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On the functions of astrocyte-mediated neuronal slow inward currents

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From the Contents

Abstract

Slow inward currents are known as neuronal excitatory currents mediated by glutamate release and activation of neuronal extrasynaptic N-methyl-D-aspartate receptors with the contribution of astrocytes. These events are significantly slower than the excitatory postsynaptic currents. Parameters of slow inward currents are determined by several factors including the mechanisms of astrocytic activation and glutamate release, as well as the diffusion pathways from the release site towards the extrasynaptic receptors. Astrocytes are stimulated by neuronal network activity, which in turn excite neurons, forming an astrocyte-neuron feedback loop. Mostly as a consequence of brain edema, astrocytic swelling can also induce slow inward currents under pathological conditions. There is a growing body of evidence on the roles of slow inward currents on a single neuron or local network level. These events often occur in synchrony on neurons located in the same astrocytic domain. Besides synchronization of neuronal excitability, slow inward currents also set synaptic strength via eliciting timing-dependent synaptic plasticity. In addition, slow inward currents are also subject to non-synaptic plasticity triggered by long-lasting stimulation of the excitatory inputs. Of note, there might be important region-specific differences in the roles and actions triggering slow inward currents. In greater networks, the pathophysiological roles of slow inward currents can be better understood than physiological ones. Slow inward currents are identified in the pathophysiological background of autism, as slow inward currents drive early hypersynchrony of the neural networks. Slow inward currents are significant contributors to paroxysmal depolarizational shifts/interictal spikes. These events are related to epilepsy, but also found in Alzheimer's disease, Parkinson's disease, and stroke, leading to the decline of cognitive functions. Events with features overlapping with slow inward currents (excitatory, N-methyl-D-aspartate-receptor mediated currents with astrocytic contribution) as ischemic currents and spreading depolarization also have a well-known pathophysiological role in worsening consequences of stroke, traumatic brain injury, or epilepsy. One might assume that slow inward currents occurring with low frequency under physiological conditions might contribute to synaptic plasticity and memory formation. However, to state this, more experimental evidence from greater neuronal networks or the level of the individual is needed. In this review, I aimed to summarize findings on slow inward currents and to speculate on the potential functions of it.

Key Words: astrocyte; cortical spreading depolarization; gliotransmission; glutamate; neural synchronization; NMDA receptor; paroxysmal depolarizational shift; slow inward current

Slow Inward Current

Definitions and basic properties of SICs

Astrocyte-driven N-methyl-D-aspartate-receptor (NMDAR)-dependent slow inward currents (SICs) were first reported in 1998 when a pioneering paper described it on a co-culture of hippocampal neurons and astrocytes (Araque et al., 1998). Since that, this phenomenon has been found in several areas of the central nervous system, recorded on slice preparations from several areas including the brainstem and spinal cord, the diencephalon, and cortical areas (**Figure 1**).

Although SICs were originally referred to as events triggered by astrocytic glutamate release and caused by neuronal extrasynaptic NMDAR activation, the nomenclature of the literature is not always consistent. There are astrocyte- and NMDAR-related events that are not called SICs but "slowly decaying inward currents" (Kang et al., 2005) or "slow transient currents" (Angulo et al., 2004). On the contrary, there are phenomena also called slow inward currents but not related to astrocytes and NMDARs. Various events are caused by the activation of different serotonin receptors, from which the excitatory ones with the fastest kinetics (named "fast inward currents") resemble to SICs (Saito and Sugimura, 2023), therefore, there is a chance to mix up the

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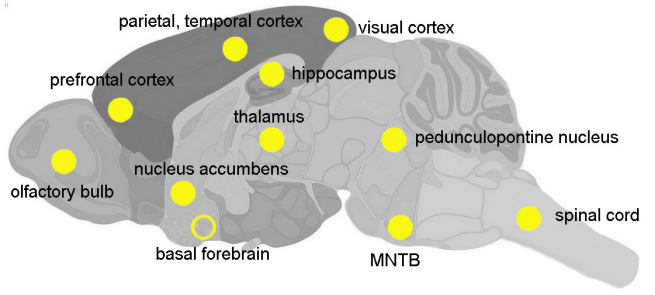
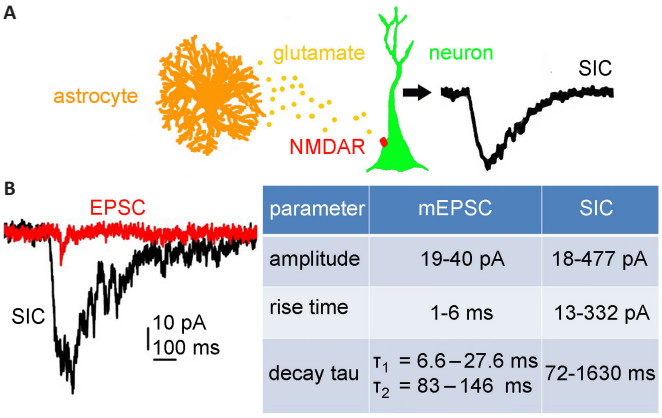


Figure 1 | **Structures of the central nervous system possessing SICs.**
Filled yellow circles: areas where SICs were confirmed by published studies, hollow circle: a brain area where SICs are found by preliminary experiments (Parri et al., 2001; Angulo et al., 2004; Kozlov et al., 2006; D'Ascenso et al., 2007; Bardoni et al., 2010; Reyes-Haro et al., 2010; Perea et al., 2014; Kovács and Pál, 2017; Csemer et al., 2023). Created with Adobe Photoshop. MNTB: Medial nucleus of the trapezoid body.



parameter	mEPSC	SIC
amplitude	19-40 pA	18-477 pA
rise time	1-6 ms	13-332 pA
decay tau	τ_1 = 6.6–27.6 ms τ_2 = 83–146 ms	72-1630 ms

Figure 2 | **Properties of slow inward currents.**
(A) Schematic illustration of the basic properties of SICs. These events are generated by astrocytic glutamate release (astrocyte: orange symbol, glutamate: yellow dots) and activation of neuronal extrasynaptic NMDA receptors (NMDA receptor: red, neuron: green). (B) Comparison of the parameters of SICs and miniature excitatory postsynaptic currents (mEPSCs). Red current trace: an example of a mEPSC, black current trace: an example of a SIC. Traces were recorded by my laboratory and modified with Adobe Photoshop. EPSC: Excitatory postsynaptic current; mEPSC: miniature excitatory postsynaptic current; NMDA:N-methyl-D-aspartate; SIC: slow inward current.

two phenomena if the receptors involved in them are not investigated. It is also worth noting that the NMDAR inhibitor D-AP5 did not always cause full blockade of SIC activity (see below), which raises the possibility that not all (although the vast majority) of the detected SICs are "canonically" elicited by astrocytic glutamate and NMDAR activation. However, a small fraction of similar currents is probably elicited by neuromodulatory actions (e.g. 5-HT3 receptor activation by serotonin; Saito and Sugimura, 2023).

SICs can be also mistaken for giant inward currents. Giant inward currents have similar kinetics (cca. 1 s half-width) and possess a component mediated by ionotropic glutamate receptors. However, these currents occur under developmental conditions (to elicit giant depolarizing potentials triggered by $GABA_A$ receptor activation), NMDA receptors have a minor contribution to them and tetrodotoxin (TTX) fully abolishes giant inward currents (Ben-Ari, 2001; Czarneczki et al., 2014). The existence of these currents is an important caveat that identification of SICs should not be solely based on the kinetical analysis of slow events because similar events with different cellular and pharmacological backgrounds also exist.

SICs can be easily distinguished from excitatory postsynaptic currents (EPSCs) based on their kinetic properties. Both the rise and decay time of SICs is significantly longer (1–6 *vs*. 13–332 ms for rise time and 6.6–146 *vs*. 72–1630 ms for decay tau of EPSCs and SICs, respectively; Csemer et al., 2023). The decay phase of EPSCs can be optimally fit with a double exponential function; whereas, for SICs, the best fit can be achieved with a single exponential function (Fellin et al., 2004; Shigetomi et al., 2008; Bardoni et al., 2010; Reyes-Haro et al., 2010). Intriguingly, human SICs are slower than murine ones, but aging shortens their duration (Csemer et al., 2023).

Of note, one can sometimes record SICs with two components: an initial faster component (which, in the human neocortex is still significantly slower than EPSCs) is followed by a prolonged slow component. Furthermore, the declining phase is often "noisy" because of the stochastic channel openings contributing to it (O'Donnell and van Rossum, 2014). In the next paragraphs, evidence underlining the NMDAR- and astrocyte-dependence of these events will be presented (**Table 1** and **Figure 2**)

Evidence for the NMDA receptor dependence

It is generally accepted that SICs are mediated by NMDARs. More specifically, extrasynaptic NMDARs that often contain GluN2B subunits are involved in SIC generation.

Properties of NMDARs in the central nervous system have been discussed by several review papers in detail (e.g. Papouin and Oliet, 2013; Ge and Wang, 2023; Ladagu et al., 2023; Yu et al., 2023). Briefly, NMDARs are present in a postsynaptic, presynaptic, and extrasynaptic location on neurons and are also found on non-neuronal cells as astrocytes or microglia (Ravikrishnan et al., 2018; Alsaad et al., 2019; Raghunatha et al., 2020; Rivas-Santisteban et al., 2021). These receptors exist as di- or triheterotetramers, which always consist of GluN1 subunits with the addition of various GluN2A-D and GluN3A-B subunits. It has been generally thought that the GluN2B-containing NMDA receptors are extrasynaptic, whereas the ones with GluN2A subunits are of synaptic location (Ladagu et al., 2023; Yu et al., 2023). However, this view was challenged by findings showing that GluN2B subunits can form synaptic NMDARs as well (Pegasiou et al., 2020), and other subunits as GluN2D can replace GluN2B in the extrasynaptic NMDARs (Bardoni et al., 2010). Furthermore, there is trafficking between extrasynaptic and synaptic NMDAR pools (McQuate and Barria, 2020; Chiu et al., 2019). Other important features of NMDARs are the virtual voltage dependence by Mg^{2+} blockade and the existence of co-activators (such as glycine and D-serine; Papouin and Oliet, 2013; Ge and Wang, 2023).

In most publications, the significance of NMDARs in SICs was tested with the application of non-specific NMDAR antagonists as D-(-)-2-amino-5-phosphonopentanoic acid (D-AP5, APV) or dizocilpine hydrogen maleate (MK801; **Table 1**). The involvement of the GluN2B subunit is based on the inhibitory actions of subunit-specific NMDAR inhibitors (1R*,2S*)-erythro-2-(4-benzylpiperidino)-1-(4-hydroxyphenyl)-1-propanol (ifenprodil) and (αR,βS)-α-(4-hydroxyphenyl)-β-methyl-4-(phenylmethyl)-1-piperidinepropanol (Ro 25-6981; Shigetomi et al., 2008; Nie et al., 2010; Reyes-Haro et al.,

Table 1 | **Experimental design of papers describing features of SICs**

Reference	NMDA receptor activation	Astrocytic activation	How were SICs elicited?
Araque et al., 1998	SIC frequency is significantly reduced by D-AP5 and CNQX and D-AP5 together abolishes SICs*	Direct electrical stimulation of astrocytes and detection of calcium waves*	
Parri et al., 2001	APV fully eliminates SICs	Spontaneous and t-ACPD (mGluR agonist)-evoked Ca^{2+} oscillations of astrocytes correlate with SIC appearance	Spontaneous Ca^{2+} wave activity of smaller astrocytic networks (up to 5). Astrocytic activity is evoked by t-ACPD (mGluR agonist). TTX did not influence the astrocytic activity.
Fellin et al., 2004	D-AP5 largely reduces SIC frequency	Calcium uncaging in astrocytes which triggered calcium wave activity in them	Hippocampal SICs had increased frequency after Schaffer collateral stimulation.
Angulo et al., 2004	Incomplete inhibition by AP5	Mechanical stimulation of astrocytes. Bafilomycin A1 (vacuolar proton pump inhibitor) treatment did not affect SICs	
Tian et al., 2005	Significant reduction of the events by APV/CNQX cocktail	Direct activation of astrocytes by filling and selective uncaging of NP-EGTA, which increased intracellular Ca^{2+} levels selectively	
Kang et al., 2005	Almost full reduction of SICs by APV/CNQX cocktail	Direct and selective activation of a single astrocyte with IP3 containing patch pipette; recording of the transporter current	
Kozlov et al., 2006	Complete inhibition of SICs by MK-801	SICs were elicited in the presence of TTX and bafilomycin A1 (vacuolar proton pump inhibitor)	Hypoosmotic environment, cyclic stretch of the blood vessels
Shigetomi et al., 2008	Decrease but not full elimination by APV and ifenprodil	Fluoroacetate preincubation diminishes SICs, BAPTA perfusion of astrocytes fully eliminated it	PAR1 receptor activation elicited increased astrocytic calcium wave activity and SICs (P2Y1 receptor activation elicits astrocytic calcium activity but not SIC activity)
Nie et al., 2010	D-AP5 fully abolishes SICs, the GluN2B-specific inhibitor Ro 25-6981 diminished them	Fluorocitrate incubation largely diminished SIC activity	Stimulation of the spinal dorsal root (sensory) fibers acutely and directly elicited SICs; AMPA receptor blockade decreases the frequency of SICs. TBOA induced tonic inward currents and SICs together.
Bardoni et al., 2010	D-AP5 diminished SIC frequency	The purinergic receptor agonist BzATP and low extracellular calcium level eliciting SICs increased astrocytic calcium waves and synchronized neuronal calcium transients	Peripheral inflammation significantly increased SIC frequency in dorsal horn lamina II neurons
Reyes-Haro et al., 2010	Blockade of SICs by APV, MK801, ifenprodil	Recorded astrocytic calcium wave activity preceding SICs	Electrical stimulation of the synapse nearby the astrocyte, which elicits an inward current and increase of calcium wave activity on astrocytes
Parri et al., 2010	Decrease of the evoked SIC occurrence by D-AP5 and ifenprodil	Generation of SICs correlates with astrocytic calcium activity. Perfusion of astrocytes with BAPTA significantly reduced SIC frequency.	Prolonged stimulation of the somatosensory and cortical inputs of the thalamus evokes increased astrocytic and SIC activity. Astrocytes are subjects of mGluR-mediated non-neuronal plasticity
Pirttimaki and Parri, 2012	Full abolishment of SICs by D-AP5		Short term synaptic stimulation failed to change SIC frequency, but prolonged stimulation increased it.
Navarrete et al., 2013	D-AP5 diminishes SIC frequency	ATP-induced astrocytic calcium waves elicit synchronized neuronal activity with delay	Spontaneous SIC activity on human neocortical and hippocampal samples from epileptic patients
Perea et al., 2014	Almost full inhibition of SIC frequency by D-AP5	Selective optogenetic stimulation of astrocytes via ChR2 and their consequential increase in calcium wave activity	
Lauderdale et al., 2015	AP-5 significantly reduced SIC frequency	SICs were resistant to bafilomycin A1; hypoosmotic solution mainly caused astrocytic and not neuronal swelling	Besides sporadical detection of spontaneous SICs, these events were elicited by hypoosmotic solutions
Pirttimaki et al., 2017	D-AP5 and ifenprodil significantly reduce SIC activity elicited by glutamate pre-exposure	Astrocytic activity synchronized neuronal calcium responses which are induced by SICs	Pre-exposure of slices with glutamate and D-aspartate.
Kovács and Pál, 2017	Not full reduction of SIC activity by D-AP5 and ifenprodil	Selective optogenetic activation of astrocytes by ChR2, consequential increase of their calcium wave activity	
Gómez-Gonzalo et al., 2018	Significant reduction of SIC frequency by D-AP5		Spontaneous or swelling-induced SICs
Wu et al., 2018	APV and MK-801 significantly reduce SIC frequency	Dialysis of astrocytes with BAPTA reduces SIC frequency. IP3R knockout mice have reduced SIC frequency	Spreading depolarization is provoked by increased extracellular K^+ concentration. Spreading depolarization evokes SICs via GABAB receptor activation and IP3R activation.
Zhang et al., 2019	Almost full inhibition by ifenprodil	Inhibition of SICs by fluorocitrate; facilitation of SICs by the mGluR I. agonist DHPG	
Mederos et al., 2019	D-AP5 almost fully inhibited SICs	Optogenetic activation of astrocytes with the G-protein-coupled melanopsin	
Walch et al., 2022	Almost full inhibition by DL-AP5	SIC activity is related to astrocytic swelling	Astrocytic swelling induced by elevated extracellular potassium concentration
Csemer et al., 2023	D-AP5 fully inhibits, ifenprodil diminished SICs in mice. The ifenprodil and the D-AP5 diminish SIC activity in humans.	Chemogenetic activation of murine astrocytes, which elicits increased Ca^{2+} wave activity. EAAT2 (astrocyte-specific glutamate transporter) inhibitor significantly increased SIC activity in humans.	Spontaneous human and murine SICs

* Under cell culture conditions. BAPTA: 1,2-Bis(2-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid; BzATP: 2'(3')-O-(4-Benzoylbenzoyl)adenosine-5'-triphosphate tri(triethylammonium) salt; ChR2: channelrhodopsin-2; CNQX: 6-cyano-7-nitroquinoxaline-2,3-dione; D-AP5: APV:D-(-)-2-amino-5-phosphonopentanoic acid; EAAT2: excitatory amino acid transporter 2; MK-801: dizocilpine hydrogen maleate; Ro-25-6981: (αR)-(4-Hydroxyphenyl)-(βS)-methyl-4-(phenylmethyl)-1-piperidinepropanol hydrochloride hydrate; SIC: slow inward current; t-ACPD: (±)-1-aminocyclopentane-trans-1,3-dicarboxylic acid; TTX: tetrodotoxin.

2010; Pirttimaki et al., 2017; Zhang et al., 2019; Csemer et al., 2023). Intriguingly, as mentioned above, some papers referred elimination of SICs by non-specific NMDAR inhibitors (Parri et al., 2001; Kozlov et al., 2006; Nie et al., 2010; Reyes-Haro et al., 2010; Pirttimaki and Parri, 2012; Csemer et al., 2023), whereas others found some remaining SICs with NMDAR inhibition (**Table 1**). The existence of NMDAR inhibitor-resistant SICs might have different reasons. First, all recordings were performed in brain slices in which the diffusion of drugs to their targets is limited compared to a cell culture or acutely isolated cells. There might be extrasynaptic NMDAR clusters that are better insulated by astrocytic processes or the extracellular matrix. Second, probably other receptors can contribute to events mimicking SICs. One candidate might be the AMPA receptor, as some studies needed AMPA receptor-NMDAR inhibitor cocktails to fully eliminate or significantly reduce SICs (Tian et al., 2005; Kang et al., 2005; Araque et al., 1998). Metabotropic glutamate receptors (mGluRs) might also directly contribute to SICs (Nicoletti et al., 2011). Activation of other neurotransmitter receptors (e.g. 5-HT3 receptor) can elicit SIC-like currents (Saito and Sugimura, 2023).

The extrasynaptic nature of the NMDARs involved in SIC generation is supported by several experimental data. Probably the most abundantly used experimental approach is the inhibition of SICs by GluN2B-specific inhibitors. Argumentation based on this is roughly valid as –even if synaptic GluN2B-containing NMDARs exist too– the majority of the GluN2B has an extrasynaptic location. It is worth mentioning that only a 30% reduction of the NMDAR-mediated EPSCs was reported when ifenprodil was applied (Ladagu et al., 2023), whereas an almost full inhibition of SICs was achieved by this drug. However, sometimes the GluN2B-specific inhibitors exert weaker action than the non-specific ones, probably because these are less effective on triheteromeric NMDARs than on diheteromers (Ge and Wang, 2023; Stroebel et al., 2018). However, the theory that SICs are always mediated by GluN2B subunits is weakened by a study in which the subunit-specific inhibitor did not affect SICs of the spinal cord (Bardoni et al., 2010). The GluN2D subunit is abundant in the spinal cord and it might form the extrasynaptic NMDARs in this region.

Another argument supporting the extrasynaptic origin of SICs is the slow and variable kinetics of these events (Angulo et al., 2004; Fellin et al., 2004) as well as the long latency of SICs evoked by synaptic stimulation (Nie et al., 2010), which indicates longer and variable diffusion pathways of glutamate than in the synapses.

An elegant demonstration that SICs have extrasynaptic origin is the stimulation of synaptic inputs and the application of the use-dependent NMDAR inhibitor MK-801. After the elimination of NMDAR-mediated evoked EPSCs by this drug, SICs could be still triggered (Nie et al., 2010).

It might also be a matter of debate, whether SICs are elicited by glutamate release. One argument supporting this is that SICs are always radically inhibited or fully eliminated in the presence of ionotropic glutamate receptor antagonists. However, NMDAR co-activators such as glycine and D-serine might also contribute to SICs. It was shown that D-serine alone can elicit a noisy inward current with magnitudes smaller amplitude than glutamate, but it is capable of boosting the amplitude of the glutamate-induced SICs (Stevens et al., 2003; Lauderdale et al., 2015).

Actions of glutamate transporter inhibitors on SICs (see below) also support the notion that SICs are elicited by glutamate release. Furthermore, it was also demonstrated that glutamate pre-exposure of the brain tissue increased SIC frequency (Pirttimaki et al., 2017).

Taken together, the role of glutamate in the formation of SICs is well supported by several experiments. However, the ultimate evidence for it could be the coincident detection of extrasynaptic glutamate release (e.g. by iGluSnFR or sniffer cells; Armbruster et al., 2020; Zielewicz et al., 2023; Koh et al., 2022) and the occurrence of SICs on neurons.

In conclusion, it is well established that SICs are mediated by extrasynaptic, GluN2B containing NMDARs activated by glutamate. However, NMDARs composed of other subunits (such as GluN2D) can occur in some regions of the central nervous system and NMDAR co-activators also have a secondary but still important role in the formation of SICs.

Evidence for astrocytic involvement

SICs are thought to be the consequence of astrocytic release of glutamate as a gliotransmitter (**Table 1**), which was shown by using various but sometimes contradictory experiments.

The contribution of astrocytes to SICs is typically demonstrated by showing a coincidence between the increase in calcium wave activity and the generation of SICs on neurons (Araque et al., 1998; Parri et al., 2001; 2010; Fellin et al., 2004; Tian et al., 2005; Bardoni et al., 2010; Navarrete et al., 2013; Perea et al., 2014; Kovács and Pál, 2017; Pirttimaki et al., 2017; Wu et al., 2018; Csemer et al., 2023). This calcium wave activity was often stimulated with various methods as calcium uncaging (Fellin et al., 2004; Tian et al., 2005), electrical stimulation of astrocytes (Araque et al., 1998), loading astrocytes with IP3 (Kang et al., 2005), or application agonists of mGluR- (Parri et al., 2001; Zhang et al., 2019), PAR1 (Shigetomi et al., 2008) and purinergic receptors (Bardoni et al., 2010; Navarrete et al., 2013). Others inhibited astrocytic calcium waves that diminished SICs. This inhibition was achieved with BAPTA loading of astrocytes (Shigetomi et al., 2008; Parri et al., 2010; Wu et al., 2018) or using IP3R knockout mice (Wu et al., 2018; also note contradictory data below by Gomez-Gonzalo et al., 2018). Inhibition of astrocytic metabolism by fluoroacetate or fluorocitrate led to a similar inhibitory action on SIC generation (Shigetomi et al., 2008; Nie et al., 2010; Zhang et al., 2019).

Another approach serving with indirect evidence on astrocytic role in SICs is to inhibit neuronal activity by TTX (Kozlov et al., 2006; Kovács and Pál, 2017) or to inhibit vacuolar proton pump by bafilomycin A1 to terminate synaptic vesicle exocytosis (Angulo et al., 2004; Kozlov et al., 2006). Interpretation of the results with vesicle release inhibitors has its caveats as these drugs reduced the surface expression of NMDA- and AMPA receptors and inhibited non-vesicular glutamate release (Woo et al., 2012, 2020).

Probably the most selective ways of astrocytic activation are the opto- or chemogenetic stimulations. For the first optogenetic experiments for astrocytic activation, channelrhodopsin-2 (ChR2) was used as an actuator (Perea et al., 2014; Kovács and Pál, 2017). As ChR2 is a non-selective cation channel causing influx of Na^+, Ca^{2+}, and H^+ and outflow of K^+, is not a physiological way of astrocytic stimulation. To provide a more physiological, G-protein coupled way of selective optogenetic activation of astrocytes, melanopsin was recently used as an actuator which also significantly increased SIC frequency (Mederos et al., 2019). Similar to this, the chemogenetic approach is the selective expression of the human modified muscarinic M3 receptor (hM3D(Gq)) on astrocytes (Csemer et al., 2023).

Excitatory amino acid transporter 2 (EAAT2) glutamate transporter inhibition is also a rather specific tool for assessing astrocytic contribution, as EAAT2 can be considered the main astrocytic glutamate transporter (Csemer et al., 2023). Using non-specific glutamate transporter inhibitors can serve as an acceptable approach for assessing the contribution of astrocytic glutamate uptake, as astrocytes are responsible for the majority of this process (Todd and Hardingham, 2020).

It has been extensively demonstrated that astrocytes significantly contribute to the generation of SICs. However, the exact ways they do it are not consistently known. The possible reason is that astrocytes elicit SICs in a complex way depending on the context of the activation, the brain area, and probably several other factors. Astrocytes possess neurotransmitter receptors which can be stimulated by neurotransmitters released as a consequence of neuronal network activity (Verkhratsky and Chvátal, 2020; Kofuji and Araque, 2021). Short-term stimulation of the somatosensory and cortical inputs of the thalamus was not related to SICs, but 20–30 minutes of long stimulation led to a long-term increase in SIC frequency (Parri et al., 2010; Pirttimaki and Parri, 2012). Stimulation of the spinal dorsal root acutely and directly drove SIC activity (Nie et al., 2010). In the hippocampus, evoked synchronized neuronal activity was likely elicited by SICs (Fellin et al., 2004). Synaptic activity activating AMPA receptors was also capable of increasing SIC activity in the medial nucleus of the trapezoid body via acting on the regional astrocytes (Reyes-Haro et al., 2010).

Pathological conditions can also increase SIC frequency. On the dorsal horn lamina II neurons, peripheral inflammation enhanced SIC activity (Bardoni et al., 2010). Spreading depolarization was also followed by an increase in SIC frequency in a $GABA_B$ receptor and IP3R-dependent way (Wu et al., 2018).

Probably one of the hot topics regarding this issue is the mechanism of how astrocytes release gliotransmitters. An increase in intracellular calcium wave activity is an important hallmark of astrocytic activation, which is shown to be linked with gliotransmitter release, more specifically glutamate release via vesicle exocytosis (Bohmbach et al., 2018; see Savtchouk and Volterra, 2018). However, numerous studies did not support the existence of this way of glutamate release (see Fiacco and McCarthy, 2018). In a recently published paper, it was elegantly demonstrated that both conclusions can be true at the same time as the vesicular exocytosis is related to a well-defined astrocytic subgroup, whereas other astrocytes cannot release glutamate in this way (de Ceglia et al., 2023).

Out of vesicular exocytosis, astrocytes release glutamate or contribute to the regulation of ambient glutamate in various ways. The diverse ways of the release include connexin/pannexin hemichannels, volume-regulated anion channels, organic anion transporters, ionotropic purinergic receptors or bestrophin-1 channels (see Pál, 2018), as well as TREK-1 channels (Woo et al., 2020). Furthermore, astrocytes can clear extrasynaptic glutamate via EAAT transporters (mostly via EAAT2 with some contribution of EAAT1; Valtcheva and Venance, 2016; Csemer et al., 2023) and inhibition of this mechanism effectively increases glutamate levels of the extrasynaptic space; thus, it might appear as glutamate "release".

It is worth noting that triggering astrocytic calcium wave activity does not always elicit SICs. PAR1 receptor activation led to an SIC frequency increase, whereas P2Y1 receptor activation –which enhanced astrocytic calcium activity in a similar way as PAR1– did not generate extra SICs compared to the control activity (Shigetomi et al., 2008).

Blockade of astrocytic calcium signaling with various methods did not fully eliminate SICs and mechanisms not closely related to calcium signaling can also provoke SIC occurrence. Mechanical stimulation of astrocytes and hypoosmotic conditions increased SIC frequency in a non-neuronal way and altered SIC parameters (Angulo et al., 2004; Lauderdale et al., 2015; Gomez-Gonzalo et al., 2017). Knocking out IP3R did not affect SIC frequency, but the hypoosmotic environment increased it (Gomez-Gonzalo et al., 2018; Walch et al., 2022). Deletion of an astrocytic VRAC channel subunit decreased SIC amplitude elicited under hypoosmotic conditions and the amplitude of a tonic NMDA current (Yang et al., 2019). Optogenetic activation of astrocytes via ChR2 likely causes astrocytic swelling, intracellular acidification, and depolarization of the neighboring neurons by the increase of extracellular K^+ concentrations besides the direct activation of calcium waves by calcium influx (Octeau et al., 2019). This multiple way of triggering astrocytic activity slowed down the kinetics of SICs evoked in this way (Kovács and Pál, 2017).

One might assume that, although SICs have a more-or-less similar appearance, at least two types of them might be distinguished. Spontaneously –and infrequently– occurring SICs might be consequences of vesicular glutamate release, whereas the prolonged events insensitive to manipulations of astrocytic calcium signaling are due to procedures associated with cell swelling and brain edema or inhibition of astrocytic glutamate clearance.

This theory is supported by a few findings: SICs elicited by a hypoosmotic environment had greater amplitude and slower kinetics than the spontaneous ones (Lauderdale et al., 2015). DL-threo-β-Benzyloxyaspartic acid (DL-TBOA; EAAT inhibitor) administration also significantly increased the decay tau of SICs (Fellin et al., 2004). Similarly, optogenetically evoked SICs by using a ChR2 actuator had significantly slower kinetical

properties (Kovács and Pál, 2017). Weakening the hypothesis, Perea et al. (2014) did not observe differences between the parameters of spontaneous and optogenetically evoked SICs.

Taken together, astrocytes can be stimulated via increased network activity and neurotransmitter release, as well as swelling and inflammation. Consequential astrocytic glutamate release via vesicle exocytosis and swelling-related mechanisms are both capable of generating SICs. However, SICs elicited by different mechanisms of glutamate release might be distinct from each other.

Functions of Slow Inward Currents at a Cellular Level

SIC as a synchronizer of neurons

SICs are generally thought to synchronize smaller populations of neighboring neurons by depolarizing them simultaneously. 53% of the paired recordings on hippocampal neurons revealed synchronized SIC activity at least once during the recording (Angulo et al., 2004). In the neocortex, hippocampus, and thalamus, neurons form groups of synchronization. An astrocyte releasing glutamate as a gliotransmitter can activate a population of local neurons and a single neuron can be activated by more astrocytes. This means that synchronized neuronal groups can overlap, i.e. a neuron can participate in more groups (Pirttimaki et al., 2011; Pirttimaki et al., 2017).

In certain structures, SICs excite (and probably synchronize) neurons in a cell type-specific way. In the olfactory bulb, SICs did not appear on mitral cells, but were found on granule cells (Kozlov et al., 2006). In the visual cortex, optogenetic astrocyte activation (which elicited SICs) had different actions on parvalbumin-positive or excitatory and somatostatin-positive neurons. In the parvalbumin-positive ones, an increase of spontaneous firing was only seen, whereas an increase and a decrease were both seen in other cell types (Perea et al, 2014). In contrast, no significant difference was seen in SIC activity on GABAergic and cholinergic neurons of the pedunculopontine nucleus (Kovács and Pál, 2017).

No synchronization by SICs was seen in the brainstem (medial nucleus of the trapezoid body, Reyes-Haro et al., 2010; pedunculopontine nucleus, Kovács and Pál, 2017). Therefore, one might assume that the synchronization of neurons by SICs in an astrocytic domain seems to be a feature of the rostral brain areas. However, this theory was challenged by the findings in the spinal cord that an increase in astrocytic calcium wave activity (triggered by factors provoking SIC activity) led to the synchronization of neuronal calcium transients (Bardoni et al., 2010).

In conclusion, SICs can directly synchronize smaller neuronal groups (**Figure 3A** and **B**). However, it is not an overall rule but seems to depend on cell type and central nervous system area.

SIC as a regulator of synaptic plasticity

It has been predicted in review papers and modeling studies that SICs are capable of influencing synaptic plasticity based on different timings between pre- and postsynaptic stimuli (Carmignoto and Fellin, 2006; Wade et al., 2011; De Pittá and Brunel, 2016).

Astrocytes (via their calcium signaling and consequential release of gliotransmitters) are known regulators of long-term synaptic plasticity evoked “canonically” by neuronal pre- and postsynaptic stimulation (Falcón-Moya et al., 2020; Naranjo et al., 2020; Pereira et al., 2021; Sancho et al., 2021; Hösli et al., 2022; Martinez-Gallego et al., 2022). Recent studies demonstrated that astrocytic activity alone was sufficient to induce long-term potentiation or depression, although synaptic plasticity by SICs was not directly described by them (Adamsky et al, 2018; Cavaccini et al, 2020; Maltsev et al, 2023).

It was also recently shown that SICs as electrical signals can elicit long-term changes in synaptic plasticity in a timing-dependent way. If SICs followed evoked EPSCs, synaptic depression was induced, if the SICs took place at the same time or preceding EPSCs, synaptic facilitation was seen. However, these observations seemed to be only valid for mice as facilitation or lack of actions can be observed in humans (Csemer et al, 2023; **Figure 3C**).

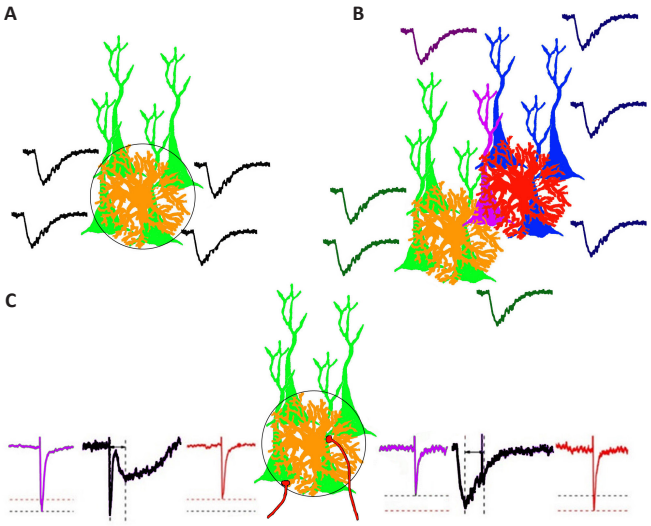


Figure 3 | **Cellular actions of SICs.**
(A) SICs simultaneously occur on neurons in the same astrocytic domain (Carmignoto and Fellin, 2006). (B) The same neuron can be activated by different astrocytes and can be a member of different synchronized neuronal groups (green: neuronal group 1; yellow: astrocyte belonging to the first neuronal group; blue: neuronal group 2; red: astrocyte belonging to the second neuronal group; purple: the neuron belonging to both groups; SICs with various colors belong to the neurons indicated with similar colors; Pirttimaki et al., 2017). (C) SICs cause timing-dependent plasticity probably depending on the position of the synapse within the astrocytic domain (red objects: excitatory inputs and synapses in the astrocytic domain). If SICs follow EPSCs, the EPSC amplitudes are reduced; whereas SICs preceding EPSCs lead to an increase in amplitude. Different timing between SICs and EPSCs might occur at different regions of the astrocytic domain (Csemer et al., 2023). Traces were recorded by my laboratory and modified with Adobe Photoshop. EPSC: Excitatory postsynaptic current; SIC: slow inward current.

SICs are subject to plasticity and aging

There is experimental evidence that SICs show certain forms of non-neuronal plasticity. Prolonged stimulation of the excitatory inputs (including the lemniscal and cortical inputs delivering somatosensory or associative information) targeting the ventrobasal thalamus increased the astrocytic Ca^{2+} wave activity and SIC frequency. Such an increase in astrocytic and

SIC activity could not be triggered by shorter stimulation of synaptic inputs indicating that acute afferent activity does not drive SIC activity (Pirttimaki et al., 2011; Pirttimaki and Parri, 2012). Of note, generation of these events happens when the thalamus has tonic activity and is distinct from the burst activity by T-type calcium channel activation. This activity might represent an astrocyte-driven activity pattern of the thalamus, by which persistent sensory stimulation can alter its responsiveness (Pirttimaki et al., 2011).

Another way of plasticity affecting SICs that these events have a refractory period of 30 seconds in the spinal cord. If two SICs appeared within this time frame, the amplitude of the second SIC was decreased (Nie et al., 2010).

SICs are also subject to aging. SICs are conserved with aging in mice, with a few age-dependent alterations. First, the frequency of SICs elicited by mGluR stimulation was reduced with age, which can be the consequence of the reduction of mGluR5 expression or the decline in the proportion of astrocytes with Ca^{2+} wave activity (Parri et al., 2001; Gomez-Gonzalo et al., 2017). Second, SIC amplitude increased and the rise time decreased during maturation of the mouse neocortex (Csemer et al., 2023). In contrast to mice, SICs were severely affected by aging in humans, as SICs had decreased charge transfer and faster kinetics with aging and fully disappeared from the age of 70 years (Csemer et al., 2023). The background of these changes is probably complex. Astrocytes undergo morphological changes with age, as the complexity and the volume of astrocytic processes are reduced (Robillard et al., 2016; Popov et al., 2021), which decreases the tortuosity of glutamate diffusion pathways leading to faster kinetics of SICs (Sykova and Vargova, 2008). Astrocytic glutamate transporter and GluN2B subunit expression was reduced with age (Potier et al., 2010; Gramuntell et al., 2021; Csemer et al., 2023) which can contribute together to the decline in SIC activity.

Significance of Slow Inward Currents on Larger-Scale Neuronal Networks

SICs are very likely to influence neuronal excitability and network properties. In the following, I aimed to present various electrical phenomena, which might be related to SICs or should be distinguished from them. Furthermore, when discussing the pathophysiological significance of the SIC-related events represented below provides insights into the pathophysiological significance of the SICs as well.

Paroxysmal depolarization shift

The paroxysmal depolarization shift (PDS) is a brief neuronal depolarizing event with slower kinetics than action potentials. An initial action potential is typically seen at the upstroke of the event, followed by a plateau-like phase with smaller spikes or membrane potential oscillations on its top. PDS is known as the cellular correlate of the interictal spikes in EEG (Kubista et al., 2019). Although the typical experimental protocol to elicit this activity is the inhibition of $GABA_A$ receptors, a set of various other ion channels as AMPA receptors (Nikolaev et al., 2021; Larushkin et al., 2023), L-type calcium channels and NMDA receptors (Kubista et al., 2019; Meyer et al., 2021) contributed to it. Despite the heterogeneity of the receptors in its background, according to certain previous studies, PDS is considered as the voltage change elicited by SICs (Kang et al., 2005; Silchenko and Tass, 2008).

Later, it was well demonstrated that these depolarizing events are AMPA receptor-dependent and only the plateau of these events was determined by NMDA receptor activation (Nikolaev et al., 2021; Larushkin et al., 2023). Furthermore, experimentally evoked ictal and interictal electrical activity under *in vitro* conditions was inhibited by TTX whereas the SICs were unaffected; indicating that SICs might only modulate but not generate epileptiform activity (Fellin et al., 2006).

Based on these findings, it can be hypothesized that PDS is not equal to the voltage change elicited by SICs, but a mixture of neuronal network activity and SICs driven by the increased activity of excitatory synapses.

Although PDSs are likely not identical with voltage changes elicited by SICs but electrical phenomena to which SICs contribute, in the following, I briefly depict the pathophysiological significance of PDSs. From these findings, the pathophysiological role of SICs might be indirectly demonstrated.

PDSs/interictal spikes are often seen between periods of epileptic discharges or preceding the onset of focal and acquired epilepsy. There is a great body of evidence supporting that these events are responsible for epileptic seizure generation; but an anti-epileptic role of them is also considered (Kubista et al., 2019). Selective deletion of VGLUT1 vesicular glutamate transporter in hippocampal astrocytes prolonged the duration of provoked epileptic seizures (de Ceglia et al., 2023).

PDS/interictal spikes (IIS) appear in Alzheimer's disease causing impairment of short-term memory and leading to cognitive disruption (Kubista et al., 2019). In a clinical study involving epileptic patients, the occurrence of IISs was correlated with cognitive impairment and memory decline (Camarillo-Rodriguez et al., 2022).

Out of these indirect demonstrations of the contribution of SICs to cognitive functions, it was directly shown that astrocytic calcium wave activity and SICs are affected by cocaine addiction (O'Donovan et al., 2021). Hallucinogens such as LSD –besides its widespread network actions– might probably also elicit SICs in the prefrontal cortex (Aghajanian, 2009).

IIS is also linked to childhood autism. IIS induction leads to autism-like symptoms in an animal model (Kubista et al., 2019). In line with this finding, it was also demonstrated that the increased SIC activity in juvenile transgenic mice overexpressing the MeCP2 gene (known as the autism model) had increased SIC activity. This increased giant SIC activity in 2 weeks old animals returned to the level of wild-type control in 3 weeks old mice. In parallel with the increased SIC activity, hypersynchrony of the neural networks was observed, whereas it turned to hyposynchrony with the development. Aberrant neuronal synchrony is well known to exist in the autistic brain (Keehn et al., 2013; d'Albis et al., 2018). It

is very likely that SICs do not cause changes in neuronal synchronization but amplify it as contributors to a hyperactive astrocyte-neuron feedback loop (as gap junction channel blockers inhibit SIC activity and hypersynchrony in juvenile animals and TTX abolished high SIC activity; Xia and Xu, 2022). Dopaminergic neurons of the substantia nigra displayed PDS during the pathogenesis of Parkinson's disease (Kubista et al., 2019). It might be in line with recent observations that knocking out astrocytic vesicular glutamate release in this area led to the enhancement of EPSC amplitude and an increase in neostriatal dopamine release (de Ceglia et al., 2023).

The spreading depolarization

Cortical spreading depolarization (or depression as an electroencephalographic term; CSD) is a traveling cortical depolarization wave from the penumbra around the lesioned cortical area. This depolarization wave has a causal role in the expansion of excitotoxic lesions (Hinzman et al., 2015; Seidel et al., 2015; Kramer et al., 2016). The ion homeostasis of neurons and astrocytes is heavily disturbed as the increase in intracellular Na^+ and Ca^{2+} concentration is accompanied by extracellular K^+ accumulation. Consequential cellular swelling also contributes to the phenomenon. Restoring the ion homeostasis and normal excitability has a high energy demand, therefore, damage to the affected cells is likely (Lauritzen et al., 2011; Kramer et al., 2016; Wu et al., 2018).

This phenomenon is related to stroke, epileptic seizures, traumatic brain injury, migraine, or subarachnoid hemorrhage, whereas it can be experimentally elicited by increased extracellular K^+ concentration, oxygen-glucose deprivation, or hypoosmotic stress (Kramer et al., 2016; Frank et al., 2021; Menyhárt et al., 2021).

A phenomenon leading to CSD is the ischemia-evoked inward current. It can be recorded on neurons under similar conditions that elicit spreading depolarization (e.g. oxygen- and glucose deprivation). The current has two components: a fast component in the nanoamper range followed by a steady state component (Soria et al., 2016; Wendt et al., 2016).

CSD and ischemia-induced inward currents share some similarities with SICs. First, these phenomena are consequences of GluN2B-containing NMDA receptor activation (Kramer et al., 2016; Soria et al., 2016; Wendt et al., 2016; Crivellaro et al., 2021; Alvarez-Merz et al., 2022). Second, astrocytic glutamate release or inhibition of astrocytic glutamate uptake has a crucial role in it (Hinzman et al., 2015; Seidel et al., 2015; Crivellaro et al., 2021; Frank et al., 2021; Alvarez-Merz et al., 2022). Third, the age dependence of SICs and CSD is similar (Hertelendy et al., 2019).

In contrast, the arguments that ischemic currents are not exaggerated SICs might be that CSD is always related to well-defined pathological conditions whereas SICs can repeatedly occur under conditions that can be considered as more-or-less physiological. Astrocytic activation is not the initial cause eliciting it, but tissue damage and extracellular potassium increase precede the generation of this phenomenon and reactive astrocytes only exacerbate its progress (Seidel et al., 2015). Furthermore, SICs and CSD might be detected together and can be well distinguished from each other (Wu et al., 2018).

Taken together, CSDs use the same "machinery" as SICs: astrocytic activation, consequential glutamate release, and activation of GluN2B-containing extrasynaptic glutamate receptors serve as backgrounds. The two events might depend on each other: after CSD generation, astrocytic calcium activity is boosted, which, in turn, increases SIC frequency (Wu et al., 2018).

Physiology of SIC, PDS, or similar depolarizing events: does it exist?

Modeling studies raised the possibility that SICs/PDSs elicit spike timing-dependent plasticity which was later supported by experimental evidence (Wade et al., 2011; De Pittá and Brunel, 2016; Csemer et al., 2023). Neighboring neurons (four neocortical pyramidal neurons per astrocytic domain) displayed synchronized activity without being synaptically coupled when SICs/PDSs affect them (Silchenko and Tass, 2008).

An argument supporting the theory of physiologically occurring SICs is that they can be recorded under control conditions in brain slices; although with a low frequency (below 0.1 Hz; Csemer et al., 2023). However, one can argue that brain slices do not represent physiological conditions as there are damaged cells on the surface of the slice, which might alter the K^+ concentration in their neighborhood. To my knowledge, there is no *in vivo* recording demonstrating that SICs exist in the intact brain. If we consider SICs as events occurring under physiological conditions as well, their possible role can be the contribution to some forms of synaptic plasticity e.g. long-term potentiation or homeostatic plasticity. However, the efficacy of this contribution can be questioned by the low occurrence rate of this event.

Optogenetic activation of astrocytes in the visual cortex elicited SICs on neurons detected under *in vitro* conditions. Using an *in vivo* approach, the same optogenetic stimulation affected neuronal firing in a cell type-specific way and altered the visual response properties. Therefore, astrocytic activation and probably also SICs might be involved in sensory information processing (Perea et al., 2014). In peripheral inflammation where mechanical allodynia and thermal hyperalgesia developed, SICs occurred in a greater frequency on dorsal horn lamina II neurons. It might indicate that SICs contribute to the control of pain transmission under inflammatory conditions by contributing to pain sensitization (Bardoni et al., 2010).

Although not directly focused on SICs, rather on the astrocytic vesicular glutamate release, a recent study might let us closer to understand the physiological significance of astrocytic glutamate signaling (and SICs, within this). If astrocytic vesicular glutamate release was knocked out in the hippocampus, the long-term potentiation of the perforant path-granule cell synapses was weakened. In line with this, mice showed impaired memory formation in the contextual fear-conditioning memory test (de Ceglia et al., 2023). This finding is in accordance with modeling and experimental

studies on SICs, where these events alone were capable of inducing synaptic plasticity (Wade et al., 2011; De Pittá and Brunel, 2016; Csemer et al., 2023) and reduction of extrasynaptic NMDA currents including SICs was accompanied with learning and memory deficits (Yang et al., 2019). However, astrocytic glutamate has widespread actions on long-term synaptic plasticity than SIC formation and probably several glutamatergic actions contribute to the recent observations on memory impairment.

Conclusions and Research Questions to Be Addressed

At the cellular or local network level, the theory, which considers astrocytes as non-neuronal excitatory "neurons", can still stand according to the recent data as well. In this model, astrocytes integrate the neuronal network activity as the long-term increase of it activates them. Astrocytes, in turn, release glutamate and elicit SICs on the neurons lying in the astrocytic domains of activated astrocytes. SICs cause synchronized depolarization in a neuronal population (Carmignoto and Fellin, 2006; Fellin et al., 2007).

In certain points, the model might be refined according to some of the recent findings. First, not only the neuronal excitability, but the synaptic strength is also set by SICs which probably further contributes to synchronization of local neuronal networks (Csemer et al., 2023). Second, the role of SICs and their dependence on long-lasting activity is not universal in the whole central nervous system and important rostrocaudal or region-specific differences might exist (Nie et al., 2010; Reyes-Haro et al., 2010; Kovács and Pál, 2017). Third, astrocytic glutamate release and SICs do not always cause synchronized excitation (Kovács and Pál, 2017). In some cases, inhibitory actions can be probably seen on the network level, or a "balancing" function of SICs is found, as the appearance of SICs provoked by actions leading to astrocytic activation are context-dependent: in case of previously high SIC activity, actions normally eliciting SICs inhibit their occurrence (Kovács and Pál, 2017; Csemer et al., 2023).

For future research on SICs, one important question is still the physiological significance of these events. All recordings were performed on brain slices and kept under physiological conditions as the model allowed it. However, these pieces of brain tissue are isolated from their inputs, networks are cut and cells are damaged. All of these manipulations might provoke some SIC activity as these events often occur in response to pathological situations. *In vivo* demonstration of the existence of SICs in an extracellular milieu with physiological Mg^{2+} concentration is also missing. Another important question is whether all SICs can be considered as the same events. There is a high variability in amplitude, kinetics, ability to synchronize neuronal networks, and probably most importantly, in ways of astrocytic activation eliciting them. Understanding SICs is also important for understanding the pathogenesis of those diseases to which SICs potentially contribute.

Search Strategy

Publications of the PubMed database with the term "slow inward current" were included regardless of the publication date. Original papers describing basic findings of the topic were included together with papers representing novel research findings. For those topics that are important for understanding the main scope of this review but not directly related to it, important and recent review papers were cited.

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