

IN a model of neurons in a brain cell assembly, changes in volume of the extracellular space affect neuronal excitability and basal metabolism. A widely applicable coefficient of excitability with respect to a variation of the volume fraction has been determined. Calculations suggest that chloride increases membrane stability by indirectly promoting an acceleration of the metabolic pumping rate as a response to a diminished extracellular volume fraction. Volume fraction changes induced by cell swelling in a compact and highly tortuous microenvironment may play a role in epilepsy and, following brain damage, in cell death and recovery.

Key words: Cell assemblies; Cell swelling; Extracellular space; Metabolic power; Non-synaptic diffusion neurotransmission; Volume fraction

Brain cell microenvironment effects on neuron excitability and basal metabolism

Gaetano L. Aiello^{1,3,CA}
and Paul Bach-y-Rita^{1,2}

¹Center for Neuroscience and ²Department of Rehabilitation Medicine, University of Wisconsin, 1300 University Ave, Room 2756, Madison, WI 53706, USA; ³Istituto di Fisica dell'Università, Via Archirafi, 36, 90123, Palermo, Italy

CA,¹Corresponding Author and Address

Introduction

Cell assemblies, or neuronal modules, consist of groups of tens of thousands to hundreds of millions of neurons.^{1,2} Many brain functions may be served by the action of cell assemblies rather than by individual neurons.^{1–3} The extracellular space (ECS) that surrounds the individual neurons participates in many functions, including non-synaptic diffusion neurotransmission (NDN).⁴ The role of ECS in brain physiology and pathology was not fully appreciated until recently: Nicholson considers that the “brain cell microenvironment came to be clearly defined in 1969”.⁵ Nicholson's diffusion studies in the living brain^{6,7} confirmed several previous morphological studies that demonstrated the existence of a large extracellular volume fraction, α , of the order of 20–30% of the volume of the brain, with significant regional differences.^{5,8} By measuring the effects of membrane depolarization on light scattering by cerebral cortical slices, Lipton⁹ concluded that “. . . changes in the intercellular K^+ concentrations of size and duration thought to occur following nervous activity in the CNS cause cell volume changes large enough to drastically reduce the intercellular volumes and so, transiently, increase extracellular molecular and ionic concentration”. Changes in the volume fraction (VF) of the ECS, (α), affect membrane excitability;¹⁰ α is reduced by neuronal^{6,7,9} and glial^{10,11} swelling. The effects of α on membrane excitability and basal metabolism of brain cells are, therefore, to be evaluated in the context of cell

assemblies embedded in neuropil, as they would be found in the living brain.³

Methods and results

In our model, the assembly comprises N identical neurons, each of volume v , all at rest. The volume of the ECS is a fraction α of the total volume of the system, i.e. $V_{ECS} = \alpha V_{sys}$. The volume of the system is the sum of the volumes of all the cells (Nv) plus V_{ECS} . As a result, V_{ECS} can be written as $V_{ECS} = Nv\alpha/(1 - \alpha)$. For each ion in the system, let n_i be the sum of all the moles inside the cells, n_{out} those in the ECS, and $n = n_i + n_{out}$ the total number of moles in the system. With these symbols, the extracellular concentration of the generic ion X can be put in the form:

$$[X]_{out} = \frac{1 - \alpha}{\alpha} \left(\frac{n}{Nv} - [X]_{in} \right) \quad (1)$$

The volume of a cell at rest is constant, i.e. the cell does not shrink or swell under mere basal metabolism. Assuming mass conservation ($n = \text{const.}$), and X_{in} a constant by virtue of metabolic pumping then, following equation 1, the extracellular concentrations can vary only by virtue of a change in the value of α . It follows that the Nernst potentials,¹² and consequently the membrane potential, will depend on α , which implies that the excitability of a cell should vary with α . In normal conditions, i.e. in the absence of any shrinking or swelling, let V_{X0} be the Nernst

potential for the ion X. Let α_0 be the volume fraction in these conditions, e.g. $\alpha_0 = 0.25$, as suggested by Nicholson.^{6,7} For $\alpha \neq \alpha_0$, $V_X \neq V_{0X}$. The difference $V_X - V_{0X}$ is exclusively due to a change in the volume fraction. It is easy to prove, with the help of equation 1, that

$$V_X - V_{0X} = Z_X^{-1} v_0 \ln \frac{1 - \alpha}{\alpha} - Z_X^{-1} v_0 \ln \frac{1 - \alpha_0}{\alpha_0} = Z_X^{-1} b(\alpha) \tag{2}$$

where Z_X is the signed valence of ion X, and $v_0 \approx 27$ mV at room temperature. The function $b(\alpha)$ is shorthand for the difference between the logarithms in equation 2, multiplied by the constant v_0 . As an example, for cations as K^+ and Na^+ , $Z_X = +1$ and V_X increases by 7.7 mV from $\alpha_0 = 0.25$ to $\alpha = 0.2$, and decreases by 1.1 mV for $\alpha = 0.3$. For anions such as Cl^- , $Z_X = -1$ and V_X decreases for $\alpha > \alpha_0$ and increases for $\alpha < \alpha_0$.

Using the Hodgkin-Huxley circuit model,¹³ the membrane potential can be written as:

$$V_m = \frac{g_K V_K + g_{Na} V_{Na} + g_{Cl} V_{Cl} + g_{Mg} V_{Mg} + g_{Ca} V_{Ca} + \dots}{g_K + g_{Na} + g_{Cl} + g_{Mg} + g_{Ca} + \dots} \tag{3a}$$

$$V_m = V_{0m} \equiv + \gamma b(\alpha) \tag{3b}$$

where the g_X s are the passive channel conductances, and V_{0m} is the voltage in normal conditions ($\alpha = \alpha_0$; $V_K \equiv V_{0K}$, $V_{Na} \equiv V_{0Na}$, $V_{Cl} \equiv V_{0Cl}$, etc...). Equation 3b states that the difference $V_m - V_{0m}$ is due to a change in volume fraction. The voltage difference is formalized by the term $\gamma b(\alpha)$, where γ is a coefficient, defined by

$$\gamma = \frac{Z_K^{-1} g_K + Z_{Na}^{-1} g_{Na} + Z_{Cl}^{-1} g_{Cl} + Z_{Mg}^{-1} g_{Mg} + Z_{Ca}^{-1} g_{Ca}}{g_K + g_{Na} + g_{Cl} + g_{Mg} + g_{Ca} + \dots} = \frac{g_K + g_{Na} - g_{Cl} + 1/2 g_{Mg} + 1/2 g_{Ca} + \dots}{g_K + g_{Na} + g_{Cl} + g_{Ca} + g_{Mg} + \dots} \tag{4}$$

From equations 3 and 4 it is evident that γ acts as an attenuator of the excitability of the cell with respect to a change in the volume fraction. The presence of free anions (such as Cl^-) makes $\gamma < 1$. Other ions, such as Mg^{2+} , Ca^{2+} act similarly ($Z_{Mg} = Z_{Ca} = 2$), the trend being toward smaller and smaller γ s as more ions are included. In contrast, in the two-ion model (K^+, Na^+) $\gamma = 1$ for any α , and no attenuation will result. Chloride ions (and, to a less extent, Mg^{2+} , Ca^{2+}), by reducing γ , have the effect of stabilizing the membrane potential. The inhibitory role of Cl^- has been noted by Smith.¹²

The attempt to maintain the membrane voltage at a constant value against the depolarizing effect of a diminished volume fraction has a cost in terms of expenditure of metabolic energy. From the standpoint of the Hodgkin-Huxley circuit, the increases in power of the ionic pumps can be easily inferred by evaluating the power losses of all the Nernst 'batteries'. Thus, with $g_X (V_X - V_m)$ being the ionic passive current, the power loss for the battery X is $g_X (V_X - V_m) V_X$. By replacing V_m as given in equation 3a, then summing over all the batteries, and finally arranging the results, the total power loss takes the form

$$W = g_{XY} (V_X - V_Y)^2 + g_{XZ} (V_X - V_Z)^2 + g_{YZ} (V_Y - V_Z)^2 + \dots \tag{5}$$

where g_{XY} stands for $g_X g_Y / (g_X + g_Y + g_Z + \dots)$. The power loss in normal conditions is obtained by replacing $V_X = V_{0X}$, $V_Y = V_{0Y}$, $V_Z = V_{0Z}$, etc. in equation 5. Replacing the Nernst potentials as given in equation 2 in $V_X - V_Y$, this difference can be written as

$$V_X - V_Y = V_{0X} - V_{0Y} + \zeta_{XY} b(\alpha) \tag{6}$$

where ζ_{XY} stands for $Z_X^{-1} - Z_Y^{-1}$. Similarly for all the other voltage differences. Replacing equation 6 in equation 5, and after carrying out the tedious algebra, the following result is obtained:

$$W = W_0 + (g_{XY} \zeta_{XY}^2 + g_{XZ} \zeta_{XZ}^2 + g_{YZ} \zeta_{YZ}^2 + \dots) b^2(\alpha) + 2[g_{XY} \zeta_{XY} (V_{0X} - V_{0Y}) + g_{XZ} \zeta_{XZ} (V_{0X} - V_{0Z}) + g_{YZ} \zeta_{YZ} (V_{0Y} - V_{0Z}) + \dots] b(\alpha) \tag{7}$$

where W_0 is the power loss in normal conditions. Despite the apparent complexity, equation 7 simply states an already familiar concept, namely, that a difference in the power loss is entirely due to a change in the volume fraction. The two extended sums in equation 7 are mere coefficients of $b^2(\alpha)$ and $b(\alpha)$ respectively, their actual values depending on the species of ions involved, and on the membrane conductances to these ions. As an example, in the three-ion model (K,Na,Cl) $\zeta_{XY} \neq 0$ only if either $X = Cl$ or $Y = Cl$. It follows that both the coefficients of $b^2(\alpha)$ and $b(\alpha)$ contain g_{Cl} as a common factor. If Cl is absent ($g_{Cl} = 0$) then both coefficients vanish and $W = W_0$ independently of α . In terms of metabolic energy, the presence of Cl^- ions makes it possible for the cell to regulate the rate of the pumps in response to a variation of the volume fraction. A mechanism that may explain the role of chloride in boosting the metabolic energy of the Na-K pump as a response to a reduced α is via the activation of the neuronal K-Cl co-transporter that maintains a low intracellular chloride level.¹⁴

Figure 1 shows the percentage variation of the membrane potential in cat motor neuron

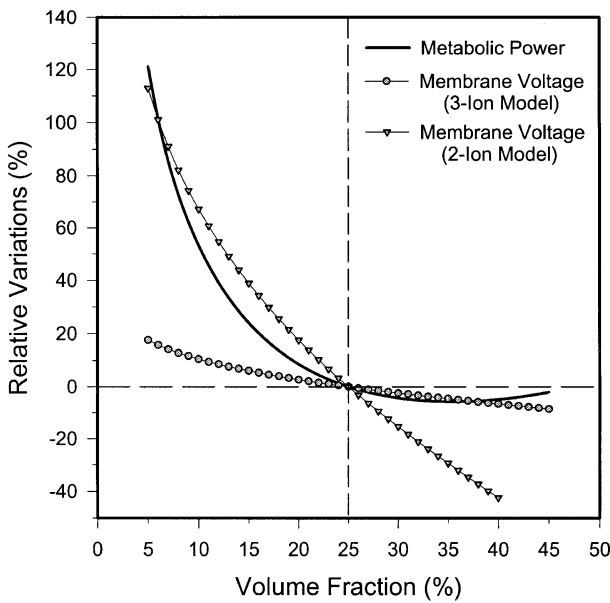


FIG. 1. Variations of membrane potential and metabolic power of a neuron at rest in a cell assembly as a response to a change in VF are graphed. In the K^+ - Na^+ model the membrane is very sensitive to a diminished volume fraction of the extracellular space (α), with a voltage rising freely toward depolarization. The addition of chloride stabilizes the membrane at a cost of an increased basal metabolism. The example refers to the cat motor neuron. The conductances were: $g_{Na}/g_K = 1/3$, $g_{Cl} = g_K$. The reference voltage is evaluated at $\alpha_0 = 0.25$.

$[V_{OK} = -88.30, V_{ONa} = +61.50, V_{OCl} = -70.28 \text{ mV}$, (cf. Ref. 12)] as a function of the volume fraction. The function plotted is $(V_m - V_{om})/|V_{om}|$, which according to equation 2 equals $\gamma b(\alpha/|V_{om}|)$. Here, the function $b(\alpha)$ is independent of the type of cell. The value of V_{om} is also irrelevant, the emphasis being on the coefficient γ , which acts as a passive gain upon $b(\alpha)$. The value of γ is actually determined by the type and numbers of free ions exchanged through the plasma membrane, and by the passive conductances of the respective transmembrane channels. In a cell model where only K^+ and Na^+ are considered, $\gamma = 1$, i.e. no attenuation on the function $b(\alpha)$ will result: the membrane voltage will rise uncontrolled towards depolarization as α decreases.

The percentage variation of the metabolic power for this neuron is also plotted in the same graph. The function plotted is $(W - W_0)/W_0$, which in this case depends directly on g_{Cl} . In absence of chloride $W = W_0$ for all values of α . The presence of chloride provides the cell with a way to oppose an α -induced depolarization by drawing on its own metabolic resources.

Discussion

In this model, changes in α are considered averaged over space and time, and caused by neuronal (and, possibly, by glial) activity outside the cell

assembly under consideration, though close enough to produce an effect as if the ionic environment were ‘concentrated’ (or diluted). In the living brain, active cells are in close proximity to inactive cells within the same assembly. During activity, the cell swells due to influx of isotonic solutions, or water.⁹ In the classical scheme of a neuron firing an action potential, the membrane permeability to Na^+ increases (voltage-gated Na -channels open up) while the permeability to K^+ decreases (voltage-gated K -channels shut off). As a result, Na^+ ions from the ECS flow into the cell, while K^+ ions are pushed away, increasing $[K^+]_{out}$. In the case of glia swelling, the cell (astrocyte) has a high K^+ conductance,¹⁴ thus K^+ ions flow in and Na^+ ions are pushed away, increasing $[Na^+]_{out}$. Delayed glia swelling prevents an excessive build up of K^+ in the small intercellular clefts after intense neuronal activity. It has been shown that $[K^+]_{out}$ increases up to 20 mM l^{-1} following normal nervous activity, and that it decays with a half-time of several seconds.⁸ Figure 2 illustrates these possible mechanisms yielding *in situ* and transient variations of the intercellular K^+ and Na^+ concentrations. As for Cl^- , its intercellular concentration would also increase, either indirectly, i.e. following influx of isotonic solutions of K^+ and Na^+ , or simply by influx of water into the active cells.

Lipton⁹ noted a 6% increase in average volume in cerebral cortical tissue slices following a 5 mM l^{-1}

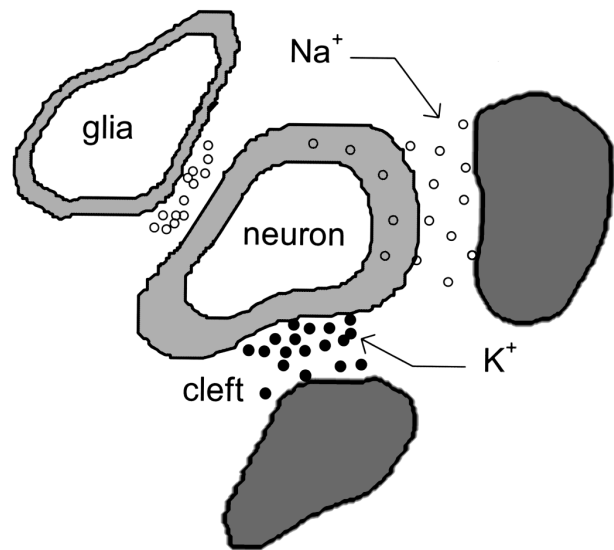


FIG. 2. A schematic representation of the local reduction of the extracellular space with neuronal activity. Three neurons and one glia cell are separated by the intracellular space, in which Na^+ and K^+ ions are represented. The middle neuron and the glia are shown to increase in volume (stippled area) with neural activity, thus reducing the intercellular distance. The dark cells are at rest. Neuron and glia swelling produce local increases of $[K^+]_{out}$ and $[Na^+]_{out}$, respectively, and indirectly of $[Cl^-]_{out}$ (see text). For clarity, K^+ and Na^+ ions are shown separately in distinct sites of the neurone membrane, although in life ions are intermixed.

increase of $[K^+]_{out}$. He concluded that "Even such a small increase would have dramatic effects upon intercellular space in the cortex". The cleft between an active cell and a cell at rest, which can be as small as 200 Å,^{6,9} is reduced following cell activity. This, together with the high tortuosity of the microenvironment,⁶ will slow down the process of diffusion of the excess K^+ (and other ions as well) in the microenvironment. Such local and transient excess of extracellular K^+ may trigger a response in an adjacent cell, e.g. if the metabolic resources available to that cell are insufficient to guarantee the power requested by the ionic pumps to face the α -induced depolarization. This, in turn, would further reduce α , thus favoring critical depolarizations in other parts of the system. As a consequence, activity may spread over the assembly in a sort of a chain-reaction that would eventually drive the entire assembly into activity. This resembles an epileptiform discharge induced by a local reduction of the volume fraction.^{16,17} Apparently, a reverberating condition thus is created in which seizure discharges decrease α , which increases membrane instability, which lowers the threshold to further discharges. The increased incidence of epileptic discharges reported following damage to the brain (e.g. after cerebral infarctions¹⁸) may be related to a reduction in the extracellular space.

Regional differences in the VF may have physiological and pathological implications: McBain *et al.*⁸ demonstrated that the VF in the hippocampus was lowest (0.12) in the CA1 region and suggested this may be related to the propensity of that region to seizure activity. They reversibly reduced the VF of the CA1 stratum pyramidale by 30% (producing spontaneous seizures) by changing the extracellular concentration of potassium from 3.5 to 8.5 mM. This equates morphological to chemical changes in the triggering of a neuronal response.

Under pathological conditions, such as anoxia and spreading depression, the VF is reduced,^{8,9,19} and it is also reduced (by up to 50%) in hyperexcitability, changes in $[K^+]_{out}$, and with epileptiform discharges.^{16,17} Hochman *et al.*¹⁰ noted that the neuronal synchronization involved in epileptiform activity can be disassociated from synaptic excitability, and proposed that "... the nonsynaptic mechanisms that underlie furosemide's (a chloride co-transporter antagonist) action are related to cell volume regulation and in particular to glial swelling".

Brain cell swelling due to anoxia and brain trauma, leading to a decreased VF, may aid in the survival of partially denervated neurons during the post-injury period of receptor upregulation, which has been shown to follow damage to the brain in animal models²⁰ as well in humans.²¹ Those cells may

respond to previously sub-threshold stimuli, either synaptically, or by NDN, which generally involves activation of membrane surface receptors.³ However, it is also possible that hyperexcitability due to a volume fraction decrease, either independently or in combination with excitotoxic activity, may increase secondary cell death following brain damage.

Glial cells have a role in neurotransmission via transmitter diffusion through the extracellular space.⁴ The present study suggests that changes in the volume of glial cells (and neurons) that alter the VF in specific areas of the brain may provide a pathway for information transmission.

Conclusions

Our previous studies have explored the diffusion neurotransmission^{4,22,23} and space and energy saving^{3,24} functional roles of the ECS, while the present study has explored the influence of the ECS volume fraction on cell membrane excitability and basal metabolism in an assembly of neurons. Chloride appears to be essential for membrane stability. A coefficient of excitability with respect to variations of the volume fraction has been determined, together with the metabolic costs of counteracting the depolarizing effects of a diminished volume fraction. Variable neuronal and glial morphology may provide a potent biophysical mechanism of brain activity regulation and information transmission, by selective effects on membrane dynamics.

References

- Freeman WJ. *Int J Bifurcat Chaos* **2**, 451-482 (1992).
- GM Edelman. *Neuron* **10**, 115-125 (1993).
- Bach-y-Rita P and Aiello GL. *NeuroReport* **7**, 1502-1504 (1996).
- Bach-y-Rita P. *Nonsynaptic Diffusion Neurotransmission and Late Brain Reorganization*. New York: Demos-Vermande, 1995.
- Nicholson C. *Ann NY Acad Sci* **481**, 43-45 (1986).
- Nicholson C and Phillips JM. *J Physiol* **321**, 225-257 (1981).
- Nicholson C. *Ann NY Acad Sci* **481** 5-70 (1986).
- McBain J, Tranelis SF and Dingledine R. *Science* **249**, 674-677 (1990).
- Lipton P. *J Physiol* **231**, 365-383 (1973).
- Hochman SC, Baraban JWM, Owens PA *et al.* *Science* **270**, 99-102 (1995).
- Kimelberg HK and Ransom BR. In: Federoff S and Vernadakes A, eds. *Astrocytes*, Vol. 3. New York: Academic Press, 1986: 129-166.
- Smith CUM. *Elements of Molecular Neurobiology*, 2nd edn. Chichester: Wiley, 1996.
- Hodgkin AL and Huxley AF. *J Physiol (Lond)* **116**, 449-472 (1952).
- Payne JA, Stevenson TJ and Donaldson LF. *J Biol Chem* **271**, 16245-16252 (1996).
- Nicholls DG. *Proteins, Transmitters and Synapses*. Oxford: Blackwell Science, 1995: 6.
- Dietzel MA, Heinemann I, Hofmeier U *et al.* *Exp Brain Res* **40**, 432-439 (1980).
- Dietzel MA and Heinemann I. *Ann NY Acad Sci* **481**, 72-86 (1986).
- Cocito L, Favale E and Reni L. *Stroke* **13**, 189-195 (1982).
- Harrevel, AV and Khallab Fl. *J Neurophysiol* **30**, 911-929 (1967).
- Westerberg E, Monaghan DT, Kalimo H *et al.* *J Neurosci* **9**, 798-805 (1989).
- De Keyser JD, Ebinger G and Vauquelin G. *Neurosci Lett* **104**, 281-285 (1989).
- Bach-y-Rita P. *Exp Neurol* **9**, 327-344 (1964).
- Bach-y-Rita P and Smith CUM. *Scand J Rehab Med* **25**, 3-6 (1993).
- Bach-y-Rita P. *Restor Neurol Neurosci* **10**, 1-3 (1996).

Received 14 January 1997;

accepted 17 January 1997