Stem Cell & Regenerative Medicine

Neural Stem Cells of Retinal Neuroepithelium Direct Retinal Ganglion Cell Axons Electrically: Galvanotropism in Embryonic Retina

Masayuki Yamashita*

Center for Medical Science, International University of Health and Welfare, Ohtawara 324-8501, Japan.

*Correspondence:
Masayuki Yamashita, Center for Medical Science, International University of Health and Welfare, Tel: +81 287 24 3088; Fax: +81 287 24 3100; Japan, E-mail: my57@iuhw.ac.jp.

Citation: Yamashita Masayuki. Neural Stem Cells of Retinal Neuroepithelium Direct Retinal Ganglion Cell Axons Electrically: Galvanotropism in Embryonic Retina. Stem Cells Regen Med. 2018; 2(1): 1-4.

ABSTRACT

Growing axons are directed not only by chemical signals but also by electric fields in a process known as galvanotropism. Axons of embryonic neurons extend along the extracellular voltage gradient towards the cathode. During embryonic development neuroepithelial cells function as neural stem cells. The neuroepithelial cell has epithelial type sodium channels (ENaC), and the sodium transport via ENaC of retinal neuroepithelial cells produces extracellular positive direct current (DC) potentials within the retinal neuroepithelium. The amplitude of the positive DC potential is large at the periphery of the embryonic retina, and almost null at the ventral part of the optic cup, where the future optic disc is formed. Retinal ganglion cells are first born at the central part of the optic cup and they extend their axons along the endogenous voltage gradient; the disruption of the DC potential by blocking ENaC results in erroneous path finding of newborn retinal ganglion cell axons. Retinal ganglion cell axons can also be oriented by exogenous electric fields in vitro. Galvanotropism may be used to reform an optic nerve from the retinal ganglion cells that are generated from ES cells and iPS cells.

Introduction

The axons of newborn retinal ganglion cells (RGCs) grow towards the future optic disc during early embryonic development. A plausible mechanism for orienting these axons might be that an attractive protein, such as netrin, guides them using its concentration gradient (chemotropism). However, the deletion of netrin or its receptor does not affect the intra-retinal trajectory of RGC axons [1]. Instead of chemotropism, it has been also known that the axons of embryonic nervous systems extend along the extracellular voltage gradient in a process termed 'galvanotropism' [2]. By applying an extracellular electric field, axons are directed towards the cathode. However, since the previous studies used culture systems to which exogenous electric fields were applied, it has been unknown whether electric fields are formed within embryonic nervous systems, or whether newborn neurons use galvanotropism to orient their axons during embryonic development.

The central nervous system (CNS), which includes the brain, spinal cord, and retina, is derived from the neural tube. The neural tube is formed of the neuroepithelium. During embryonic CNS development, neuroepithelial cells act as neural stem cells by renewing themselves and producing neurons. Neuroepithelial cells have a polarized structure: their apical process faces the ventricle, while the furthest portion of their basal process makes contact with the basement membrane. In the retina, the apical process of retinal neuroepithelial cells makes the outer limiting membrane, and the basement membrane is the inner limiting membrane, along which the axons of newborn RGCs extend.

During CNS development, the neurons that issue long-distance travelling axons, such as descending fibers and optic nerves, are born first. These axons start to extend in their own defined directions almost simultaneously at various parts of the CNS [3]. After the discovery of netrin, long-range chemoattractants were supposed to guide long distance axons [4]. However, the mechanism for orienting long-distance axons has been unknown, since the existence of long-range chemoattractants was denied; the chemical effect is haptotactic acting as a short-range cue [1,5,6]. The present review article points to the electrical activity of

neuroepithelial cells in the embryonic retina and the role for the electric field in RGC axon orientation.

Neuroepithelial cells produce the extracellular positive DC potential

The neuroepithelial cell has various types of ion channels [7-9], including epithelial type sodium channels (ENaC) [10]. In the course of electrophysiological studies of retinal neuroepithelial cells and newborn RGCs [7], the extracellular potential was recorded from the retinal neuroepithelium in an optic cup isolated from chick embryos [10,11]. Upon penetration of the inner limiting membrane from the vitreous side with a fine glass microelectrode [12], a positive direct current (DC) potential was recorded. Na^+ ions enter the neuroepithelial cell through ENaC from the apical side, and are extruded by Na^+-K^+ pumps in the basal region (Figure 1). The application of a blocker for ENaC (amiloride) reversibly reduces the amplitude of the positive DC potential [10]. Since the extracellular electrical resistance is extremely high due to the tight structure of the epithelium [Supplementary Discussion in 11], and the inner limiting membrane has also high electrical resistance [10], the positive charge is accumulated inside the retinal neuroepithelium. Thus, the sodium transport by neuroepithelial cells generates the extracellular positive DC potential in the basal region of the retinal neuroepithelium.
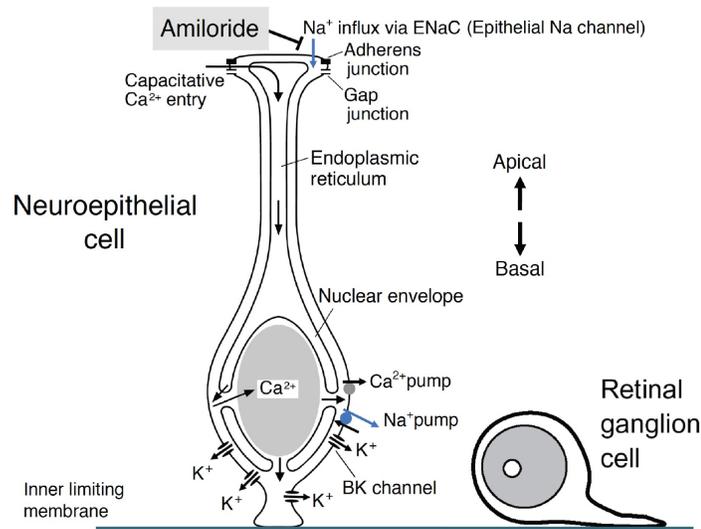

Figure 1: Neuroepithelial cells produce the extracellular positive DC potential. Na^+ enters through epithelial sodium channels (ENaC) from the apical side, and is excluded by Na^+ pump in the basal region. This figure is cited from [Supplementary Discussion in 11].

Endogenous electric fields in embryonic retina

The amplitude of the extracellular positive DC potential is largest at the dorsal part of the opti cup, and also large at the nasal and temporal parts, but almost null at the ventral part [11]. As a result, extracellular voltage gradients are formed within the retinal neuroepithelium in the direction from the periphery to the ventral part (Figure 2). The strength of the electric field estimated from the voltage gradient is 15 mV/mm at the central part of the optic cup. As the positive DC potential is generated by the sodium transport

of neuroepithelial cells, the highest potential is established at the dorsal part of the optic cup, where the neuroepithelial cells most actively proliferate increasing cell densities [13]. On the other hand, the cells located ventrally in the optic cup are triangular-shaped [1], similar to the wedge-shaped floor plate cell in the neural tube [14]. Since the ventrally located cells have the ion channels that are not responsible for the sodium transport [15], positive potentials are not generated at the ventral part of the developing CNS including the retina. The future optic disc is formed at the ventral part of the optic cup (Figure 2).
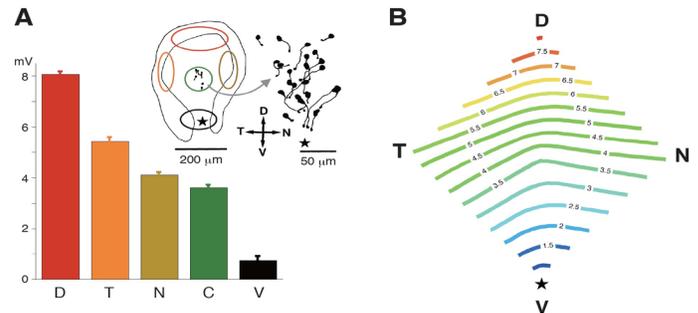
Figure 2: Voltage gradients in embryonic chick retina. (A) Amplitudes of positive DC potential recorded from dorsal (D), temporal (T), nasal (N), central (C), and ventral (V) parts of optic cup, indicated as mean ± S.E.M., numbers of recordings: 41 (D); 52 (T); 72 (N); 53 (C); 33 (V). Inset shows the five recording areas in optic cup, and the first axon-bearing ganglion cells, which appear at the central part. All of the axons grow ventrally towards the future optic disc (★). (B) The direction of the voltage gradient is indicated by rainbow scale. This figure is cited from [11].

The endogenous electric field directs RGC axons towards the future optic disc

RGCs, the first type of retinal neurons, are born at the central part of the optic cup (Figure 2). They extend their axons in the basal region of the retinal neuroepithelium towards the future optic disc. The disruption of the endogenous voltage gradient by applying amiloride to the optic vesicle of chick embryos and to the medium of the organotypic culture of optic cups results in erroneous path-finding of newborn RGC axons [11]. These results strongly suggest that the endogenous electric field is the guidance cue for orienting RGC axons.

Galvanotropic behavior of RGC axons in constant electric field culture

A culture system was built to apply constant electric fields to retinal strips for quantitative studies of the effect of electric fields on the growth of RGC axons [16]. The orientation of RGC axons growing in vitro mirrors the pattern of axon growth during normal development in vivo [17]. When retinal strips are explanted from the segment that is originally dorsal to the optic nerve head, numerous axons emerge from the ventral side of the retinal strip after 24 hours in vitro (Figures 3A and 3B). It is likely that these ventrally growing axons are already oriented before the explant [17]. On the dorsal side of the retinal strip, outgrowing axons are rarely observed (Figures 3A, and 3B). However, by applying electric fields in the direction opposite to the endogenous field,

many axons extend from the dorsal side (Figure 3C). The reverse, dorsally directed electric field can orient RGC axons at the field strength quite weaker than the endogenous electric field [16]. On the contrary, the ventrally directed field enhances the ventral growth (Figure 3D).
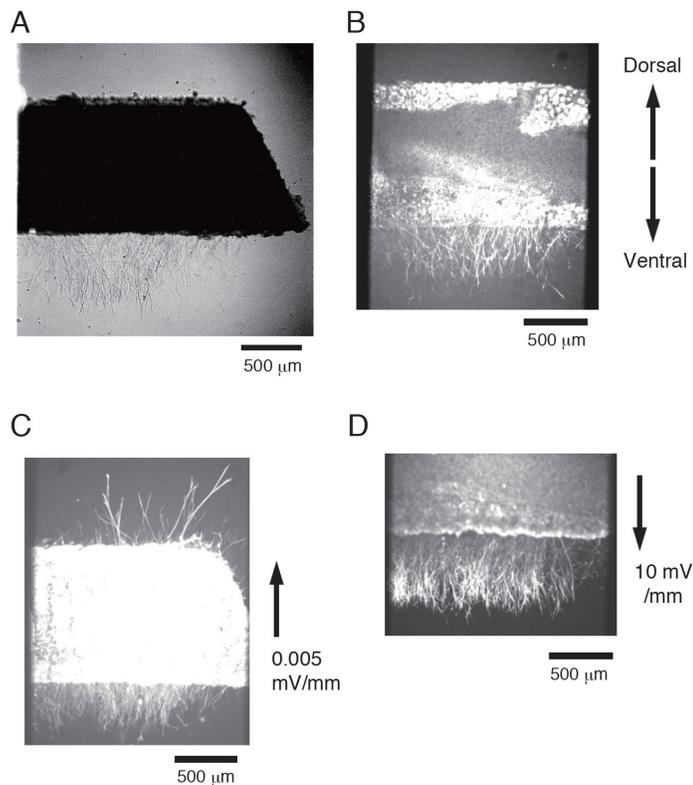
Figure 3: Effects of exogenous electric fields on RGC axon growth in vitro. (A) A retinal strip cultured for 24 hours without exogenous electric fields (DIC image). (B) Fluorescence image of (A) stained with calcein-AM. (C, D) Retinal strips cultured for 24 hours in exogenous electric fields. Directions and strengths of electric fields are indicated on the right side of each panel. (C) Outgrowing axons on the dorsal side. (D) Outgrowing axons on the ventral side. Modified from figures 1 and 2 in [16].

Molecular mechanisms for electric axon guidance

McCaig proposed a model for cathodal orientation of growing axons [2]. In this model, membrane receptors, such as nicotinic acetylcholine receptors, accumulate towards the cathode-facing side of growth cones, which leads to cathodal steering of the growth cone through cytoplasmic Ca^{2+} elevation. However, it remained to be answered whether the asymmetric redistribution of receptors is required for cathodal orientation. The transmembrane potential would be depolarized at the cathode-facing side, and hyperpolarized at the anode-facing side. These changes in the membrane potential may cause asymmetric activation of voltage-dependent Ca^{2+} channels. However, its contribution to axon steering is controversial [18]. McCaig also proposed that voltage-sensitive phosphatases act as voltage sensors in electric fields [19]. However, these voltage-sensing molecules have a transmembrane voltage-sensing domain, and a significant difference in the transmembrane potential between the anode- and cathode-facing sides is required for asymmetric activation of these voltage-sensitive molecules.

From the fact that weak electric fields can cause galvanotropic behavior [2,16], it seems unlikely that the asymmetric activation of voltage-sensing molecules is responsible for axon steering. McCaig's group demonstrated roles for the GTPases Rac, Cdc42 and Rho [20], and for microtubules and microfilaments [21] in axon steering by electric fields. However, it remained unknown what is the cell surface molecule that is asymmetrically activated in electric fields. A preliminary study suggested the involvement of integrins in electric axon guidance [22]. Further studies are required to reveal the molecular mechanism for electric axon orientation.

Future directions

It is possible to generate an optic cup, layered retinae, and RGCs with functional axons from ES cells and iPS cells [23-25]. However, optic nerves are not formed in these stem cell cultures. The reason for the lack of optic nerves may be that there is no guidance cue for the generated RGC axons. To guide these RGC axons, galvanotropism may be used. By applying electric fields, RGC axons could be gathered to form an optic nerve. Galvanotropism has been already applied to injured spinal cords for axon regeneration [19]. Restoration of vision by transplantation of the generated retina and RGCs from stem cells to degenerated retinae depends on the reformation of optic nerves.

References

1. Deiner MS, Kennedy TE, Fazeli A, et al. Netrin-1 and DCC mediate axon guidance locally at the optic disc: Loss of function leads to optic nerve hypoplasia. Neuron. 1997; 19: 575-589.
2. McCaig CD, Rajnicek AM, Song B, et al. Controlling cell behavior electrically: Current views and future potential. Physiol. Rev. 2005; 85: 943-978.
3. Windle WF, Austin MF. Neurofibrillar development in the central nervous system of chick embryos up to 5 days' incubation. J. Comp. Neurol. 1936; 63: 431-463.
4. Tessier-Lavigne M, Goodman CS. The molecular biology of axon guidance. Science. 1996; 274: 1123-1133.
5. MacLennan AJ, McLaurin DL, Marks L, et al. Immunohistochemical localization of netrin-1 in the embryonic chick nervous system. J Neurosci. 1997; 17: 5466-5479.
6. Brankatschk M, Dickson BJ. Netrins guide *Drosophila* commissural axons at short range. Nature Neurosci. 2006; 9: 188-194.
7. Yamashita M. Synchronous Ca^{2+} oscillation emerges from voltage fluctuations of Ca^{2+} stores. FEBS J. 2008; 275: 4022-4032.
8. Yamashita M. Ion channel activities in neural stem cells of the neuroepithelium. Stem Cells Int. 2012; doi: org/10.1155/2012/247670.
9. Yamashita M. From neuroepithelial cells to neurons: Changes in the physiological properties of neuroepithelial stem cells. Arch. Biochem. Biophys. 2013; 534: 64-70.
10. Yamashita M. Epithelial sodium channels (ENaC) produce extracellular positive DC potentials in the retinal

neuroepithelium. Data in Brief. 2016; 6: 253-256.

11. Yamashita M. Electric axon guidance in embryonic retina: Galvanotropism revisited. Biochem. Biophys. Res. Commun. 2013; 431: 280-283.

12. Yamashita M. Electrophysiological recordings from neuroepithelial stem cells. Stem Cell Renewal and Cell-Cell Communication (ed. Turksen K.) Methods in Molecular Biology, Springer Protocols 2015; 1212: 195-200.

13. Prada C, Puga J, Pérez-Méndez L, et al. Spatial and temporal patterns of neurogenesis in the chick retina. European Journal of Neuroscience. 1991; 3: 559-569.

14. Smith JL, Schoenwolf GC. Notochordal induction of cell wedging in the chick neural plate and its role in neural tube formation. J Exp Zool. 1989; 250: 49-62.

15. Frischknecht F, Randall AD. Voltage- and ligand-gated ion channels in floor plate neuroepithelia of the rat. Neuroscience. 1998; 85: 1135-1149.

16. Yamashita M. Weak electric fields serve as guidance cues that direct retinal ganglion cell axons in vitro. Biochem Biophys Rep. 2015; 4: 83-88.

17. Halfter W, Newgreen DF, Sauter J, et al. Oriented axon outgrowth from avian embryonic retinae in culture. Dev Biol. 1983; 95: 56-64.

18. Palmer AM, Messerli MA, Robinson KR. Neuronal galvanotropism is independent of external Ca^{2+} entry or internal Ca^{2+} gradients. J Neurobiol. 2000; 45: 30-38.

19. McCaig CD, Song B, Rajnicek AM. Electrical dimensions in cell science. J Cell Sci. 2009; 122: 4267-4276.

20. Rajnicek AM, Foubister LE, McCaig CD. Temporally and spatially coordinated roles for Rho, Rac, Cdc42 and their effectors in growth cone guidance by a physiological electric field. J Cell Sci. 2006; 119: 1723-1735.

21. Rajnicek AM, Foubister LE, McCaig CD. Growth cone steering by a physiological electric field requires dynamic microtubules, microfilaments and Rac-mediated filopodial asymmetry. J Cell Sci. 2006; 119: 1736-1745.

22. Yamashita M. Electric axon guidance in embryonic retina: Involvement of integrins. J Neurol Neurophysiol. 2017; 8: 39.

23. Nakano T, Ando S, Takata N, et al. Self-formation of optic cups and storable stratified neural retina from human ESCs. Cell Stem Cell. 2012; 10: 771-785.

24. Tanaka T, Yokoi T, Tamalu F, et al. Generation of retinal ganglion cells with functional axons from human induced pluripotent stem cells. Scientific Reports. 2015; 5: 1-11.

25. Tanaka T, Yokoi T, Tamalu F, et al. Generation of retinal ganglion cells with functional axons from mouse embryonic stem cells and induced pluripotent stem cells. Invest Ophthalmol. Vis. Sci. 2016; 57: 3348-3359.