



Synaptic transmission and excitability during hypoxia with inflammation and reoxygenation in hippocampal CA1 neurons

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ARTICLE INFO

Article history:

Received 17 January 2018

Received in revised form

19 April 2018

Accepted 8 May 2018

Keywords:

Hypoxia

Inflammation

Reoxygenation

Synaptic transmission

Hyperexcitability

Hyperpolarization-activated cation current

(I_h)

ABSTRACT

Although a number of experimental and clinical studies have shown that hypoxia typically accompanies acute inflammatory responses, the combinatorial effect of the two insults on basic neural function has not been thoroughly investigated. Previous studies have predominantly suggested that hypoxia reduces network activity; however, several studies suggest the opposite effect. Of note, inflammation is known to increase neural activity. In the current study, we examined the effects of limited oxygen in combination with an inflammatory stimulus, as well as the effects of reoxygenation, on synaptic transmission and excitability. We observed a significant reduction of both synaptic transmission and excitability when hypoxia and inflammation occurred in combination, whereas reoxygenation caused hyperexcitability of neurons. Further, we found that the observed reduction in synaptic transmission was due to compromised presynaptic release efficiency based on an adenosine-receptor–dependent increase in synaptic facilitation. Excitability changes in both directions were attributable to dynamic regulation of the hyperpolarization-activated cation current (I_h) and to changes in the input resistance and the voltage difference between resting membrane potential and action potential threshold. We found that zatebradine, an I_h current inhibitor, reduced the fluctuation in excitability, suggesting that it may have potential as a drug to ameliorate reperfusion brain injury.

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1. Introduction

Sufficient oxygen supply is necessary for appropriate brain functions, such as consciousness, voluntary movement, and higher-level brain function (Gao et al., 2014; Tzour et al., 2017; Lennox et al., 1935; Rossen et al., 1943; Sugar and Gerard, 1938). However, despite numerous studies on the mechanisms of cell death resulting from the effects of the hypoxia (Pfisterer and Khodosevich, 2017; Walton et al., 1999), the impact of these pathophysiological conditions on basic neural functions, such as synaptic transmission and excitability, remains rather controversial (Doolette and Kerr, 1995; Fujiwara et al., 1987; Zanelli et al., 2015). In addition, the physiological mechanisms by which brain damage is exacerbated during subsequent restoration of oxygen supply are

still unclear (The NINDS t-PA Stroke Study Group, 1997).

Neuroinflammation is one of the most common phenomena in many neurological disorders (Block and Hong, 2005), including epilepsy (Vezzani, 2014); degenerative diseases, such as Alzheimer's disease (Remarque et al., 2001), Parkinson's disease (Kannarkat et al., 2013), multiple sclerosis (Centonze et al., 2009), and Huntington's disease (Zoghbi, 2006); and traumatic brain injury (Makinde et al., 2017; Webster et al., 2017). In particular, in hypoxia, the activity of the oxygen-sensing enzyme prolylhydroxylase (PHD) is inhibited by decreased oxygen availability (Kaelin and Ratcliffe, 2008), which in turn increases the stability of transcription factors, such as hypoxia-inducible factor (HIF) and nuclear factor- κ B (NF- κ B), that regulate inflammation (Eltzschig and Carmeliet, 2011; Eltzschig and Eckle, 2011; Taylor, 2008). Consequently, hypoxia increases inflammatory responses. In fact, even brief (5–10 min) and focal ischemic insults have been shown to induce inflammatory responses (Gelderblom et al., 2009; Zhan et al., 2010). On the other hand, accumulating lines of evidence have suggested that such inflammatory responses, in turn, alter the blood-brain barrier (BBB) in disruptive and non-disruptive

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Abbreviations

I _h	Hyperpolarization-activated cation current
PHD	Prolylhydroxylase
HIF	Hypoxia-inducible factor
NF-κB	Nuclear factor-κB
BBB	Blood-brain barrier
LPS	Lipopolysaccharide
TNF-α	Tumor-necrosis factor-alpha
SD	Interleukin-1 beta (IL-1β) Sprague-Dawley
aCSF	Artificial cerebrospinal fluid
IR-DIC	Infrared differential interference contrast
Hypo-Inf	Hypoxia with inflammation
EPSCs	Excitatory postsynaptic currents
TTX	Tetrodotoxin
AP	Action potential
RRP	Readily releasable pool

DPCPX	8-Cyclopentyl-1,3-dipropylxanthine
VGCC	Voltage-gated Ca ²⁺ channel
mEPSC	Miniature EPSC
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
NMDA	N-methyl-D-aspartate
AMPA receptors	AMPA receptors
EPSC _{AMPA}	EPSC mediated by AMPA receptors
EPSC _{NMDA}	EPSC mediated by NMDA receptors
RMP	Resting membrane potential
R _{in}	Input resistance
Δ; V _{thresh}	Voltage difference between the RMP and AP threshold
ISI	Inter-spike intervals
HCN channel	Hyperpolarization-activated cyclic nucleotide-gated channel

manners (Varatharaj and Galea, 2017). Further, hypoxia often occurs with the reduced cerebral blood flow that accompanies many inflammatory brain states, such as neurodegeneration (Peers et al., 2009).

Studies show that ischemic hypoxia decreases synaptic transmission and excitability in CA1 pyramidal neurons *in vivo* and *in vitro* (Boissard and Gribkoff, 1993; Fujiwara et al., 1987; Fujimura et al., 1997; Chi and Xu, 2000; Lipton and Whittingham, 1982). In contrast, studies have indicated that neuroinflammation enhances neuronal network activity (Gao et al., 2014; Pickering et al., 2005; Tancredi et al., 1992; Tzour et al., 2017). In fact, neuroinflammation induced by lipopolysaccharide (LPS) application to hippocampal brain slices reduced the Kv7-dependent potassium current, resulting in increased excitability (Tzour et al., 2017). Further, pro-inflammatory cytokines alter gating properties of voltage-gated ion channels, leading to hyperexcitability (for review Vezzani and Viviani, 2015), and reduced release of pro-inflammatory cytokine interleukin-1 beta (IL-1β) decreases acute seizure activity (Ravizza et al., 2006). Inflammation has also been shown to contribute to increased excitatory synaptic transmission. In particular, the proinflammatory cytokine tumor-necrosis factor-alpha (TNF-α) has been shown to increase excitatory but not inhibitory synaptic transmission by increasing the number of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors at synapses (Beattie et al., 2002; Stellwagen, 2005; Tancredi et al., 1992; Pickering et al., 2005). Moreover, proinflammatory cytokines slow AMPA receptor closure (Centonze et al., 2009, 2010) and increase the Ca²⁺ permeability of N-methyl-D-aspartate (NMDA) receptors (Viviani et al., 2003), which collectively causes greater depolarization upon synaptic transmission. Therefore, an evaluation of the combined effects of hypoxia and inflammation is still needed to enable estimation of the true net effect of ischemic hypoxia on neuronal functions.

In addition to the initial damage a limited oxygen supply causes to brain tissue, further damage occurs upon reoxygenation (Choi, 1988; Eltzschig and Eckle, 2011; Sanderson et al., 2013; Zhang and Murphy, 2007). Studies suggest that reperfusion-induced cellular injury is mediated by an abrupt increase in the intracellular Ca²⁺ concentration and excitability of neurons (Choi, 1985; Doolette and Kerr, 1995; Fan et al., 2008; Noh et al., 2005; Yellon and Hausenloy, 2007). However, the precise causal mechanism of this hyperexcitability remains elusive, which obstructs development of protective measures that can be put in place before reperfusion to prevent further brain damage.

In the present study, we examined the net effect of simultaneous hypoxia and inflammation and of reoxygenation on excitatory synaptic transmission and excitability.

2. Methods

2.1. Slice preparation

Acute hippocampal slices were prepared from Sprague-Dawley (SD) rats aged 12–21 postnatal days (n = 64) and ~8–9 weeks (young adult) (n = 10). All animal care and treatment protocols were approved by the Animal Care and Use Committee of the Korea Brain Research Institute. Rats were decapitated after euthanasia with CO₂; subsequently, the brains were rapidly removed and placed in an ice-cold artificial cerebrospinal fluid (aCSF) solution of the following composition (in mM): 119 NaCl, 2.5 KCl, 26 NaHCO₃, 1.25 NaH₂PO₄, 20 glucose, 2 CaCl₂, 1 MgSO₄, 0.4 ascorbic acid, and 2 pyruvic acid. Coronal slices (300 μm) were prepared using a vibratome (Leica, Germany) and were incubated at 32 °C for at least 30 min. During preparation of acute slices, the solution was oxygenated with 95% O₂ and 5% CO₂.

2.2. Electrophysiological recordings

The acute hippocampal slices were transferred to a submerged recording chamber with a continuous flow of aCSF saturated with carbogen (95% O₂, 5% CO₂) for whole-cell patch-clamp recordings. Slices were visualized by infrared differential interference contrast (IR-DIC) microscopy using a BX51WI upright microscope (Olympus, Japan) through a water-immersed 40× objective lens (numerical aperture 0.8). Patch electrodes with 3–5 MΩ tip resistances were prepared using a pipette puller (Shutter Instrument, USA) and filled with an internal solution as follows. For the current clamp experiment, we used an internal solution containing (in mM) 20 KCl, 125 K-gluconate, 10 HEPES, 4 NaCl, 0.5 EGTA, 4 ATP, 0.3 TrisGTP, and 10 phosphocreatine (pH 7.2, ~290–300 mOsm); for the voltage clamping experiment, we used an internal solution containing (in mM) 135 CsMS, 0.2 EGTA, 10 HEPES, 0.9 MgCl₂, 10 Tetraethylammonium Chloride (TEA-Cl), 0.9 Mg²⁺ ATP, 0.3 Na⁺ GTP, 20 phosphocreatine (pH 7.2, ~290–300 mOsm), and 100 μM Picrotoxin in the bath.

2.2.1. Synaptic transmission

To evoke synaptic activity in the Schaffer collateral pathway, we

electrically stimulated (0.2 Hz for basal transmission and 10 Hz for short-term plasticity measurement) with a stimulation electrode placed in the CA1 stratum radiatum. To maintain recording quality during hypoxia with inflammation (Hypo-Inf) and reperfusion, small voltage steps (−5 mV, 100 ms) were applied periodically to measure the seal resistance, and recordings were discarded if the seal resistance was changed by >30% from the initial measurement. To establish the relationship between the paired-pulse ratio and the size of excitatory postsynaptic currents (EPSCs), two consecutive stimuli were delivered with 100 ms intervals every 5 s during Hypo-Inf and reperfusion.

To measure spontaneous synaptic transmission, neurons were voltage clamped at −70 mV in the presence of 0.5 μ M tetrodotoxin (TTX). The paired-pulse ratio throughout the course of hypoxia and reoxygenation was measured using two consecutive stimuli delivered 100 ms apart and was calculated as the ratio of the EPSC amplitude produced by the second stimulus to that produced by the first stimulus.

2.2.2. Excitability

To measure the excitabilities of hippocampal CA1 neurons, various current pulses (−200 to 200 pA) were injected for 600 ms each through recording pipettes, and the resulting changes in membrane potential were assessed. Subthreshold excitabilities were monitored by observing voltage sags that are typically generated by activation of I_h channels, and suprathreshold excitabilities were monitored by measuring repetitive action potential (AP) frequencies and AP threshold during injection of positive-current pulses.

All electrophysiological data were recorded using an Axopatch 700B amplifier (Molecular Devices, Foster City, CA), and command pulse generation was performed using Digidata 1550A (Molecular Devices, Foster City, CA).

2.2.3. Hypoxia with inflammation (Hypo-Inf)

Hypo-Inf was stimulated by perfusion with 10 μ g/ml LPS containing aCSF saturated with 8% O_2 , 5% CO_2 , and 87% N_2 . Once EPSCs or excitability was stabilized (~15–20 min), the perfusion solution was changed back to normally oxygenated aCSF (95% O_2 and 5% CO_2) to examine the effects of reoxygenation.

2.3. Data analysis and statistics

Data acquisition and analysis were performed using Clampfit 10.4 (Axon Instruments, Foster City, CA) and AxoGraph X (AxoGraph, Canberra, Australia). Igor Pro software (Wavemetrics, Lake Oswego, OR), Matlab (Mathworks, Natick, MA), or MS Excel (Microsoft, Redmond, WA) software were used for further data analysis and to determine statistical significance. The relationship between the paired-pulse ratio and the initial amplitude of EPSCs was described as a quadratic polynomial curve using a *fit* function, and 95% prediction intervals were calculated using the *predint* function in Matlab (Mathworks, Natick, MA). Template-matching event detection was used to detect mEPSCs. Events with slow activation ($\tau_{\text{activation}} > 2$ ms) and wide half-widths (>10 ms) were removed from statistical analysis.

For statistical analysis of EPSCs, we used the Wilcoxon Signed Rank test, paired *t*-test, or Smirnov-Kolmogorov test. Statistical significance of neuronal excitabilities (frequency, resting membrane potential, threshold, etc.) were examined using the paired *t*-test. Statistical significance of the effects of zatebradine on hypoxia and reoxygenation was tested using the paired *t*-test, two-way ANOVA, or one-way ANOVA, as indicated in the figure legends. In all cases, significance was accepted for *p* values <0.05, and *p* values <0.01 or <0.001 are indicated on the data plots. Error bars in

all figures indicate standard error unless noted otherwise.

3. Results

Hypoxia and inflammation are known to be mutually inducible (Huang et al., 2006; Iadecola et al., 2004; Zlokovic, 2011). However, few studies have examined the net effect of the two insults on basic neural function (Batti and O'Connor, 2010; Mukandala et al., 2016; Zhang et al., 2014). Thus, in the present study, we aimed to evaluate the net effect of the combined insults of hypoxia with inflammation and of reoxygenation on synaptic transmission and excitability.

We first examined the combinatorial effect of hypoxia and inflammation on basal synaptic transmission. CA1 pyramidal neurons in the hippocampus were voltage clamped at −70 mV, while synaptic transmission was elicited by electrical stimulation of Schaffer collateral axons at the stratum radiatum. After establishing a stable baseline of EPSCs, we applied LPS (10 μ g/ml) in hypoxic aCSF (aCSF saturated with 8% O_2 instead of 95%; this combination is denoted as Hypo-Inf, hereafter). This dose of LPS has previously been shown to increase excitatory synaptic transmission and excitability through acutely increased inflammatory cytokines and inhibition of Kv7/M channels (Gao et al., 2014; Tzour et al., 2017). As shown previously (Zhang et al., 2014), we found that the amplitude of EPSCs was reduced by $81.6 \pm 2.0\%$ and reached a plateau within ~10 min (Fig. 1). EPSC amplitude recovered to the control level ~10 min after reoxygenation (Fig. 1).

Reduction of synaptic transmission could be due to at least three phenomena: (1) reduced neurotransmitter-release efficiency, (2) decreased number of AMPA receptors at the postsynaptic density, and/or (3) decreased excitability of neurons; in the last case, electrical stimulation of the same strength would cause fewer APs, thereby evoking reduced synaptic transmission. To differentiate between these possible mechanisms, we first examined the effects of Hypo-Inf on presynaptic neurotransmitter-release efficiency.

3.1. Reversible reduction in presynaptic release efficiency by Hypo-Inf

We first analyzed short-term synaptic plasticity to determine if the reduced synaptic transmission originated from presynaptic release efficiency (Fig. 2 and Supplementary Fig. 1). Short-term plasticity represents the balance between synaptic facilitation by residual Ca^{2+} at the synaptic nanodomain and depression by depletion of the readily releasable pool (RRP) of synaptic vesicles (Schneggenburger and Rosenmund, 2015; Regehr, 2012). If Hypo-Inf decreases the release probability, a slower depletion of the RRP would be expected, thereby resulting in a more pronounced facilitation during tetanic stimulation. Alternatively, if the reduced EPSC was due to postsynaptic factors, no change of short-term plasticity would be expected. Short-term synaptic plasticity during 10 Hz tetanic stimuli was examined both under the control condition and under the Hypo-Inf condition (Fig. 2). Under the control condition EPSCs were facilitated by ~2–3 stimuli, then reduced steadily, in corroboration with previous reports (Jackman et al., 2016); in contrast, we observed dramatically enhanced synaptic facilitation with minimal depression in the presence of the Hypo-Inf insult (Fig. 2A and B). EPSCs under Hypo-Inf were facilitated for the initial ~5–7 stimuli, and maximum facilitation was significantly greater than that under the control condition (Fig. 2A and B). Cumulative normalized EPSCs during tetanic stimulation illustrated the difference more dramatically (Supplementary Fig. 1).

To rule out the possibility that this altered short-term plasticity profile may have been due to a reduced signal-to-noise ratio, we measured the short-term plasticity with increased stimulation strength. For this, once the EPSC was stabilized in the presence of

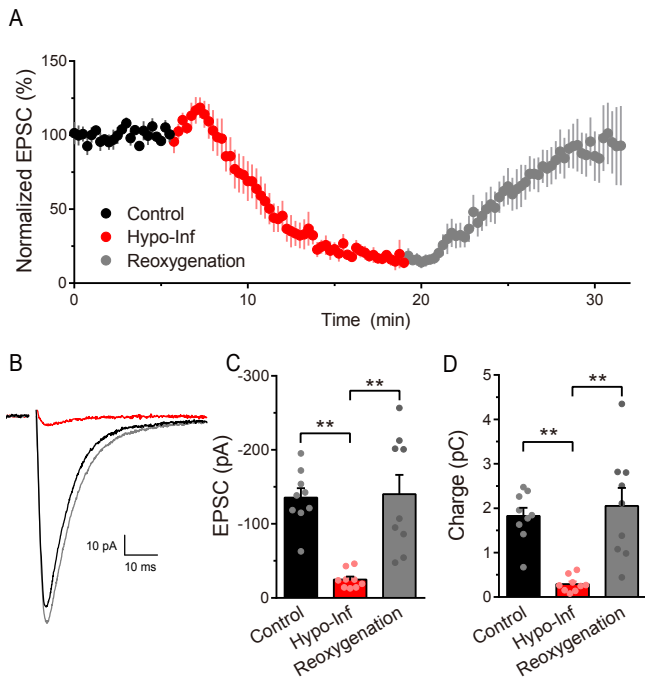


Fig. 1. Hypo-Inf-induced decrease of basal synaptic transmission. (A) Normalized EPSC during Hypo-Inf and reoxygenation. Perfusion of aCSF with LPS (10 μ g/ml) saturated with reduced oxygen concentration (8% O_2) decreased synaptic EPSCs by $81.6 \pm 2.98\%$. The depression was fully reversed after reperfusion with normal aCSF (saturated with 95% O_2). (B) Sample traces were taken from three averaged points: control (black), Hypo-Inf (red), and reoxygenation (gray). Scale bars represent 10 pA and 10 ms (C and D). Mean EPSC amplitudes and charges measured during control, Hypo-Inf, and Reoxygenation (** $p < 0.01$, Wilcoxon signed rank test).

Hypo-Inf, the sizes of the EPSCs were restored back to the control level by increasing the stimulation strength, and the short-term plasticity was remeasured. Under this condition, the same pattern of short-term plasticity was observed; hence the data were pooled (Fig. 2B). Short-term plasticity measured after recovery of the EPSC amplitude was indistinguishable from that under the control condition (Fig. 2B), suggesting that indeed the reduction of EPSC was due to reduced release probability and not to non-specific changes in the recording condition.

Previous studies showed that synaptic depression in hypoxia is mediated by the presynaptic neuromodulator adenosine (Brust et al., 2006; Choi et al., 2015; Dunwiddie and Masino, 2001; Gervitz et al., 2001; Mukandala et al., 2016). To examine if adenosine plays the same role in Hypo-Inf, we tested whether 8-Cyclopentyl-1,3-dipropylxanthine (DPCPX), a selective adenosine A1 receptor antagonist, prevents reduction of synaptic transmission (Supplementary Fig. 2A and B). Indeed, the Hypo-Inf-induced synaptic depression was significantly attenuated ($60.6 \pm 12.8\%$ of EPSC) in the presence of 200 nM DPCPX. Furthermore, enhanced synaptic facilitation by Hypo-Inf was restored by DPCPX (Supplementary Fig. 2C). These findings strongly suggest that reduced synaptic transmission is, at least in part, mediated by the A1 Receptor. Of note, A1 adenosine receptors inhibit voltage-gated Ca^{2+} channels (VGCC) through the $G_{i/o}$ -coupled signaling pathway (McCool and Farroni, 2001; Palmer and Stiles, 1995) and thereby reduce efficiency of synaptic transmission in hypoxia (Gervitz et al., 2001). We did not find a significant change in the rate of spontaneous neurotransmitter release (miniature EPSC or mEPSC) in spite of the dramatic reduction in EPSC amplitude (Supplementary Fig. 3). Since inhibition of VGCC does not affect mEPSC frequency (Vyleta and Smith, 2011) but reduces evoked EPSCs, our finding supports the hypothesis that the depression we observed can be

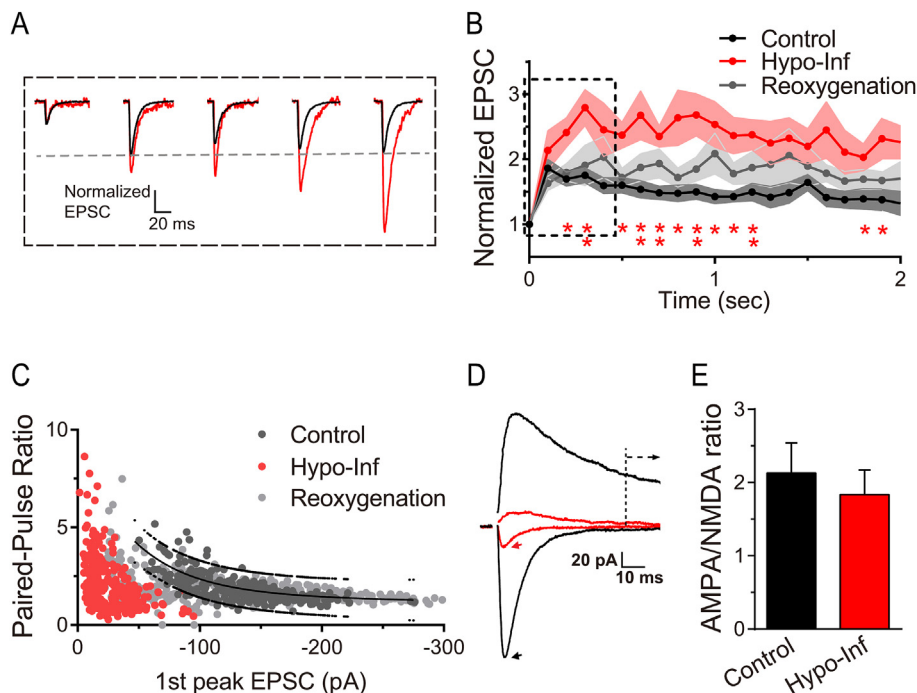


Fig. 2. Hypo-Inf-increased synaptic facilitation without significant difference in EPSC_{AMPA}/EPSC_{NMDA} ratio. (A) Representative sample traces of the first five normalized EPSC traces during 10 Hz stimulation in control (black) and Hypo-Inf (red). Scale bar = 20 ms and normalized initial EPSC amplitude. (B) Mean normalized amplitudes of EPSCs during 10 Hz stimulation in control (black), Hypo-Inf (red), and reoxygenation (gray) (** $p < 0.01$, * $p < 0.05$, paired t -test). (C) Relationship between the EPSC amplitude and paired-pulse ratio of control (black), Hypo-Inf (red), and reoxygenation (gray). Black solid line and dots are quadratic polynomial curve fit with 95% confidence bounds. (D) Sample EPSC traces at -70 mV (inward) for measuring EPSC_{AMPA} current (solid line arrows) and at +40 mV holding (outward) for estimating EPSC_{NMDA} current (dotted line arrow) in control (black) and in Hypo-Inf (red). Scale bars: 20 pA, 10 ms. EPSC_{AMPA} were measured as the peak current of EPSCs, while the neuron was voltage-clamped at -70 mV. EPSC_{NMDA} was defined by mean current at 40 mV between 50 and 70 ms after the peaks of EPSC_{AMPA}. (E) Average EPSC_{AMPA}/EPSC_{NMDA} ratio of D.

attributed to A1 adenosine receptor–dependent VGCC inhibition.

If the reduced synaptic transmission is solely due to presynaptic release efficiency, then the paired-pulse ratio in the Hypo-Inf condition should be in line with the relationship between EPSCs and the paired-pulse ratio of the baseline condition. To test this, two consecutive stimuli were delivered with a 100 ms interval while measuring the amplitude of EPSCs under control, Hypo-Inf, and reoxygenation conditions. When paired-pulse ratio was plotted as a function of the first amplitude of the two EPSCs, EPSCs and the paired-pulse ratio under the basal condition showed a strong negative relationship (black solid line in Fig. 2C, see section 2.3: Data analysis and Statistics). On the other hand, the relationship between EPSCs and the paired-pulse ratio measured during the Hypo-Inf insult formed a discrete cluster that was significantly left-shifted compared with the 95% prediction interval (Fig. 2C). These results indicate that abridged release efficacy significantly contributes to, but may not be the sole cause of, the reduced synaptic transmission induced by Hypo-Inf.

To examine if dynamic trafficking of postsynaptic AMPA receptors (AMPARs) may be involved in synaptic depression induced by Hypo-Inf (Zhang et al., 2014), we examined the relative contribution of AMPARs to the EPSCs. The amplitude of AMPAR-mediated synaptic current cannot be directly compared across different slices because the numbers of stimulated synapses can vary even if stimulation strength is set to the same value. Thus, we assessed the relative post-synaptic responses mediated by AMPA receptors (EPSC_{AMPA}) to that mediated by NMDA receptors (EPSC_{NMDA}). The ratio of EPSC_{AMPA} to EPSC_{NMDA} forms a linear correlation with postsynaptic strength (Takumi et al., 1999; Nusser et al., 1998; Freund et al., 1985) because the number of AMPARs is dynamically regulated by the history of activity, whereas the number of NMDA receptors remains relatively static (Matsuzaki et al., 2001; Kidd and Isaac, 2001). Therefore, the relative number of AMPARs at synapses can be assessed using EPSC_{NMDA} as an internal control. Further, if reduction of transmission is partly due to a reduced number of AMPARs at synapses, then a smaller EPSC_{AMPA}-to-EPSC_{NMDA} ratio is expected, independent of the number of synapses stimulated by electrical field stimulation. We found no significant difference in the EPSC_{AMPA}-to-EPSC_{NMDA} ratio, suggesting that the reduced synaptic current induced by the Hypo-Inf insult was not due to a reduced number of AMPARs at the synapses (Fig. 2D and E). This conclusion was further supported by unchanged mEPSC amplitude, charge, and kinetics (Supplementary Fig. 3C–G).

Thus, the decreased synaptic transmission induced by the Hypo-Inf insult is due to reduced efficacy of presynaptic neurotransmitter release but not to AMPAR internalization.

3.2. Reduced excitability induced by hypoxia-inflammation and hyperexcitability induced by reoxygenation

We examined possible changes in the excitability of neurons induced by Hypo-Inf and reoxygenation. Membrane properties and excitability under the control condition were in agreement with results from previous studies of pyramidal neurons in the hippocampal CA1 (Yang et al., 2013). Under the control condition, the resting membrane potential (RMP) was -63.1 ± 0.7 mV and the input resistance (R_{in}), calculated as the slope of voltage change as a function of injected current, was 102.4 ± 3.9 M Ω . Upon 100 pA depolarizing current injection, we observed a series of mildly adapting APs with relatively regular firing frequency (15.7 ± 1.0 Hz, Fig. 3A and B, Supplementary Table 1).

In corroboration with a previous studies (Fujiwara et al., 1987; Gao et al., 2014; Tzour et al., 2017), we found that 10 μ g/ml LPS

increased the excitability of hippocampal neurons acutely (Supplementary Fig. 4), whereas hypoxia alone decreased excitability (Supplementary Table 2). Upon Hypo-Inf treatment, we found that the AP frequency evoked by the same current injection was reduced to $\sim 1/3$ with similar time course with reduction of basal transmission (6.1 ± 1.5 Hz, $p < 0.01$, Fig. 3B). Further, subsequent reoxygenation caused a dramatic increase in the AP frequency compared with the control condition (22.6 ± 1.1 Hz, $p < 0.01$, Fig. 3B).

Hyperexcitability in response to reperfusion after hypoxia alone was reported previously (Levin et al., 2010; Levin and Godukhin, 2009; Schiff and Somjen, 1985; Turovsky et al., 2013). To examine the cause of this change in excitability during hypoxia and reoxygenation, we measured RMP, AP threshold, and R_{in} (Fig. 3D). During hypoxia, the R_{in} was reduced to 94.5 ± 5.1 M Ω ($p < 0.01$, Fig. 3D; Supplementary Table 1) and the membrane was hyperpolarized to -66.7 ± 0.9 mV ($p < 0.01$), leading to an increased voltage change requirement for evoking an AP (voltage difference between the RMP and AP threshold, called ΔV_{thresh} hereafter), which was 19.5 ± 0.6 mV in the control condition and 22.7 ± 1.2 mV in the Hypo-Inf condition ($p < 0.01$, Fig. 3E). Upon reoxygenation, in drastic contrast, ΔV_{thresh} was significantly reduced (17.6 ± 1.1 mV in the reperfusion condition, $p < 0.05$, Fig. 3D) and the R_{in} was significantly increased (reperfusion: 109.5 ± 6.0 M Ω ; control: 102.4 ± 3.9 M Ω ; $p < 0.01$, Fig. 3D) compared with the control condition. As expected based on the changes in ΔV_{thresh} and R_{in} , the mean injected current required to evoke an AP changed: 70 ± 6.6 pA in control, 113.3 ± 7.7 pA in Hypo-Inf. During reoxygenation, the smallest current injection examined (50 pA) evoked one or more APs in all cases (Supplementary Table 1).

We next examined whether changes in these parameters were in temporal agreement with the alterations in AP frequency. Indeed, variations of R_{in} and ΔV_{thresh} were found to correlate strongly with changes in AP frequency (Fig. 3B and E). We also examined shape and adaptation pattern of repetitive APs during Hypo-Inf and reoxygenation compared with the control condition. To visualize the shapes of the APs, we plotted the voltage of AP against its time derivative (hereafter phase-plane plot) (Supplementary Fig. 5A) (Jenerick, 1963). This plot showed a hyperpolarized resting membrane potential under the Hypo-Inf condition (downward arrows). A lowered AP threshold (downward arrows in Supplementary Fig. 5A inset) and a reduced spike potential were evident under the reoxygenation condition (upward arrows in Supplementary Fig. 5A and B). Further, we noticed that the half-width of the AP was lengthened under the Hypo-Inf condition (Supplementary Fig. 5C). To describe this pattern of adaptation, the inter-spike intervals (ISI) of the initial 10 APs were measured. Significantly greater adaptation was observed in the Hypo-Inf condition and less adaptation was observed in the reoxygenation condition (Supplementary Fig. 5D and E). However, a mildly adapting firing pattern remained unchanged in both conditions.

Because our results to this point were acquired from very young animals (12–21 postnatal days), we next examined whether the observed changes in excitability and synaptic transmission were age-specific. In particular, we examined if the effects of Hypo-Inf and reoxygenation on neural excitability were similar in young adult animals (8–9 weeks). Indeed, we observed very similar phenomena between these two age groups, namely decreased excitability induced by Hypo-Inf and hyperexcitability induced by reoxygenation (Supplementary Fig. 6). These results suggest that the observed changes in excitability are a general response to Hypo-Inf and reoxygenation, rather than age-specific responses.

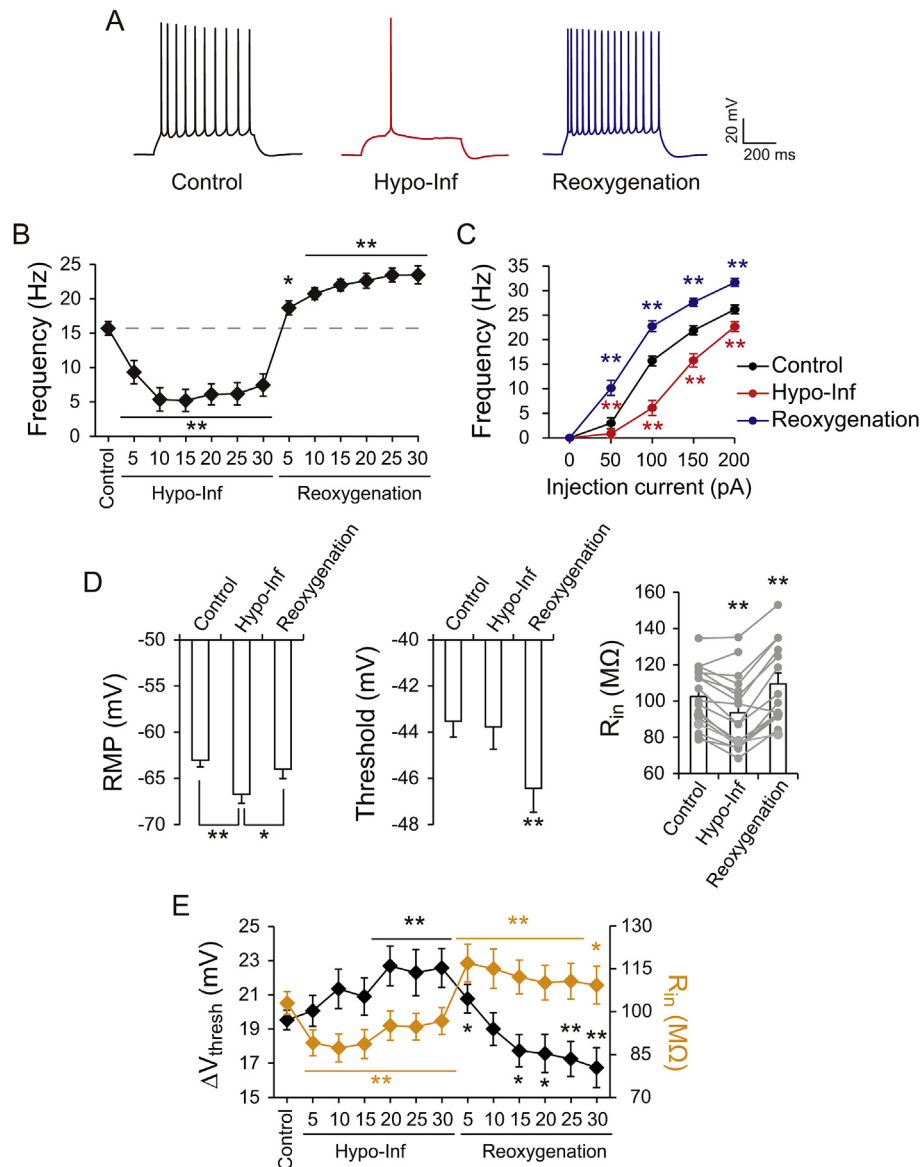


Fig. 3. Effects of Hypo-Inf and reoxygenation on neuronal excitability in hippocampal CA1 pyramidal neurons. (A) Sample traces of evoked APs by 100-pA current injections for 600 ms. Each trace shows control (left), 20 min after Hypo-Inf washing-in (middle), and 20 min after washing-in of normally oxygenated aCSF (right). Scale bars: 20 mV, 200 ms. (B) The frequency of AP induced by 100-pA current injection as a function of time, during control, Hypo-Inf, and reoxygenation. (C) AP frequencies induced by various injected currents for 600 ms. (D) Average resting membrane potentials (RMPs), threshold of AP measured from the first AP by current injection, and input resistance (R_{in}). (E) Voltage difference (black) between RMP and AP threshold (ΔV_{thresh} , black) as a function of time during Hypo-Inf, followed by reoxygenation. The orange color shows changes of R_{in} . Significance was determined using the paired *t*-test (**p* < 0.05, ***p* < 0.01 compared with control).

3.3. I_h current in Hypo-Inf and reoxygenation

We observed a pronounced depolarizing voltage sag in response to hyperpolarizing current injection, as well as a hyperpolarizing sag on termination of the current injection (Fig. 4A). A previous study has indicated that depolarizing/hyperpolarizing voltage sags result from hyperpolarization-activated cyclic nucleotide-gated channel (HCN channel) activation and deactivation, respectively (Robinson and Siegelbaum, 2003). Corroborating this previous study, the voltage sags we observed with the initiation and cessation of hyperpolarizing current injection were both eliminated by the selective HCN channel antagonist, zatebradine (10 μ M, Fig. 5), in accordance with previous reports (Gill et al., 2004; Shahi et al., 2014; Stieber, 2006; Zhang et al., 2016).

We also tested whether dynamic regulation of I_h activity

contributes to the observed AP frequency change. HCN channel activation during re-polarization of AP has been reported to provide the inward current that drives successive AP generation (Robinson and Siegelbaum, 2003), which subsequently determines discharge patterns and frequency in hippocampal CA1 neurons (Gasparini and DiFrancesco, 1997) and in other types of neurons (Lüthi and McCormick, 1998). If I_h is the source of the AP frequency change, then an I_h current amplitude of that covaries with AP frequency can be expected. To test this idea, we compared the I_h current produced by a 200 pA hyperpolarizing current injection. The I_h amplitude was defined by subtracting the mean steady state current from the peak amplitude of the sag current. The voltage sag amplitude was dramatically reduced in the presence of Hypo-Inf and then increased upon reoxygenation (8.8 ± 0.6 mV in control, 6.3 ± 0.7 mV in Hypo-Inf, *p* < 0.01, and 9.90 ± 0.87 mV in

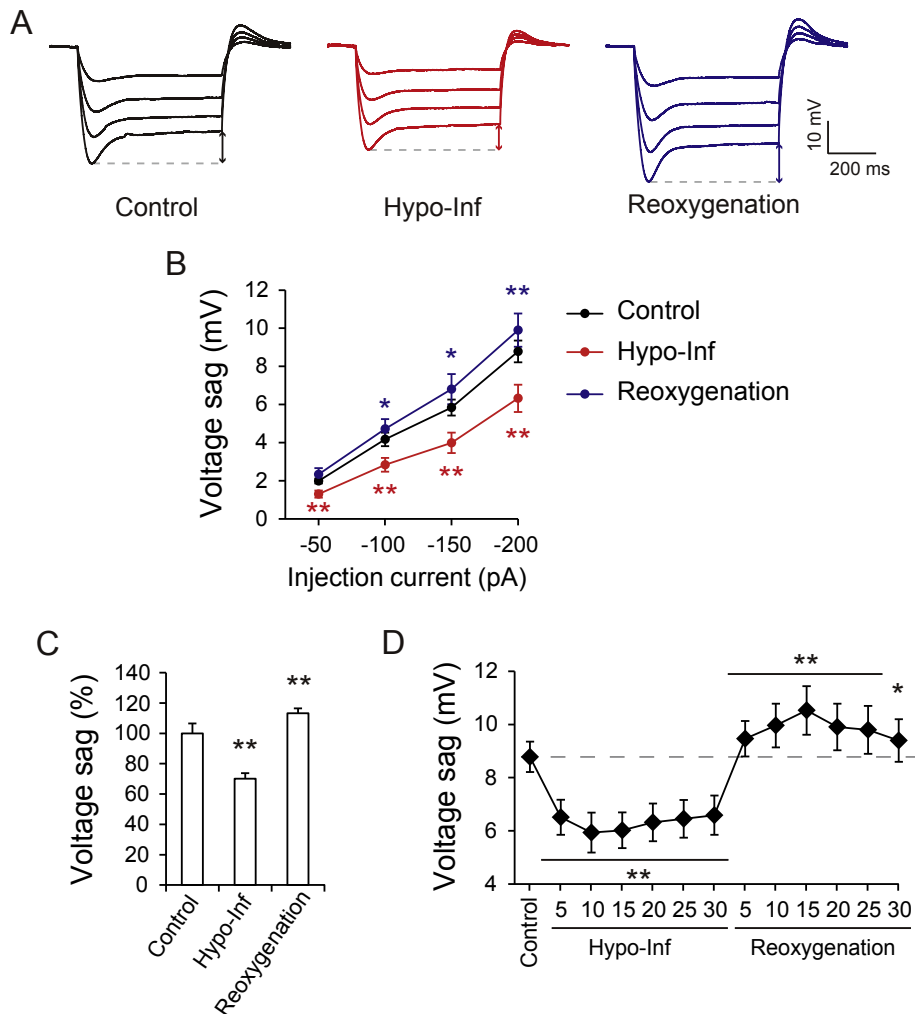


Fig. 4. Voltage sag regulation by Hypo-Inf and reoxygenation. (A) Sample voltage traces induced by negative current pulse (from -200 pA to -50 pA with 50 pA steps for 600 ms). Double-headed arrows indicate the size of voltage sag. Scale bars: 10 mV, 200 ms. (B) I_h current measured by voltage sags as a function of injected current, in control (black), Hypo-Inf (red), and reoxygenation (blue). (C) Amplitude of voltage sag during Hypo-Inf and reoxygenation. (D) Normalized voltage sags in control, Hypo-Inf, and reoxygenation. Error bars represent standard error. Statistical significance is presented as $*p < 0.05$ or $**p < 0.01$ (paired t -test compared with control).

reoxygenation; $p < 0.01$, Fig. 4A–C, Supplementary Table 2).

The change of I_h amplitude coincident with AP frequency shown in Fig. 4D indicated that I_h current contributes to the determination of AP frequency during Hypo-Inf and reoxygenation. To test this hypothesis more directly, we examined AP frequency changes in the presence of zatebradine. We first determined whether zatebradine could block I_h in all experimental conditions (Hypo-Inf, reoxygenation, and control condition). Pretreatment with $10 \mu\text{M}$ zatebradine for 20 min maintained the I_h amplitude at a minimal level with all sizes of current injection tested, lasting for the duration of the whole-cell recording (>1 h) (Supplementary Table 3 and Fig. 5). Contrary to our expectations, in spite of effective blockage of the I_h current, zatebradine did not reduce basal excitability of the neurons (Fig. 6, baseline). Furthermore, the magnitude of excitability reduction by Hypo-Inf was significantly ameliorated in the presence of zatebradine (Fig. 6B, $p < 0.01$). We believe these observations resulted because the effective voltage accumulation due to increased input resistance counteracts the effect of the I_h block (R_{in} was $183.7 \pm 15.1 \text{ M}\Omega$ in baseline with zatebradine, 113.3 ± 7.0 in control; $153.0 \pm 16.2 \text{ M}\Omega$ in Hypo-Inf with zatebradine pretreatment and $94.5 \pm 5.1 \text{ M}\Omega$ in Hypo-Inf without zatebradine, Fig. 6D).

We next examined whether blockage of I_h reinstated the over-excitability observed upon reoxygenation. In support of our hypothesis that increased I_h is a critical determinant of the hyper-excitability that occurs during reoxygenation, the AP frequency during reperfusion in the presence of zatebradine returned to levels observed under the control condition ($18.0 \pm 1.8 \text{ Hz}$ with zatebradine, $22.6 \pm 1.1 \text{ Hz}$ without zatebradine pretreatment, $p < 0.01$, Fig. 6A–C). Blockage of I_h also restored ΔV_{thresh} by hyperpolarizing RMP ($\Delta V_{\text{thresh}} = 25.5 \pm 1.4 \text{ mV}$ with zatebradine, $17.6 \pm 1.1 \text{ mV}$ without zatebradine pretreatment, $p < 0.01$, Fig. 6D and E).

These results demonstrate that increased I_h during reperfusion plays a major role in rebounding hyperexcitability after Hypo-Inf and suggests the use of zatebradine as a novel potential reagent to reduce reperfusion brain injury.

4. Discussion

Despite our increased understanding of the molecular mechanisms involved in oxidative stress and cell death during hypoxia (Pfisterer and Khodosevich, 2017; Walton et al., 1999), the effects on basic neuronal functions, such as synaptic transmission and excitability, remain controversial (Centonze et al., 2009; Fan et al., 2008;

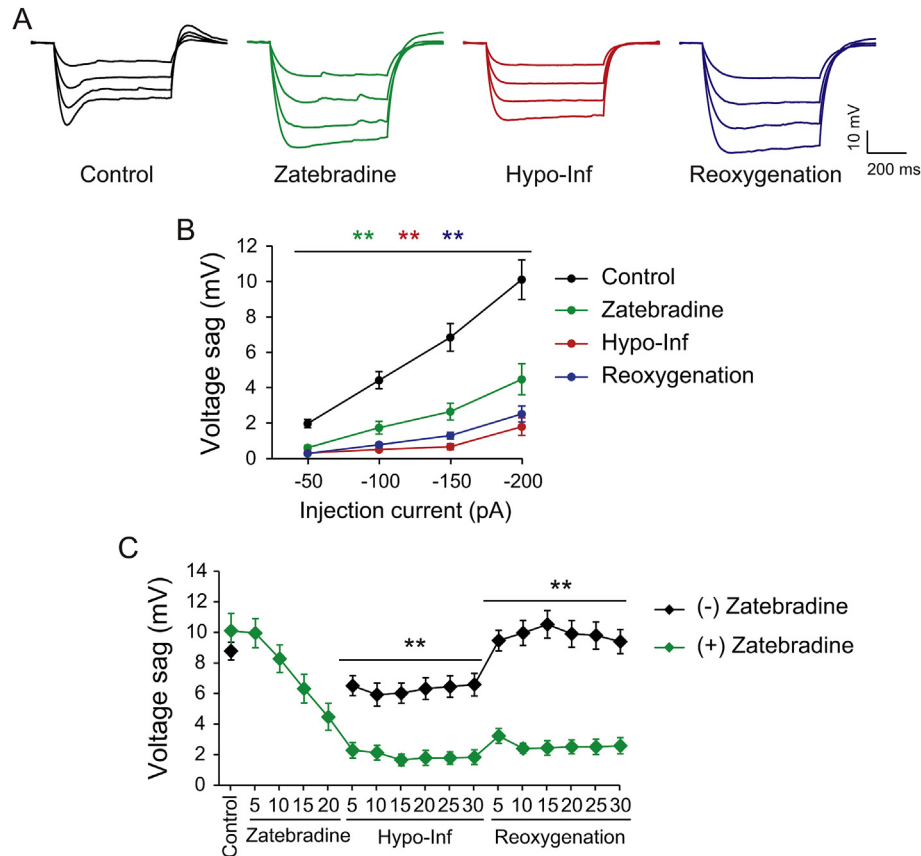


Fig. 5. Effects of zatebradine on voltage sag. (A) Sample trace showing voltage sags induced by the indicated negative current injection for 600 ms. Scale bars: 10 mV, 200 ms. (B) Size of voltage sags induced by indicated current injection in control (black), zatebradine pretreatment (green), Hypo-Inf (red), and reoxygenation combined with zatebradine pretreatment (blue). Significance was determined using paired *t*-test (***p* < 0.01 compared with control). (C) Size of voltage sags in the presence (green) or absence (black) of zatebradine pretreatment. Voltage sags in Hypo-Inf and reoxygenation, with or without zatebradine, were compared with two-way ANOVA followed by the Tukey Honest significant differences test (***p* < 0.01).

Gao et al., 2014; Levin et al., 2010; Levin and Godukhin, 2009; Schiff and Somjen, 1985; Turovsky et al., 2013; Tzour et al., 2017; Zanelli et al., 2015). The observed discrepancies could have multiple explanations. In particular, many experiments performed *in vitro* may fail to mimic pathological conditions. For example, the drastic accompanying inflammatory responses, which show very different effects on basic neuronal functions, are ignored in most studies (but (Batti and O'Connor, 2010; Zhang et al., 2014)). On the other hand, *in vivo* studies are often performed after transient interruption of cerebral blood flow, and effects of hypoxia cannot be clearly dissected from the reperfusion effect.

In the current study, we examined the effects of simultaneous limited oxygen supply and inflammation and the effects of reoxygenation on synaptic transmission and excitability. We observed that the excitability of neurons decreased rapidly due to a greater voltage difference between the RMP and AP threshold in addition to reduced membrane resistance (Fig. 3). These findings can be explained by the strong expression of K_{ATP} channels in CA1 pyramidal neurons, the gating of which is regulated by the ATP/ADP ratio (Sun and Feng, 2013; Sun and Hu, 2010; Thomzig et al., 2005; Zawar et al., 1999). Hypoxia-induced decreases in the ATP/ADP ratio lead to the opening of K_{ATP} channels, which in turn hyperpolarizes neurons and shuts down their activity to prevent further energy consumption (Sun et al., 2006, 2007, but see (Nisticò et al., 2007)). Of note, in our study decreased I_h current, a critical regulator of excitability during hypoxia, contributed to the reduced excitability (Fig. 4).

Concurrent with reduced excitability, we observed an attenuation of synaptic transmission (Fig. 1). Together, the enhanced short-term facilitation during high-frequency synaptic transmission and the DPCPX-sensitivity of this facilitation (Fig. 2 and Supplementary Fig. 2) support the hypothesis of a reduced efficiency of neurotransmitter release. Our observation of reduced synaptic transmission with increased short-term facilitation could have been caused by a reduced excitability or I_h . If the number of axons stimulated decreased due to reduced excitability induced by Hypo-Inf, then smaller EPSC can be expected. Moreover, considering that I_h exerts a dampening effect on voltage integration (Berger et al., 2001; Magee, 1999; Williams and Stuart, 2000), reduced I_h due to Hypo-Inf could lead to increased short-term facilitation, as observed in hippocampal neurons lacking TRIP8b (Brager et al., 2013), an auxiliary protein required for functional HCN channels (Lewis et al., 2009; Santoro et al., 2009). However, DPCPX sensitivity and the same pattern of short-term plasticity with increased stimulation cannot be readily explained by reduced excitability alone. Therefore, the notion that reduced release probability contributes to reduced circuit activity, and thereby affects cognitive function and sedation during hypoxia, remains the most straightforward interpretation. Neurotransmitter release efficiency is determined by a number of factors, including association of vesicles with the soluble N-ethylmaleimide-sensitive factor attachment receptor (SNARE) complex, available number of synaptic vesicles in RRP, and Ca^{2+} sensed by Ca^{2+} sensors upon AP (Südhof, 2013). Among these factors, the SNARE complex factor, which provides a

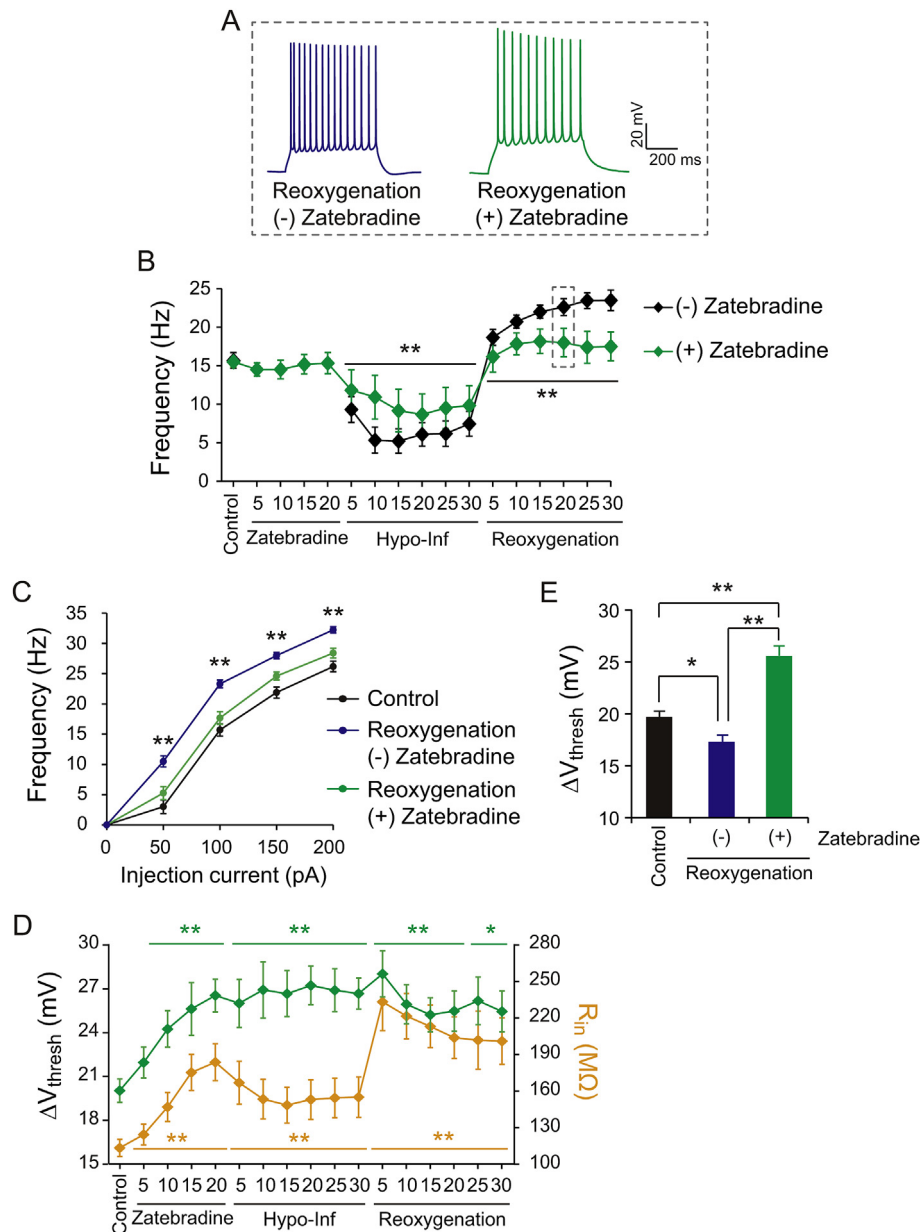


Fig. 6. Reduced excitability changes induced by zatebradine pretreatment. (A) Sample traces of APs obtained by current injections (100 pA/600 ms) in reoxygenation (blue) and in reoxygenation with 10 μ M zatebradine pretreatment (blue). Scale bars: 20 mV, 200 ms. (B) Average AP frequencies with (green) or without (black) I_h channels blocked by zatebradine (two-way ANOVA, $**p < 0.01$). (C) AP frequencies with various magnitudes of current injection for 600 ms; frequencies depicted as averages of measurements taken between 20 and 30 min after washing-in. Control (black), reoxygenation (blue), and reoxygenation with zatebradine (green). Significance was determined using one-way ANOVA, followed by Bonferroni post hoc test, $**p < 0.01$. (D) Average voltage differences (ΔV_{thresh} , green) and input resistance (R_{in} , orange) of zatebradine-pretreated neurons as a function of time during Hypo-Inf and reoxygenation (paired t -test, $*p < 0.05$, $**p < 0.01$). (E) ΔV_{thresh} in control (black), reoxygenation (blue), and reoxygenation with zatebradine pretreatment (blue). One-way ANOVA, $*p < 0.05$, $**p < 0.01$. Error bars represent standard error.

force capable of overcoming repulsive charges between fusing membranes (Gerber et al., 2008; Südhof, 2009; Zhou et al., 2013), and RRP size are key determinants of mEPSC frequency (Deak et al., 2006; Guzman et al., 2010; Stein et al., 2009). On the other hand, the rise in Ca^{2+} concentration is critical for AP-dependent, Ca^{2+} -evoked neurotransmitter release (Elmqvist and Feldman, 1965; Katz and Miledi, 1965; Wheeler et al., 1994) but not for spontaneous membrane fusion. Of note, mEPSC frequency is not affected by blocking voltage-gated Ca^{2+} channels (VGCC) (Vyleta and Smith, 2011). Therefore, the observed increase in synaptic facilitation without a concurrent change in mEPSC frequency (Supplementary Fig. 3) indicates that synaptic depression occurs through VGCC

inhibition by endogenously released adenosine (Brust et al., 2006; Choi et al., 2015; Dunwiddie and Masino, 2001; Gervitz et al., 2001).

Bath-applied TNF- α increases basal synaptic transmission on a similar time scale with our experiment through an increase in the number of AMPA receptors at postsynaptic densities (Beattie et al., 2002; Tancredi et al., 1992). We did not detect any significant changes in postsynaptic strength (Figs. 2 and 3) despite the fact that the concentration of LPS in our bath solution was sufficient to increase excitability (Supplementary Fig. 4). These findings suggest that the initial synaptic depression that occurs with Hypo-Inf employs a distinct mechanism from that occurring with the long-lasting depression triggered by reoxygenation after Hypo-Inf, as

observed by Zhang et al. (2014). In contrast to Zhang and colleagues, we did not find long-lasting effects of synaptic depression associated with reperfusion. We believe this discrepancy is due to different experimental conditions across the two studies. Whereas LPS was kept in the recording solution in the experiment by Zhang et al., we washed LPS out together with the anoxic bath solution. Of note, when we measured EPSCs in conditions in which we retained LPS in the bath solution during reoxygenation, we also observed long-lasting depression of synaptic transmission (Supplementary Fig. 7).

The changes we observed in excitability induced by hypoxia in combination with LPS were similar to those induced by hypoxia alone, but the effect was reinforced by the addition of LPS (Supplementary Table 2). For example, the increased ΔV_{thresh} along with reduced R_{in} during hypoxia without LPS was not as dramatic as that we observed under Hypo-Inf. This finding is contradictory to the findings that LPS alone increased excitability by reducing ΔV_{thresh} (Fujiwara et al., 1987; Vezzani and Viviani, 2015 and Supplementary Fig. 4). One possible explanation for this finding is that inflammatory insult has an additive effect and worsens the effects of hypoxia (Varatharaj and Galea, 2017), rather than having its own role in hyperexcitability.

In their early study on cerebral anoxia in healthy humans using a cervical pressure cuff, Rossen et al. described a “seizure occurred after release of pressure in the cuff, and rarely or never during the acute cerebral anoxia” (Rossen et al., 1943). In addition, reduced excitability induced by antagonists of NMDA receptors or of Ca^{2+} -permeable AMPARs, as well as that induced by antiepileptic drugs, such as lamotrigine, improves cerebral ischemic damage (Albers et al., 1989; Crumrine et al., 1997; Noh et al., 2005). Clinically, hypoxic ischemia is a known cause of infant seizure (Ronen et al., 2007; Tekgul, 2006). Corroborating previous findings regarding post-transient ischemia (Levin et al., 2010; Levin and Godukhin, 2009; Schiff and Somjen, 1985; Turovsky et al., 2013), we observed a dramatic increase in excitability on account of reduced ΔV_{thresh} and increased membrane resistance (Fig. 3D and E). Further, dynamic regulation of neuronal excitability by HCN1 channel conductance and/or surface expression have been reported previously (Jung et al., 2010; Noam et al., 2010).

Our results indicate that the excitability of neurons in response to Hypo-Inf and reoxygenation relies on dynamic regulation of the I_h current, as well. Of note, the HCN channel blocker zatebradine can ameliorate the reperfusion-induced increase in excitability, whilst excitability of neurons in normoxia was not significantly affected (Fig. 6B). Furthermore, the decrease in excitability induced by Hypo-Inf was ameliorated in the presence of zatebradine (Fig. 6B). Our results indicate that zatebradine has two opposing effects on neuronal excitability. Zatebradine blocks I_h (Fig. 5) and increases ΔV_{thresh} (Fig. 6D), both of which make AP generation more difficult. On the other hand, zatebradine increases R_{in} (Fig. 6D), which makes voltage accumulation more efficient and thereby eases AP generation. We believe the unchanged excitability of neurons in normoxia with zatebradine results from a balance of opposites. Similarly, the ameliorated excitability reduction observed during Hypo-Inf in the presence of zatebradine can be explained by increased R_{in} . Normally, Hypo-Inf increases ΔV_{thresh} in neurons and induces smaller R_{in} and I_h , which would mean a greater voltage change is required to reach AP and the membrane is leaky. Furthermore, even when an AP is generated, the current that drives successive APs through I_h is smaller and, therefore, AP frequency drops rapidly. In instances of Hypo-Inf in the presence of zatebradine, on the other hand, ΔV_{thresh} is greater and I_h is blocked, and R_{in} is increased even more so than under normoxic conditions. Therefore, addition of zatebradine decreases the reduction in excitability observed with Hypo-Inf.

In the current study, whole-cell patch clamping was used to monitor excitability along with other electrophysiological parameters, and zatebradine treatment was used prior to induction of hypoxia for the sake of experimental ease. However, if zatebradine were to be used in patients, the administration would occur after hypoxia and before reperfusion. Therefore, we believe the reduction of reperfusion-induced hyperexcitability caused by zatebradine indicates that this could be an important therapeutic tool to reduce reperfusion brain injury. Further study is required to confirm that zatebradine could reduce reperfusion brain injury *in vivo*.

Conflicts of interest

Authors have no conflicts of interest to disclose.

Acknowledgement

This work was supported by the KBRI Research Program of the Ministry of Science and ICT (18-BR-01-05 and 18-BR-04-01), Brain Research Program and Bio medical technology development program through the National Research Foundation of Korea funded by the Ministry of Science and ICT (NRF-2017M3C7A1048086 and NRF-2017M3A9G8084463).

Appendix A. Supplementary data

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.neuropharm.2018.05.011>.

References

- Albers, G.W., Goldberg, M.P., Choi, D.W., 1989. N-methyl-D-aspartate antagonists: ready for clinical trial in brain ischemia? *Ann. Neurol.* 25, 398–403. <https://doi.org/10.1002/ana.410250412>.
- Batti, L., O'Connor, J.J., 2010. Tumor necrosis factor- α impairs the recovery of synaptic transmission from hypoxia in rat hippocampal slices. *J. Neuroimmunol.* 218, 21–27. <https://doi.org/10.1016/j.jneuroim.2009.11.002>.
- Beattie, E.C., Stellwagen, D., Morishita, W., Bresnahan, J.C., Ha, B.K., Von Zastrow, M., Beattie, M.S., Malenka, R.C., 2002. Control of synaptic strength by glial TNF α . *Science* 295, 2282–2285. <https://doi.org/10.1126/science.1067859>.
- Berger, T., Larkum, M.E., Lüscher, H.R., 2001. High I_h channel density in the distal apical dendrite of layer V pyramidal cells increases bidirectional attenuation of EPSPs. *J. Neurophysiol.* 85, 855–868. <https://doi.org/10.1152/jn.2001.85.3.855>.
- Block, M.L., Hong, J.S., 2005. Microglia and inflammation-mediated neurodegeneration: multiple triggers with a common mechanism. *Prog. Neurobiol.* 76, 77–98. <https://doi.org/10.1016/j.pneurobio.2005.06.004>.
- Boissard, C.G., Gribkoff, V.K., 1993. The effects of the adenosine reuptake inhibitor solufazine on synaptic potentials and population hypoxic depolarizations in area CA1 of rat hippocampus in vitro. *Neuropharmacology* 32, 149–155. [https://doi.org/10.1016/0028-3908\(93\)90095-K](https://doi.org/10.1016/0028-3908(93)90095-K).
- Brager, D.H., Lewis, A.S., Chetkovich, D.M., Johnston, D., 2013. Short- and long-term plasticity in CA1 neurons from mice lacking h-channel auxiliary subunit TRIP8b. *J. Neurophysiol.* 110, 2350–2357. <https://doi.org/10.1152/jn.00218.2013>.
- Brust, T.B., Cayabyab, F.S., Zhou, N., MacVicar, B.A., 2006. p38 mitogen-activated protein kinase contributes to adenosine A1 receptor-mediated synaptic depression in area CA1 of the rat hippocampus. *J. Neurosci.* 26, 12427–12438. <https://doi.org/10.1523/JNEUROSCI.4052-06.2006>.
- Centonze, D., Muzio, L., Rossi, S., Cavanini, F., De Chiara, V., Bergami, A., Musella, A., D'Amelio, M., Cavallucci, V., Martorana, A., Bergamaschi, A., Cencioni, M.T., Diamantini, A., Butti, E., Coni, G., Bernardi, G., Cecconi, F., Battistini, L., Furlan, R., Martino, G., 2009. Inflammation triggers synaptic alteration and degeneration in experimental autoimmune encephalomyelitis. *J. Neurosci.* 29, 3442–3452. <https://doi.org/10.1523/JNEUROSCI.5804-08.2009>.
- Centonze, D., Muzio, L., Rossi, S., Furlan, R., Bernardi, G., Martino, G., 2010. The link between inflammation, synaptic transmission and neurodegeneration in multiple sclerosis. *Cell Death Differ.* 17, 1083–1091. <https://doi.org/10.1038/cdd.2009.179>.
- Chi, X.X., Xu, Z.C., 2000. Differential changes of potassium currents in CA1 pyramidal neurons after transient forebrain ischemia differential changes of potassium currents in CA1 pyramidal neurons after transient forebrain ischemia. *J. Neurophysiol.* 84, 2834–2843.
- Choi, D.W., 1988. Calcium-mediated neurotoxicity: relationship to specific channel types and role in ischemic damage. *Trends Neurosci.* 11, 465–469. [https://doi.org/10.1016/0166-2236\(88\)90046-6](https://doi.org/10.1016/0166-2236(88)90046-6).

- org/10.1016/0166-2236(88)90200-7.
- Choi, D.W., 1985. Glutamate neurotoxicity in cortical cell culture is calcium dependent. *Neurosci. Lett.* 58, 293–297. [https://doi.org/10.1016/0304-3940\(85\)90069-2](https://doi.org/10.1016/0304-3940(85)90069-2).
- Choi, I.S., Cho, J.H., Lee, M.G., Jang, I.S., 2015. Enzymatic conversion of ATP to adenosine contributes to ATP-induced inhibition of glutamate release in rat medullary dorsal horn neurons. *Neuropharmacology* 93, 94–102. <https://doi.org/10.1016/j.neuropharm.2015.01.020>.
- Crumrine, R.C., Bergstrand, K., Cooper, A.T., Faison, W.L., Cooper, B.R., 1997. Lamotrigine protects hippocampal CA1 neurons from ischemic damage after cardiac arrest. *Stroke* 28, 2230–2236 discussion 2237. <https://doi.org/10.1161/01.STR.28.11.2230>.
- Deak, F., Shin, O.-H., Kavalali, E.T., Sudhof, T.C., 2006. Structural determinants of synaptobrevin 2 function in synaptic vesicle fusion. *J. Neurosci.* 26, 6668–6676. <https://doi.org/10.1523/JNEUROSCI.5272-05.2006>.
- Doolette, D.J., Kerr, D.I.B., 1995. Hyperexcitability in CA1 of the rat hippocampal slice following hypoxia or adenosine. *Brain Res.* 677, 127–137. [https://doi.org/10.1016/0006-8993\(95\)00139-H](https://doi.org/10.1016/0006-8993(95)00139-H).
- Dunwiddie, T.V., Masino, S.A., 2001. The role and regulation of adenosine in the central nervous system. *Annu. Rev. Neurosci.* 24, 31–55.
- Elmqvist, D., Feldman, D.S., 1965. Calcium dependence of spontaneous acetylcholine release at mammalian motor nerve terminals. *J. Physiol.* 181, 487–497. <https://doi.org/10.1113/jphysiol.1965.sp007777>.
- Eltzschig, H.K., Carmeliet, P., 2011. Hypoxia and inflammation. *N. Engl. J. Med.* 364, 656–665. <https://doi.org/10.1056/NEJMra0910283>.
- Eltzschig, H.K., Eckle, T., 2011. Ischemia and reperfusion - from mechanism to translation. *Nat. Med.* 17, 1391–1401. <https://doi.org/10.1038/nm.2507.Ischemia>.
- Fan, Y., Deng, P., Wang, Y.-C., Lu, H.-C., Xu, Z.C., Schulz, P.E., 2008. Transient cerebral ischemia increases CA1 pyramidal neuron excitability. *Exp. Neurol.* 212, 415–421. <https://doi.org/10.1016/j.expneurol.2008.04.032>.
- Freund, T.F., Martin, K.A.C., Somogyi, P., Whitteridge, D., 1985. Innervation of cat visual areas 17 and 18 by physiologically identified X- and Y- type thalamic afferents. 11. In: *Identification of Postsynaptic Targets by GABA Immunocytochemistry and Golgi Impregnation* 291.
- Fujimura, N., Tanaka, E., Yamamoto, S., Shigemori, M., Higashi, H., 1997. Contribution of ATP-sensitive potassium channels to hypoxic hyperpolarization in rat hippocampal CA1 neurons in vitro. *J. Neurophysiol.* 77, 378–385. <https://doi.org/10.1016/0101-0001.1997.01.01.00.01>.
- Fujiwara, Shimoji, K., Yoshimura, M., 1987. Effects of hypoxia on rat hippocampal neurones in vitro. *J. Physiol.* 131–151.
- Gao, F., Liu, Z., Ren, W., Jiang, W., 2014. Acute lipopolysaccharide exposure facilitates epileptiform activity via enhanced excitatory synaptic transmission and neuronal excitability in vitro. *Neuropsychiatric Dis. Treat.* 10, 1489–1495. <https://doi.org/10.2147/NDT.S65695>.
- Gasparini, S., DiFrancesco, D., 1997. Action of the hyperpolarization-activated current (I_h) blocker ZD 7288 in hippocampal CA1 neurons. *Pflugers Arch. Eur. J. Physiol.* 435, 99–106. <https://doi.org/10.1007/s004240050488>.
- Gelderblom, M., Leyboldt, F., Steinbach, K., Behrens, D., Choe, C.U., Siler, D.A., Arumugam, T.V., Orthey, E., Gerloff, C., Tolosa, E., Magnus, T., 2009. Temporal and spatial dynamics of cerebral immune cell accumulation in stroke. *Stroke* 40, 1849–1857. <https://doi.org/10.1161/STROKEAHA.108.534503>.
- Gerber, S.H., Rah, J., Min, S., Liu, X., Wit, H., De Verhage, M., Rosenmund, C., Südhof, T.C., 2008. Conformational switch of Syntaxin-1. *Science* 320, 1507–1510. <https://doi.org/10.1126/science.1163174>.
- Gervitz, L.M., Lutherer, L.O., Davies, D.G., Pirch, J.H., Fowler, J.C., 2001. Adenosine induces initial hypoxic-ischemic depression of synaptic transmission in the rat hippocampus in vivo. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 280, R639–R645.
- Gill, C.H., Randall, A., Bates, S.A., Hill, K., Owen, D., Larkman, P.M., Cairns, W., Yusuf, S.P., Murdock, P.R., Stribos, P.J.L.M., Powell, A.J., Benham, C.D., Davies, C.H., 2004. Characterization of the human HCN1 channel and its inhibition by capsazepine. *Br. J. Pharmacol.* 143, 411–421. <https://doi.org/10.1038/sj.bjp.0705945>.
- Guzman, R.E., Schwarz, Y.N., Rettig, J., Bruns, D., 2010. SNARE force synchronizes synaptic vesicle fusion and controls the kinetics of quantal synaptic transmission. *J. Neurosci.* 30, 10272–10281. <https://doi.org/10.1523/JNEUROSCI.1551-10.2010>.
- Huang, J., Upadhyay, U.M., Tamargo, R.J., 2006. Inflammation in stroke and focal cerebral ischemia. *Surg. Neurol.* 66, 232–245. <https://doi.org/10.1016/j.surneu.2005.12.028>.
- Iadecola, C., Cho, S., Feuerstein, G.Z., Hallenbeck, J., 2004. Cerebral ischemia and inflammation. In: *Stroke: Pathophysiology, Diagnosis, and Management*, pp. 883–893. <https://doi.org/10.1016/B0-44-306600-0/50052-3>.
- Jackman, S.L., Turecek, J., Belinsky, J.E., Regehr, W.G., 2016. The calcium sensor synaptotagmin 7 is required for synaptic facilitation. *Nature* 529, 88–91. <https://doi.org/10.1038/nature16507>.
- Jenerick, H., 1963. Phase plane trajectories of the muscle spike potential. *Biophys. J.* 3, 363–377. [https://doi.org/10.1016/S0006-3495\(63\)86827-7](https://doi.org/10.1016/S0006-3495(63)86827-7).
- Jung, S., Bullis, J.B., Lau, I.H., Jones, T.D., Warner, L.N., Poolos, N.P., 2010. Down-regulation of dendritic HCN channel gating in epilepsy is mediated by altered phosphorylation signaling. *J. Neurosci.* 30, 6678–6688. <https://doi.org/10.1523/JNEUROSCI.1290-10.2010>.
- Kaelin, W.G., Ratcliffe, P.J., 2008. Oxygen sensing by metazoans: the central role of the HIF hydroxylase pathway. *Mol. Cell* 30, 393–402. <https://doi.org/10.1016/j.molcel.2008.04.009>.
- Kannarkat, G.T., Boss, J.M., Tansey, M.G., 2013. The role of innate and adaptive immunity in Parkinson's disease. *J. Parkinson's Dis.* 3, 493–514. <https://doi.org/10.3233/JPD-130250>.
- Katz, B., Miledi, R., 1965. The effect of calcium on acetylcholine release from motor nerve terminals. *Proc. R. Soc. Lond. B Biol. Sci.* 161, 496–503. <https://doi.org/10.1098/rspb.1965.0017>.
- Kidd, F.L., Isaac, J.T., 2001. Kinetics and activation of postsynaptic kainate receptors at thalamocortical synapses: role of glutamate clearance. *J. Neurophysiol.* 86, 1139–1148.
- Lennox, W.G., Gibbs, F.A., Gibbs, E.L., 1935. Relationship of unconsciousness to cerebral blood flow and to anoxemia. *Arch. Neurol. Psychiatr.* 34, 1001–1013. <https://doi.org/10.1001/archneurpsyc.1935.02250230073006>.
- Levin, S.G., Godukhin, O.V., 2009. Comparative roles of ATP-sensitive K⁺ channels and Ca²⁺-activated BK⁺ channels in posthypoxic hyperexcitability and rapid hypoxic preconditioning in hippocampal CA1 pyramidal neurons in vitro. *Neurosci. Lett.* 461, 90–94. <https://doi.org/10.1016/j.neulet.2009.06.018>.
- Levin, S.G., Shamsutdinova, A.A., Godukhin, O.V., 2010. Apamin, a selective blocker of SKCa channels, inhibits posthypoxic hyperexcitability but does not affect rapid hypoxic preconditioning in hippocampal CA1 pyramidal neurons in vitro. *Neurosci. Lett.* 484, 35–38. <https://doi.org/10.1016/j.neulet.2010.08.012>.
- Lewis, A.S., Schwartz, E., Savio Chan, C., Noam, Y., Shin, M., Wadman, W.J., James Surmeier, D., Baram, T.Z., Macdonald, R.L., Chetkovich, D.M., 2009. Alternatively spliced isoforms of TRIP8b differentially control h channel trafficking and function. *J. Neurosci.* 29, 6250–6265. <https://doi.org/10.1523/JNEUROSCI.0856-09.2009>.
- Lipton, P., Whittingham, T.S., 1982. Reduced ATP concentration as a basis for synaptic transmission failure during hypoxia in the in vitro Guinea-pig hippocampus. *J. Physiol.* 325, 51–65. <https://doi.org/10.1113/jphysiol.1982.sp014135>.
- Lüthi, A., McCormick, D.A., 1998. H-current: properties of a neuronal and network pacemaker. *Neuron* 21, 9–12. [https://doi.org/10.1016/S0896-6273\(00\)80509-7](https://doi.org/10.1016/S0896-6273(00)80509-7).
- Magee, J.C., 1999. Dendritic I(h) normalizes temporal summation in hippocampal CA1 neurons. *Nat. Neurosci.* 2, 508–514. <https://doi.org/10.1038/9158>.
- Makeinde, H.M., Just, T.B., Cuda, C.M., Perlman, H., Schwulst, S.J., 2017. The role of microglia in the etiology and evolution of chronic traumatic encephalopathy. *Shock* 1. <https://doi.org/10.1097/SHK.0000000000000859>.
- Matsuzaki, M., Ellis-Davies, G.C.R., Nemoto, T., Miyashita, Y., Iino, M., Kasai, H., 2001. Dendritic spine geometry is critical for AMPA receptor expression in hippocampal CA1 pyramidal neurons. *Nat. Neurosci.* 4, 1086–1092. <https://doi.org/10.1038/nn736>.
- McCool, B.A., Farroni, J.S., 2001. A1 adenosine receptors inhibit multiple voltage-gated Ca²⁺ channel subtypes in acutely isolated rat basolateral amygdala neurons. *Br. J. Pharmacol.* 132, 879–888. <https://doi.org/10.1038/sj.bjp.0703884>.
- Mukandala, G., Tynan, R., Lanigan, S., O'Connor, J.J., 2016. The effects of hypoxia and inflammation on synaptic signaling in the CNS. *Brain Sci.* 6 (6). <https://doi.org/10.3390/brainsci6010006>.
- Nisticò, R., Piccirilli, S., Sebastianelli, L., Nisticò, G., Bernardi, G., Mercuri, N.B., 2007. The blockade of K⁺-ATP channels has neuroprotective effects in an in vitro model of brain ischemia. *Int. Rev. Neurobiol.* 82, 383–395. [https://doi.org/10.1016/S0074-7742\(07\)80201-6](https://doi.org/10.1016/S0074-7742(07)80201-6).
- Noam, Y., Zha, Q., Phan, L., Wu, R.L., Chetkovich, D.M., Wadman, W.J., Baram, T.Z., 2010. Trafficking and surface expression of hyperpolarization-activated cyclic nucleotide-gated channels in hippocampal neurons. *J. Biol. Chem.* 285, 14724–14736. <https://doi.org/10.1074/jbc.M109.070391>.
- Noh, K.-M., Yokota, H., Mashiko, T., Castillo, P.E., Zukin, R.S., Bennett, M.V.L., 2005. Blockade of calcium-permeable AMPA receptors protects hippocampal neurons against global ischemia-induced death. *Proc. Natl. Acad. Sci. U. S. A.* 102, 12230–12235. <https://doi.org/10.1073/pnas.0505408102>.
- Nusser, Z., Luján, R., Laube, G., Roberts, J.D.B., Molnar, E., Somogyi, P., 1998. Cell type and pathway dependence of synaptic AMPA receptor number and variability in the hippocampus. *Neuron* 21, 545–559. [https://doi.org/10.1016/S0896-6273\(00\)80565-6](https://doi.org/10.1016/S0896-6273(00)80565-6).
- Palmer, T.M., Stiles, G.L., 1995. Review: neurotransmitter receptors VII, adenosine receptors. *Neuropharmacology* 34, 683–694.
- Peers, C., Dallas, M.L., Boycott, H.E., Scragg, J.L., Pearson, H.A., Boyle, J.P., 2009. Hypoxia and neurodegeneration. *Ann. N. Y. Acad. Sci.* 1177, 169–177. <https://doi.org/10.1111/j.1749-6632.2009.05026.x>.
- Pfisterer, U., Khodosevich, K., 2017. Neuronal survival in the brain: neuron type-specific mechanisms. *Cell Death Dis.* 8 e2643–14. <https://doi.org/10.1038/cddis.2017.64>.
- Pickering, M., Cumiskey, D., O'Connor, J.J., 2005. Actions of TNF- α on glutamatergic synaptic transmission in the central nervous system. *Exp. Physiol.* 90, 663–670. <https://doi.org/10.1113/expphysiol.2005.030734>.
- Ravizza, T., Lucas, S.M., Balosso, S., Bernardino, L., Ku, G., Noé, F., Malva, J., Randle, J.C.R., Allan, S., Vezzani, A., 2006. Inactivation of caspase-1 in rodent brain: a novel anticonvulsive strategy. *Epilepsia* 47, 1160–1168. <https://doi.org/10.1111/j.1528-1167.2006.00590.x>.
- Regehr, W.G., 2012. Short-term presynaptic plasticity. *Cold Spring Harb. Perspect. Biol.* 4 a005702. <https://doi.org/10.1101/cshperspect.a005702>.
- Remarque, E.J., Bollen, E.L.E.M., Weverling-Rijnsburger, A.W.E., Latereveer, J.C., Blauw, G.J., Westendorp, R.G.J., 2001. Patients with Alzheimer's disease display a pro-inflammatory phenotype. *Exp. Gerontol.* 36, 171–176. [https://doi.org/10.1016/S0531-5565\(00\)00176-5](https://doi.org/10.1016/S0531-5565(00)00176-5).
- Robinson, R.B., Siegelbaum, S.A., 2003. Hyperpolarization-activated cation currents: from molecules to physiological function. *Annu. Rev. Physiol.* 65, 453–480. <https://doi.org/10.1146/annurev.physiol.65.092101.142734>.

- Ronen, G.M., Buckley, D., Penney, S., Streiner, D.L., 2007. Long-term prognosis in children with neonatal seizures: a population-based study. *Neurology* 69, 1816–1822. <https://doi.org/10.1212/01.wnl.0000279335.85797.2c>.
- Rossen, R., Kabat, H., Anderson, J.P., 1943. Acute arrest of cerebral circulation in man. *Arch. Neurol. Psychiatr.* 50, 510–528. <https://doi.org/10.1001/archneurpsyc.1943.02290230022002>.
- Sanderson, T.H., Reynolds, C.A., Kumar, R., Przyklenk, K., Hüttemann, M., 2013. Molecular mechanisms of ischemia-reperfusion injury in brain: pivotal role of the mitochondrial membrane potential in reactive oxygen species generation. *Mol. Neurobiol.* 47 (1), 9–23. <https://doi.org/10.1007/s12035-012-8344-z>.
- Santoro, B., Piskrowski, R.A., Pian, P., Hu, L., Liu, H., Siegelbaum, S.A., 2009. TRIP8b splice variants form a family of auxiliary subunits that regulate gating and trafficking of HCN channels in the brain. *Neuron* 62, 802–813. <https://doi.org/10.1016/j.neuron.2009.05.009>.
- Schiff, S.J., Somjen, G.G., 1985. Hyperexcitability following moderate hypoxia in hippocampal tissue slices. *Brain Res.* 337, 337–340. [https://doi.org/10.1016/0006-8993\(85\)90071-X](https://doi.org/10.1016/0006-8993(85)90071-X).
- Schneggenburger, R., Rosenmund, C., 2015. Molecular mechanisms governing Ca²⁺ regulation of evoked and spontaneous release. *Nat. Neurosci.* 18, 935–941. <https://doi.org/10.1038/nn.4044>.
- Shahi, P.K., Choi, S., Zuo, D.C., Kim, M.Y., Park, C.G., Kim, Y.D., Lee, J., Park, K.J., So, I., Jun, J.Y., 2014. The possible roles of hyperpolarization-activated cyclic nucleotide channels in regulating pacemaker activity in colonic interstitial cells of Cajal. *J. Gastroenterol.* 49, 1001–1010. <https://doi.org/10.1007/s00535-013-0849-3>.
- Stein, A., Weber, G., Wahl, M.C., Jahn, R., 2009. Helical extension of the neuronal SNARE complex into the membrane. *Nature* 460, 525–528. <https://doi.org/10.1038/nature08156>.
- Stellwagen, D., 2005. Differential regulation of AMPA receptor and GABA receptor trafficking by tumor necrosis factor- α . *J. Neurosci.* 25, 3219–3228. <https://doi.org/10.1523/JNEUROSCI.4486-04.2005>.
- Stieber, J., 2006. Bradycardic and proarrhythmic properties of sinus node inhibitors. *Mol. Pharmacol.* 69, 1328–1337. <https://doi.org/10.1124/mol.105.020701>.
- Südhof, T.C., 2013. Neurotransmitter release: the last millisecond in the life of a synaptic vesicle. *Neuron* 80, 675–690. <https://doi.org/10.1016/j.neuron.2013.10.022>.
- Südhof, T.C., 2009. Membrane Fusion : grappling with. *Science* 323 (5913), 474–477. <https://doi.org/10.1126/science.1161748>.
- Sugar, O., Gerard, R.W., 1938. Anoxia and brain potentials. *J. Neurophysiol.* 1, 558–572.
- Sun, H., Feng, Z., 2013. Neuroprotective role of ATP-sensitive potassium channels in cerebral ischemia. *Acta Pharmacol. Sin.* 34, 24–32. <https://doi.org/10.1038/aps.2012.138>.
- Sun, H., Feng, Z., Miki, T., Seino, S., French, R.J., Feng, Z., Miki, T., Seino, S., French, R.J., 2006. Enhanced neuronal damage after ischemic insults in mice lacking Kir6.2-containing ATP-sensitive K ϵ channels. *J. Neurophysiol.* 95, 2590–2601. <https://doi.org/10.1152/jn.00970.2005>.
- Sun, H.S., Feng, Z.P., Barber, P.A., Buchan, A.M., French, R.J., 2007. Kir6.2-containing ATP-sensitive potassium channels protect cortical neurons from ischemic/anoxic injury in vitro and in vivo. *Neuroscience* 144, 1509–1515. <https://doi.org/10.1016/j.neuroscience.2006.10.043>.
- Sun, X.L., Hu, G., 2010. ATP-sensitive potassium channels: a promising target for protecting neurovascular unit function in stroke. *Clin. Exp. Pharmacol. Physiol.* 37, 243–252. <https://doi.org/10.1111/j.1440-1681.2009.05190.x>.
- Takumi, Y., Ramírez-León, V., Laake, P., Rinvik, E., Ottersen, O.P., 1999. Different modes of expression of AMPA and NMDA receptors in hippocampal synapses. *Nat. Neurosci.* 2, 618–624. <https://doi.org/10.1038/10172>.
- Tancredi, V., D'Arcangelo, G., Grassi, F., Tarroni, P., Palmieri, G., Santoni, A., Eusebi, F., 1992. Tumor necrosis factor alters synaptic transmission in rat hippocampal slices. *Neurosci. Lett.* 146, 176–178. [https://doi.org/10.1016/0304-3940\(92\)90071-E](https://doi.org/10.1016/0304-3940(92)90071-E).
- Taylor, C.T., 2008. Interdependent roles for hypoxia inducible factor and nuclear factor- κ B in hypoxic inflammation. *J. Physiol.* 586, 4055–4059. <https://doi.org/10.1111/jphysiol.2008.157669>.
- Tekgul, H., 2006. The current etiologic profile and neurodevelopmental outcome of seizures in term newborn infants. *Pediatrics* 117, 1270–1280. <https://doi.org/10.1542/peds.2005-1178>.
- The NINDS t-PA Stroke Study Group, 1997. Intracerebral hemorrhage after intravenous t-PA therapy for ischemic stroke. *Stroke* 28, 2109–2118. <https://doi.org/10.1161/01.STR.28.11.2109>.
- Thomzig, A., Laube, G., Prüss, H., Veh, R.W., 2005. Pore-forming subunits of K-ATP channels, Kir6.1 and Kir6.2, display prominent differences in regional and cellular distribution in the rat brain. *J. Comp. Neurol.* 484, 313–330. <https://doi.org/10.1002/cne.20469>.
- Turovsky, E.A., Turovskaya, M.V., Kononov, A.V., Zinchenko, V.P., 2013. Short-term episodes of hypoxia induce posthypoxic hyperexcitability and selective death of GABAergic hippocampal neurons. *Exp. Neurol.* 250, 1–7. <https://doi.org/10.1016/j.jexpneurol.2013.09.006>.
- Tzour, A., Leibovich, H., Barkai, O., Biala, Y., Lev, S., Yaari, Y., Binshtok, A.M., 2017. K ν 7/M channels as targets for lipopolysaccharide-induced inflammatory neuronal hyperexcitability. *J. Physiol.* 595, 713–738. <https://doi.org/10.1113/jpp272547>.
- Varatharaj, A., Galea, I., 2017. The blood-brain barrier in systemic inflammation. *Brain Behav. Immun.* 60, 1–12. <https://doi.org/10.1016/j.bbi.2016.03.010>.
- Vezzani, A., 2014. Epilepsy and inflammation in the brain: overview and pathophysiology. *Epilepsy Current* 14, 3–7. <https://doi.org/10.5698/1535-7511-14.s2.3>.
- Vezzani, A., Viviani, B., 2015. Neuromodulatory properties of inflammatory cytokines and their impact on neuronal excitability. *Neuropharmacology* 96, 70–82. <https://doi.org/10.1016/j.neuropharm.2014.10.027>.
- Viviani, B., Bartsaghi, S., Gardoni, F., Vezzani, A., Behrens, M.M., Bartfai, T., Binaglia, M., Corsini, E., Di Luca, M., Galli, C.L., Marinovich, M., 2003. Interleukin-1 β enhances NMDA receptor-mediated intracellular calcium increase through activation of the Src family of kinases. *J. Neurosci.* 23, 8692–8700. <https://doi.org/10.1523/JNEUROSCI.23.25.8692>.
- Vyleta, N.P., Smith, S.M., 2011. Spontaneous glutamate release is independent of calcium influx and tonically activated by the calcium-sensing receptor. *J. Neurosci.* 31, 4593–4606. <https://doi.org/10.1523/JNEUROSCI.6398-10.2011>.
- Walton, M., Connor, B., Lawlor, P., Young, D., Sirimanne, E., Gluckman, P., Cole, G., Dragunow, M., 1999. Neuronal death and survival in two models of hypoxic-ischemic brain damage. *Brain Res. Brain Res. Rev.* 29, 137–168.
- Webster, K.M., Sun, M., Crack, P., O'Brien, T.J., Shultz, S.R., Semple, B.D., 2017. Inflammation in epileptogenesis after traumatic brain injury. *J. Neuroinflammation* 14 (10). <https://doi.org/10.1186/s12974-016-0786-1>.
- Wheeler, D., Randall, A., Tsien, R., 1994. Roles of N-type and Q-type Ca²⁺ channels in supporting hippocampal synaptic transmission. *Science* 264 (5155), 107–111. <https://doi.org/10.1126/science.7832825>.
- Williams, S.R., Stuart, G.J., 2000. Site independence of EPSP time course is mediated by dendritic I $_h$ in neocortical pyramidal neurons. *J. Neurophysiol.* 83, 3177–3182. <https://doi.org/10.1006/jn.2000.0617>.
- Yang, Y.S., Kang, M.S., Kim, S.H., Kim, S.J., Eun, S.Y., Kwon, O., Bin, Jung, S.C., 2013. I $_h$ channels prevent overexcitability of early developmental CA1 neurons showing high input resistance in rats. *Brain Res. Bull.* 91, 14–20. <https://doi.org/10.1016/j.brainresbull.2012.11.009>.
- Yellon, D.M., Hausenloy, D.J., 2007. Myocardial reperfusion injury. *N. Engl. J. Med.* 357, 1121–1135. <https://doi.org/10.1056/NEJMr071667>.
- Zanelli, S.A., Rajasekaran, K., Grosebaugh, D.K., Kapur, J., 2015. Increased excitability and excitatory synaptic transmission during in vitro ischemia in the neonatal mouse hippocampus. *Neuroscience* 310, 279–289. <https://doi.org/10.1016/j.neuroscience.2015.09.046>.
- Zawar, C., Plant, T.D., Schirra, C., Konnerth, A., Neumcke, B., 1999. Cell-type specific expression of ATP-sensitive potassium channels in the rat hippocampus. *J. Physiol.* 514 (2), 327–341. <https://doi.org/10.1111/j.1469-7793.1999.315ae.x>.
- Zhan, X., Ander, B.P., Jickling, G., Turner, R., Stamova, B., Xu, H., Liu, D., Davis, R.R., Sharp, F.R., 2010. Brief focal cerebral ischemia that simulates transient ischemic attacks in humans regulates gene expression in rat peripheral blood. *J. Cerebr. Blood Flow Metabol.* 30, 110–118. <https://doi.org/10.1038/jcbfm.2009.189>.
- Zhang, J., Malik, A., Choi, H.B., Ko, R.W.Y., Dissing-Olesen, L., MacVicar, B.A., 2014. Microglial CR3 activation triggers long-term synaptic depression in the hippocampus via NADPH oxidase. *Neuron* 82, 195–207. <https://doi.org/10.1016/j.neuron.2014.01.043>.
- Zhang, K., Xu, T., Yuan, Z., Wei, Z., Yamaki, V.N., Huang, M., Haganir, R.L., Cai, X., 2016. Essential roles of AMPA receptor GluA1 phosphorylation and presynaptic HCN channels in fast-acting antidepressant responses of ketamine. *Sci. Signal.* 9, 1–25. <https://doi.org/10.1126/scisignal.aai7884>.
- Zhang, S., Murphy, T.H., 2007. Imaging the impact of cortical microcirculation on synaptic structure and sensory-evoked hemodynamic responses in vivo. *PLoS Biol.* 5, 1152–1167. <https://doi.org/10.1371/journal.pbio.0050119>.
- Zhou, P., Bacaj, T., Yang, X., Pang, Z.P., Südhof, T.C., 2013. Lipid-anchored SNAREs lacking transmembrane regions fully support membrane fusion during neurotransmitter release. *Neuron* 80, 470–483. <https://doi.org/10.1016/j.neuron.2013.09.010>.
- Zlokovic, B.V., 2011. Neurovascular pathways to neurodegeneration in Alzheimer's disease and other disorders. *Nat. Rev. Neurosci.* 12, 723–738. <https://doi.org/10.1038/nrn3114>.
- Zoghbi, H.Y., 2006. Trinucleotide repeat disorders. *Princ. Mol. Med.* 1114–1122. https://doi.org/10.1007/978-1-59259-963-9_116.