

Review Article

Extracellular Electric Signals Regulate Neuroblast Behaviours in Adult Brain

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Abstract

The existence of electrical signals in the extracellular space of mammalian brain has been known for many decades, for example in hippocampus and cerebellum. But their biological significance is poorly understood. Recently, we discovered that endogenous electrical signals serve as a guidance cue for neuroblast migration from the subventricular zone (SVZ) to the olfactory bulb (OB). We identified the existence of naturally occurring electrical currents ($1.5 \pm 0.6 \mu\text{A}/\text{cm}^2$) which generate average electrical field strength of 3-5mV/mm between the SVZ and the OB in adult mouse brain. The currents enter the brain from the lateral ventricles and flow out from the OB. In addition, the cortical surface was ~ 5.5 mV positive to the ventricle. This represents a flow of electrical current from SVZ to OB with a return path from the pia mater membrane to the ependymal epithelium of the lateral ventricle. Intriguingly, a similarly sized applied electric field (EF) of 5mV/mm induced cathodally directed migration of neuroblasts, both in isolated culture and in brain slices. This suggests that naturally occurring electrical signals serve as a novel long distance guidance vectors that promote directed neuronal migration along the rostral migratory stream. In addition, an applied EF guided the direction of neural outgrowth from the neural tube. Therefore, an endogenous extracellular EF may play an important role in neurogenesis as one of the extracellular cues in the microenvironment. Here we review how endogenous extracellular electrical signals are generated, their functional role in neuroblast migration and the resulting clinical potential.

ABBREVIATIONS

EF: Electric Field; **SVZ:** Subventricular Zone; **OB:** Olfactory Bulb; **RMS:** Rostral Migratory Stream; **LV:** Lateral Ventricle; **ECS:** Extracellular Space.

INTRODUCTION

In adult mammalian brain, thousands of new neuronal precursors are born every day in the subventricular zone (SVZ), and some of these type A migrating neuronal precursor cells and neuroblasts will migrate from the SVZ through the rostral migratory stream (RMS) to the olfactory bulb (OB) [1-4]. There they differentiate into interneurons and are integrated into local circuits [4]. Extracellular cues dictate the continual process of directional migration of neuroblasts to their final destination and for normal RMS migration these include the spatially localised expression of polysialated neuronal cell adhesion molecule (PSA-NCAM), integrin signalling, secreted SLIT ligands and their receptors, and the ROBO family of proteins [4]. In addition, biophysical cues appear to play a role in the rostral migration of neuronal cells. Sawamoto et al reported an important role for cerebrospinal fluid flow in guiding neuronal migration [5]. Our

recent work has identified that the extracellular electric field (EF) is an additional biophysical cue for guiding long distance migration of neuroblasts in brain [6,7]. Gradients of electrical potential exists extracellularly in many areas of our body and although these have been detected and measured directly in brain, the biological roles and significance of such extracellular potentials are not well understood. Here, we focus on two questions, how are endogenous extracellular electric currents generated? And what is their functional role with regard to neurogenesis in the brain?

Extracellular Electrical signals in adult brain

The cortical surface has been shown to be 0.5 to 5.5 mV positive with respect to the ventricles [8]. Bures also studied the ontogenetic development of such a polarization gradient between a normal cortical surface and a necrotic area of cortex or sciatic nerve in the rat. They found that the potential difference increased with age, being only 1 to 1.6 mV at age 5 days, but 20 mV in the mature rat [9]. Synchronous volleys of neuronal action potentials within highly laminar structures such as the hippocampus also generated substantial extracellular field potentials with an extracellular EF ranging from 6 to 31

mV/mm (average 17 mV/mm; S.E. = 2.9; n = 8) [10]. Moreover, short DC pulses (25-250 ms, 5-70 mV/mm) were detected to influence the amplitude of population spikes recorded in the dentate granule cell layer when the extracellular fields were parallel to the dendrosomatic axis of the neurons [11]. Winson et al considered that the rhythmic slow activity or theta potentials of neurons in the hippocampus generates fields of 3-4 mV/mm [12]. In addition, Chan and Nicholson found an electrical field ranging from 13 to 43 mV/mm in cerebellum [13]. These data indicate the widespread presence of extracellular electrical fields in brain associated with modification of the neuronal membrane potential (action potential firing) [11].

We detected current flow at the lateral ventricle (LV) walls and at the surface of the OB using a vibrating probe system, which measures electric currents non-invasively with high spatial and quantitative resolution [6]. The average magnitude of the inward electric currents (I_i) were $I_i = -1.6 \pm 0.4 \mu\text{A}/\text{cm}^2$ on the surface of LV, whereas at the OB, five measuring positions showed uniformly outward currents ($I_o = 1.5 \pm 0.6 \mu\text{A}/\text{cm}^2$). Furthermore, we measured the electric potential in the interstitial spaces along the rostral migratory pathway directly, using a voltmeter. This voltage divided by the distance between the measuring points along the rostral migratory pathway from the SVZ to the OB, gave an average voltage drop of $5.7 \pm 1.2 \text{mV}/\text{mm}$ [6]. It is clear therefore that gradients of electrical potential exist naturally along this important neuronal migratory pathway.

Mechanisms of EF generation in brain

The concentration gradient of ions, such as K^+ , Na^+ , Cl^- and Ca^{2+} , in different locations within the extracellular space defines the strength of the steady electrical field [14]. However, how the ionic gradients form within brain is less clear. Firstly, the extracellular space occupies as much as 20% of brain volume in rat [15]. The extracellular space (ECS) plays a very important functional role as a system of interconnected channels bounded by cell membranes and filled with an ionic solution [16,17]. The extracellular space (ECS) mediates intercellular communication and the transport of nutrients and metabolites [18], and forms a reservoir of ions that establish the resting potential of cells and mediate ionic fluxes across cell membranes. It also serves to deliver therapeutic substances to cells over long distances [19]. All these processes are primarily mediated by diffusion and are affected by action potential firing. During action potential propagation, a negative and self-sustained wave of potential shift in the ECS is one of the main characteristics of extracellular ionic change, together with intense variations in ionic dynamics [20]. Therefore, the extracellular space and tortuous diffusion pathways set fundamental constraints on the movement of ions and other substances in the mammalian brain [21]. A further consideration is the asymmetric distribution of ionic pumps: e.g. Na^+/K^+ -ATPases [14]. The high level apical localization of the pumps at the OB would drive electric currents (flow of positive charges) outwards and thus generate a voltage sink in the OB [22]. In the SVZ, the ependymal membranes covering the apical surface have little or no ATPase activity [6]. However, very strong ATPase reaction product is present in the slender processes terminating on the basal laminae of perivascular spaces [6,23]. Inward flow of current into brain (flux of positive ions by convention) has been

detected at the SVZ and inhibition of Na^+/K^+ -ATPases reduced this current effectively [6]. The difference in distribution of Na^+/K^+ -ATPase on the surface of the OB and on the basal side at the SVZ may lead to a Na^+ gradient between these two areas due to the $3\text{Na}^+ / 2\text{K}^+$ exchange. So far there is no direct evidence to identify ionic gradient formation between SVZ and OB in extracellular space. But other work supports this hypothesis. 1) The apparent diffusion coefficient (ADC) of Na^+ in rat brain is $1.15 \pm 0.09 \mu\text{m}^2/\text{ms}$, which is 61% of the aqueous Na^+ free diffusion coefficient (D^{free}) at 37°C ($1.9 \pm 0.6 \mu\text{m}^2/\text{ms}$) [24]. The average distance from the SVZ to the OB in adult mice is $\sim 3 \text{mm}$. This indicates that for Na^+ to diffuse to OB from SVZ will take around ~ 7 hours. This would be necessary to form a Na^+ gradient. 2) Using sodium magnetic resonance imaging (MRI), the concentrations of sodium are 10.6 ± 2.2 and $11.7 \pm 2.2 \text{mM}$ in grey and white matter of human brain [25]. This indicates that a Na^+ gradient exists between cortex of OB (grey matter) and RMS (white matter). In summary, the asymmetric distribution of Na^+/K^+ -ATPase on the pia mater (OB) and ependymal epithelium (SVZ) act as a power source in this electrical pathway (Figure 1A to C). Thirdly, we measured an electrical potential difference between pia mater and ventricle [8] and these regions are connected through the cerebrospinal fluid (with a current of $1.5 \pm 0.6 \mu\text{A}/\text{cm}^2$ outward from OB and inward to LV). This constitutes a complete current loop, which is necessary for the formation of a DC continuous current. Finally, the gradient of ions establishes a circulating flow of electrical charges creating EF strength of 3-5 mV/mm between the SVZ to the OB (Figure 1D).

The extracellular EF signal regulated directed migration of neuroblasts and differentiation of embryonic stem cells

Many types of cells respond to very small EFs with directed polarization, growth and migration (galvanotropism, galvanotaxis /electrotaxis) [26,27]. For example, Schwann cells from chick embryo migrate directionally in an EF as small as 3 mV/mm [28]. We have shown that an endogenous electrical gradient exists along the rostral migratory pathway and clearly this could regulate neuroblast migration from the SVZ to the OB [6]. In an applied EF, we found that the expression of N-Cadherin and β -Catenin were increased in SVZ neuroblasts and that this induced the neuroblasts to form chain-like migration (Figure 2) [7]. An applied EF as low as 3.5 mV/mm induced significant directional migration of neuroblasts towards the cathode. This is the EF strength which we have measured along the rostral migratory pathway in adult mouse brains. Thus a physiological EF exists between the SVZ and OB and is capable of inducing directed migration of SVZ neuroblasts along the rostral migratory path.

An applied EF of 6-8 mV/mm induced more neuroblast differentiation in *Xenopus laevis* embryos *in vitro* [29] which suggests that the electrical signal may act as a "trophic factor", increasing cell survival and differentiation [30]. Additionally, an applied EF may promote the differentiation of embryonic stem cells. An electrical field with field strengths of 250 and 500 V/m applied for 90s to cardiomyocyte increased both the number of embryoid bodies differentiating as beating foci of cardiomyocytes and the size of the beating foci [31]. Treatment of ES cell-derived

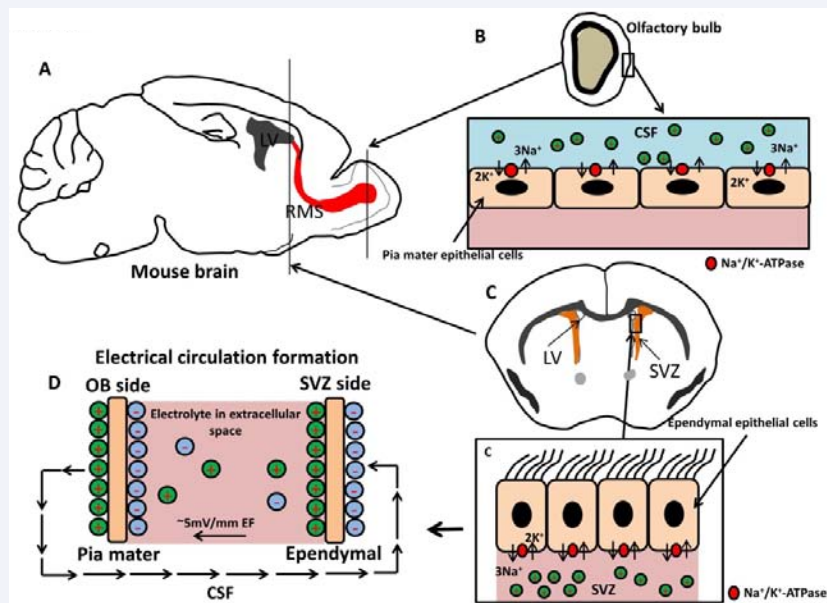


Figure 1 The mechanisms responsible for development of the endogenous EF between the SVZ and the OB. A. anatomical diagram of mouse brain showing location of RMS. B. The Na^+/K^+ -ATPase is distributed on the apical side (CSF side) of pia mater epithelial cells. This will transport more Na^+ into CSF. C. The distribution of Na^+/K^+ -ATPase located on the basal side of ependymal epithelial cells (SVZ side) of the lateral ventricle (LV). This will transport more Na^+ into the SVZ. D. There is an ion gradient and electrical circulation formed due to the asymmetric distribution of ion channels/pump, e.g. Na^+/K^+ -ATPase. Arrows indicate the direction of the endogenous EF.

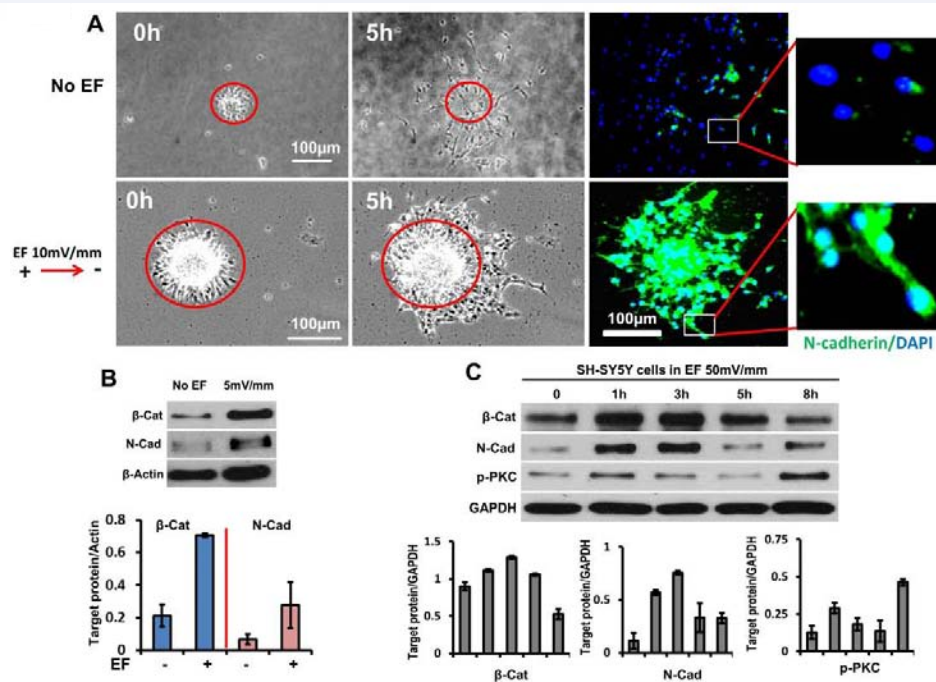


Figure 2 An applied EF induced chain migration of neuroblasts. A. Neurospheres from mouse SVZ were cultured in electrotoxicity detection chambers and an EF of physiological strength (10mV/mm) was applied for 5h. Upper row - no EF control, neuroblasts migrate in random directions and do so largely separated from and independent of each other (see enlarged image). Lower row - EF applied with cathode to the right. EF-stimulated neurospheres remain closely adherent to each other and migrate together in chains. Neurosphere cells were fixed and stained immunofluorescently with an N-Cadherin antibody. Cultures exposed to a physiological EF of 10mV/mm (lower row) show strikingly enhanced N-Cadherin staining compared to no EF controls (upper row). B. An applied EF increased the expression of N-Cadherin and β -Catenin (shown by Western blotting). Actin is a loading control. The ratio of protein expression/actin is shown below the western blots. (C) Western blotting showed that the applied EF effectively increased the expression of N-Cadherin and β -Catenin at 1 hour after EF exposure. PKC was activated significantly after 8 hours treatment in an applied EF. GAPDH is a loading control. Lower panel - The ratio of protein expression/GAPDH is shown below the western blots.

embryoid bodies with field strengths ranging from 250 V/m to 750 V/m, applied for 60 s, increased the capillary area staining positive for the endothelial-specific marker platelet endothelial cell adhesion molecule-1 in a dose-dependent manner [32]. This indicates that applied EF stimulation induced differentiation and angiogenesis of endothelial cells.

Extracellular EF signals are transduced into intracellular signals

Electrical fields (EFs) in the physiological range enhance migration including the speed and directedness of different neural cell types from different species. However, it is still unclear how extracellular electrical signals are translated into intracellular signalling and result in directed migration. The most likely sequence of events include:

Regulation of cytokine secretion, VEGF and extracellular ATP. An applied EF may upregulate growth-factor secretion, which would give rise to chemical gradients, e.g. VEGF release from endothelial cells was upregulated by exposure to a physiological EF [32]. Extracellular ATP acts as a neurotransmitter and neuromodulator in the CNS. ATP release was dependent on a finite trans-epithelial potential (TEP, extracellular electrical signal) in urothelium [33].

Activation of receptors on the cell membrane, e.g. acetylcholine (ACh) receptors (AChRs), EGFR and tyrosine protein kinase transmembrane receptor (ROR2). EFs cause asymmetric relocation of membrane-bound receptors such as the neuronal nicotinic acetylcholine receptor (nAChRs) which accumulate on the cathodal side. Activation of nAChRs leads to elevation of intracellular Ca^{2+} as nAChRs are permeable to Ca^{2+} , which, in turn, is essential for cathodal migration [26]. Electric-field-enhanced directional migration also correlates well with the expression level of EGF receptors (EGFR/ErbB1) [34]. In addition, we reported that ROR2 mediated the activation of ERK1/2 and LKB1 by an applied EF in intestinal epithelial cells [35].

Downstream signalling pathways, e.g. MAPK/ERK pathway, PTEN/PI3K pathway. MAPK/ERK and PTEN/PI3K signalling pathways were involved in the EF-directed migration at the wound edge in electrically driven wound healing [36,37]. In addition, EFs trigger the redistribution of the PI3K effector PIP3 and its co-localization with actin at the leading edge of the cell, which results in transduction of electrical gradient sensing in stem cells [38].

Regulation of endogenous EF and applied EFs in clinical treatment

Because there are steady electrical signals in damaged brain [11] and because both neuronal and glial precursor cells show directed migration in response to such EFs, there is hope for the development of new electrically-based therapeutic strategies to treat brain injury and disease. However, the high complexity of the CNS is an obstacle to establishing well worked out methods for applying EFs to damaged brain to promote neurogenesis. Regulating endogenous electrical signals also is promising as a treatment option through enhancing the activation of Na^+/K^+ -ATPases using chemical and physical methods. For example, a

synchronization modulation stimulation (SMS) using a train of electric pulses of 2V can synchronize the Na^+/K^+ pump activity to increase the endogenous direct current electric field driven by epithelial cells [39]. In addition, it has been reported that agonists of Na^+/K^+ -ATPases, e.g. beta-adrenergic agonist, glutamatergic, extracellular purines (ATP, ADP), regulate the Na^+/K^+ -ATPase activation [40,41]. These also are potential modulators for endogenous EFs. Although DC EFs are effective in guiding neuron migration *in vitro*, the application of EFs in the complicated *in vivo* brain microenvironment remains a significant bioengineering challenge. The development of biomaterials and electronic technologies are expected to provide promising methods for the application of EFs *in vivo*.

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