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Endogenous electric currents might guide rostral migration of neuroblasts

Lin Cao^{1,2*}, Dongguang Wei^{2,3*}, Brian Reid^{1,2,4}, Siwei Zhao⁵, Jin Pu⁶, Tingrui Pan⁵, Ebenezer Yamoah^{2,3}
& Min Zhao^{1,2,4,7+}

¹Department of Dermatology, ²Center for Neuroscience, ³Department of Anesthesiology and Pain Medicine, University of California, Davis, California, USA, ⁴Institute of Regenerative Cures, School of Medicine, University of California, Davis, California, USA, ⁵Department of Biomedical Engineering, University of California, Davis, California, USA, ⁶School of Medical Sciences, University of Aberdeen, Aberdeen, Scotland, UK, and ⁷Department of Ophthalmology, University of California, Davis, California, USA

Mechanisms that guide directional migration of neuroblasts from the subventricular zone (SVZ) are not well understood. We report here that endogenous electric currents serve as a guidance cue for neuroblast migration. We identify the existence of naturally occurring electric currents ($1.5 \pm 0.6 \mu\text{A}/\text{cm}^2$, average field strength of $\sim 3 \text{ mV}/\text{mm}$) along the rostral migration path in adult mouse brain. Electric fields of similar strength direct migration of neuroblasts from the SVZ in culture and in brain slices. The purinergic receptor P2Y1 mediates this migration. The results indicate that naturally occurring electric currents serve as a new guidance mechanism for rostral neuronal migration.

Keywords: neuroblast; rostral migration; electric field; electrical corridors; purinergic receptor

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INTRODUCTION

During development, some neurons migrate long distances from their origin to their final destination. Migration of some types of new-born neuron persists in adult mammalian brains [1–4]. One well-studied example in the brain is the highly directional

migration of neuroblasts from the subventricular zone (SVZ) to the olfactory bulb (OB). This pathway spans more than several millimetres or even more and is called the rostral migratory pathway or rostral migratory stream [1,3,5–11]. Defects in neuronal migration might lead to important diseases, including lissencephaly, epilepsy and mental retardation [12–14].

Genetic and molecular analyses have suggested several molecules that might guide neuronal migration. These molecules form extracellular molecular gradients (for example gradients of stromal-derived factor-1, slits and reelin) and participate in guiding the rostral migration [2,15–17]. In addition, biophysical cues might have a role in the rostral migration. A recent elegant work demonstrated an important role of flow of cerebro spinal fluid in guiding neuronal migration. The beating cilia of ependymal cells generates directional flow of cerebro spinal fluid, which helps to build slit gradients that contribute to the rostral migration of neuroblasts [18].

Another potential candidate cue for guiding long distance migration is the electric field (EF). Directional fluxes of ions generate electrical potential gradients, endogenous EFs, which have been measured in the embryo and at wounds [19–26]. Many types of cells respond to small EFs by directional polarization, growth and migration (galvanotropism, galvanotaxis or electrotaxis) [20,27–32]. Schwann cells from chick embryo, for example, migrate directionally in an EF as low as $3 \text{ mV}/\text{mm}$ [33]. Electric potential gradients have also been reported in the normal and injured brains. The cortical surface in adult rabbit was measured 0.5 to 5.5 mV positive to the ventricle [34]. The potential difference increased with age, being only $1 \sim 1.6 \text{ mV}$ at age 5 days but 20 mV in the mature rat [35]. Injury induces electrical potential gradients between normal cortical surface and a damaged area of cortex in the rat [35]. Infarction of the brain generates large negative extracellular potential changes lasting on the order of minutes [36,37].

We suggest that there are endogenous currents along the rostral migration pathway that contribute to the guidance of neuroblasts to migrate from the SVZ to the OB.

¹Department of Dermatology, University of California, Davis, 3301 C Street, Sacramento, California 95816, USA

²Center for Neuroscience, University of California, Davis, 1544 Newton Court, Davis, California 95618, USA

³Department of Anesthesiology and Pain Medicine, University of California, Davis, 4150 V Street, Sacramento, California 95817, USA

⁴Institute of Regenerative Cures, School of Medicine, University of California, Davis, 2315 Stockton Blvd, Sacramento, California 95817, USA

⁵Department of Biomedical Engineering, University of California, One Shields Avenue, Davis, California 95616, USA

⁶School of Medical Sciences, University of Aberdeen, IMS Building, Ashgrove Road West, Foresterhill, Aberdeen AB25 2ZD, Scotland, UK

⁷Department of Ophthalmology, University of California, Davis, 4860 Y Street, Sacramento, California 95817, USA

*Corresponding author. Tel: +1 916 703 9381; Fax: +1 916 703 9384;

E-mail: minzhao@ucdavis.edu

+These author contributed equally to this work.

Fig 1 | Naturally occurring electric currents flow from the SVZ to the OB. (A,B) An inward electric current of $-1.6 \pm 0.4 \mu\text{A}/\text{cm}^2$ and an outward electric current of $1.5 \pm 0.6 \mu\text{A}/\text{cm}^2$ were detected at the lateral ventricular wall (LV1–5) and the surface of olfactory bulb (OB1–5), respectively, with the vibrating probe system. Scale bar, 1 mm. (C) Na^+/K^+ -ATPases distribute at the apical side of epithelial layer of OB (c,d), whereas those electrogenic molecules distribute at the basal side of the lateral ventricular wall (g,h). Myosin was stained in green (b,f). Nuclei blue (a,e). Scale bars, 100 μm . (D) Treatment with Ouabain significantly decreased both the inward currents at the LV and outward currents at the OB. Data were from three or more independent experiments and are presented as mean $\pm$ s.e.m. DAPI, 4,6-diamidino-2-phenylindole; OB, olfactory bulb; SVZ, subventricular zone.

RESULTS AND DISCUSSION

Endogenous currents in rostral migratory pathway

We first mapped the electric current flow at the lateral ventricle walls, and at the surface of the OB, using a vibrating probe system, which measures electric currents noninvasively with high sensitivity and spatial resolution [38,39]. At lateral ventricle of adult mouse brain, very consistent inward currents were detected at all five measuring positions from frontal to occipital horn (see detailed Methods in Supporting Materials). The average magnitudes of the inward electric currents (I_i) were $I_i = -1.6 \pm 0.4 \mu\text{A}/\text{cm}^2$, whereas at the OB, five measuring positions showed uniformly outward currents ($I_o = 1.5 \pm 0.6 \mu\text{A}/\text{cm}^2$) (Fig 1A,B).

We next used the four-point probe technique with Ag/AgCl electrodes to measure the direct current (DC) electrical resistance

in brain tissues (Fig 2A) [40,41]. A DC resistance of $5.8 \pm 1.4 \text{ k}\Omega$ existed between the SVZ and the OB. The extracellular voltage is the product of the resistance of interstitial fluid and the electric currents. The voltage can be calculated according to Ohm's law: $V = R \times I$ ($I = J \times A_{\text{out}}$), where J is the ionic current density measured using vibrating probe; R is interstitial resistance measured with the four-point probes; and A_{out} is the total surface area of the OB. The average distance from the SVZ to the OB in adult mice is 3 mm, therefore an EF of $\sim 2 \text{ mV}/\text{mm}$ exists naturally along the rostral migration pathway.

Third, we directly measured the electric potential in the interstitial spaces along the rostral migration pathway, using a voltmeter. The Ag/AgCl electrodes were insulated except the tips

(diameter $100 \mu\text{m}$). The detection electrode was inserted in brain tissue $\sim 200 \mu\text{m}$ under the lateral ventricle (LV) wall at different positions, in the centre of the OB, or in the centre of the rostral migration pathway. The reference electrode was placed in artificial cerebral spinal fluid, which bathed the hemisphere with same distance to different measurement points (Fig 2B). The SVZ had a positive potential: $7.9 \pm 5.3 \text{ mV}$ and the centre of OB had a negative potential: $-2.0 \pm 5.6 \text{ mV}$ in relation to the bathing artificial cerebral spinal fluid. The voltage drop between SVZ and OB thus is $9.9 \pm 1.8 \text{ mV}$ (Fig 2C). This voltage drop divided by the distance between the measuring points in the SVZ and the OB gave an average voltage gradient of $\sim 3.3 \text{ mV/mm}$. We also measured the voltage drop when detection electrodes were

Fig 2 | Measurement of electric potentials and resistance in the mouse brain. (A) The four-point probe was used to measure and calculate the bulk resistance of brain tissue between the SVZ and OB. One voltmeter (blue) was used to measure the voltage drop in brain tissue between the test probes (blue). Another voltmeter and a power supply with a variable resistor (R) provided a calibration voltage through the blue electrodes. This method gave a value of $5.8 \pm 1.4 \text{ K}\Omega$ for the tissue resistance between the SVZ and the OB. (B) A pair of Ag/AgCl electrodes was used to measure the electrical potential difference at the four points (P1–P4) as shown in upper image between SVZ and the OB. The reference electrode was located in artificial cerebrospinal fluid. (C) The voltage difference of $9.9 \pm 1.8 \text{ mV}$ between the SVZ and OB was detected with AgCl electrodes. The reference electrode was located in artificial cerebral spinal fluid. (D) On the basis of direct measurement and calculation, an electric potential difference of $\sim 3 \text{ mV/mm}$ existed between the SVZ and the OB in the mouse brain. Data were grouped from three or more independent experiments and are presented as mean $\pm$ s.e.m. DC, direct current; OB, olfactory bulb; SVZ, subventricular zone.

simultaneously inserted into the SVZ and OB, respectively. These showed a voltage drop of $5.7 \pm 1.2 \text{ mV/mm}$. We measured the voltage at several points along the rostral migratory pathway; consistent positive potentials were measured along the rostral migration path relative to the potential at the OB (Fig 2C). Electrical potential gradients therefore exist naturally along the rostral migratory pathway (Fig 2D).

How is the voltage between the SVZ and the OB generated? The extracellular space might occupy as much as 20% of brain volume [42,43]. The concentration of ions (K^+ , Na^+ , Ca^{2+} , and so on) in the extracellular space contributes to the strength of the extracellular fields [44–46]. The field can provide a built-in driving force to move ion currents through cells and organs, and these currents are integrally involved in all of our sensory systems [19]. Electrogenic pumps such as Na^+/K^+ -ATPases are expressed at high levels at the superficial layers of the OB [47]. We used antibodies to label the $\beta 1$ subunits of Na^+/K^+ -ATPases, and confirmed very strong and exclusive apical expression (Fig 1C). The apical localization of these pumps would drive electric currents (flow of positive charges) outwards, generating a voltage sink in the OB. At the ependyma of the lateral ventricles, a layer of multi-ciliated epithelial cells separates the SVZ from the ventricular lumen. The apical surfaces of these ependymal cells, including the microvilli, had little or no ATPase activity. Very strong ATPase reaction product was found in the slender processes terminating on the basal laminae of perivascular spaces [48]. Our immunofluorescence staining verified the basal location of the Na^+/K^+ -ATPases, which would drive currents into the brain (Fig 1C). Ouabain, an inhibitor of Na^+/K^+ -ATPases, effectively reduced the electric current at both ventricular wall and the OB (Fig 1D). Thus, flow of electric currents into the brain tissue (flux of positive ions) at the SVZ and outward flow of currents at the OB orchestrate in establishing gradients of ions and EFs of $3\text{--}5 \text{ mV/mm}$ along the rostral migratory pathway from the SVZ to the OB (Fig 2C,D).

Applied EFs guide neuroblast migration

We then tested the response of neuroblasts from the SVZ to EFs of similar strength that we detected in the brain.

Fig 3 | EFs direct neuroblasts to migrate towards the cathode. (A) Cells from the SVZ expressed Nestin as shown with immunofluorescence staining. Scale bars, 20 μm . (B) EF-directed neuroblast migration towards the cathode (EF = 50 mV/mm). Scale bars, 40 μm for upper channel; 60 μm for lower channel. (C,D) Angular histograms. Red lines indicate the direction and magnitude of the mean migration vector. The length of the mean resultant vector is a crucial quantity for the measurement of directedness of cell migration that is close to 1 with significant migration to the cathode. The analysis of cell migration shows the voltage-dependent directional migration of SVZ neuroblasts towards the cathode (the right) for a period of 5 h. An EF as small as 3.5 mV/mm guided migration of neuroblasts with significant directedness towards the cathode. One-factor ANOVA was used to analyse the significant difference between the EF applied group and no-EF control. (E) The EF-directed neuroblast migration was voltage dependent. Increasing the voltage significantly enhanced the directionality and the migration rate. The data were grouped from 40 to 80 cells from three or more independent experiments. The data are shown as mean $\pm$ s.e.m. * $P < 0.05$, ** $P < 0.01$ when compared with the control values using one-way ANOVA. ANOVA, analysis of variance; DAPI, 4,6-diamidino-2-phenylindole; EFs, electric fields; SVZ, subventricular zone.

We confirmed that the cells obtained from the SVZ expressed neuroblast markers (Fig 3A). These neuroblasts migrated actively in culture (Fig 3B–E). In an applied EF, SVZ neuroblasts migrated more actively with significantly increased migration rate (Fig 3E). Both trajectory and displacement rate (Tt/t and Td/t) increased significantly in an applied EF of 10, 50 or 250 mV/mm (Fig 3E). Most importantly, the SVZ neuroblasts migrated directionally towards the cathode (Fig 3B–D; supplementary Movies S1–S3 online). This is the direction of the endogenous electrical potential gradients from the SVZ to the OB (see Fig 2D). An EF as low as 3.5 mV/mm induced significant directional migration of neuroblasts towards the cathode (Fig 3C,D). This is the EF strength naturally occurring along the rostral migratory pathway in adult mouse brains. The physiological EFs that exist between the SVZ and OB therefore might direct and stimulate migration of the SVZ neuroblasts along the rostral migratory path.

Electrotaxis of neuroblasts in brain slices

We next investigated migration of neuroblasts transplanted into brain slices. SVZ neuroblasts from adult mice were labelled with

cell membrane dye DiI. We transplanted the neuroblasts into the SVZ in the brain of neonatal mice (P3–P7). After recovery of 1–2 days, brain slices were obtained from the neonatal mice for tracking migration of transplanted cells (Fig 4A) (see supplementary information online for details). We monitored migration of transplanted cells with fluorescence time-lapse video imaging (MetaMorph Imaging System) [29]. In the brain slices, the primary migratory direction of neuroblasts was towards the OB and towards the cortex (Fig 4B). We used an established index—directedness—to quantify how directional the neuroblasts migrated towards the OB. The brain slices were placed in a defined orientation. If the cells migrated in random directions, the average directedness value would be 0. If all the cells migrated perfectly towards the right (cathode), the average directedness value would be 1. If cells migrated preferentially towards the right, an average directedness would be larger than 0 and approaching 1 [29]. When the brain slices were orientated as in Fig 4B and no EF was applied, labelled neuroblasts migrated with a directedness value of 0.24, which indicated moderately directional migration towards the right (towards the OB) (Fig 4B). When the brain slices were subjected

Fig 4 | EFs guided migration of neuroblasts in brain slices. (A) Cultured neuroblasts were stained with DiI and injected into the SVZ of neonatal mice (p3–p7). Transplanted cells were visualized under a microscope. Scale bars, 120 μm . (B) An applied EF-directed migration of transplanted cells towards the cathode (the right panel). The directedness of cell migration to the right is 0.48 and 0.6 under 10 and 50 mV/mm of EF, indicating significantly increased directional migration compared with that of transplanted cells in the control condition without an applied EF. Scale bar, 40 μm . The directedness values ($\cos\theta$) shown are mean $\pm$ s.e.m. (C) Reversal of an applied EF reversed the migration direction of neuroblasts in brain slices. A neuroblast (a, white arrowhead) was directed to migrate to the cathode (to the right) for 70 min. The polarity of the field was reversed, the cell reversed the direction and migrated back to the left. Another cell (b, yellow arrowhead) came into the focal plane and also migrated to the left. Note, the cells migrated in and out of the focal plane, therefore show different size and shape. Scale bar, 15 μm . EF = 50 mV/mm. EF, electric field; OB, olfactory bulb; SVZ, subventricular zone.

to EFs of 10 or 50 mV/mm, the transplanted neuroblasts migrated more pronouncedly towards the right (the cathode). In EF, the directedness value of migration was 0.48 and 0.6, significantly higher (2–2.5-fold) than that of no-EF control (Fig 4B). Trajectories of cell migration showed significantly more directional migration in an applied EF towards the cathode (Fig 4B). Reversal of the field polarity reversed the migration direction of neuroblasts in the brain slices (Fig 4C; supplementary Movie S4 online).

P2Y1 mediates EF-guided neuroblast migration

We finally investigated possible molecular mediators of the electrically guided migration. We tested purinergic receptors P2Y1 as these receptors are transiently and specifically expressed in neuroblasts that migrate out of the SVZ. Extracellular ATP signalling is essential for the migration of intermediate neuronal progenitors and formation of the SVZ. P2Y1 receptors mediate Ca^{2+} fluctuations in neuronal progenitors at the time they leave the SVZ and are

required for the formation of the SVZ [49,50]. ATP signalling might also contribute to neuronal differentiation and process outgrowth; both contributed to the radial migration of postmitotic neurons [51].

We used two inhibitors of P2Y1 receptor: a general inhibitor for purinergic receptor suramin and a more specific P2Y1 receptor inhibitor MSR2179. Suramin significantly reduced EF-directed migration of neuroblasts. Suramin at concentration of 5 μM significantly inhibited the directional migration without obvious effect on migration rate. Cells migrated in an EF of 50 mV/mm with a directedness value of 0.28, significantly lower than the control (Fig 5A,D; supplementary Figs S1A,S2 online and supplementary Movie S5 online).

As suramin is a general inhibitor of purinergic receptors, we used two approaches to narrow down specific type of receptors: (1) pharmacological inhibitor MSR2179, a specific P2Y1 inhibitor, and (2) knockdown with small interfering RNA (siRNA)^{P2Y1}. MSR2179 inhibited the EF-directed migration in a dose-dependent

Fig 5 | P2Y1 receptor mediated the directional migration of SVZ neuroblasts in an EF. (A) Suramin (general purinergic receptor inhibitor, 5 μ M) did not affect cell motility rate. However, the direction of EF induced migration was significantly inhibited when cells were treated with 5 μ M suramin. (B) A specific inhibitor of P2Y1 (MSR2179, 120 μ M) significantly inhibited the directedness of neuroblasts in an EF without affecting migration rate significantly. (C) Knockdown of P2Y1 with siRNA (P2Y1 KD) significantly reduced the directedness of neuroblast migration. (D) Suramin, MSR2179 and siRNA^{P2Y1} significantly reduced the directedness (Cos θ) of neuroblasts migration in an applied EF. The western blot inset shows downregulation of P2Y1 expression by siRNA^{P2Y1}. Trajectories of cell migration are shown for 5 h. The directedness value (Cos θ) is mean $\pm$ s.e.m. Total number of cells $n = 45-55$. ** $P < 0.01$ (compared with that exposed to an EF without drug or siRNA treatment with unpaired t -test). Scale bars, 100 μ m. EF, electric field; siRNA, small interfering RNA; SVZ, subventricular zone.

manner without significantly affecting cell migration rate (Fig 5B,D; supplementary Fig S1B,C online, supplementary Fig S3 online and supplementary Movie S6 online). siRNA^{P2Y1} reduced P2Y1 expression level significantly as confirmed with western blot (Fig 5D). Knockdown of P2Y1 with siRNA resulted in significant reduction in the directedness of neuroblasts migration in an EF (Fig 5C,D; supplementary Fig S1C online).

In summary, we detected with different techniques the existence of DC EF gradients between the SVZ and the OB. Applied EFs of the same strength guided migration of the SVZ neuroblasts towards the cathode. P2Y1 receptors that are expressed transiently in the SVZ neuroblasts seem to mediate the EF-guided migration. Our data suggest that the naturally occurring electric potential gradients are a long-distance directional signal for neuroblast rostral migration. This mechanism might offer new approaches in guiding neuroblast migration in stem cell therapies. These results indicate an alternative view of cell migration that complements, but is not mutually exclusive from, the present well known roles of chemokines, matrix metalloproteinases and adhesion molecules in the rostral migration stream.

METHODS

Endogenous EFs were measured with a vibrating probe, a four-point probe system, and microelectrodes. Migration of neuroblasts was recorded with an imaging system. For detailed Methods, please see supplementary Information online.

Supplementary information is available at EMBO reports online (<http://www.emboreports.org>).

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Author contributions: M.Z. conceived and designed the project. L.C., D.W., B.R. and S.Z. performed the experiments and prepared figures. M.Z. and L.C. wrote the main manuscript text. T.P. and E.Y. provided facilities and commented on the manuscript. All authors reviewed the manuscript.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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