Journal Club

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# Glutamate Induces Blood–Brain Barrier Permeability through Activation of *N*-Methyl-D-Aspartate Receptors

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Review of Vazana et al.

The blood–brain barrier (BBB) acts as a control point for the entry of blood-borne molecules and cells into the CNS. It is formed by tight junctions between the endothelial cells that line blood vessels, astrocytic endfeet, and a basement membrane (Abbott et al., 2010). BBB disruption is a key element in the pathogenesis of many neurological and neurodegenerative disorders, including epilepsy, stroke, and Alzheimer's disease. Furthermore, the ability to cross the BBB is a crucial consideration for drugs targeting the brain via delivery through the bloodstream. Therefore, advances in our understanding of the structure, function, and integrity of the BBB are key to developing effective treatments for a wide variety of neurological diseases (Sandoval and Witt, 2008). In a recent study published in *The Journal of Neuroscience*, Vazana et al. (2016) demonstrate a novel mechanism governing BBB permeability related to the neuronal release of glutamate. In addition, they show that this mechanism is involved in the bidirectional modulation of brain endothelial permeability.

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To monitor BBB permeability, Vazana et al. (2016) intravenously injected the fluorescent tracer sodium fluorescein and used *in vivo* optical imaging to record the extent of dye extravasation in the rat cerebral cortex. They found that glutamate release increased vascular permeability, and this effect was attributed to activation of the N-methyl-D-aspartate type of glutamate receptors (NMDARs). Direct perfusion of glutamate and seizure induction, which leads to an increase in glutamate release, increased vessel permeability. This increase in BBB opening was mimicked by NMDA perfusion and blocked by glutamate codelivered with an NMDAR antagonist D-AP-5. This suggests that neuronal release of glutamate acts specifically on NMDARs.

To further elucidate how glutamate stimulation of NMDARs increases vessel permeability, the authors examined molecular changes in endothelial cells. Endothelial cells express NMDARs, and activation of these receptors produces a calcium influx that results in nitric oxide (NO) synthesis by the calcium-dependent enzyme nitric oxide synthase (NOS). Released NO diffuses into adjacent endothelial cells, where it activates guanylyl cyclase to generate cyclic guanosine monophosphate (cGMP). Increased intracellular levels of cGMP lead to a signaling cascade that causes BBB opening through rearrangement of tight junction proteins away from cell–cell contact regions (De Bock et al., 2013). Consistent with this, glutamate increased intracellular calcium levels in endothelial cells and subsequently resulted in greater levels of NO around microvessels. Together, these results support a mechanism whereby glutamate activates NMDARs in endothelial cells, which leads to calcium signaling and downstream NO production to promote BBB permeability (Fig. 1).

A limitation of this study is that imaging was focused on the microvasculature, effectively excluding the broader neurovascular unit from analysis. The endothelium together with astrocytes, pericytes, neurons, and the extracellular matrix comprises a neurovascular unit that is critical for the maintenance of BBB function. Previous reports have demonstrated that NMDARs are expressed by a variety of cells implicated in the modulation of BBB integrity, including astrocytes, pericytes, and microglia (Kaindl et al., 2012; Hall et al., 2014; Hogan-Cann and Anderson, 2016). Microglia can be activated by glutamate through NMDARs to produce NO (Kaindl et al., 2012) and may become further activated following BBB opening (Khatri et al., 2012). NO production in response to excess glutamate accumulation potentiates the release of glutamate from astrocytes, feeding the release of additional vesicular glutamate (Bal-Price et al., 2002). In addition, glutamate may stimulate NO release from neurons themselves by activating NMDARs (Attwell et

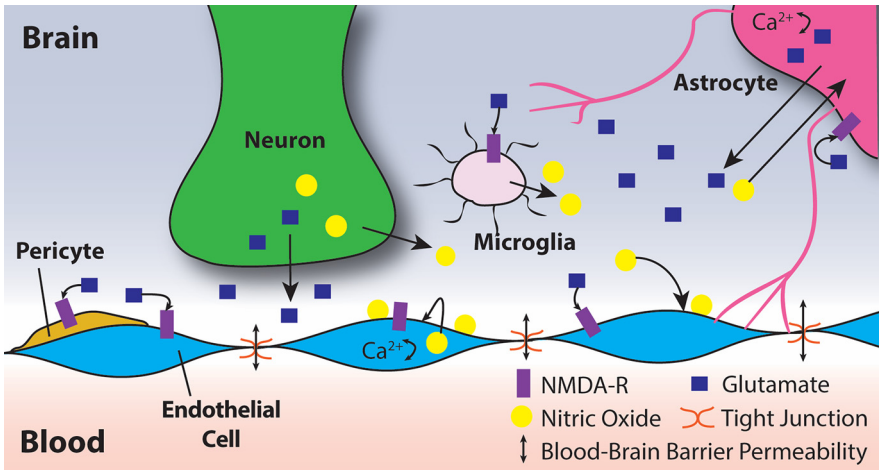


**Figure 1.** A proposed mechanism of glutamate-induced BBB disruption. Vazana et al. (2016) demonstrate that activation of neurons can induce glutamate release, which acts via NMDARs on brain endothelial cells to increase intracellular calcium levels and production of NO, leading to increased BBB permeability. Future work should investigate the contributions of other cell types in close proximity to microvascular endothelial cells, including astrocytes, pericytes, and microglia, which all possess NMDARs and may contribute to the production of NO and BBB opening.

al., 2010). These additional pathways have the potential to increase the exposure of vascular endothelial cells to NO (Fig. 1). The specific role of endothelial cells, astrocytes, and microglia in NO production and disruption of the BBB could be investigated through selective inhibition or genetic knockout of NMDARs or NOS in each cell type. If glutamate-induced vessel permeability is largely mediated by a given cell type, no BBB opening should occur after glutamate delivery in mice lacking NMDARs in that cell type.

Vazana et al. (2016) investigated whether the mechanism described above mediates bidirectional control of BBB permeability by glutamate. They found that perfusion of the NMDAR antagonist D-AP-5 reduced barrier permeability in the peri-ischemic cortex of a rat model of focal cerebral ischemia. This finding is in line with previous work related to the use of NMDAR blockers to prevent glutamate-mediated excitotoxicity after ischemia (Muir, 2006), and suggests that NMDAR blockers exert their therapeutic effect in part by promoting BBB closure. However, BBB permeability was only monitored for 60 min after photothrombosis. Future work should elucidate the time course over which D-AP-5 acts to reduce BBB permeability. This would help define the therapeutic window of D-AP-5 treatment to facilitate BBB closure after stroke.

In their final set of experiments, Vazana et al. (2016) demonstrate the use of transcranial magnetic stimulation (TMS) to increase BBB permeability for drug delivery. TMS is a nonsurgical technique that stimulates neuronal activity and increases glutamate release (Pell et al., 2011). Preclinical work using electrocortigram recordings in the rat cortex showed that TMS facilitated delivery of intravenously administered penicillin, which enhances neuronal excitability upon entry into the CNS. The authors also conducted a pilot clinical study to assess the effects of TMS on BBB integrity in humans. TMS increased BBB permeability in 10 of 15 patients with malignant brain tumors, using dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) to quantitatively monitor barrier permeability. Overall, these results confirm the therapeutic potential of TMS-mediated BBB opening for drug delivery.

Intriguingly, the preclinical experiments demonstrated that 1 Hz repetitive TMS (rTMS) induced BBB permeability, but 10 Hz failed to show the same effect. Previous studies have shown that rTMS at ≥5 Hz increases neuronal excitability and glutamate release, whereas low-frequency stimulation (~1 Hz) may suppress neuronal activity (Funke and Benali, 2011; Pell et al., 2011). The authors suggest that rTMS mediates BBB permeability through neuronal activation by showing no change in vascular permeability after rTMS combined with administration of the sodium channel blocker tetrodotoxin and synaptic transmission blockers. However, tetrodotoxin has also been shown to block sodium channels of astrocytes (Black and Waxman, 2013). Sodium ion flux through these channels affects intracellular signaling, which in turn regulates various cellular targets that are critical for astrocyte function (Parpura and Verkhratsky, 2012). These results suggest a potential role of astrocyte activity in low-frequency rTMS-induced BBB permeability. Indeed, astrocytic endfeet are an essential component of the BBB, and TMS has been suggested to modulate astrocyte activity through gap junction signaling (Oliviero et al., 2011). The role of astrocytes in TMS-induced glutamate release and vascular permeability could be further investigated by applying gap junction inhibitors, such as a connexin 43 mimetic peptide (Danesh-Meyer et al., 2012).

An important consideration when disrupting the BBB is to prevent substantial vascular compromise, leading to insufficient blood supply that would result in ischemic injury and cell death. To address this concern, Vazana et al. (2016) examined whether glutamate-induced BBB opening triggered an ischemic response. Administration of 4-AP to stimulate neuronal glutamate release and BBB opening caused a transient increase, not a decrease, in tissue oxygen levels. This outcome indicates that glutamate-mediated BBB permeability is not accompanied by ischemic injury. Furthermore, the rapid change in tissue oxygenation levels suggests that opening is highly controlled, supporting the utility of this mechanism for drug delivery. Increased brain tissue oxygenation, however, may also have negative consequences. For example, oxygen reperfusion following ischemia can stimulate the production of neurotoxic reactive oxygen species (ROS), such as NO, and inflammation (Khatri et al., 2012). Vazana et al. (2016) demonstrated an increase in ROS production as part of the mechanism of BBB opening. When produced in excessive amounts, however, NO can cause extensive cellular damage or death by oxidizing proteins, lipids, and DNA (Crowe et al., 2011).

In addition to potentially affecting oxygen levels, increased permeability of the BBB may lead to brain damage by allow circulating leukocytes, including monocytes and neutrophils, to enter the brain parenchyma, thereby causing a local immune response. BBB disruption is further accompanied by coagulation and fibrinolysis, which promote glial cell activation, inflammation, and BBB restoration (Bardehle et al., 2015). An accurate assessment of the time course of BBB opening following TMS treatment would provide insight into the extent of tissue injury, or lack thereof, and the potential toxicity of prolonged BBB disruption. For instance, the degree of TMS-induced opening may not be substantial enough to induce processes, such as fibrinolysis. The time course of TMS-induced per-

meability in humans could be monitored with follow-up DCE-MRI scans.

Overall, the results of this study support the hypothesis that glutamate plays an important role in mediating BBB function and can be exploited for clinical translation. The findings presented by Vazana et al. (2016) provide direct experimental evidence that the neuronal release of glutamate modulates BBB function, through activation of NMDARs. Transient modulation of BBB integrity has important implications for facilitating BBB repair and maintenance in neurological disorders, such as epilepsy and stroke, as well as enhancing BBB permeability for the delivery of therapeutics into the brain.

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