Commentary 4267

# Electrical dimensions in cell science

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#### **Summary**

Cells undergo a variety of physiological processes, including division, migration and differentiation, under the influence of endogenous electrical cues, which are generated physiologically and pathologically in the extracellular and sometimes intracellular spaces. These signals are transduced to regulate cell behaviours profoundly, both in vitro and in vivo. Bioelectricity influences cellular processes as fundamental as control of the cell cycle, cell proliferation, cancer-cell migration, electrical signalling in the adult brain, embryonic neuronal cell migration, axon outgrowth, spinal-cord repair, epithelial wound repair, tissue regeneration and establishment of left-right

body asymmetry. In addition to direct effects on cells, electrical gradients interact with coexisting extracellular chemical gradients. Indeed, cells can integrate and respond to electrical and chemical cues in combination. This Commentary details how electrical signals control multiple cell behaviours and argues that study of the interplay between combined electrical and chemical gradients is underdeveloped yet necessary.

Key words: Directed migration, Electric field, Polarity, Regeneration, Wound healing

#### Introduction

The existence of steady (direct current; DC) electrical signals within the extracellular spaces of plants, animals and humans has been known for two centuries. Roles for these electrical signals have been demonstrated in development, physiology, regeneration and pathology (Piccolino, 1998; Borgens et al., 1979; McCaig et al., 2005), yet many cell biologists are unaware of them. Others dismiss them as epiphenomena, without understanding them fully. By contrast, the physiological bases of the electrocardiogram and the electroencephalogram, which record dynamic extracellular electrical signals from thousands of action potentials, are understood widely. It is curious that scientists accept dynamic electrical signalling, but often ignore the coexisting steady electrical signals. Using electrical stimulation to regulate cell physiology (e.g. cardiac pacemakers) is an important modern clinical therapy but, in the past, bogus electrical therapies to 'cure' ailments ranging from impotence to baldness were common. 'Electric air baths', for example, were a popular Victorian spa treatment and, when Mary Shelley was writing Frankenstein, public demonstrations using electrical shocks to raise corpses were popular for their theatrical impact. Much of the bad reputation associated with bioelectricity is rooted in this quackery.

In almost all systems that have been studied, crucial behaviours such as cell division, cell migration and cell differentiation take place within an extracellular milieu in which standing voltage gradients persist for several hours or even days (Levin, 2007; McCaig et al., 2005). Mostly, these electrical signals arise from spatial variations in the functioning of ion pumps, or leaks across individual cells or across layers of cells, such as an ion-transporting epithelium (Fig. 1). The resulting ionic gradients drive extracellular ionic current flow and this establishes the voltage gradients. Two reviews published in the last few years have addressed in depth the origins of steady DC extracellular electrical signals, and their integral roles in developmental and regenerative physiology (Levin 2007; McCaig et al., 2005).

This Commentary aims to show that much work on extracellular electrical signals is rigorous and credible; that chemical, physical and electrical gradients coexist inside and outside cells, so these gradients must interact; and that cells simultaneously read and integrate responses to both chemical and electrical cues. We discuss developments and new directions in electrical signalling in the brain, in spinal-cord repair, in neuronal migration, in wound repair, in control of the cell cycle, cell proliferation and cancer, and in establishing left-right body asymmetry. We argue that an electrical physiology exists alongside and interacts with biochemical and molecular signalling, and caution that dismissing this area of science means ignoring an integral part of any biological system.

#### **Electrical guidance of nerves**

Identifying the cues that direct axonal outgrowth during development and following injury would improve our understanding of the development of neuronal networks, and might foster new therapies for neurodevelopmental disorders and brain injuries. Most studies concentrate on the principles of axon guidance by gradients of morphogens or growth factors (chemotropism), but there is also evidence that DC electrical fields can affect axonal growth. Interestingly, shortly after the invention of tissue culture, application of a DC electrical field (EF) to neurons in culture was shown to direct their growth (Ingvar, 1920) in vitro. Since then, EFs also have been applied in vivo in efforts to promote regeneration both in the peripheral and central nervous systems (e.g. Borgens, 1999; Shapiro et al., 2005). Claims of success in vitro and in vivo are about as frequent as claims that EF application does not improve nerve growth (McCaig, 1988; McCaig et al., 2005; Robinson, 1985). Many negative results can be ascribed to insufficient understanding of the biophysics or the cell biology of the system, and the subsequent failure to determine the physiologically appropriate experimental conditions. One notable exception is the extensive in vivo work of Borgens' group, who have shown that the application of a DC field to damaged spinal nerves can promote regeneration and some functional recovery (Box 1).

Some impetus for the studies of Borgens and colleagues comes from work that showed profound nerve growth and guidance effects of applied EFs with strengths as low as 7 mV/mm (Hinkle et al., 1981; McCaig et al., 2005) [Fig. 2; also see movies 1 and 2 in

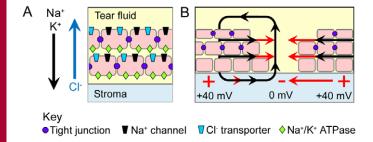


Fig. 1. Naturally occurring EFs in tissues arise from polarised ion transport. The mammalian corneal epithelium model is shown. (A) Tight junctions (purple dots) in the upper layers of the intact epithelium seal neighbouring cells to each other as well as restricting lateral mobility of membrane proteins within the plasma membrane of individual cells. The apical domain of each polarised cell is therefore enriched in Na<sup>+</sup> channels (black) and Cl<sup>-</sup> transporters (blue), whereas the basolateral domain contains Na<sup>+</sup>-K<sup>+</sup> ATPases (green); this polarised distribution results in net movement of Na<sup>+</sup> and K<sup>+</sup> into the stromal layer and net movement of Cl<sup>-</sup> into the tear fluid (arrows). (B) The consequent separation of charge across the tightly sealed, multilayered epithelium results in a measurable transepithelial potential (TEP) difference of ~40 mV, with the stroma positive relative to the tear fluid. Upon injury, the TEP collapses instantaneously to zero at the wound centre, but it remains ~40 mV distally (~1 mm), where ion transport is unaffected. This voltage gradient establishes an EF (red arrows) in the corneal tissues that has a vector parallel to the epithelial surface and the wound centre as the cathode (Vanable, 1989; Reid et al., 2005). Another consequence of breaching epithelial integrity is leakage of Na<sup>+</sup> and K<sup>+</sup> ions out of the wound (down their concentration gradient). The movement of positive ions constitutes the physiological injury current. The black arrows indicate positive ion (and current) flow through tissues and the return path through the tear fluid. In addition to directing neuronal growth, the EF controls cell migration during re-epithelialisation by steering cells towards the cathodal wound centre (Box 2).

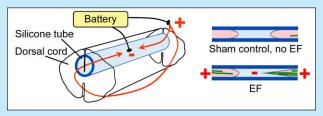
Rajnicek et al. (Rajnicek et al., 2006a)]. In these studies, neuronal growth cones (the motile tips of growing nerves) of dissociated *Xenopus* embryonic spinal-cord neurons (the predominant in vitro model of chemotropic axon-guidance studies) rapidly and dramatically changed direction to migrate towards the cathode in response to these EFs. They also branched more frequently towards the cathode, migrated more rapidly towards the cathode and advanced relatively slowly in the direction of the anode (or retracted). We envisage electrical signals as co-regulators of developmental and regenerative nerve growth and guidance, because physiological electrical signals exist in the central nervous system (CNS) in vivo (see below) and evoke subtle voltage- and time-dependent responses that are enhanced or suppressed by interaction of the electrical signals with other extracellular guidance cues. For example, cathodal attraction is enhanced by the neurotrophin brain-derived neurotrophic factor (BDNF), is suppressed by the endogenous cannabinoid anandamide and, at low EF strengths, is reversed by the neurotrophin NT3 to become anodal attraction (McCaig et al., 2000; Berghuis et al., 2007). Moreover, because the relative rate of retraction for anode-facing neurites is less than the rate of enhanced cathodal attraction, switching the EF polarity might promote growth in both directions over a prolonged period, as is required for regeneration of both ascending (sensory) and descending (motor) tracts in spinal-cord injury (McCaig, 1987). The Borgens group exploited this latter observation to design an oscillating stimulator for the dog and human spinal-injury studies referred to in Box 1, in which the polarity of the DC EF is switched every 30 minutes.

There has been no new therapeutic advance in the treatment of spinal-cord injuries for several decades. The relevance of Borgens'

# Box 1. Application of an EF to the damaged mammalian spinal cord

Borgens and colleagues showed that steady electrical signals are a natural consequence of spinal-cord transection in larval lampreys, and that an applied EF that offsets these injuryinduced signals improves regeneration (Borgens et al., 1980; Borgens et al., 1981). In adult guinea pig, they cut the dorsal part of the spinal cord, which carries sensory information to the brain, and showed that the cut sensory dorsal column fibres never recovered in the absence of EF application, and nor did an accompanying spinal reflex (the cutaneous trunci muscle reflex, which is commonly used by horses and cattle to rapidly contract their skin to dislodge flies). However, passing 35  $\mu A$  across the hemisected dorsal cord for 4 weeks (EF ~300-400 μV/mm, cathode rostral) induced recovery of the reflex in 25% of animals. Both sets of electrodes (cathode and anode) were placed close to the spinal column, outside the spinal cord. Behavioural-reflex recovery was associated with regeneration of appropriate ascending and descending fibres (Borgens et al., 1987).

In other experiments [figure below redrawn from Borgens (Borgens, 1999)], a silicone tube (6 mm long  $\times$  1 mm diameter) was implanted in the severed dorsal cord with the cathode (–) inserted in the lumen of the tube [the anode (+) remained outside the vertebral column]. Red arrows indicate the current path and EF direction inside the tube. With no current passing (sham control, no EF), tissue plugs formed (pink) but no significant nerve growth was observed in either end of the tube after 1-2 months. This is expected, because adult mammalian spinal-cord axons do not normally regenerate. In the presence of an electric field, however, significant regenerative axonal growth (green lines) was stimulated. Axons were attracted towards the tube and penetrated several millimetres into it (Borgens, 1999).



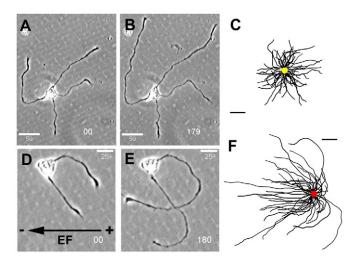
In dogs, significant improvement in several neurological parameters was seen when variably damaged spinal cords (accidental injuries) were exposed to a DC EF (Borgens et al., 1999).

Finally, a Phase 1 human clinical trial has shown that quadriplegic patients tolerated electrical implants for 3 months and that, just 1 year after removal, nine of the ten patients had recovered some sensory (but no motor) function (Shapiro et al., 2005). It is planned to extend these encouraging results with a larger clinical trial that will implant electrical stimulators in hundreds of spinal-cord-injured patients. No information is available on this as yet.

Notably, combined application of a neurotrophic chemical (inosine) with electrical stimulation was more effective than either treatment alone in recovery of spinal reflexes in the lesioned guinea-pig model (Bohnert et al., 2007). This first attempt to combine neurotrophic molecules and electrical stimulation in treating spinal-cord lesions is based on tissue-culture studies that demonstrated striking additive effects (McCaig et al., 2000).

work for human disability remains controversial, and the next few years will be defining for electrical treatments of spinal-cord injuries.

Studies by members of our laboratory (Rajnicek, 2006a; Rajnicek, 2006b) have added to our understanding of the signalling elements



**Fig. 2.** *Xenopus* spinal neurons grow towards the cathode in vitro. (A,B) First and last frames from time-lapse sequences of growing neurons in the absence of an EF [reproduced with permission from movies 1 and 2 in Rajnicek et al. (Rajnicek et al., 2006a)]. Elapsed time (minutes) is shown in the lower right corner. (C) Composite drawing, in which the cell bodies (yellow dot in centre) of several neurons have been superimposed to show the path of neurite outgrowth over 5 hours (no EF). (D,E) Neurons in the presence of an EF of 150 mV/mm for 3 hours, with the EF vector shown in D [reproduced with permission from movie 2 in Rajnicek et al. (Rajnicek et al., 2006a)]. Growth cones start to turn cathodally within minutes and one growth cone crosses another neurite to migrate towards the cathode. (F) Composite diagram for cells exposed to a 150 mV/mm EF for 5 hours. Neurite paths curve dramatically towards the cathode and neurites extend faster cathodally than anodally. Scale bars: 50 μm (A,B); 100 μm (C,F); 25 μm (D,E). (C,F) Modified from figure 1 in Rajnicek et al. (Rajnicek et al., 2006a), with permission.

that transduce electrical guidance in nerves. Using the *Xenopus* spinal neuron model, we showed that inhibition of the small GTPases RhoA, Rac1 and Cdc42 attenuated guidance of growth cones towards the cathode. An attractive model is that high cathodal Cdc42 and Rac activity (with low Rho activation) and high anodal Rho activity (with low Cdc42 and Rac activity) controls regional assembly and disassembly of the growth-cone cytoskeleton and promotes turning towards the cathode (Rajnicek et al., 2006a, Rajnicek et al., 2006b). From this work and other studies (Rajnicek et al., 2007; Rajnicek et al., 2008), we derived two important additional conclusions – namely, that the molecular mechanisms underpinning cathodal guidance in neurons and in epithelial cells (see below) differ, and that chemical and electrical gradients activate some common and some divergent signalling cascades.

The receptors that transduce the EF differ according to cell type. One model for detection and transduction of DC EFs states that EFs drive the asymmetry of charged receptors within the plane of the plasma membrane, and that this enhances signalling on one side of the cell because downstream second-messenger asymmetries are triggered (McCaig et al., 2005). Asymmetric distribution of receptors for neurotrophins and for the neuronal nicotinic acetylcholine receptor might be crucial for electrically mediated growth-cone guidance, whereas asymmetric epidermal growth factor (EGF) receptors and vascular endothelial growth factor (VEGF) receptors might transduce epithelial and endothelial responses to EFs (Erskine and McCaig, 1995; Zhao et al., 1999; Zhao et al., 2002, Zhao et al., 2003; Fang et al., 1999; Pullar et al., 2006).

Signalling pathways downstream from these different receptor molecules also differ according to cell type. Common signalling elements that are used either in EF-directed epithelial cells (below) or in chemotactic guidance of neutrophils, such as phosphoinositide 3-kinase (PI3K), p38MAPK and MEK1/2 (MEK1 and MEK2), are not involved in neuronal growth-cone guidance (Rajnicek et al., 2006a). There is much detail to be unravelled regarding the molecular signals that underpin EF-directed growth. However, the above discussion envisages 'signalling' purely in terms of biochemical pathways. In addition to these recognised biochemical reactions, 'signalling' might involve parallel physical and electrical signals that are carried by the endogenous polymers of the extraand intracellular spaces (see below).

Because embryonic zebrafish growth cones did not respond directionally to a DC EF in vitro, it has been suggested that EF-directed neuronal growth might be a peculiarity of *Xenopus* neurons (Cormie and Robinson, 2007). However, Cormie and Robinson's zebrafish study tested a single EF strength (100 mV/mm), a single culture substratum (laminin), neurons from one developmental period (16- to 17-somite stage), and cultures in which nerves were grown for only 6 hours. Consequently, further studies are needed to determine unequivocally whether the neurons are able to respond. There is also a need for a robust in vitro mammalian model in which EF-directed neuronal guidance can be characterised, and we are testing EF responses of a range of dissociated rodent CNS neurons and three-dimensional brain slice cultures. Although developing a mammalian in vitro neuronal model has proven challenging, experiments using mammalian systems confirm that nerve growth in vivo is directed by endogenous electrical cues (Boxes 1 and 2, Fig. 2).

#### **Electrical control of neuronal migration**

It is clear that growth cones of differentiated neurons can be steered electrically in development and perhaps also following brain injury; however, differentiating neurons and neuronal stem cells migrate from areas of neurogenesis to their final locations, and EFs might also have a role in this process.

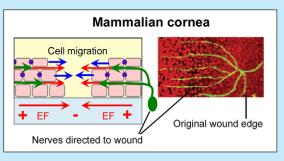
Some of the chemical cues that direct neuronal migration are known, and these can differ according to the neuronal population (Hatten, 2002). In the tangential chain migration of subventricular zone (SVZ) neurons to the olfactory bulb via the rostral migratory stream, Slit proteins, ephrins and Eph receptors, and polysialylated NCAM have guidance roles, whereas neurons migrating from the germinal zones to the outer cortical layers along radial glial fibres initially use the extracellular-matrix molecule reelin as a guidance cue (Hatten, 2002; Ghashghaei et al., 2007). By contrast, in adult neurogenesis, stromal-cell-derived factor  $1\alpha$ , which is transduced by the chemokine receptor CXCR4 on neuronal stem cells, directs neurons to sites of brain injury (Imitola et al., 2004).

Intriguingly, most cell migrations in developing and damaged brain probably occur through tissues in which steady electrical signals exist. For example, there is a large voltage gradient across the wall of the *Xenopus* neural tube (between the central canal and the extracellular space) during early neurogenesis, when outward migration of neurons occurs (Hotary and Robinson, 1991). Epileptic seizure, stroke, ischaemia, migraine and acute damage to the hippocampus all induce extracellular electrical signals in brain that persist for hours (Leiao, 1944; Marshall, 1959; Jefferys, 1981; Hadjikhani et al., 2001; Strong et al., 2002; Reid et al., 2007). In addition to reading chemical gradients as molecular guidance cues, migrating neurons might also read the electrical 'language' in the confined extracellular spaces of brain. Newborn rat hippocampal neurons and progenitor neurons from the lateral ganglionic eminence

#### Box 2. Endogenous EFs direct nerve growth in vivo

#### Nerve growth in mammalian cornea is directed by a naturally occurring EF

Nerve bundles grow at right angles directly towards a wound edge in mammalian cornea (Rosza et al., 1983; Beurmann and Rosza, 1984). However, the cues guiding this oriented nerve growth had not been explored. Therefore, we manipulated the wound-generated EF pharmacologically to test the hypothesis that the wound-induced EF directed nerves to the wound edge (Song et al., 2004). Irrespective of the drug used or the cellular mechanism of its action, more nerves sprouted when the EF was enhanced; sprouts also appeared earlier and grew more directly towards the wound edge. Directed sprouting of trigeminal nerves is depicted by green arrows in the cartoon (left panel on figure; red arrows show the EF and blue arrows the direction of cell migration) and by green immunofluorescence detecting growth-associated

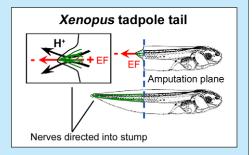


protein 43 (GAP43), a marker of regenerating nerve fibres, in the fluorescence image [right panel; reproduced with permission (Song et al., 2004)]. By contrast, collapsing the EF permitted only sparse nerve sprouting, and this was no longer directed towards the wound edge (Song et al., 2004).

Wound healing is poor in individuals with sensory neuropathies (Friend and Thoft, 1984). In diabetic individuals, for example, repeated attempts to reepithelialise corneal wounds fail because the simultaneous sensory re-innervation required for successful re-epithelialisation is poor. Neurotrophic interactions between the epithelial cells and the sensory nerve sprouts are therefore a requirement for robust re-epithelialisation (Beuerman and Schimmelpfenning, 1980). Perhaps the diabetic cornea has compromised electrical signals that contribute to its poor innervation, and consequently to poor wound healing.

#### Xenopus tadpole tail and spinal-cord regeneration

Levin and colleagues have studied Xenopus tadpoles, which (with the exception of a period between stages 45-47 - that is, between limbbud formation and tail resorption) can regenerate their tail fully, including the nerves (green lines in the figure) of the spinal cord (Adams et al., 2007; Beck et al., 2003). Tail regeneration and proper routing of nerves into the regenerating stump require activation of the H<sup>+</sup> V-ATPase in cells at the amputation plane (dashed line). This pump repolarises cells that were depolarised upon amputation. Sustained depolarisation of cells at the amputation site induced by a loss of V-ATPase function (by genetic or pharmacological manipulation) prevented tail regeneration, and nerves were misrouted. Because the V-ATPase is activated in epithelial and mesenchymal cells to repolarise tail-bud cells, this also causes an efflux of protons (black arrows) from the cut stump, which establishes a steady voltage gradient (red arrow), with the cathode distal. This voltage gradient is thought to direct the parallel, ordered projection of nerves into the distal tail bud [image redrawn with permission (Adams et al., 2007)].



both migrate cathodally and, for rat hippocampal neurons, this requires activation of both PI3K and Rho kinase (ROCK) as signalling elements. The receptor transduction mechanisms upstream of PI3K are unexplored (Yao et al., 2008; Li et al., 2008).

The involvement of PI3K in electrically directed neuronal migration might be important. Both PI3K and its negative regulator phosphatase and tensin homologue (PTEN) drive electrically regulated epithelial-cell migration and wound healing (Zhao et al., 2006) (see below). Emigration of neurons from the SVZ in rat is sensitive to PTEN, and mice lacking PTEN have several brain disorders including gliomas, macrocephaly and mental retardation; in each case, the lesion involves disrupted cell proliferation, cell migration and synaptogenesis (Li et al., 2002; Kwon et al., 2007). The discovery that EFs can control migration of neurons and neuronal stem cells as well as the extent of neuronal sprouting in vivo, and that these events might involve tumour suppressor genes, raises questions regarding the potential involvement of faulty extracellular electrical signalling in the genesis of neurodevelopmental diseases such as schizophrenia, autism, mental retardation and brain tumours (Box 3).

#### The future for electrically driven neuronal migration

Recent excitement in the field of neuronal migration centres on the cerebrospinal fluid (CSF) as a source of guidance information. CSF flow might set up a chemical gradient of guidance molecules that directs the early emigration of neurons from the SVZ (Sawamoto et al., 2006). Intriguingly, electrical signals might coexist in this area too. There are electrical gradients within the CSF, and the ependymal cells that line the CSF-filled ventricles of the brain and spinal cord also act as a selective ion-transporting epithelium with tight-junction-mediated electrical seals between the cells (Hornbein and Sorensen, 1972; Jarvis and Andrew, 1988; Lippoldt et al., 2000). We speculate that electrical gradients along the ependymal lining layers might regulate the beating rate of ependymal cilia and so control the establishment of gradients of guidance molecules within the flowing CSF. In addition, an electrical gradient across the ventricular wall might act as a cue to regulate the axis of cell division or cell proliferation of neuroblasts (Song et al., 2002), or to 'kickstart' neuronal migrations from the germinal layers that line the ventricle. Again, the challenge is to outline respective roles for chemical and electrical cues, and to determine their interactions.

Similar to neurons, astrocytes and Schwann cells undergo oriented growth in response to an EF, and Schwann cells migrate rapidly anodally in EFs as low as 3 mV/mm (Moriarty and Borgens, 2001; McKasson et al., 2008). Glial and neuronal cells are tightly related functionally and are often interconnected by communicating gap junctions. How the effects of an EF are integrated between neurons and glial cells is completely unexplored.

# Electrical control of wound healing

It has been clear for 20 years that electrical signals have a role in amphibian and mammalian wound healing (Rajnicek et al., 1988; Vanable, 1989). Three studies further our understanding of electrical control over wound healing (Zhao et al., 2006; Pullar, 2006; Pullar et al., 2007). Two genes (encoding PI3K and PTEN) that regulate electrically driven wound healing have been identified using wounded rat cornea and skin (Zhao et al., 2006) (Fig. 1 and Box 2). In keratinocytes from PI3K $^{-/-}$  mice ( $p110\gamma$  gene deletion), EF-induced signalling was decreased, directed movement of healing epithelium was abolished and electrotaxis was impaired. In addition, tissue-specific deletion of the gene encoding PTEN, a negative regulator of PI3K, enhanced EF-induced signalling and directed keratinocyte migration.

We have tested the potency of an applied EF in the context of coexisting cues in a scratch monolayer model of wound healing [see movie 1 in Zhao et al. (Zhao et al., 2006)]. The default wound-healing cues that drive closure of scratched monolayers are assumed to include the ability of cells to identify open space (release from contact inhibition) and the establishment of chemical gradients of growth factors or cytokines released at the wound edge. (Importantly, an electrical signal arises the instant the epithelium is breached, whereas chemical gradients can take many minutes or hours to establish.) When an EF with a polarity that mimicked that at a corneal wound (cathode at wound) was applied, scratch wounds closed more quickly. When the electrical signal was applied with a reversed ('wrong') polarity, the default wound-healing cues failed to drive wound closure and, remarkably, the wound opened up. One interpretation of these data is that a physiological electrical cue can override pre-existing chemical and physical wound-healing cues [see movie 1 in Zhao et al. (Zhao et al., 2006)]. Finally, we showed that the migration of epithelial cells, nerves, fibroblasts and neutrophils is directed in each case by a wound-induced EF (Zhao et al., 2006). Intriguingly, each of these cell behaviours is required coordinately for effective wound healing. Perhaps the instantaneous wound-induced electrical signal 'kickstarts' several cellular processes that are crucial for wound healing. These might include the upregulation of growth-factor receptors [the EGF receptor is upregulated in corneal epithelial cells by an EF (Zhao et al., 1999; Zhao et al., 2002)] and the upregulation of growth-factor secretion, which would give rise to chemical gradients [secretion of VEGF by endothelial cells is upregulated by exposure to a physiological EF (Zhao et al., 2003)].

The work of Pullar and colleagues has added two molecular players –  $\beta 4$  integrins and  $\beta$ -adrenergic receptors – to those involved in EF-driven wound healing. They showed that keratinocytes with truncated  $\beta 4$  integrins were blinded to an applied EF in the absence of EGF, and that addition of EGF together with functioning  $\beta 4$  integrins enhanced EF-directed cell migration (Pullar, 2006). As an explanation for this phenomenon, the authors suggested that cooperativity between EF-activated EGF signalling and  $\beta 4$ -integrin signalling through the small GTPase Rac might occur, perhaps at focal adhesions. Pullar and colleagues have also shown that activation of  $\beta$ -adrenergic receptors partially blinds keratinocytes to a physiological EF and slows corneal wound healing. By contrast,  $\beta$ -adrenergic-receptor antagonists enhanced the migration response to an EF and accelerated corneal wound healing (Pullar et al., 2007).

#### The future for electrical wound healing

Because EF-induced wound healing depends on known ionic transport mechanisms, there is growing interest in approaching wound-healing therapies with topically applied agents that target the enhancement or suppression of ion channels or pumps to regulate the transepithelial potential difference. Electrodes embedded in a hydrogel whose ionic or pharmacological content

#### Box 3. Extracellular electricity in the brain

Cell recruitment to sites of brain damage is known to involve damage-induced growth factors, cytokines and chemokines. However, electrical signals and chemical cues share the extracellular space and inevitably interact. In the brain, EFs of 50 mV/mm arise from the firing of hippocampal granule cells and these EFs persist for minutes to hours (Lomo, 1971). In models of epilepsy, synchronous firing of neurons also creates steady voltage gradients of 10-50 mV/mm (Jefferys, 1981). Brain trauma and focal ischaemia cause recurrent waves of cortical spreading depression [CSD; a moving wave that severely depolarises cells to the extent that they cannot fire action potentials and large areas of brain tissue are therefore transiently silenced: first described by Leiao (Leiao, 1944)]. Ischaemia results in depolarisations of 20-50 mV, which persist for many minutes, and recur up to five times per hour (Marshall, 1959). CSD has been implicated in the pathophysiology at border zones in injured human brain (Strong et al., 2002) and in migraine aura in the human visual cortex (Hadjikhani et al., 2001).

Both CSD and the uncontrolled firing of bursts of action potentials that occur in epilepsy also cause profound changes in the concentration of extracellular  $K^+$  ( $[K^+]_o$ ). Experimental CSD increased  $[K^+]_o$  from 4 mM to 60-80 mM for several minutes and caused a 37% increase in cell swelling in mouse cortical neurons (Takano et al., 2007). Natural variations in anatomical packing between different areas of the hippocampus cause extracellular resistance changes of three- to fivefold (Faber and Korn, 1989) and it is likely that transient or sustained cell swelling would have similarly profound effects. Phasic increases in extracellular resistance therefore occur in normal and damaged brain. An important consequence is that, for a given level of ionic current flow, a greater EF strength would result in these areas of increased extracellular resistance.

Applied EFs have been used to suppress seizure-like electrical activity in brain, and the firing frequency of CA1 pyramidal neuron networks can be synchronised by extracellular gradients as small as 140  $\mu\text{V/mm}$ . Importantly, networks of neurons seem to be more sensitive to applied EFs than single cells (Francis et al., 2003).

In conclusion, electrical influences impinge on the control of neuronal proliferation, neuronal differentiation, neuronal migration and growth-cone pathfinding. Thus, the potential impact of extracellular electricity in the brain is vast and remains underexplored.

enhances epithelial-driven wound currents would represent a novel approach to wound-healing therapies. A new device (the trade name of which is the Dermacorder) that measures electrical fields at wounds in human skin also has been developed and is likely to be of significant clinical use (Nuccitelli et al., 2008).

#### EFs and cancer

The involvement of electrical signals in cancer has been investigated sporadically for around 50 years (Ambrose, 1956). Three electrical approaches have been applied recently to cancer-cell biology.

#### High-frequency AC EFs

In alternating current (AC) EFs, all polar molecules are subject to alternating forces; consequently, ionic flows and dipole rotations oscillate. Because of the slow kinetics of bioelectrical events, AC EFs above 100 kHz were thought to have no biological effect apart from heating. However, recent work challenges this view by showing that AC EFs (1 V/cm; 100-300 kHz) inhibit the growth

of cultured tumour cells, of solid mouse melanomas, and of highly malignant human glioblastoma tumours in clinical trials (Kirson et al., 2004; Kirson et al., 2007). The mechanism is proposed to involve electrical focussing at the cleavage furrow, which causes interference with microtubule polymerisation, leading to disrupted mitotic-spindle formation and function (Kirson et al., 2004).

#### High-intensity pulsed EFs

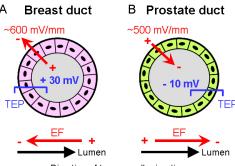
Recently, solid mouse melanomas have been exposed to ultrashort, very strong, pulsed EFs (Nuccitelli et al., 2006; Nuccitelli et al., 2009). The application of 400 (40 kV/cm) pulses of 300 ns duration caused nuclei within tumour cells to shrink by 50% within minutes and by around 70% in 3 hours. Tumour blood flow stopped in many cases and melanomas shrank by 90% within 2 weeks. A second treatment resulted, in many cases, in complete remission. These are remarkable responses to an EF with a cumulative duration of only a few hundred µs and they are not thermally based. These pulses penetrate the cell and very briefly permeabilise intracellular organelles. The EF is thought to cause rapid electromechanical deformation of the nucleus, which damages DNA associated with the nuclear membrane.

#### Physiological DC EFs

In addition to using very high EFs (see above), tumours also have been treated with physiological DC EFs. Potential gradients in the extracellular space between cancerous and normal tissues can be measured at the tissue surface and have been used clinically to diagnose early-onset breast cancer (Cuzick et al., 1998). The voltage drop between cancerous and normal tissue might have its origins in the depolarisation of the membrane potential of most tumour cells (Ambrose et al., 1956; Binggeli and Weinstein, 1986), which causes a drop in the transepithelial potential difference in areas in which tumour cells are dividing rapidly. A more polarised epithelium is maintained in normal tissues because epithelial cells remain more hyperpolarised (Faupel et al., 1997). We and others have sought to mimic the endogenous DC EFs between tumour and normal tissue, and to study electrical methods of preventing the directed migration of tumour cells that is an early feature of metastasis.

Interestingly, cells of a highly metastatic rat prostate-cancer cell line responded strongly to a DC EF (by migrating cathodally) but those of a weakly metastatic cell line did not respond to a similar EF. Cathodal migration of the highly metastatic cells was blocked with tetrodotoxin (which blocks voltage-gated Na<sup>+</sup> channels), implicating these channels in EF-directed migration (Djamgoz et al., 2001; Mycielska and Djamgoz, 2004). The electrical-potential difference across the lumen of rat prostate glands is around –10 mV [negative with respect to the serosal (blood) side] (Szatkowski et al., 2000). Therefore, a steady DC EF of 500 mV/mm (5 V/cm) exists across the 20-µm-thick lumenal wall (in which the lumen is cathodal); this is sufficient to attract ingrowth of metastasizing prostate epithelial cells (cathodally) (Fig. 3) (Djamgoz et al., 2001).

Metastasis of breast-cancer cells requires directed cell migration and might also have an electrical dimension. DC EFs stimulate and direct migration of human breast-cancer cells. This is more pronounced in highly metastatic cells (Pu et al., 2007). EF-directed migration correlated well with levels of expression of the EGF receptor Erb1, and involved downstream signalling via tyrosine kinases, PI3K, Rho GTPases and ERK1/2 (extracellular-signal-regulated kinases 1 and 2) (Pu et al., 2007). Normal breast epithelium has a transepithelial potential difference of +30 mV (duct lumen positive), which is distributed across a lumenal wall that is 50 μm wide. This represents a voltage drop of 600 mV/mm,



Direction of tumour cell migration

**Fig. 3.** The polarity of the transepithelial potential (TEP), and hence the polarity of the endogenous EF of mammary and prostate ducts, correlates with the direction of their breast- or prostate-tumour cell migration relative to an EF in vitro. (A) The human breast-duct lumen has a potential of 30 mV (positive) with respect to the surrounding tissue; therefore, the lumen represents an anode (Faupel et al., 1997). (B) Conversely, the rat prostate-duct lumen is 10 mV (negative) relative to the surrounding tissue and is therefore a cathode (Szatkowski et al., 2000). In tissue culture, metastatic breast-tumour cells migrate towards the anode but prostate-tumour cells migrate towards the cathode. The EF strength required to stimulate directed migration in each case was lower than that of the EF that would exist across the duct epithelium (calculated as TEP divided by total thickness of epithelium). The initial migration during metastasis is into the lumen in both tissues, so the endogenous EF might contribute to metastasis.

about ten times greater than is needed to direct these cells (Pu et al., 2007) (Fig. 3). It might be important that the potential difference across prostate and breast ducts differs in polarity, whereas transformed epithelial cells from these tissues also differ in the polarity of their migration in an applied EF (Fig. 3). In summary, metastasis might have an electrically directed dimension and discovering ways to offset this might offer new therapeutic potential.

#### A future for electrical control of cancer?

Both the cell cycle and the mitotic spindle are targets for physiological extracellular EFs (Wang et al., 2003; Wang et al., 2005; Song et al., 2002), and regulating these might have a profound impact on the control of tumours. For example, cultured rat lens epithelial cells and bovine endothelial cells show markedly reduced proliferation rates in a physiological EF. The EF suppresses cyclin-E expression and increases expression of p27kip1 (an inhibitor of the cyclin-E-Cdk2 complex), and consequently cells fail to make the G1-S-phase transition and undergo cell-cycle arrest (Wang et al., 2003; Wang et al., 2005). In rat corneal epithelial cells in vivo, however, an endogenous EF enhanced proliferation and oriented the mitotic spindle (Song et al., 2002). Increasing or reducing the wound-induced EF in this tissue (see Box 2) increased and reduced epithelial-cell proliferation close to the wound edge and made the orientation of the mitotic spindle either more or less parallel to the EF vector (Song et al., 2002).

In summary, little is known about how EFs control important cell-biological events such as proliferation, spindle orientation, cell differentiation and cell-lineage selection. Delineating and targeting these control mechanisms is likely to offer novel therapeutic approaches for a host of pathologies, including several cancers.

# New cellular and extracellular targets of EF

Left-right determination

The Levin laboratory has introduced the concept that H<sup>+</sup> flux that is driven by the plasma-membrane V-ATPase pump might be crucial

for creating extracellular voltage gradients that drive directed regeneration of spinal-cord axons and tadpole-tail regeneration (Adams et al., 2007) (Box 2). Interestingly, this group also showed that H<sup>+</sup> gradients created across a syncytium of gap-junction-coupled midline cells in early chick and amphibian embryos give rise to a left-right intracellular voltage gradient. Gradients of 20 mV were measured, with cells on the left side of the midline being depolarised with respect to those on the right side (Levin et al., 2002). This induces an intracellular gradient of voltage within the syncytium, which in turn (through electrophoresis) establishes a gradient of serotonin within cells of the midline, and this is thought to regulate left-right determination (Adams et al., 2006; Esser et al., 2006). This is clear evidence that an EF can establish intracellular gradients of relevant signalling molecules.

#### Limb development

During limb development in amphibian embryos, ionic current flows out of the precise site on the flank skin at which the limb bud appears a day or so later (Borgens et al., 1983; Robinson, 1983). This predictive current also appears in chick and mouse, and reversing it in chick causes major limb abnormalities, indicating that it is required in limb development (Altizer et al., 2001). Injecting a fluorescently tagged charged protein (albumin) into the extracellular space of nonlimb-bud flank skin resulted in radial diffusion. However, when albumin was injected into the limb bud, the distribution was cometlike, presumably because of induced electrophoresis of charged albumin within the extracellular spaces (Messerli and Robinson, 1997). Therefore, the ionic-current-flow-induced EF controls the distribution of charged proteins. This is likely to include key morphogenic molecules that orchestrate limb development.

#### Voltage sensing by phosphatases

The first voltage-sensitive membrane phosphatase has been discovered recently. This voltage-sensor-containing phosphatase, which was discovered in Caenorhabditis intestinalis and is therefore called Ci-VSP, has a transmembrane voltage-sensing domain that is homologous to segments of known voltage-sensitive channels and a cytoplasmic domain that is similar to the signalling molecule PTEN (Murata et al., 2005). It displays channel-like gating currents and can convert the second messenger phosphatidylinositol (3,4,5)trisphosphate [PtdIns $(3,4,5)P_3$ ] to PtdIns $(4,5)P_2$  as a direct consequence of changes in membrane potential. Clearly, therefore, voltage sensing can function independently of channel proteins. Intriguingly, Ci-VSP is transiently expressed in developing mouse brain and spinal cord, and might be important in many of the neuronal functions discussed above. Taken together with the discovery that PTEN is involved in transducing the EF-induced wound-healing response of epithelial cells (see above), this discovery places PTEN and its relatives at the centre of a host of possible cell responses to EFs.

## A coexisting electrical dimension in cell biology

In this Commentary, we have stressed the concept of extracellular gradients of voltage that are induced and maintained by gradients of specific ions. One can picture these gradients in a similar way to gradients of morphogens, in that they vary in magnitude, position and duration. Considering the interactions through which endogenous voltage gradients establish, amplify or nullify coexisting gradients of charged chemicals, it is inevitable that – even before the receptor transduction stage – EFs will shape the molecular gradients that cells create and sense.

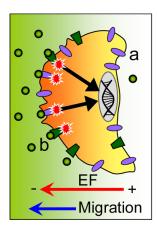


Fig. 4. A model in which directed cell migration is controlled by integrated extracellular voltage and/or chemical gradients (green shading) and intracellular electrochemical signals. This schematic shows a non-neuronal cell, but the principles should also apply to neuronal growth cones. Here, we consider a cell that is stimulated to migrate up the chemical gradient or towards the cathode. The red arrow indicates the direction of the chemoattractant gradient and the voltage gradient (EF). The blue arrow indicates the direction of cell migration. Two membrane-receptor configurations are shown: (a) receptors that are uniformly distributed within the membrane (purple), and (b) receptors that are concentrated at the leading edge (green), possibly by the EF itself. For a cell in an extracellular EF, uniformly distributed receptors (a) lead to asymmetric cytoplasmic signalling (shaded cell interior) if the EF perturbs the plasmamembrane potential sufficiently to activate voltage-sensitive channels on the cathode-facing membrane. Alternatively, for a cell in a gradient of a soluble extracellular ligand (green dots), symmetric distribution of ligand-specific receptors (b) leads to asymmetric cytoplasmic signalling because of the increased probability that a receptor on the high side of the gradient would be activated (stars) by ligand. It is probable that electrical and chemical signalling gradients interact. In addition to a role for naturally occurring EFs in establishing, maintaining or amplifying gradients of charged molecules. activation of receptors for chemoattractive ligands depolarises neuronal membranes (and chemorepulsive ligands hyperpolarise membranes) (Nishiyama et al., 2008), which might activate voltage-sensitive channels and amplify the gradient of signalling activity across the cell. Signal transduction via the cytoskeleton is also likely to control migration. Membrane receptors for neurotransmitters or growth factors associate with microtubules or microfilaments via linker molecules. Receptor activation might stimulate electrical signalling (black arrows) directly along the cytoskeletal polymer networks to the nucleus. Because DNA is an electrically conducting polymer, this might have direct consequences for gene expression. Collectively, therefore, electrical and chemical signalling gradients reinforce each other and might amplify gradients of intracellular signals, leading to controlled cell migration.

The ability of an EF both to induce gradients of ligands in the microenvironment and to drive asymmetric distribution of receptors within the plasma membrane suggests that these two processes might act as a positive-feedback amplifier. In other words, high concentrations of receptors at the leading edge would be activated by the highest concentrations of the ligand, both imposed by the electrical gradient. Moreover, there is scope for further interactive feedback between electrical and chemical signals. Neuronal growth cones respond to a gradient of extracellular GABA through asymmetric redistribution of their GABAA receptors towards the high gradient source (Bouzigues et al., 2007). This redistribution is microtubuledependent and amplifies a downstream gradient of intracellular Ca<sup>2+</sup>. This raises the possibility that EF-driven asymmetry of receptors and creation of extracellular ligand gradients might be self-enhancing, because the ligand gradient might trigger further receptor asymmetry. Coexisting electrical and chemical signals would thus be acting synergistically in an electrochemical interplay (Fig. 4).

Cytoskeletal polymers are electrical conductors and have been likened to biological electrical wires. Both actin filaments and microtubules conduct electricity, with roughly the same conduction velocity along filamentous actin as in nerves (20 m/s) (Tuszynski et al., 2004; Priel et al., 2006). Actin filaments and microtubules are intimately associated with the anchoring of voltage-sensitive membrane receptors and channels, and intact microtubules and actinfilament networks are required for directional EF responses (Rajnicek et al., 2006b). Thus, in considering the biochemical signaltransduction steps that follow activation of a neurotransmitter or a growth-factor receptor, perhaps we should consider the possibility that there is direct activation of cytoskeleton-based electrical conduction to the nucleus in parallel with the known biochemical cascades. Direct mechanical linkage from the plasma membrane to the nucleus and its role in selective gene activation has been demonstrated (Maniotis et al., 1997; Nelson et al., 2005). Intriguingly, DNA also conducts over long distances and certain base-pair sequences can interrupt this, perhaps acting as on-off switches (Merino et al., 2008). So, in addition to thinking about steady gradients of morphogens or of growth factors in the extracellular spaces, which are transduced by receptors into a cascade of biochemical signalling molecules, we should consider the possibility of continuous electrical signalling from the extracellular matrix, which is transduced by voltage-gated mechanisms that activate electrically conducting actin filaments, microtubules and even DNA (Fig. 4).

#### New techniques to map extra- and intracellular EFs

The ability to visualize EFs, both inside and outside cells, is crucial for their study. Fluorescent probes are used widely to visualise and quantify the potential difference across the membranes of cells or of intracellular organelles such as mitochondria. However, direct visualisation of the time course and the dynamics of EFs within extracellular spaces has proven more challenging. One promising approach involves the use of E-PEBBLES, which are made by encapsulating voltage-reporting molecules such as the fluorescent dye di-4-ANEPPS inside lipid microspheres. They can be targeted to the cell interior, where they are released and report quantitatively on EFs generated by mitochondria and by plasma membranes with nanometer-to-micrometer spatial resolution (Tyner et al., 2007). Surprisingly, very high voltage gradients extend out from these structures for several micrometers into the cytoplasm, not merely for nanometers as had been thought previously. This type of probe is also suitable for the visualization and mapping of voltage gradients within extracellular spaces.

#### Conclusions and perspectives

We have shown that EFs regulate some of the most fundamental cell behaviours (proliferation, migration and differentiation) in multiple tissues (brain, epithelia and various cancers). The overlap of cellular regulation by chemical and electrical gradients is profound and wide-reaching. As cell biologists, we should integrate steady electrical gradients into our standard thinking about cell function. Areas of study can fall out of fashion when developments of technology shift the focus of research. In the case of electrophysiology, the development of intracellular microelectrodes and patch-clamp electrodes shifted attention away from extracellular voltage signals and towards transmembrane potentials and the recording of electrical signals associated with single ion channels, respectively. For this reason, the physiological significance of spatial voltage gradients in the extra-and intracellular space, which was well recognised in the early 1900s, has attracted only sporadic interest in the last half-century.

Re-focussing on this could have wide-reaching potential. By applying modern probes that visualise steady electrical signals, there is the real prospect of re-invigorating an important field of study.

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