit properties, then the study conducted in [58] provides a possible mechanism. The symport normally engages in Na^+-driven HCO_3^- transport into the astrocyte due to the astrocytic depolarization induced by the rise in $[K^+]$ in the ECS immediately following neuronal depolarization [121]. DIDS blocks this action from occurring, leading to the two main effects found in the study: a greater peak alkalization of the ECS and a reduction in stimulus-induced ECS volume shrinkage. Of these effects, the only local anti-epileptic effect of DIDS was the reduction in activity-induced ECS shrinkage. This further highlights the importance that ECS volume dynamics has on ictogenesis.

Volume Transmission, GLT-1, and Ambient Glutamate

The interplay between the glutamate transporter family and the ECS is a primary determinant in determining the dynamics of volume transmission—a physiological phenomenon that promotes synaptic crosstalk across neurons. The main member of the glutamate transporter family that is responsible for 90% of glutamate reuptake in the brain is GLT-1 (also known as EAAT2) [122, 123]. GLT-1 is present on astrocytes and allows for the secondary active transport of glutamate by bringing a Na^+ down its concentration gradient into the astrocyte. Almost all synapses in the brain have some degree of astrocytic ensheathment around the synapse, which forces neurotransmitter that escapes the synaptic cleft to interact with astrocytic membranes [124]. During normal transmission in a glutamatergic synapse, glutamate will travel through the cleft and eventually concentrate around the perisynaptic astrocytic membrane. The GLT-1 in the perisynaptic membrane will then allow for the uptake of glutamate and Na^+ into the astrocyte, providing the main clearance mechanism of glutamate from the ECS. If the glutamate concentration becomes too high, it can overwhelm the kinetics of the GLT-1 transporters and escape beyond the perisynaptic space, potentially reaching other glutamatergic sites on distant cells. This phenomenon can represent normal physiological volume transmission—a mechanism in which synapses are non-isolated and distant neurons can communicate with each other through neurotransmitters—or it can be a consequence of excessive glutamate transmission, such as during seizure [51, 62, 125, 126].

Because of GLT-1's role in glutamate clearance, its presence around the synapse is important in determining synaptic isolation and the degree of spillover that can occur from glutamatergic synapses. Different synapses have differing degrees of glial ensheathment and have concentrations of extracellular matrix that help trap glutamate around GLT-1 transporters to allow for clearance to occur [127]. This means that the isolation of synapse can be modulated simply by changing the proximity of the astrocytic membrane to the synaptic cleft. Indeed, this phenomenon is demonstrated in the supraoptic nucleus (SON) of rats during lactation. During lactation, astrocytic processes retract away from glutamatergic synapses in this region, causing GLT-1 to be overwhelmed, glutamate to spill over, and ultimately accumulate in the ECS [128]. Spillover from synapses such as these and accumulation

of glutamate can have multiple consequences related to seizures. First, an environment rich in ambient glutamate can excite multiple neurons in a single point in time. Second, excess glutamate in an environment is responsible for the downstream consequences of seizure: promoting excitotoxicity, and ultimately neuronal death [129].

While this type of synaptic crosstalk does not inherently cause seizures, the presence of ambient glutamate in the ECS does assist in promoting hyperexcitability and synchrony. As discussed previously, manipulating the ECS by washing on hypotonic ACSF led to an increase in neuronal excitability and action-potential frequency. Further experiments have shown that hypotonic ACSF also promote slow inward currents (SICs), which are neuronal and induced by enhanced glutamate binding to extrasynaptic NMDA receptors during cell swelling induced by the addition of ACSF [76]. This phenomenon matches the time course of the excitatory postsynaptic potentials that occur during osmotic edema induced by hypotonic ACSF, making it a possible link between ECS shrinkage and neuronal excitability. Ambient glutamate in the ECS can have several main sources, such as volume transmission and astrocytic glutamate release [130]. Therefore, if there is an ambient glutamate concentration present and any ECS shrinkage occurs, then there is the potential to generate SICs and start down the path to a seizure. GLT-1 has also been known to participate in glial swelling, as it can accommodate water and therefore cause swelling when sufficient glutamate needs to be cleared from the ECS. Sufficient glutamate in the ECS can therefore also cause swelling of astrocytes, promoting further concentration of the excitatory substance.

Conclusions and Future Directions

The relationship between the ECS, neuronal physiology, and the propagation of seizures is dominated by a variety of cellular components that control ionic gradients. Both astrocytic and neuronal transporters exert control over neuronal firing, either through modification of the ECS volume fraction, alteration of ionic or neurotransmitter activity, or both. Summaries of the mechanisms that affect neuronal firing either through mediation of the ECS or otherwise are shown in Figs. 3 and 4. Of the transporters discussed, all of them can affect ECS volume through alteration of ionic gradients and induction of cellular swelling, which can ultimately lead to a seizure. However, NKCC1/KCC2, Kir 4.1, AQP4, and GLT-1 are all likely to promote or depress neuronal excitability through other means, such as modulation of $[K^+]$ concentrations or through alteration of neurotransmitter activity. NBCe1, because of its direct implication in modulation of stimulus-induced ECS shrinkage, and because blockade has no other direct anti-epileptic effect, is likely to be the main transporter to participate in seizure propagation through ECS volume changes. This induction of cellular swelling is likely to promote seizures through multiple mechanisms (summarized in Figs. 3, 4), such as the concentration of glutamate and ECS K^+ or the enhancement of ephaptic interactions. At the time that such a volume change

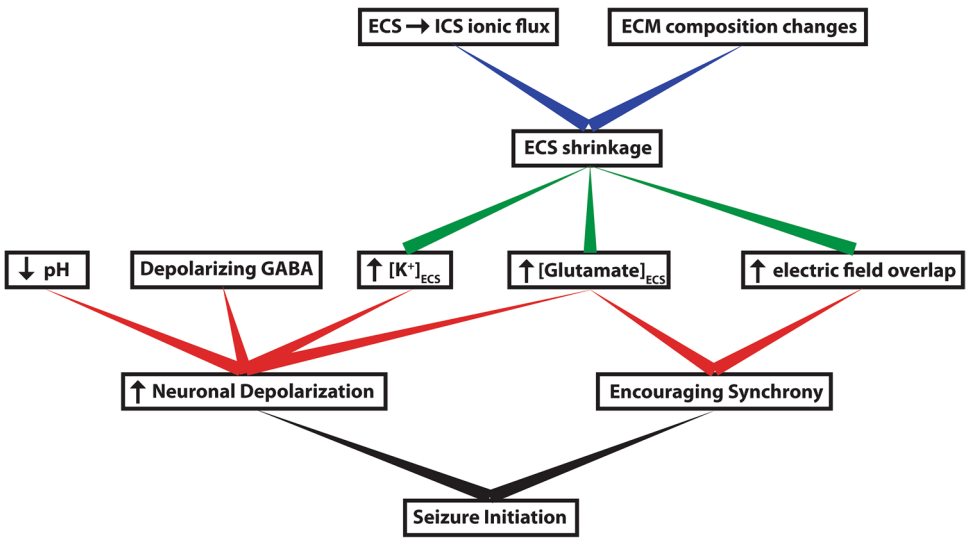

Fig. 3 A flow chart showing the mechanisms that can ultimately lead to seizures. Two main conditions need to occur to induce seizures: excessive neuronal depolarization and a synchronously firing neuronal circuit. Many mechanisms can lead to these consequences. ECS shrinkage is the main mechanism involving the ECS that can ultimately lead to seizure propagation through the indicated intermediary steps (green). The blue elements point to mechanisms that can assist in seizure propagation, but only purely by leading to ECS shrinkage. Red elements would, by themselves lead to seizure propagation through the indicated mechanism. (Color figure online)

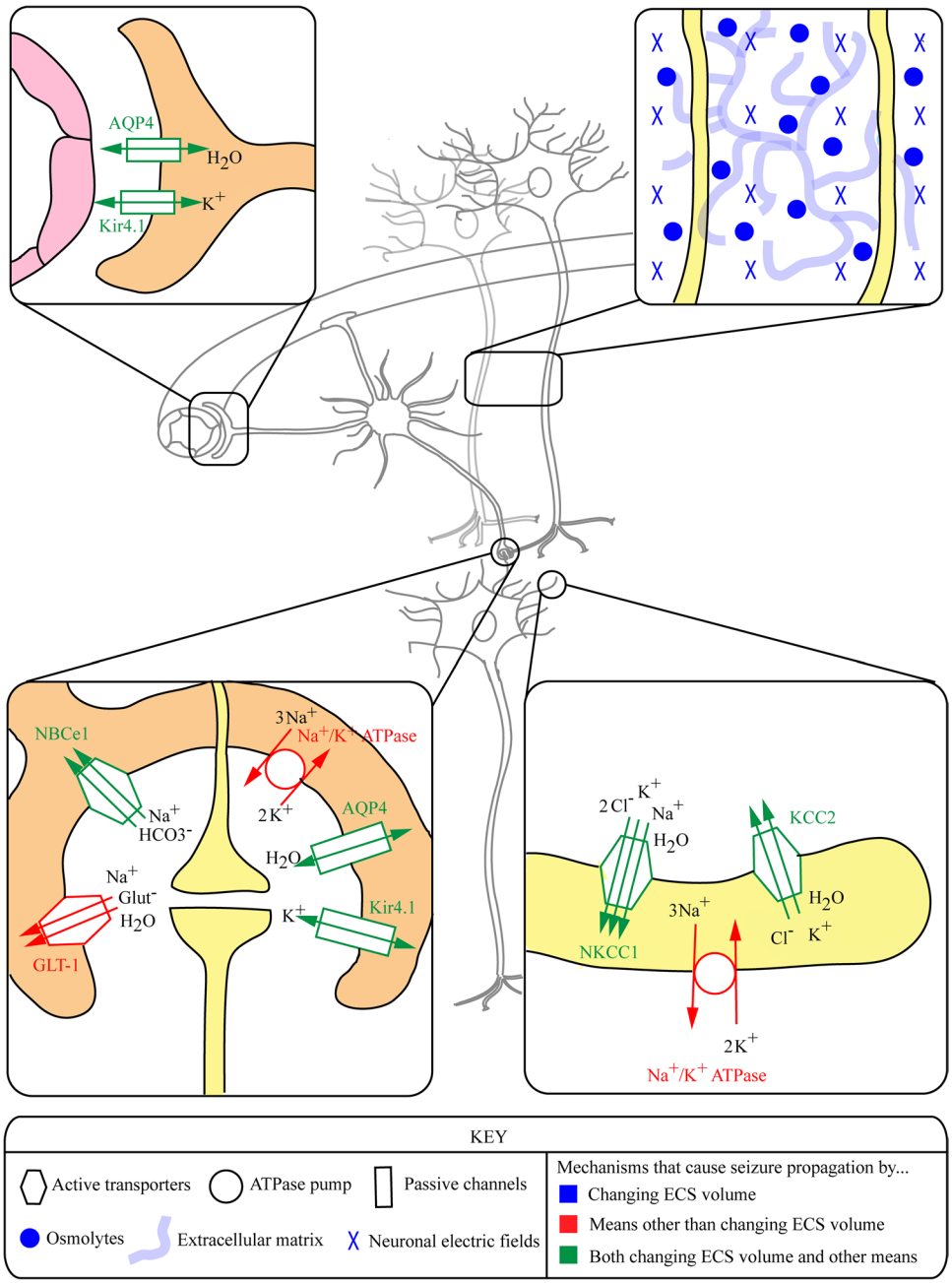

Fig. 4 A graphical summary of the mechanisms discussed in the review and how they can ultimately affect the likelihood of seizure propagation. Four main highlighted regions—the perivascular endfeet (top-left box), the synapse and perisynaptic area (bottom-left box), the soma-dendrite system (bottom-right box), and the ECS surrounding neuronal axons (top-right box)—contain a variety of highlighted elements that alter neuronal excitation and/or synchrony by ECS and non-ECS volume-altering mechanisms

occurs, the composition of the interstitial fluid is therefore of prime importance, as it could already have a high concentration of pro-excitation forces, such as excess glutamate from a source of volume transmission.

All of these excitable forces that relate to the ECS can serve as potential new therapeutic targets to treat drug resistant epilepsies. However, other fundamental questions must still be explored to understand the phenomenon. The NBCe1 is a likely transporter to mediate ECS volume changes, but it remains unclear whether it itself allows for some water transport through the channel itself. If not, then what is the main path for water to get into the astrocyte following an osmotic shift, and can this path also be targetable mechanism to inhibit seizure activity? Continuing to trace this mechanism can lead to the elucidation of a complete pathway, and therefore provide many avenues to explore for treatment, especially if blockade of one of the steps turns out to be unviable or ineffective. Furthermore, while it has been shown that the ECS shrinks during seizures, the temporal and spatial resolution of the techniques used to show this so far have been somewhat limited. Are there more transient volume changes that occur during a seizure that correspond closely to the oscillation of neuronal firing, or is there simply a baseline swelling that occurs throughout the tissue? A

cellular view of the process would also be important, as it would be important to definitively elucidate what cell types are swelling. Is this purely an astrocytic problem, or do the neurons themselves contribute to the cellular swelling? If these issues can be addressed in future experiments, then it can help guide any future therapeutic developments that are designed to treat current cases of drug-resistant epilepsies.

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References

1. Behr C, Goltzene MA, Kosmalski G, Hirsch E, Ryvlin P (2016) Epidemiology of epilepsy. Rev Neurol (Paris) 172(1):27–36. https://doi.org/10.1016/j.neurol.2015.11.003
2. Brodie MJ, Barry SJ, Bamagous GA, Norrie JD, Kwan P (2012) Patterns of treatment response in newly diagnosed epilepsy. Neurology 78(20):1548–1554. https://doi.org/10.1212/WNL.0b013e3182563b19
3. Bromfield EB, Cavazos JE, Sirven JI (2006) An introduction to epilepsy. American Epilepsy Society, West Hartford
4. Staley K (2015) Molecular mechanisms of epilepsy. Nat Neurosci 18(3):367–372. https://doi.org/10.1038/nn.3947
5. Pitkanen A, Lukasiuk K, Dudek FE, Staley KJ (2015) Epileptogenesis. Cold Spring Harb Perspect Med. https://doi.org/10.1101/cshperspect.a022822
6. Goldenberg MM (2010) Overview of drugs used for epilepsy and seizures: etiology, diagnosis, and treatment. P T 35(7):392–415
7. Korogod N, Petersen CC, Knott GW (2015) Ultrastructural analysis of adult mouse neocortex comparing aldehyde perfusion with cryo fixation. Elife. https://doi.org/10.7554/eLife.05793
8. Syková E (2004) Extrasynaptic volume transmission and diffusion parameters of the extracellular space. Neuroscience 129(4):861–876. https://doi.org/10.1016/j.neuroscience.2004.06.077
9. Kamali-Zare P, Nicholson C (2013) Brain extracellular space: geometry, matrix and physiological importance. Basic Clin Neurosci 4(4):282–286
10. Syková E, Nicholson C (2008) Diffusion in brain extracellular space. Physiol Rev 88(4):1277–1340. https://doi.org/10.1152/physrev.00027.2007
11. Wolak DJ, Thorne RG (2013) Diffusion of macromolecules in the brain: implications for drug delivery. Mol Pharm 10(5):1492–1504. https://doi.org/10.1021/mp300495e
12. Nicholson C, Phillips JM, Gardner-Medwin AR (1979) Diffusion from an iontophoretic point source in the brain: role of tortuosity and volume fraction. Brain Res 169(3):580–584
13. Dityatev A, Seidenbecher CI, Schachner M (2010) Compartmentalization from the outside: the extracellular matrix and functional microdomains in the brain. Trends Neurosci 33(11):503–512. https://doi.org/10.1016/j.tins.2010.08.003
14. Almond A (2005) Towards understanding the interaction between oligosaccharides and water molecules. Carbohydr Res 340(5):907–920. https://doi.org/10.1016/j.carres.2005.01.014
15. Galtrey CM, Fawcett JW (2007) The role of chondroitin sulfate proteoglycans in regeneration and plasticity in the central nervous system. Brain Res Rev 54(1):1–18. https://doi.org/10.1016/j.brainresrev.2006.09.006
16. Hrabetova S, Masri D, Tao L, Xiao F, Nicholson C (2009) Calcium diffusion enhanced after cleavage of negatively charged components of brain extracellular matrix by chondroitinase ABC. J Physiol 587(Pt 16):4029–4049. https://doi.org/10.1113/jphysiol.2009.170092
17. Yamaguchi Y (2000) Lecticans: organizers of the brain extracellular matrix. Cell Mol Life Sci 57(2):276–289. https://doi.org/10.1007/PL00000690
18. Oohashi T, Edamatsu M, Bekku Y, Carulli D (2015) The hyaluronan and proteoglycan link proteins: organizers of the brain extracellular matrix and key molecules for neuronal function and plasticity. Exp Neurol 274(Pt B):134–144. https://doi.org/10.1016/j.expneurol.2015.09.010
19. Zimmermann DR, Dours-Zimmermann MT (2008) Extracellular matrix of the central nervous system: from neglect to challenge. Histochem Cell Biol 130(4):635–653. https://doi.org/10.1007/s00418-008-0485-9
20. Ruoslahti E (1996) Brain extracellular matrix. Glycobiology 6(5):489–492
21. Novak U, Kaye AH (2000) Extracellular matrix and the brain: components and function. J Clin Neurosci 7(4):280–290. https://doi.org/10.1054/jocn.1999.0212
22. Thorne RF, Legg JW, Isacke CM (2004) The role of the CD44 transmembrane and cytoplasmic domains in co-ordinating adhesive and signalling events. J Cell Sci 117(Pt 3):373–380. https://doi.org/10.1242/jcs.00954
23. Carulli D, Rhodes KE, Brown DJ, Bonnert TP, Pollack SJ, Oliver K, Strata P, Fawcett JW (2006) Composition of perineuronal nets in the adult rat cerebellum and the cellular origin of their components. J Comp Neurol 494(4):559–577. https://doi.org/10.1002/cne.20822
24. Wyckoff RW, Young JZ (1956) The motorneuron surface. Proc R Soc Lond B Biol Sci 144(917):440–450
25. Van Harreveld A, Crowell J, Malhotra SK (1965) A study of extracellular space in central nervous tissue by freeze-substitution. J Cell Biol 25:117–137
26. Rall DP, Oppelt WW, Patlack CS (1962) Extracellular space of brain as determined by diffusion of inulin from the ventricular system. Life Sci 1(2):43–48
27. Tønnesen J, Inavalli V, Nägerl UV (2018) Super-resolution imaging of the extracellular space in living brain tissue. Cell 172(5):1108–1121 e1115. https://doi.org/10.1016/j.cell.2018.02.007
28. Hrabetova S, Nicholson C (2007) Biophysical properties of brain extracellular space explored with ion-selective microelectrodes, integrative optical imaging and related techniques. In: Michael AC, Borland LM (eds) Electrochemical methods for neuroscience. Frontiers in Neuroengineering, Boca Raton
29. Nicholson C (1993) Ion-selective microelectrodes and diffusion measurements as tools to explore the brain cell microenvironment. J Neurosci Methods 48(3):199–213
30. Syková E, Hník P, Vyklický L (1981) Ion-selective microelectrodes and their use in excitable tissues. Plenum, New York
31. Kaur G, Hrabetova S, Guilfoyle DN, Nicholson C, Hrabe J (2008) Characterizing molecular probes for diffusion measurements in the brain. J Neurosci Methods 171(2):218–225. https://doi.org/10.1016/j.jneumeth.2008.03.007
32. Nicholson C, Phillips JM (1981) Ion diffusion modified by tortuosity and volume fraction in the extracellular microenvironment of the rat cerebellum. J Physiol 321:225–257
33. Nicholson C, Hrabetova S (2017) Brain extracellular space: the final frontier of neuroscience. Biophys J 113(10):2133–2142. https://doi.org/10.1016/j.bpj.2017.06.052
34. Odackal J, Colbourn R, Odackal NJ, Tao L, Nicholson C, Hrabetova S (2017) Real-time iontophoresis with tetramethylammonium to quantify volume fraction and tortuosity of brain extracellular space. J Vis Exp. https://doi.org/10.3791/55755

35. Voipio JPM, MacLeod K (1994) Ion-sensitive microelectrodes. In: Ogden D (ed) Microelectrode techniques—the plymouth workshop handbook, 2nd edn. The Company of Biologists Limited, Cambridge, pp 275–316
36. Dietzel I, Heinemann U, Hofmeier G, Lux HD (1980) Transient changes in the size of the extracellular space in the sensorimotor cortex of cats in relation to stimulus-induced changes in potassium concentration. Exp Brain Res 40(4):432–439
37. Hrabetova S, Cognet L, Rusakov DA, Nagerl UV (2018) Unveiling the extracellular space of the brain: from super-resolved microstructure to *in vivo* function. J Neurosci 38(44):9355–9363. https://doi.org/10.1523/JNEUROSCI.1664-18.2018
38. Kume-Kick J, Mazel T, Vořísek I, Hrabetova S, Tao L, Nicholson C (2002) Independence of extracellular tortuosity and volume fraction during osmotic challenge in rat neocortex. J Physiol 542(Pt 2):515–527
39. Lehmenkühler A, Syková E, Svoboda J, Zilles K, Nicholson C (1993) Extracellular space parameters in the rat neocortex and subcortical white matter during postnatal development determined by diffusion analysis. Neuroscience 55(2):339–351
40. Syková E, Vořísek I, Antonova T, Mazel T, Meyer-Luehmann M, Jucker M, Hájek M, Ort M, Bureš J (2005) Changes in extracellular space size and geometry in APP23 transgenic mice: a model of Alzheimer's disease. Proc Natl Acad Sci USA 102(2):479–484. https://doi.org/10.1073/pnas.0408235102
41. Syková E, Mazel T, Hasenohrl RU, Harvey AR, Šimonová Z, Mulders WH, Huston JP (2002) Learning deficits in aged rats related to decrease in extracellular volume and loss of diffusion anisotropy in hippocampus. Hippocampus 12(2):269–279. https://doi.org/10.1002/hipo.1101
42. Svoboda J, Syková E (1991) Extracellular space volume changes in the rat spinal cord produced by nerve stimulation and peripheral injury. Brain Res 560(1–2):216–224
43. Xie L, Kang H, Xu Q, Chen MJ, Liao Y, Thiyagarajan M, O'Donnell J, Christensen DJ, Nicholson C, Iliff JJ, Takano T, Deane R, Nedergaard M (2013) Sleep drives metabolite clearance from the adult brain. Science 342(6156):373–377. https://doi.org/10.1126/science.1241224
44. Ding F, O'Donnell J, Xu Q, Kang N, Goldman N, Nedergaard M (2016) Changes in the composition of brain interstitial ions control the sleep-wake cycle. Science 352(6285):550–555. https://doi.org/10.1126/science.aad4821
45. Sherpa AD, Xiao F, Joseph N, Aoki C, Hrabetova S (2016) Activation of β-adrenergic receptors in rat visual cortex expands astrocytic processes and reduces extracellular space volume. Synapse 70(8):307–316. https://doi.org/10.1002/syn.21908
46. Hodgkin AL, Huxley AF, Katz B (1952) Measurement of current-voltage relations in the membrane of the giant axon of *Loligo*. J Physiol 116(4):424–448
47. Lux HD, Heinemann U, Dietzel I (1986) Ionic changes and alterations in the size of the extracellular space during epileptic activity. Adv Neurol 44:619–639
48. Murphy TR, Binder DK, Fiacco TA (2017) Turning down the volume: astrocyte volume change in the generation and termination of epileptic seizures. Neurobiol Dis 104:24–32. https://doi.org/10.1016/j.nbd.2017.04.016
49. Dudek FE, Snow RW, Taylor CP (1986) Role of electrical interactions in synchronization of epileptiform bursts. Adv Neurol 44:593–617
50. Vargová L, Syková E (2014) Astrocytes and extracellular matrix in extrasynaptic volume transmission. Philos Trans R Soc Lond B Biol Sci 369(1654):20130608. https://doi.org/10.1098/rstb.2013.0608
51. Vargová L, Syková E (2008) Extracellular space diffusion and extrasynaptic transmission. Physiol Res 57(Suppl 3):S89–S99
52. Arranz AM, Perkins KL, Irie F, Lewis DP, Hrabe J, Xiao F, Itano N, Kimata K, Hrabetova S, Yamaguchi Y (2014) Hyaluronan deficiency due to *Has3* knock-out causes altered neuronal activity and seizures via reduction in brain extracellular space. J Neurosci 34(18):6164–6176. https://doi.org/10.1523/JNEUROSCI.3458-13.2014
53. Syková E (1997) The extracellular space in the CNS: Its regulation, volume and geometry in normal and pathological neuronal function. Neuroscientist 3(1):28–41. https://doi.org/10.1177/107385849700300113
54. Weiss SA, Faber DS (2010) Field effects in the CNS play functional roles. Front Neural Circuits 4:15. https://doi.org/10.3389/fncir.2010.00015
55. Ransom BR, Yamate CL, Connors BW (1985) Activity-dependent shrinkage of extracellular space in rat optic nerve: a developmental study. J Neurosci 5(2):532–535
56. Connors BW, Ransom BR, Kunis DM, Gutnick MJ (1982) Activity-dependent K + accumulation in the developing rat optic nerve. Science 216(4552):1341–1343
57. Haj-Yasein NN, Jensen V, Ostby I, Omholt SW, Voipio J, Kaila K, Ottersen OP, Hvalby O, Nagelhus EA (2012) Aquaporin-4 regulates extracellular space volume dynamics during high-frequency synaptic stimulation: a gene deletion study in mouse hippocampus. Glia 60(6):867–874. https://doi.org/10.1002/glia.22319
58. Larsen BR, MacAulay N (2017) Activity-dependent astrocyte swelling is mediated by pH-regulating mechanisms. Glia 65(10):1668–1681. https://doi.org/10.1002/glia.23187
59. Larsen BR, Assentoft M, Cotrina ML, Hua SZ, Nedergaard M, Kaila K, Voipio J, MacAulay N (2014) Contributions of the Na^+/K^+-ATPase, NKCC1, and Kir4.1 to hippocampal K^+ clearance and volume responses. Glia 62(4):608–622. https://doi.org/10.1002/glia.22629
60. Ammann D (1986) Ion-selective microelectrodes. principles, design, and application. Springer-Verlag, Berlin
61. Raimondo JV, Burman RJ, Katz AA, Akerman CJ (2015) Ion dynamics during seizures. Front Cell Neurosci 9:419. https://doi.org/10.3389/fncel.2015.00419
62. Šlais K, Vořísek I, Zoremba N, Homola A, Dmytrenko L, Syková E (2008) Brain metabolism and diffusion in the rat cerebral cortex during pilocarpine-induced status epilepticus. Exp Neurol 209(1):145–154. https://doi.org/10.1016/j.expneurol.2007.09.008
63. Traynelis SF, Dingledine R (1989) Role of extracellular space in hyperosmotic suppression of potassium-induced electrographic seizures. J Neurophysiol 61(5):927–938. https://doi.org/10.1152/jn.1989.61.5.927
64. de Curtis M, Uva L, Gnatkovsky V, Librizzi L (2018) Potassium dynamics and seizures: why is potassium ictogenic? Epilepsy Res 143:50–59. https://doi.org/10.1016/j.eplepsyres.2018.04.005
65. Gardner-Medwin AR (1983) Analysis of potassium dynamics in mammalian brain tissue. J Physiol 335:393–426
66. During MJ, Spencer DD (1993) Extracellular hippocampal glutamate and spontaneous seizure in the conscious human brain. Lancet 341(8861):1607–1610
67. Pena F, Tapia R (2000) Seizures and neurodegeneration induced by 4-aminopyridine in rat hippocampus *in vivo*: role of glutamate- and GABA-mediated neurotransmission and of ion channels. Neuroscience 101(3):547–561
68. Kaila K, Ruusuvuori E, Seja P, Voipio J, Puskarjov M (2014) GABA actions and ionic plasticity in epilepsy. Curr Opin Neurobiol 26:34–41. https://doi.org/10.1016/j.conb.2013.11.004
69. Zhang M, Ladas TP, Qiu C, Shivacharan RS, Gonzalez-Reyes LE, Durand DM (2014) Propagation of epileptiform activity can be independent of synaptic transmission, gap junctions, or diffusion and is consistent with electrical field transmission. J

Neurosci 34(4):1409–1419. https://doi.org/10.1523/JNEUROSCI.3877-13.2014
70. Chang BS, Lowenstein DH (2003) Epilepsy. N Engl J Med 349(13):1257–1266. https://doi.org/10.1056/NEJMra022308
71. McBain CJ, Traynelis SF, Dingledine R (1990) Regional variation of extracellular space in the hippocampus. Science 249(4969):674–677
72. Murphy TR, Davila D, Cuvelier N, Young LR, Lauderdale K, Binder DK, Fiacco TA (2017) Hippocampal and cortical pyramidal neurons swell in parallel with astrocytes during acute hypoosmolar stress. Front Cell Neurosci 11:275. https://doi.org/10.3389/fncel.2017.00275
73. Andrew RD, Fagan M, Ballyk BA, Rosen AS (1989) Seizure susceptibility and the osmotic state. Brain Res 498(1):175–180
74. Ballyk BA, Quackenbush SJ, Andrew RD (1991) Osmotic effects on the CA1 neuronal population in hippocampal slices with special reference to glucose. J Neurophysiol 65(5):1055–1066. https://doi.org/10.1152/jn.1991.65.5.1055
75. Kilb W, Dierkes PW, Syková E, Vargová L, Luhmann HJ (2006) Hypoosmolar conditions reduce extracellular volume fraction and enhance epileptiform activity in the CA3 region of the immature rat hippocampus. J Neurosci Res 84(1):119–129. https://doi.org/10.1002/jnr.20871
76. Lauderdale K, Murphy T, Tung T, Davila D, Binder DK, Fiacco TA (2015) Osmotic edema rapidly increases neuronal excitability through activation of NMDA receptor-dependent slow inward currents in juvenile and adult hippocampus. ASN Neuro. https://doi.org/10.1177/1759091415605115
77. Weigel PH (2015) Hyaluronan synthase: the mechanism of initiation at the reducing end and a pendulum model for polysaccharide translocation to the cell exterior. Int J Cell Biol 2015:367579. https://doi.org/10.1155/2015/367579
78. Saghyan A, Lewis DP, Hrabe J, Hrabetova S (2012) Extracellular diffusion in laminar brain structures exemplified by hippocampus. J Neurosci Methods 205(1):110–118. https://doi.org/10.1016/j.jneumeth.2011.12.008
79. Toole BP (2001) Hyaluronan in morphogenesis. Semin Cell Dev Biol 12(2):79–87. https://doi.org/10.1006/scdb.2000.0244
80. Pivovarov AS, Calahorro F, Walker RJ (2018) Na^+/K^+-pump and neurotransmitter membrane receptors. Invert Neurosci 19(1):1. https://doi.org/10.1007/s10158-018-0221-7
81. Thomas RC (1972) Electrogenic sodium pump in nerve and muscle cells. Physiol Rev 52(3):563–594. https://doi.org/10.1152/physrev.1972.52.3.563
82. Holm TH, Lykke-Hartmann K (2016) Insights into the pathology of the α3 Na^+/K^+-ATPase ion pump in neurological disorders; lessons from animal models. Front Physiol 7:209. https://doi.org/10.3389/fphys.2016.00209
83. Vaillend C, Mason SE, Cuttle MF, Alger BE (2002) Mechanisms of neuronal hyperexcitability caused by partial inhibition of Na^+-K^+-ATPases in the rat CA1 hippocampal region. J Neurophysiol 88(6):2963–2978. https://doi.org/10.1152/jn.00244.2002
84. Grisar T, Franck G, Schoffeniels E (1980) Glial control of neuronal excitability in mammals: II. Enzymatic evidence: two molecular forms of the (Na^+,K^+)-ATPase in brain. Neurochem Int 2C:311–320
85. Larsen BR, Stoica A, MacAulay N (2016) Managing brain extracellular K^+ during neuronal activity: the physiological role of the Na^+/K^+-ATPase subunit isoforms. Front Physiol 7:141. https://doi.org/10.3389/fphys.2016.00141
86. Stoica A, Larsen BR, Assentoft M, Holm R, Holt LM, Vilhardt F, Vilsen B, Lykke-Hartmann K, Olsen ML, MacAulay N (2017) The α2β2 isoform combination dominates the astrocytic Na^+/K^+-ATPase activity and is rendered nonfunctional by the α2.G301R familial hemiplegic migraine type 2-associated mutation. Glia 65(11):1777–1793. https://doi.org/10.1002/glia.23194
87. Larsen BR, Stoica A, MacAulay N (2019) Developmental maturation of activity-induced K^+ and pH transients and the associated extracellular space dynamics in the rat hippocampus. J Physiol 597(2):583–597. https://doi.org/10.1113/JP276768
88. Attwell D, Laughlin SB (2001) An energy budget for signaling in the grey matter of the brain. J Cereb Blood Flow Metab 21(10):1133–1145. https://doi.org/10.1097/00004647-200110000-00001
89. Lees GJ, Lehmann A, Sandberg M, Hamberger A (1990) The neurotoxicity of ouabain, a sodium-potassium ATPase inhibitor, in the rat hippocampus. Neurosci Lett 120(2):159–162
90. Macaulay N, Zeuthen T (2012) Glial K^+ clearance and cell swelling: key roles for cotransporters and pumps. Neurochem Res 37(11):2299–2309. https://doi.org/10.1007/s11064-012-0731-3
91. de Los Heros P, Alessi DR, Gourlay R, Campbell DG, Deak M, Macartney TJ, Kahle KT, Zhang J (2014) The WNK-regulated SPAK/OSR1 kinases directly phosphorylate and inhibit the K^+-Cl^- co-transporters. Biochem J 458(3):559–573. https://doi.org/10.1042/BJ20131478
92. Kelley MR, Deeb TZ, Brandon NJ, Dunlop J, Davies PA, Moss SJ (2016) Compromising KCC2 transporter activity enhances the development of continuous seizure activity. Neuropharmacology 108:103–110. https://doi.org/10.1016/j.neuropharm.2016.04.029
93. Dzhala V, Staley KJ (2015) Acute and chronic efficacy of bumetanide in an *in vitro* model of posttraumatic epileptogenesis. CNS Neurosci Ther 21(2):173–180. https://doi.org/10.1111/cns.12369
94. Edwards DA, Shah HP, Cao W, Gravenstein N, Seubert CN, Martynyuk AE (2010) Bumetanide alleviates epileptogenic and neurotoxic effects of sevoflurane in neonatal rat brain. Anesthesiology 112(3):567–575. https://doi.org/10.1097/ALN.0b013e3181cf9138
95. Mazarati A, Shin D, Sankar R (2009) Bumetanide inhibits rapid kindling in neonatal rats. Epilepsia 50(9):2117–2122. https://doi.org/10.1111/j.1528-1167.2009.02048.x
96. Mares P (2009) Age- and dose-specific anticonvulsant action of bumetanide in immature rats. Physiol Res 58(6):927–930
97. Margineanu DG, Klitgaard H (2006) Differential effects of cation-chloride co-transport-blocking diuretics in a rat hippocampal slice model of epilepsy. Epilepsy Res 69(2):93–99. https://doi.org/10.1016/j.eplepsyres.2006.01.005
98. Moore YE, Kelley MR, Brandon NJ, Deeb TZ, Moss SJ (2017) Seizing control of KCC2: a new therapeutic target for epilepsy. Trends Neurosci 40(9):555–571. https://doi.org/10.1016/j.tins.2017.06.008
99. Hochman DW (2012) The extracellular space and epileptic activity in the adult brain: explaining the antiepileptic effects of furosemide and bumetanide. Epilepsia 53(Suppl 1):18–25. https://doi.org/10.1111/j.1528-1167.2012.03471.x
100. Holthoff K, Witte OW (1996) Intrinsic optical signals in rat neocortical slices measured with near-infrared dark-field microscopy reveal changes in extracellular space. J Neurosci 16(8):2740–2749
101. Hamann S, Herrera-Perez JJ, Bundgaard M, Alvarez-Leefmans FJ, Zeuthen T (2005) Water permeability of Na^+-K^+-2Cl^- cotransporters in mammalian epithelial cells. J Physiol 568(Pt 1):123–135. https://doi.org/10.1113/jphysiol.2005.093526
102. Zhang Y, Chen K, Sloan SA, Bennett ML, Scholze AR, O'Keeffe S, Phatnani HP, Guarnieri P, Caneda C, Ruderisch N, Deng S, Liddelow SA, Zhang C, Daneman R, Maniatis T, Barres BA, Wu JQ (2014) An RNA-sequencing transcriptome and splicing database of glia, neurons, and vascular cells of the cerebral cortex. J Neurosci 34(36):11929–11947. https://doi.org/10.1523/JNEUROSCI.1860-14.2014
103. Uwera J, Nedergaard S, Andreasen M (2015) A novel mechanism for the anticonvulsant effect of furosemide in rat hippocampus

in vitro. Brain Res 1625:1–8. https://doi.org/10.1016/j.brainres.2015.08.014102
104. MacAulay N, Zeuthen T (2010) Water transport between CNS compartments: contributions of aquaporins and cotransporters. Neuroscience 168(4):941–956. https://doi.org/10.1016/j.neuroscience.2009.09.016
105. Nagelhus EA, Mathiisen TM, Ottersen OP (2004) Aquaporin-4 in the central nervous system: cellular and subcellular distribution and coexpression with KIR4.1. Neuroscience 129(4):905–913. https://doi.org/10.1016/j.neuroscience.2004.08.053
106. Binder DK, Yao X, Zador Z, Sick TJ, Verkman AS, Manley GT (2006) Increased seizure duration and slowed potassium kinetics in mice lacking aquaporin-4 water channels. Glia 53(6):631–636. https://doi.org/10.1002/glia.20318
107. Zhang H, Verkman AS (2008) Aquaporin-4 independent Kir4.1 K^+ channel function in brain glial cells. Mol Cell Neurosci 37(1):1–10. https://doi.org/10.1016/j.mcn.2007.08.007
108. Ruiz-Ederra J, Zhang H, Verkman AS (2007) Evidence against functional interaction between aquaporin-4 water channels and Kir4.1 potassium channels in retinal Muller cells. J Biol Chem 282(30):21866–21872. https://doi.org/10.1074/jbc.M703236200
109. Das A, Holmes C, McDowell ML, Smith JA, Marshall JD, Bonilha L, Edwards JC, Glazier SS, Ray SK, Banik NL (2012) Hippocampal tissue of patients with refractory TLE is associated with astrocyte activation, inflammation, and altered expression of channels and receptors. Neuroscience 220:237–246. https://doi.org/10.1016/j.neuroscience.2012.06.002
110. Heuser K, Eid T, Lauritzen F, Thoren AE, Vindedal GF, Tauboll E, Gjerstad L, Spencer DD, Ottersen OP, Nagelhus EA, de Lanerolle NC (2012) Loss of perivascular Kir4.1 potassium channels in the sclerotic hippocampus of patients with mesial temporal lobe epilepsy. J Neuropathol Exp Neurol 71(9):814–825. https://doi.org/10.1097/NEN.0b013e318267b5af
111. Heuser K, Nagelhus EA, Tauboll E, Indahl U, Berg PR, Lien S, Nakken S, Gjerstad L, Ottersen OP (2010) Variants of the genes encoding AQP4 and Kir4.1 are associated with subgroups of patients with temporal lobe epilepsy. Epilepsy Res 88(1):55–64. https://doi.org/10.1016/j.eplepsyres.2009.09.023
112. Reichold M, Zdebik AA, Lieberer E, Rapedius M, Schmidt K, Bandulik S, Sterner C, Tegtmeier I, Penton D, Baukrowitz T, Hulton SA, Witzgall R, Ben-Zeev B, Howie AJ, Kleta R, Bockenhauer D, Warth R (2010) KCNJ10 gene mutations causing EAST syndrome (epilepsy, ataxia, sensorineural deafness, and tubulopathy) disrupt channel function. Proc Natl Acad Sci USA 107(32):14490–14495. https://doi.org/10.1073/pnas.1003072107
113. Eid T, Lee TS, Thomas MJ, Amiry-Moghaddam M, Bjornsen LP, Spencer DD, Agre P, Ottersen OP, de Lanerolle NC (2005) Loss of perivascular aquaporin 4 may underlie deficient water and K^+ homeostasis in the human epileptogenic hippocampus. Proc Natl Acad Sci USA 102(4):1193–1198. https://doi.org/10.1073/pnas.0409308102
114. Coulter DA, Steinhauser C (2015) Role of astrocytes in epilepsy. Cold Spring Harb Perspect Med 5(3):a022434. https://doi.org/10.1101/cshperspect.a022434
115. Yao X, Hrabetova S, Nicholson C, Manley GT (2008) Aquaporin-4-deficient mice have increased extracellular space without tortuosity change. J Neurosci 28(21):5460–5464. https://doi.org/10.1523/JNEUROSCI.0257-08.2008
116. Larsen BR, MacAulay N (2014) Kir4.1-mediated spatial buffering of K^+: experimental challenges in determination of its temporal and quantitative contribution to K^+ clearance in the brain. Channels (Austin) 8(6):544–550. https://doi.org/10.4161/19336950.2014.970448
117. Bevensee MO, Weed RA, Boron WF (1997) Intracellular pH regulation in cultured astrocytes from rat hippocampus. I. Role Of HCO3. J Gen Physiol 110(4):453–465
118. Theparambil SM, Naoshin Z, Thyssen A, Deitmer JW (2015) Reversed electrogenic sodium bicarbonate cotransporter 1 is the major acid loader during recovery from cytosolic alkalosis in mouse cortical astrocytes. J Physiol 593(16):3533–3547. https://doi.org/10.1113/JP270086
119. Sinning A, Hubner CA (2013) Minireview: pH and synaptic transmission. FEBS Lett 587(13):1923–1928. https://doi.org/10.1016/j.febslet.2013.04.045
120. Yang T, Lin Z, Xie L, Wang Y, Pan S (2017) 4,4′-Diisothiocyanatostilbene-2,2′-disulfonic acid attenuates spontaneous recurrent seizures and vasogenic edema following lithium-pilocarpine induced status epilepticus. Neurosci Lett 653:51–57. https://doi.org/10.1016/j.neulet.2017.05.015
121. Theparambil SM, Ruminot I, Schneider HP, Shull GE, Deitmer JW (2014) The electrogenic sodium bicarbonate cotransporter NBCe1 is a high-affinity bicarbonate carrier in cortical astrocytes. J Neurosci 34(4):1148–1157. https://doi.org/10.1523/JNEUROSCI.2377-13.2014
122. Danbolt NC (2001) Glutamate uptake. Prog Neurobiol 65(1):1–105
123. Haugeto O, Ullensvang K, Levy LM, Chaudhry FA, Honore T, Nielsen M, Lehre KP, Danbolt NC (1996) Brain glutamate transporter proteins form homomultimers. J Biol Chem 271(44):27715–27722
124. Heller JP, Rusakov DA (2015) Morphological plasticity of astroglia: understanding synaptic microenvironment. Glia 63(12):2133–2151. https://doi.org/10.1002/glia.22821
125. Bradford HF (1995) Glutamate, GABA and epilepsy. Prog Neurobiol 47(6):477–511
126. Piet R, Bonhomme R, Theodosis DT, Poulain DA, Oliet SH (2003) Modulation of GABAergic transmission by endogenous glutamate in the rat supraoptic nucleus. Eur J Neurosci 17(9):1777–1785
127. Celio MR, Spreafico R, De Biasi S, Vitellaro-Zuccarello L (1998) Perineuronal nets: past and present. Trends Neurosci 21(12):510–515
128. Oliet SH, Piet R, Poulain DA (2001) Control of glutamate clearance and synaptic efficacy by glial coverage of neurons. Science 292(5518):923–926. https://doi.org/10.1126/science.1059162
129. Whetsell WO Jr (1996) Current concepts of excitotoxicity. J Neuropathol Exp Neurol 55(1):1–13
130. Abdullaev IF, Rudkouskaya A, Schools GP, Kimelberg HK, Mongin AA (2006) Pharmacological comparison of swelling-activated excitatory amino acid release and Cl^- currents in cultured rat astrocytes. J Physiol 572(Pt 3):677–689. https://doi.org/10.1113/jphysiol.2005.103820