The phenomenon known as neural flow coupling (NFC) occurs at the capillary level where there are no known pressure controlling structures. Recent developments in advanced magnetic resonance imaging technologies have made possible in vivo direct investigations of water physiology that have shed new insight on the water dynamics of the cortical pericapillary space and their complex functionality in relation to NFC. Neural activities initiate a chain of events that ultimately affect NFC. First, neural activities generate extracellular acidification. Extracellular acidosis in turn produces inhibition of aquaporin-4 (AQP-4) located at the end feet of pericapillary astrocytes, the water channel which regulates water influx into the pericapillary space and, hence, interstitial flow. Reduction of pericapillary water pressure results in a negative balance between pericapillary and intraluminal capillary pressure, allowing for capillary caliber expansion. Proton permeability through the tight junctions of the blood brain barrier is significantly high owing to the Grothuss proton “tunneling” mechanism and, therefore, carbonic anhydrase (CA) type IV (CA-IV) anchored to the luminal surface of brain capillaries functions as scavenger of extracellular protons. CA-IV inhibition by acetazolamide or carbon dioxide results in the accumulation of extracellular protons, causing AQP-4 inhibition and a secondary increase in rCBF.

Keywords: neuro flow coupling, aquaporin 4, interstitial flow, protons, neural activation.

Introduction
Recent studies have shown that the classic circulation model of cerebrospinal fluid (CSF) is incomplete. Production of CSF is not only dependent on choroid plexus but also on water flux in the pericapillary (Virchow Robin) space. Historically, CSF flow through the pericapillary space is known as interstitial flow, and is considered to play a role equivalent to the systemic lymphatic system. Advancements in modern magnetic resonance imaging (MRI) technologies have allowed for the noninvasive investigation of water flow in vivo. These studies revealed that water dynamics of the pericapillary space, ie, interstitial flow, is controlled by aquaporin-4 (AQP-4), the main subset of the aquaporin water channel family in the brain. It has also been demonstrated that inhibition of AQP-4 is strongly coupled with an increase in regional cerebral blood flow (rCBF). These observations have led to a better understanding of the architectural significance and functionality of the cerebrovascular system. This article is a concise review of the modern concept of neural flow coupling (NFC) and its relationship to water dynamics in the pericapillary space.

Cerebral Autoregulation: Upstream Control
Cerebral autoregulation signifies an intrinsic ability of the cerebral vasculature to maintain cerebral blood flow at a relatively constant rate of approximately 50 ml per 100 g brain tissue per minute in the face of blood pressure changes. Autoregulation generally functions between mean blood pressures of 60 to 150 mmHg. It is maintained in parasympathetically and/or sympathetically denervated animals and the system is independent from extrinsic neural control. Instead, intrinsic neural nitric oxide (NO) control and release of vasoactive substrates by the brain are believed to play essential roles in maintaining constant cerebral perfusion. Perfusion is held constant by means of the cerebral vasculature smooth muscle that constricts and dilates in response to elevated and decreased systemic pressure, respectively. Although this “upstream” control of inflow pressure appears to be rather straightforward, the physiologic mechanisms underlying NFC, neural activity-associated rCBF increase, were until now poorly understood.

Virchow Robin Space and Interstitial Flow: Cerebral Lymphatic Equivalent
Fluid-filled canals surrounding perforating arteries and veins in the parenchyma of the brain were recognized in early modern medicine and described in detail by Rudolph Virchow and Charles Philippe Robin. The space is commonly referred to as the Virchow Robin space. It is now clearly understood that the fluid in the Virchow Robin space constitutes interstitial flow that drains into the CSF system (Fig 1). Virchow Robin interstitial flow is believed to play a role similar to systemic lymphatics.

The basic function of systemic lymphatics is drainage of cellular debris subjected to molecular scrutiny before returning...
Virchow Robin space and interstitial flow. The ventricles and β is blood viscosity, R is pressure loss (differences in inflow and outflow ∼ it is the fluid pressure R μ 4 1/Δ1 β Therefore, AQP-4 activities play a role in β and constant venous drainage of CSF. By contrast, AQP-4 is a closely packed water channel and is involved in preventing β-amyloid aggregation and senile plaque formation. Active water influx into the CSF system from the blood stream has been shown to be regulated by AQP-4, not AQP-1, indicating that interstitial flow plays an important role in CSF dynamics.

As cerebral equivalent of the systemic lymphatic system, interstitial flow dysfunction can be expected to result in reduction of β-amyloid clearance. Indeed, senile plaque bearing transgenic mice showed significant decline of water influx into the CSF system, to the extent similar to that found in AQP-4 knockout mice. Positron emission tomography studies in AD patients have shown virtually identical results.

NFC and rCBF
Increased rCBF associated with brain activation is a well-recognized phenomenon that is known as NFC. Since this is a micro-, rather than macroenvironmental event occurring within an area limited to 250 μm around the site of neural activity, the regulatory mechanism for NFC should be within the capillaries.

Considering blood flow to be steady, laminar flow within a long cylindrical pipe (Fig 2), the Hagen-Poiseuille equation gives volumetric blood flow, Φ, as

$$\Phi = \frac{\pi \Delta P}{\eta L} R^4$$

where ΔP is pressure loss (differences in inflow and outflow pressure), L is the length of the vessel tube, η is blood viscosity, and R is the radius of the vessel.

Given that steady inflow pressure is rigorously controlled by upstream arterial autoregulation and constant venous pressure, under physiological conditions, cerebral blood flow is virtually determined by the radius of the vessel and increases parallel to its fourth power

$$\Phi \sim R^4$$

The relationship implies that even small changes in capillary caliber have significant effects on rCBF.

Capillaries are devoid of muscle and, hence, are not under neural control. The perforating vessels of the cerebral cortex are surrounded by a fluid-filled perivascular (Virchow Robin) space. At the capillary level, fluid pressure within the vessel lumen is directly opposed by pericapillary fluid pressure. Therefore, the parameter that determines capillary caliber is the pressure balance between luminal (intracapillary) and outer (pericapillary) fluid pressures. Since intracapillary pressure reflects the inner pressure of arterioles, and is therefore a function of upstream arterial autoregulation, it is the fluid pressure of the pericapillary space that inversely determines cerebral capillary caliber changes and, hence, rCBF, as follows:

$$\Phi \sim R^4 \sim \frac{1}{P_{peri-capillary}}$$

AQP-4 controls the water dynamics of the pericapillary space in the brain. Therefore, AQP-4 activities play a role in controlling rCBF. Simply put, under physiological conditions, rCBF correlates inversely to AQP-4 activities.

$$rCBF \sim \frac{1}{AQP-4\ activities}$$
Fig 2. Vessel diameter is determined by tension of smooth muscle in artery, arteriole, venule, and vein (Brain Vessels). Capillaries are devoid of muscle and in capillaries with tight endothelium such as brain capillaries, capillary caliber is determined by the pressure balance between luminal and outer fluid pressures (Brain Capillary). For capillaries with leaky endothelium (Common Capillary), pressure balance is quickly equalized without capillary caliber changes.

Fig 3. Pericapillary water dynamics. Water permeability of brain capillaries is restricted due to the tight endothelium, presence of tight junctions and active suppression of AQP-1. By contrast, significant water flow is present in the Virchow Robin space (interstitial flow) and is supported by active water inflow through AQP-4. Although it has not been clearly confirmed (?), interstitial flow may similarly be present along the medullary and subependymal veins.

Indeed, reduced AQP-4 activities by its inhibitor TGN-020 effectively increased rCBF in mice. Under physiological conditions, AQP-4 is believed to be inhibited by extracellular protons similar to other AQP isoforms. Therefore, rCBF is predicted to correlate with extracellular proton density (Figs 3 and 4).

\[ \text{rCBF} \sim [H^+]_{\text{extra}} \]

The steps outlined above provide a fresh understanding of the underpinnings of NFC.

Neural Activities and Extracellular Acidosis

Since the original description by Urbanics et al extracellular (interstitial) acidification associated with neural activities has been extensively studied by various investigators. Modern MRI technologies demonstrated unequivocally that regional neural activities in humans are accompanied by extracellular acidosis found in a virtually identical distribution as the neural activity-induced increase in rCBF detected by blood oxygenation level-dependent contrast. Therefore, at least from a phenomenological standpoint, neural activity-induced extracellular acidification plays a role in NFC. Although the precise underlying mechanisms remain to be elucidated, it appears clear that neural activity-induced interstitial acidification, and the resultant inhibition of AQP-4, is indeed a main mediator of neural activity-associated rCBF increase. Further support for this concept comes from the “Diamox effect.” Acetazolamide (Diamox) is a carbonic anhydrase (CA) inhibitor and a powerful agent for increasing rCBF. This “Diamox effect” is well known to be accompanied by interstitial acidosis in the brain.

Within the large CA family, CA type IV (CA-IV) represents the dominant CA in the cerebral cortex and is anchored to the luminal surface of cerebral capillaries. It has been shown that interstitial CA activity in the brain is attributable to CA-IV. The human NBC1 sodium bicarbonate cotransporter directly interacts with CA-IV. The tethering of intracellular CA type II (CA-II) and extracellular CA-IV in proximity to the NBC1 HCO\(_3\)\(^-\) transport site maximizes the transmembrane HCO\(_3\)\(^-\) gradient local to NBC1 and thereby activates the transport rate.

Since proton permeability through the tight junctions is significantly higher than for other small molecules, owing to the Grotthuss proton tunneling mechanism, capillary CA-IV with NBC1 and CA-II effectively function as scavenger of extracellular protons generated by neural activation (Fig 5). CA inhibition by acetazolamide or excess of carbon dioxide (CO\(_2\)) in capillary blood results in accumulation of extracellular protons which in turn inhibit water flux through AQP-4. The resultant negative pressure relation with respect to intraluminal capillary pressure affects capillary dilatation and an increase in rCBF.
Fig 4. Neural activation. Neural activation produces extracellular acidification accompanied by increase in rCBF and astrocyte swelling. Proton inhibition of AQP-4 results in a reduction of water flow from astrocytes into the pericapillary Virchow Robin space, astrocyte swelling and capillary expansion due to reduction of pericapillary fluid pressure.

Fig 5. CA-IV system. Complex of CA-IV anchored to luminal surface of cerebral capillary, human NBC1 sodium bicarbonate cotransporter and intracellular CA-II. Their proximity maximizes the transmembrane HCO$_3^-$ gradient local to NBC1 and thereby activates the transport rate. Because of the high proton permeability through tight junctions, capillary CA-IV with NBC1 and CA-II effectively function as scavenger of extracellular protons generated by neural activation. CA inhibition by acetazolamide or excess of CO$_2$ in capillary blood results in accumulation of extracellular protons.

Summary: A New Concept

Technological advancements, especially those in the field of MRI, have led to a new level of understanding of the physiologic underpinnings of neural activation-induced rCBF increase, a phenomenon known as NFC. The main player mediating NFC is the proton. Neural activities produce interstitial acidification. Excess protons inhibit AQP-4 activities in the pericapillary Virchow Robin space, resulting in a reduction in the pericapillary pressure. The negative balance between pericapillary and intraluminal capillary pressure induces dilatation of capillaries and an increase in rCBF. Acetazolamide or excess CO$_2$ blocks active clearance of interstitial protons which are ordinarily highly permeable through the tight junctions and, similar to neural activities, causes interstitial acidification and an increase in rCBF.

The precise molecular mechanism of extracellular acidification associated with neural activities remains to be elucidated. Such acidification has been shown to be associated with
intracellular alkalization of astrocytes. Active proton extrusion by astrocytes appears to be the most attractive explanation. The functional significance of NFC has been linked to elimination of heat production brought about by neuronal activities. A heat-sensitive voltage-gated proton channel similar to neutrophil Hv1 may play a role, although much remains to be investigated.

The work was supported by grants from the Ministry of Education, Culture, Sports, Science, and Technology [Japan].

References
1. Orešković D, Klarica M. The formation of cerebrospinal fluid: nearly a hundred years of interpretations and misinterpretations. Brain Res Rev 2010;64:241-62.
2. Igarashi H, Tsuji M, Kwee IL, et al. Water influx into Cerebrospinal fluid (CSF) is primarily controlled by aquaporin-4, not by aquaporin-1: O-17 JJVCPE MRI study in knockout mice. Neuroreport 2014;25:39-43.
3. Iliff JJ, Wang M, Liao Y, et al. A paravascular pathway facilitates CSF flow through the brain parenchyma and the clearance of interstitial solutes, including amyloid. Sci Transl Med 2012;4:147ra111.
4. Esiri MM, Gay D. Immunological and neuropathological significance of the Virchow-Robin space. J Neurol Sci 1980;50:8-15.
5. Weller RO, Pathology of cerebrospinal fluid and interstitial fluid of the CNS: significance for Alzheimer disease, prion disorders and multiple sclerosis. J Neuropathol Exp Neurol 1998;57:885-94.
6. Johnston M, Papaionomou C. Cerebrospinal fluid transport: a lymphatic perspective. News Physiol Sci 2002;17:227-30.
7. Abbott NJ. Evidence for bulk flow of brain interstitial fluid: significance for physiology and pathology. Neurochem Int 2004;45:545-52.
8. Weller RO, Djuanda E, Yow HY, et al. Lymphatic drainage of the brain and the pathophysiology of neurological disease. Acta Neurophysiol 2009;117:1-14.
9. Weller RO, Kida S, Zhang ET. Pathways of fluid drainage from the brain – morphological aspects and immunological significance in rat and man. Brain Pathol 1992;2:277-84.
10. Haj-Yasein NW, Jensen V, Ostby I, et al. Aquaporin-4 regulates extracellular space volume dynamics during high-frequency synaptic stimulation: a gene deletion study in mouse hippocampus. Glia 2012;60:867-74.
11. Kitaura H, Tsuji M, Huber VJ, et al. Activity-dependent gial swelling is impaired in aquaporin-4 knockout mice. Neurosci Res 2009;64:208-12.
12. Huber VJ, Tsuji M, Nakada T. Aquaporins in drug discovery and pharmacotherapy. Mol Aspects Med 2012;33:691-703.
13. Igarashi H, Tsuji M, Huber VJ, et al. Inhibition of Aquaporin-4 significantly increases regional cerebral blood flow. NeuroReport 2013;24:324-8.
14. Nakada T. Virchow-Robin space and aquaporin-4: new insights on an old friend. Croat Med J 2014;55:328-36.
15. Lulu X, Kang H, Xu Q, et al. Sleep drives metabolite clearance from the adult brain. Science 2013;342:373-7.
16. Nakada T, Igarashi H, Suzuki Y, et al. Alzheimer patients show significant disturbance in water influx into CSF space strongly supporting β-amyloid clearance hypothesis. American Academy of Neurology Annual Meeting 2014;S58.001.
17. Paulson OB, Strandgaard S, Edvinsson L. Cerebral autoregulation. Cerebrovasc Brain Metab Rev 1990;2:161-92.
18. Heistad DD, Kontos HA. In: Berne RM, Sperelakis N, eds. Handbook of Physiology. Chapter: The Cardiovascular System III. Bethesda, MD: American Physiological Society; 1979:137-82.
19. Mellander S. Functional aspects of myogenic vascular control. J Hypertens 1989;7:521-30.
20. Osel G, Berekke JF, McElroy-Yaggy K, et al. Myogenic tone, reactivity, and forced dilatation: a three-phase model of in vitro arterial myogenic behavior. Am J Physiol Heart Circ Physiol 2002;283:H2260-7.
21. Busija EW, Heistad DD. Factors involved in the physiological regulation of the acerbroculear circulation. Rev Physiol Biochem Pharmacol 1984;101:161-211.
22. Talman WT, Nitschke Dragon D. Neuronal nitric oxide mediates cerebral vasodilatation during acute hypertension. Brain Res 2007;1139:126-32.
23. Virchow R. Ueber die Erweiterung kleinerer Gefässe. Arch Pathol Anat Physiol Klin Med 1851;3:427-62.
24. Robin C. Recherches sur quelques particularites de la structure des capillaires de l’encephale, J Physiol Homme Animaux 1859;2:537-48.
25. Parikh MS, Brewer GJ. Amyloid-β as a modulator of synaptic plasticity. J Alzheimers Dis 2010;22:741-63.
26. Li X, Buxbaum JN. Transthyretin and the brain re-visited: is neuronal synthesis of transthyretin protective in Alzheimer’s disease? Neurodegeneration 2011;16:79.
27. Nielsen S, Smith BL, Christensen El, et al. Distribution of the aquaporin CHIP in secretory and resorptive epithelia and capillary endothelia. Proc Natl Acad Sci USA 1993;90:7275-9.
28. Dolman D, Drndarski S, Abbott NJ, et al. Induction of aquaporin 1 but not aquaporin 4 messenger RNA in rat primary brain microvessel endothelial cells in culture. J Neurochem 2005;93:825-33.
29. Igarashi H, Suzuki Y, Kwee IL, et al. Water influx into cerebrospinal fluid is significantly reduced in senile plaque bearing transgenic mice, supporting β-amyloid clearance hypothesis of Alzheimer disease. Neurological Res 2014;36:1094-8.
30. Silver IA. Cellular microenvironment in relation to local blood flow. In: Elliott K, O’Connor M, eds. Ciba Foundation Symposium 56 – Cerebral Vascular Smooth Muscle and Its Control. Chichester, UK: John Wiley & Sons; 2008: Ch. 5.
31. Faber TE. Fluid Dynamics for Physicists. Cambridge: Cambridge University Press, 1995.
32. Frick A, Järva M, Törnroth-Horsefield S. Structural basis for pH gating of plant aquaporins. FEBS Letters 2013;587:989-93.
33. Urbanics R, Leniger-Follert E, Litibers DW. Time course of changes of extracellular H+ and K+ activities during and after direct electrical stimulation of the brain cortex. Pfuegers Arch 1978;378:47-53.
34. Kraig RP, Ferreira-Filho CR, Nicholson C. Alkaline and acid transients in cerebellar microenvironment. J Neurophysiol 1983;49:831-51.
35. Chester M. Regulation and modulation of pH in the brain. Physiol Rev 2003;83:1183-221.
36. Chester M, Kraig RP. Intracellular pH transitions of mammalian astrocytes. J Neurosci 1989;9:2011-9.
37. Magnotta VA, Heo HY, Dlouhy BJ, et al. Detecting activity-evoked pH changes in human brain. Proc Natl Acad Sci 2012;109:8270-3.
38. Vorstrup S, Henriksen L, Paulson OB. Effect of acetazolamide on cerebral blood flow and cerebral metabolic rate for oxygen. J Clin Invest 1984;74:1634-9.
39. Ghandour MS, Langley OK, Zhu XL, et al. Carbonic anhydrase IV on brain capillary endothelial cells: a marker associated with the blood-brain barrier. Proc Natl Acad Sci USA 1992;89:6823-7.
40. Tong C-K, Brion LP, Suarez C, et al. Interstitial carbonic anhydrase (CA) activity in brain is attributable to membrane-bound CA type IV. J Neurosci 2000;20:8247-33.
41. Alvarez BV, Loiselle FB, Supuran CT, et al. Direct extracellular interaction between carbonic anhydrase IV and the human NBC1 sodium/bicarbonate co-transporter. Biochemist 2003;42:12321-9.
42. Angelow S, Kim KJ, Yu ASL. Claudin-8 modulates paracellular permeability to acid and basic ions in MDCK II cells. J Physiol 2006;571.1:15-26.
43. Fujisawa Y, Kurokawa T, Takeshi K, et al. The cytoplasmic coiled-coil mediates cooperative gating temperature sensitivity in the voltage-gated H+ channel Hv1. Nat Commun 2012;3:816.