IBBJ

Autumn 2017, Vol 3, No 4

Mini Review

The Bimodal Nature of Neurovascular Coupling

Morris Henry Baslow*, David Nigel Guilfoyle

Center for Biomedical Imaging and Neuromodulation, Nathan Kline Institute for Psychiatric Research, 140 Old Orangeburg Road, Orangeburg, NY, 10962, USA.

Submitted 25 Jun 2017; Accepted 30 Jul 2017; Published 26 Aug2017

Neurons, by virtue of their complex and continuously changing signaling roles in brain, must be able to regulate access to energy in order to maintain their ability to communicate meaningful frequency-encoded information. This is accomplished by release of neurotransmitters to astrocytes that in turn signal the vascular system to increase cerebral blood flow (CBF). This process has been termed "neurovascular coupling" (NVC). It has also been observed that NVC is bimodal in that there are two separate mechanisms for control of CBF. One type is rapid [phasic] in response to changes in glutamatergic synaptic activity and release of glutamate (Glu), K^+ and nitric oxide (NO). Uptake of free Glu and K^+ by astrocytes induces Ca^{2+} waves activating regional astrocyte syncytium's to liberate prostaglandins which in turn dilate capillaries by relaxing surrounding pericytes. The NO dilates arterioles by relaxing surrounding smooth muscle cells. These agents acting in concert sharply increase CBF within 1-3 seconds. The other type is slow [tonic] reflecting ongoing neuronal metabolic activity of all neuron types independent of changes in synaptic activity or astrocyte Ca^{2+} waves and eliciting modest oscillations in CBF in 10's of seconds. In this review, we describe two neuronal signaling mechanisms that match the criteria for phasic and for tonic regulation of CBF. The difference being the nature of the "Glu" released by neurons and of their targeted astrocyte receptors. Dependence on synaptic activity limits phasic responses to gray matter, but tonic responses can regulate CBF in both gray matter and white matter and may be the primary regulator of CBF in white matter.

Keywords: Brain energy metabolism, cerebral blood flow, glucose, glutamate, N-acetylaspartylglutamate, neurovascular coupling, ionotropic, metabotropic

Neuronal signaling and metabolism

The function of neurons is communication and to do this efficiently, neurons must maintain a constant readiness. This entails two separate processes; housekeeping activities to maintain their structural and metabolic integrity, and second, maintaining an ability to spike as required. Much progress has been made in understanding the encoding of spike-generated neuronal languages. These sometimes very complicated and specific signal trains require adequate amounts of adenosine tri-phosphate (ATP) for neurons to perform at any level of required synaptic activity. Each spike and recovery period lasts about 1 ms and individual neurons may spike at up to 800-900 spikes/s (Hz). The spike is generated by depolarization of the cell body and axonal plasma membranes with K^+ leaving the neuron and Na^+ entering the neuron, making the interior somewhat less negative. The membrane is rapidly repolarized after each spike via Na^+/K^+

*Correspondence: Center for Biomedical Imaging and Neuromodulation, Nathan Kline Institute for Psychiatric Research, 140 Old Orangeburg Road, Orangeburg, NY, 10962, USA. E-mail: baslow@nki.rfmh.org

ATPase using ATP to restore the internal to external negative potential (1). This produces adenosine di-phosphate (ADP) as a byproduct which must then be regenerated into ATP. To do this, neurons take up and oxidize D-glucose (Glc) using O_2, both of which are supplied by the vascular system. Since the total energy supply available to the brain is limited (2), it is vital for neurons to be able to divert scarce energy supplies to areas of high metabolic need and/or increased spiking activity. Neuron cell bodies and their axons have sufficient supplies of stored ATP for repolarization to send meaningful messages for only several minutes. Therefore, it is important to understand how neurons communicate with the vascular system for supply of sufficient energy to maintain their complex, rapid, and continuously changing signaling roles. This activity to regulate and divert cerebral blood flow (CBF) as needed involves interaction between neurons, astrocytes and the vascular system and the process has been termed neurovascular coupling (NVC).

The bimodal nature of neurovascular coupling

In brain, it has been observed that there are two types of NVC that control changes in CBF (3). One type is rapid [phasic] in response to increased glutamatergic neuron synaptic activity and characterized by release of nitric oxide (NO) generated by neuron nitric oxide synthase (nNOS) (4) and liberation of K^+ and free glutamate (Glu) to extracellular fluid (ECF). Astrocytes, a component of the "tripartite synapse", take up Glu and K^+ via specific channel transporters: the high affinity sodium-dependent ionotropic Glu AMPA transporter subunits 1-4 (iGluA1-4) (5,6) and the K^+ weakly rectifying (Kir4.1) transporter respectively (7), inducing astrocyte Ca^{2+} currents and then Ca^{2+} waves that activate regional astrocyte syncytium's. These Ca^{2+} activated astrocytes synthesize and release second messengers to the vascular system via cyclooxygenase-1 (COX-1) and the secondary action of terminal prostaglandin synthases (8). Prostaglandin E_2 is reported to dilate capillaries by relaxing capillary endothelial-associated pericytes and capillary dilation appears to account for about 84% of the increase in CBF (9). The neuronal NO (nNO) along with astrocyte NO (aNO) and vascular endothelial NO (eNO) relax smooth muscles and dilate arterioles (4). Acting together, NO and prostaglandins generate a phasic response to increased synaptic firing, a response characterized by an increase in CBF and a rapid positive blood oxygenation level dependent (BOLD) magnetic resonance (MR) response. Increases in BOLD and in cerebral blood volume (CBV) are initiated in 1-3 s by arteriole dilation (10) which appear to precede astrocyte Ca^{2+} waves that occur in 3-6 s (11). The second type is slow [tonic], independent of synaptic firing, without triggering astrocyte Ca^{2+} waves and characteristic of resting state brain activity operating over minutes (3). These authors estimate that about 50% of brain vasodilation is controlled by the tonic system. Whereas several trigger molecules were known to control rapid phasic NVC, how the brain accomplished slow tonic NVC remained obscure. The observed characteristics of phasic and tonic NVC are shown in table 1.

Table 1. Characteristics of phasic and tonic NVC in brain

Characteristic	Rapid phasic NVC	Slow tonic NVC
Synaptic firing	Dependent	Independent
Astrocyte Ca^{2+} waves	Yes	No
Timeframe	1-3 Seconds	10's of Seconds
BOLD response	Rapid large increases	Slow small oscillations
Capillary dilation	Yes	Yes
Arteriole dilation	Yes	No

A candidate for control of slow tonic NVC

While a trigger for phasic NVC had been identified with the neurotransmitter Glu reaching the astrocyte ionotropic iGluA1-4 receptor, the nature of the tonic neurotransmitter and its astrocyte receptor was unknown. The physiological role of the neurotransmitter N-acetylaspartylglutamate (NAA G), with its bound Glu, and its targeted astrocyte metabotropic glutamate receptor 3 (mGluR3), was also unknown (12-15). A hypothesis based on the independent findings that NAAG peptidase that cleaves NAAG into N-acetylaspartate (NAA) and Glu was highly expressed only in astrocytes (16) and that NAA acylase that cleaves NAA into aspartate and acetate for recycling was highly expressed only in oligodendrocytes [13] suggested that NAAG might play a role in neuron-glial signaling for the purpose of regulating CBF. Neurons produce approximately 1 molecule of NAAG for every 400 molecules of Glc oxidized (17). Based on the specific characteristics of the slow tonic trigger (3) and listed in table 1, it was recently proposed that NAAG was the astrocyte-targeted neurotransmitter for regulation of tonic control of CBF (18). NAAG fits the description closely in that it is directly tied to the rate of Glc oxidation rather than to synaptic events, and can be liberated to ECF via a non-synaptic

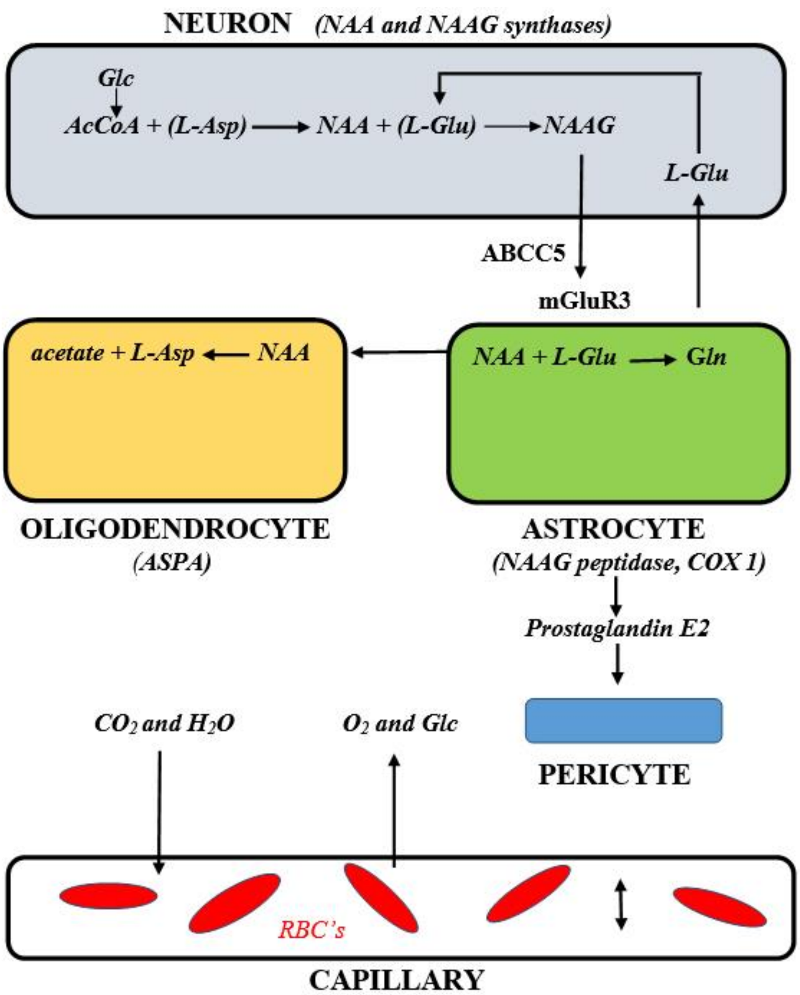

Figure 1. Cartoon of the tri-cellular metabolism and proposed function of the NAA-NAAG system in slow tonic NVC. NAAG is a neurotransmitter targeted to the astrocyte surface mGluR3 receptor. It can be released non-synaptically from neurons via the ABCC5 export transporter and upon hydrolysis by astrocyte NAAG peptidase, Glu can activate astrocyte COX-1 upregulating prostaglandin synthesis and release. Prostaglandins relax capillary-surrounding pericytes resulting in capillary dilation and an increase in focal blood flow. The Glu is transformed into glutamine (Gln) by astrocytes and recycled to neurons. NAA is released to ECF and scavenged by oligodendrocytes where it is hydrolyzed by ASPA and its products metabolized thus completing the metabolic sequence.

mechanism, perhaps associated with the neuron membrane ATP-binding cassette subfamily C, member 5 (ABCC5) NAAG efflux transporter (19). Also, its dedicated metabotropic receptor, mGluR3 is a G-protein Gi/Go bound receptor negatively coupled to adenylate cyclase that does not trigger Ca^{2+} increases in astrocytes, thus excluding its involvement in rapid synaptic events that trigger astrocyte Ca^{2+} waves and release of other NVC agents (20). In addition, evidence of a connection between NAAG and CBF was previously obtained by inhibiting astrocyte mGluR3-associated NAAG peptidase activity in mice with 2-(phosphonomethyl) pentanedioic acid (2-PMPA) and observing that there was a prolonged global drop in the BOLD signal of about 3% (21). The proposed relationship between NAAG metabolism and CBF is graphically illustrated in figure 1.

The nature of the BOLD signal

The BOLD signal is an MR water signal that is diminished by an increase in red blood cell (RBC) paramagnetic deoxyhemoglobin (Hb) resulting from the drawdown of O_2 from RBC oxyhemoglobin (HbO_2) by activated neurons (17). Thus, the BOLD signal varies inversely with RBC Hb levels, and the signal increases as CBF increases bringing a fresh supply of HbO_2 and reducing Hb levels. Therefore, the decrease in the BOLD signal in the case of inhibiting the action of NAAG peptidase was interpreted as a lack of increase in CBF and a sign that a normal NVC mechanism had been uncoupled to some degree by blocking the release of Glu at the astrocyte surface (21). In the absence of vascular dilating mechanisms in brain (3) and with limited availability of energy supplies (2), a default condition would occur where distribution of energy is a function of static physical dimensions of blood vessels rather than need-based vessel dilation.

Uncovering the multicellular genesis for obtaining sufficient energy and oxygen during rest and any level of spiking activity

The brain is the most complex organ in the body and the physiological function of neurons is to transmit meaningful information in the form of encoded spike frequencies. In order to do this neurons must maintain a state of constant readiness. The brain while only about 2% of body weight uses approximately 25% of its daily energy intake (22). In addition, the heterogeneity of neuronal cells and regions that comprise the brain is such that the needs of even very small regions of brain may change quickly over time and in a highly variable temporal fashion. To deal with such a complex organization both locally and regionally due to simultaneous, coordinated and spatially separated activations, it is vital that neurons which have limited energy stores are able to continuously signal their needs to the vascular system. As described, they do this by liberating specific neurotransmitters to astrocytes whose end-feet are in close contact with both neurons and the vascular system endothelial cells. The mechanism for rapid "phasic" changes in focal CBF has been identified with glutamatergic synaptic release of Glu and K^+ to astrocytes. A second method "tonic" has also been identified (Table 1) that does not depend on spiking, and is associated with housekeeping activities such as synthesis of proteins and the myriad metabolites that sustain their ability to carry out their signaling functions (3). In this short review, evidence is presented that phasic changes in brain CBF are a function of glutamatergic synaptic release of K^+ and of Glu that is targeted to an astrocyte ionotropic Glu receptor, and that tonic changes in CBF are a function of non-synaptic release of peptide-bound Glu by many neuron types in the form of NAAG targeted to an astrocyte metabotropic Glu receptor where the Glu is liberated by the action of NAAG peptidase. This process is highly complex and involves the coordinated activities of neurons, astrocytes, pericytes, smooth muscle cells, vascular endothelial cells, and oligodendrocytes. The multicellular genesis of these two NVC control mechanisms is presented in table 2.

Table 2. Multicellular genesis of slow tonic and rapid phasic NVC and their respective metabotropic and ionotropic "Glu" receptor control

Component	Slow tonic NVC (50%)	Rapid phasic NVC (50%)	
Control mechanism	*Metabotropic Glu receptor (mGluR3)*	*Ionotropic Glu receptor (iGluA1-4)*	
Sites of action	*Capillaries (100%)*	*Capillaries (84%)*	*Arterioles (16%)*
Neurons			
Trigger	rate of Glc oxidation	rate of firing	rate of firing
Timeframe	10's of seconds	3-6 seconds	1-3 seconds
Number of neurons	each as individual	2 or more synapsed	2 or more synapsed
Neurotransmitters	NAAG**	Glu***, K^+	nNO
Source	NAAG non-synaptic efflux (ABCC5)	synaptic leakage (tripartite synapse)	neuron NOS
Target cells	Astrocytes	Astrocytes	Smooth muscle
Astrocytes			
Receptors	mGluR3	iGluA1-4, Kir4.1	astrocyte NOS
Enzymes	NAAG peptidase		NO
Products	NAA, Glu		
Ca^{2+} waves	no	yes	
Activators	Cox-1	Cox-1	
Messengers	prostaglandins	prostaglandin E2	aNO
Target cells	Pericytes	Pericytes	Smooth muscle
NVC			
Response	slow change in HbO_2	rapid change in HbO_2	rapid change in HbO_2
Measure	BOLD	BOLD	BOLD
Inhibitors	2-PMPA	firing inhibitors	firing inhibitors
	Cox-1 inhibitors	Cox-1 inhibitors	NOS inhibitors
Brain regions served	Gray and white matter	Gray matter	Gray matter

* Table is generated from literature cited in this review, ** All neuron types can synthesize NAA. NAA is the only precursor of NAAG, *** Glutamatergic neurons.

Conclusions

In this mini review we present evidence of two separate mechanisms used by neurons to communicate their needs for increased energy. One is phasic in response to rapid changes in signaling activity that results in increases in CBF in 1-3 s. The other is tonic that results in increased CBF in 10's of seconds to minutes. Both appear to use neuronal "Glu" transmitted to juxtaposed astrocyte endfeet that in turn signal a neuron's metabolic requirements to the vascular system. Phasic NVC uses Glu leaked from synapses and activates the astrocyte ionotropic iGluA1-4 receptor, initiating astrocyte Ca^{2+} waves and release of prostaglandins and NO that rapidly increase CBF in a region of increased spiking. While the nature of the tonic transmitter is still open, we proposed that the non-synaptic release of NAAG, a non-excitatory form of Glu targeted to the astrocyte metabotropic mGluR3 receptor, matches the criteria for the tonic transmitter as shown in table 1. After docking with the mGluR3 receptor, NAAG is cleaved by astrocyte NAAG peptidase forming Glu which then can activate astrocytes without initiating Ca^{2+} waves, to release prostaglandins that increase CBF. This bimodal mechanism is unusual in that it appears to use two distinct forms of the neurotransmitter “Glu”, two different release mechanisms and two types of Glu receptors in order to signal astrocytes to increase CBF. In gray matter, the actions of these two systems cannot be separated in time or space and both systems may interact with astrocytes at all times. However, in white matter, the dearth of synapses precludes strong phasic responses to signaling and it is likely that only the tonic system is responsible for maintaining substantial neuron axon metabolic requirements via an “axon-glia-vascular unit” (23). Failure of either the phasic or the tonic system to supply adequate levels of energy to neurons and their axons in a timely manner could lead to a chronic lack of energy and inability to transmit a full range of meaningful frequency-encoded information. The functions of NAAG, the mGluR3 receptor and NAAG peptidase have recently been associated with several human brain disorders including Alzheimer’s disease, Parkinson’s disease. Huntington’s disease, cognitive loss, and neuropsychiatric disorders, and are current targets for therapeutic drug interventions (24, 25). Availability of adequate energy in both gray and white matter is the critical factor for normal neuron and brain function. We hope that this review is helpful in understanding the many facets of this developing story and that it leads to new approaches to understand the etiology of brain disorders. In summary we postulate:

- There are two mechanisms controlling NVC, one rapid [phasic] and one slow [tonic].
- Phasic NVC is associated with the rate of synaptic spiking and tonic NVC is associated with the rate of neuron Glc oxidation.
- Both mechanisms use the neurotransmitter “Glu”; phasic in the form of free Glu, and tonic as NAAG bound Glu.
- Both neurotransmitters target astrocytes, the key component in NVC.
- They are targeted to different Glu receptors on astrocytes, phasic to an ionotropic receptor and tonic to a metabotropic receptor.
- Both mechanisms can operate in gray matter, but only tonic in white matter.
- The NVC neurotransmitter in white matter is likely NAAG which is present in highest concentrations in axons and can be released to astrocytes non-synaptically at nodes of Ranvier.

Failure of either mechanism to supply adequate energy as needed may be reflected in a variety of brain signaling and metabolic disorders.

Conflict of interest

The authors declared no conflict of interest.

References

1. Dinuzzo M, Giove F, Maraviglia B, et al. Computational Flux Balance Analysis Predicts that Stimulation of Energy Metabolism in Astrocytes and their Metabolic Interactions with Neurons Depend on Uptake of K+ Rather than Glutamate. Neurochem Res. 2017;42:202-16.

2. Lennie P. The cost of cortical computation. Curr Biol. 2003;13:493-7.

3. Rosenegger D G, Tran C H, Wamsteeker Cusulin J I, et al. Tonic Local Brain Blood Flow Control by Astrocytes Independent of Phasic Neurovascular Coupling. J Neurosci. 2015;35:13463-74.

4. Toth P, Tarantini S, Davila A, et al. Purinergic glio-endothelial coupling during neuronal activity: role of P2Y1 receptors and eNOS in functional hyperemia in the mouse somatosensory cortex. Am J Physiol Heart Circ Physiol. 2015;309:H1837-45.

5. Dzamba D, Honsa P, Valny M, et al. Quantitative Analysis of Glutamate Receptors in Glial Cells from the Cortex of

GFAP/EGFP Mice Following Ischemic Injury: Focus on NMDA Receptors. Cell Mol Neurobiol. 2015;35:1187-202.

6. Hadzic M, Jack A, Wahle P. Ionotropic glutamate receptors: Which ones, when, and where in the mammalian neocortex. J Comp Neurol. 2017;525:976-1033.

7. Wanke E, Gullo F, Dossi E, et al. Neuron-glia cross talk revealed in reverberating networks by simultaneous extracellular recording of spikes and astrocytes' glutamate transporter and K+ currents. J Neurophysiol. 2016;116:2706-19.

8. Niwa K, Haensel C, Ross M E, et al. Cyclooxygenase-1 participates in selected vasodilator responses of the cerebral circulation. Circ Res. 2001;88:600-8.

9. Hall C N, Reynell C, Gesslein B, et al. Capillary pericytes regulate cerebral blood flow in health and disease. Nature. 2014;508:55-60.

10. Yu X, He Y, Wang M, et al. Sensory and optogenetically driven single-vessel fMRI. Nat Methods. 2016;13:337-40.

11. Nizar K, Uhlirova H, Tian P, et al. In vivo stimulus-induced vasodilation occurs without IP3 receptor activation and may precede astrocytic calcium increase. J Neurosci. 2013;33:8411-22.

12. Coyle J T. The nagging question of the function of N-acetylaspartylglutamate. Neurobiol Dis. 1997;4:231-8.

13. Baslow M H. Functions of N-acetyl-L-aspartate and N-acetyl-L-aspartylglutamate in the vertebrate brain: role in glial cell-specific signaling. J Neurochem. 2000;75:453-9.

14. Baslow M, Guilfoyle D. A Breakthrough in Understanding the Nature of Canavan Disease, a Human Spongiform Leukodystrophy due to Inborn Errors in the Gene Encoding for Aspartoacylase. Brain Disord Ther. 2015;4:170-1.

15. Vornov J J, Hollinger K R, Jackson P F, et al. Still NAAG'ing After All These Years: The Continuing Pursuit of GCPII Inhibitors. Adv Pharmacol. 2016;76:215-55.

16. Berger U V, Luthi-Carter R, Passani L A, et al. Glutamate carboxypeptidase II is expressed by astrocytes in the adult rat nervous system. J Comp Neurol. 1999;415:52-64.

17. Baslow M H, Guilfoyle D N. Using proton magnetic resonance imaging and spectroscopy to understand brain "activation". Brain Lang. 2007;102:153-64.

18. Baslow M, Guilfoyle D. Evidence that N-acetylaspartylglutamate is the astrocyte-targeted neurovascular coupling agent that regulates slow tonic control of brain blood flow. J. Glycomics Metab. 2016;1:32-4.

19. Jansen R S, Mahakena S, De Haas M, et al. ATP-binding Cassette Subfamily C Member 5 (ABCC5) Functions as an Efflux Transporter of Glutamate Conjugates and Analogs. J Biol Chem. 2015;290:30429-40.

20. Sun W, Mcconnell E, Pare J F, et al. Glutamate-dependent neuroglial calcium signaling differs between young and adult brain. Science. 2013;339:197-200.

21. Baslow M H, Dyakin V V, Nowak K L, et al. 2-PMPA, a NAAG peptidase inhibitor, attenuates magnetic resonance BOLD signals in brain of anesthetized mice: evidence of a link between neuron NAAG release and hyperemia. J Mol Neurosci. 2005;26:1-15.

22. Attwell D, Laughlin S B. An energy budget for signaling in the grey matter of the brain. J Cereb Blood Flow Metab. 2001;21:1133-45.

23. Hayakawa K, Lo E H. Brain-peripheral cell crosstalk in white matter damage and repair. Biochim Biophys Acta. 2016;1862:901-8.

24. Ribeiro F M, Vieira L B, Pires R G, et al. Metabotropic glutamate receptors and neurodegenerative diseases. Pharmacol Res. 2017;115:179-91.

25. Acosta C, Anderson H D, Anderson C M. Astrocyte dysfunction in Alzheimer disease. J Neurosci Res. 2017.