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Previews

Eyes on glutamate in angiogenesis and barrier formation

Yosuke Hashimoto[1] and Matthew Campbell[1,2,*]
[1]Smurfit Institute of Genetics, Trinity College Dublin, D02 VF25 Dublin, Ireland
[2]FutureNeuro, Science Foundation Ireland Research Centre for Chronic and Rare Neurological Diseases, Royal College of Surgeons in Ireland, University of Medicine and Health Sciences, D02 YN77 Dublin, Ireland
*Correspondence: matthew.campbell@tcd.ie
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In this issue of *Neuron*, Biswas et al.[1] demonstrate the roles of glutamatergic neuronal activity in the regulation of angiogenesis and retinal vascular endothelial barrier maturation using three different knockout mouse models. Their findings shed light on how aberrant glutamatergic activity can impact neural vascular function and barrier integrity.

Given the very-high-energy demand of the mammalian retina, a dense and extensive capillary network is required to maintain homeostasis and optimum phototransduction. Indeed, the human retina has the highest energy demand per weight of any tissue in the body. During retinal development, neurons guide retinal capillary formation and drive retinal endothelial cells to evolve strong tight junctions and low rates of fluid phase transcytosis. In effect, these retinal endothelial cells form a tight barrier termed the inner blood-retina barrier (iBRB), which strictly regulates what gets into and out of the retina on a daily basis.

Much like endothelial cells of the blood-brain barrier (BBB), retinal endothelial cells are dependent on surrounding cells, such as pericytes and glial cells, to maintain their integrity. In fact, the retina is part of the central nervous system (CNS), and many studies to date have found disruptions to the integrity of the iBRB and BBB as being associated with ophthalmological and neurological disorders, respectively. Added to this, identifying pathological disease mechanisms that drive BBB dysfunction can assist and inform studies on iBRB pathologies and vice versa.

For example, in our own work, we have recently demonstrated that patients with a missense mutation in the claudin-5 protein, which is the major component of BBB-associated tight junctions, show complex neurological dysfunction comprising hemiplegia, epilepsy, brain microcephaly, and brain calcification.[2] There is now growing evidence that posits that the crosstalk between neural activity and endothelial cell integrity will identify therapeutic targets for neurological and ophthalmological diseases. In this issue of *Neuron*, Biswas et al. detail the role of glutamatergic activity in the retina evoked by photoreceptors in regulating retinal angiogenesis and vascular maturation *in vivo*.[1]

In order to examine the role of glutamatergic neuronal activity on the retinal vasculature, Biswas et al. used three distinct animal models in their studies: (1) *Vglut1*$^{-/-}$ mice in which retinal photoreceptors fail to release glutamate even when they are depolarized in the dark; (2) *Gnat1*$^{-/-}$ mice in which rod photoreceptors remain constitutively depolarized and release excess glutamate; and (3) *Chrnb2*$^{-/-}$ mice in which the time course of glutamatergic neural activities in the retina is impacted by the absence of cholinergic neural activity.[3] No differences were observed in any of these models in developmental angiogenic responses in the superficial capillary plexus, i.e., those vessels that are readily visible in *en face* views of the neural retina, when compared to wild-type mice (Figure 1A). Intriguingly, however, vascular development in the deep capillary plexus (i.e., within the retinal layers) (Figure 1B) were "stunted" in *Vglut1*$^{-/-}$ mice and "accelerated" in *Gnat1*$^{-/-}$ mice when compared to wild-type or *Chrnb2*$^{-/-}$ mice, indicating that glutamatergic, not cholinergic, neural activities promote developmental angiogenesis in this region. This regional difference can be explained by glutamatergic neuronal activity not being initiated when physiological angiogenesis in the superficial capillary plexus has been completed. Furthermore, Biswas et al. examined the permeability status of the retinal vasculature using biocytin-tetramethylrhodamine (870 Da) in both the superficial and deep capillary plexuses and showed that paracellular permeability was limited by an upregulation of claudin-5 in a glutamatergic, but not cholinergic, -dependent manner.

The authors went on to reveal how angiogenesis and barrier maturation occur by these glutamatergic neural activities and cues using single-cell RNA sequencing. Indeed, several pro-angiogenic genes such as *Htra1* and *Aplnr*, the effectors of β-catenin signaling such as *Cttnb1* and *lef1*, and genes associated with tight junction formation such as *Cldn5* and *Tjp1* were significantly downregulated in endothelial cells in *Vglut1*$^{-/-}$ mice compared to wild-type mice. Genes associated with transcellular barrier formation such as *Cav1* and *Mfsd2a* were, however, not significantly changed, likely due to transcellular barrier development preceding glutamatergic activity initiation. Based on these data, Biswas et al. hypothesized that Norrin/β-catenin signaling evoked by neural-cell-derived glutamatergic activity may regulate retinal angiogenesis and barrier maturation through the upregulation of genes such as *Aplnr* and *Cldn5* (Figure 1C). In addition to being an adhesion junction protein, β-catenin, which is degraded by glycogen

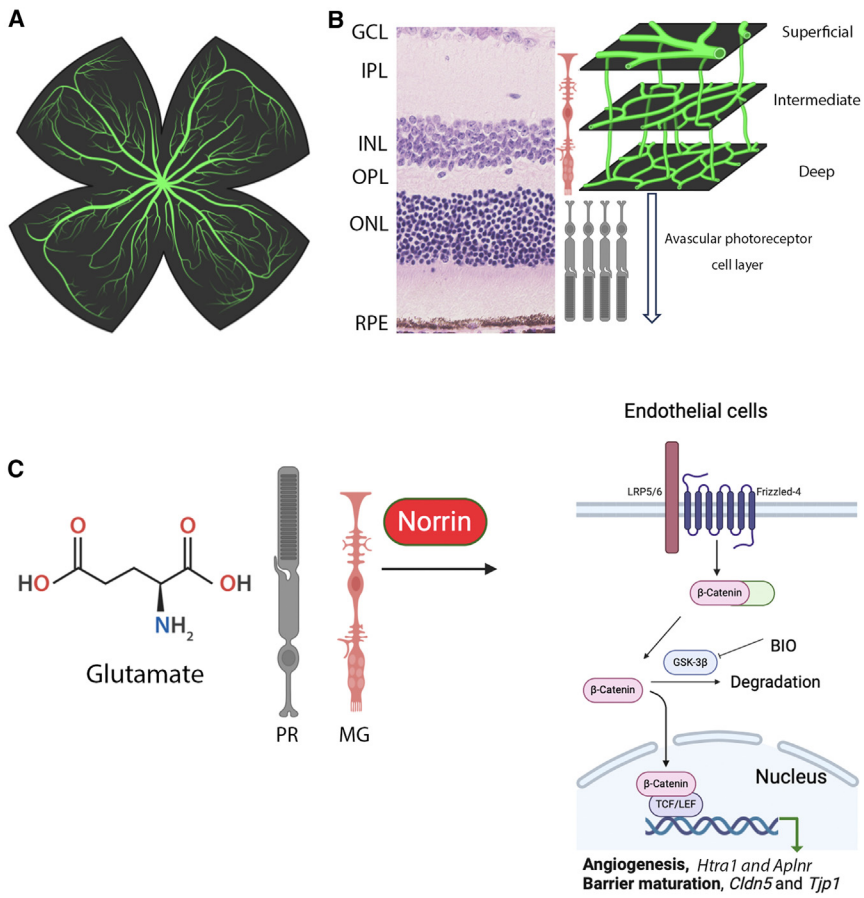

Figure 1. Glutamate-mediated angiogenesis and barrier maturation through Norrin/β-catenin signaling

(A) *En face* view of the flat, mounted retinal vasculature of an adult mouse.

(B) Histological profile of the mouse retina showing the laminar structure of the tissue and the schematic localization of the superficial, intermediate, and deep vascular plexus. The photoreceptor cell layer and outer nuclear layer remain avascular. GCL, ganglion cell layer; IPL, inner plexiform layer; INL, inner nuclear layer; OPL, outer plexiform layer; ONL, outer nuclear layer; RPE, retinal pigment epithelium.

(C) Glial cells and neurons are activated by released glutamate and then Norrin is secreted from these cells. β-catenin signaling is activated by Norrin to promote angiogenesis and barrier maturation in retinal endothelial cells. In endothelial cells associated with the superficial capillary plexus, glutamatergic-activity-mediated Norrin/β-catenin signaling mainly serves to tighten the paracellular barrier as glutamate release from photoreceptors is initiated after the physiological angiogenesis is completed in these retinal regions.

synthase kinase-3β (GSK-3β), can act as a transcription factor by complexing with lymphoid enhancer factor-1/T cell factor (LEF-1/TCF) members in the nucleus. In order to move from the plasma membrane to the cytosol, β-catenin is released into the cytoplasm upon binding of Norrin and various Wnt ligands to their receptors, Frizzled-4 and LRP-5/6 (low-density lipoprotein-related receptors-5/6). Interestingly, a database of single-cell RNA sequencing created by another group[4] showed a wide range of neurons and glial cells in the inner nuclear layer (INL) of the retina, where the deep capillary plexus is present, expresses high levels of Norrin. In addition to this, Norrin expression in the INL was downregulated in *Vglut1*$^{-/-}$ mice and upregulated in *Gnat1*$^{-/-}$ mice.

In order to further explore this concept, Biswas et al. used a pharmacological agent, (2′Z,3′E)-6-Bromoindirubin-3′-oxiome (BIO), an inhibitor of GSK-3β, to activate β-catenin signaling and showed that BIO treatment was able to rescue the defects in retinal angiogenesis/barrier maturation in *Vglut1*$^{-/-}$ mice. The β-catenin/TCF-4/LEF-1 complex can directly bind to the promoter region of *Cldn5*. This promoter binding can likely increase the transcriptional activity of *Cldn5*, but it should also be noted that the β-catenin/TCF-4 complex stabilizes the association of FoxO1 in the promoter region of *Cldn5* to suppress its expression.[5] LEF-1 may compete with FoxO1 or TCF-4 to interact with β-catenin in this region. FoxO1 is not present in the nucleus because of its phosphorylation through Akt in the cytosol after the adherens junctions are established. Therefore, the different localization status of FoxO1 may actually be a direct switch for retinal angiogenesis and barrier maturation through Norrin/β-catenin signaling.

The glutamatergic neuronal activities mediated by Norrin/β-catenin signaling have important functions in the early and late phases of vascular development, as demonstrated by Biswas et al., but they almost certainly have many other important physiological functions once retinal vascular development is completed and the retinal vasculature is established.

Intriguingly, iBRB properties have been shown to change depending on the time of day (morning or night), which is likely induced by exposure of light to the retina. In this regard, the iBRB appears more "leaky" in the evening across multiple species (mice, non-human primates, and humans). This aligns strongly with circadian-mediated changes in the levels of claudin-5.[6] Interestingly, *Cldn5* can be directly regulated by *Bmal1/Clock*,[7] and the data obtained by Biswas et al. suggest that claudin-5 expression in retinal endothelial cells may be recovered by the harmony of *BMAL1/CLOCK*-mediated and Norrin/β-catenin-mediated *Cldn5* upregulation during the night when there is no light exposure.

The influence of neuron-derived glutamatergic activity on endothelial barrier maturation and maintenance likely also occurs in the brain, as astrocytes associated with brain endothelial cells also express high levels of Norrin.[8] Norrin- or Frizzled-4-deficient mice have been shown to develop a leaky iBRB/BBB in a region-dependent manner.[9,10] This important work by Biswas et al. has revealed key molecular guidance cues for neural endothelial cells influenced by glutamatergic neuronal activities. Future studies addressing the molecular

crosstalk between neuronal activity and endothelial cells will likely reveal key regional differences in BBB/iBRB integrity. In the context of the retina, this will be important in identifying underlying mechanisms of pathobiology in conditions such as retinopathy of prematurity, diabetic retinopathy, retinitis pigmentosa, Coats' disease, and macular telangiectasia to name a few. Identifying therapeutic targets for neurological diseases and ophthalmological pathologies focused on barrier-regulating agents may change the landscape of how these conditions are treated and managed clinically in the future and may represent a brand-new class of drug.

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DECLARATION OF INTERESTS

M.C. is a member of the scientific advisory board of NeuVasq Biotechnologies.

REFERENCES

1. Biswas, S., Shahriar, S., Bachay, G., Arvanitis, P., Jamoul, D., Brunken, W.J., and Agalliu, D. (2024). Glutamatergic neuronal activity regulates angiogenesis and blood-retinal barrier maturation via Norrin/β-catenin signaling. Neuron *112*, 1978–1996.e6. https://doi.org/10.1016/j.neuron.2024.03.011.

2. Hashimoto, Y., Poirier, K., Boddaert, N., Hubert, L., Aubart, M., Kaminska, A., Alison, M., Desguerre, I., Munnich, A., and Campbell, M. (2022). Recurrent *de novo* mutations in *CLDN5* induce an anion-selective blood-brain barrier and alternating hemiplegia. Brain *145*, 3374–3382. https://doi.org/10.1093/brain/awac215.

3. Bansal, A., Singer, J.H., Hwang, B.J., Xu, W., Beaudet, A., and Feller, M.B. (2000). Mice lacking specific nicotinic acetylcholine receptor subunits exhibit dramatically altered spontaneous activity patterns and reveal a limited role for retinal waves in forming ON and OFF circuits in the inner retina. J. Neurosci. *20*, 7672–7681. https://doi.org/10.1523/JNEUROSCI.20-20-07672.2000.

4. Macosko, E.Z., Basu, A., Satija, R., Nemesh, J., Shekhar, K., Goldman, M., Tirosh, I., Bialas, A.R., Kamitaki, N., Martersteck, E.M., et al. (2015). Highly parallel genome-wide expression profiling of individual cells using nanoliter droplets. Cell *161*, 1202–1214. https://doi.org/10.1016/j.cell.2015.05.002. https://www.sciencedirect.com/science/article/pii/S0092867415005498.

5. Morini, M.F., Giampietro, C., Corada, M., Pisati, F., Lavarone, E., Cunha, S.I., Conze, L.L., O'Reilly, N., Joshi, D., Kjaer, S., et al. (2018). VE-cadherin-mediated epigenetic regulation of endothelial gene expression. Circ. Res. *122*, 231–245. https://doi.org/10.1161/CIRCRESAHA.117.312392.

6. Hudson, N., Celkova, L., Hopkins, A., Greene, C., Storti, F., Ozaki, E., Fahey, E., Theodoropoulou, S., Kenna, P.F., Humphries, M.M., et al. (2019). Dysregulated claudin-5 cycling in the inner retina causes retinal pigment epithelial cell atrophy. JCI Insight *4*, e130273. https://doi.org/10.1172/jci.insight.130273.

7. Westgate, E.J., Cheng, Y., Reilly, D.F., Price, T.S., Walisser, J.A., Bradfield, C.A., and FitzGerald, G.A. (2008). Genetic components of the circadian clock regulate thrombogenesis in vivo. Circulation *117*, 2087–2095. https://doi.org/10.1161/CIRCULATIONAHA.107.739227.

8. Vanlandewijck, M., He, L., Mäe, M.A., Andrae, J., Ando, K., Del Gaudio, F., Nahar, K., Lebouvier, T., Laviña, B., Gouveia, L., et al. (2018). A molecular atlas of cell types and zonation in the brain vasculature. Nature *554*, 475–480. https://doi.org/10.1172/jci.insight.130273.

9. Wang, Y., Rattner, A., Zhou, Y., Williams, J., Smallwood, P.M., and Nathans, J. (2012). Norrin/Frizzled4 signaling in retinal vascular development and blood brain barrier plasticity. Cell *151*, 1332–1344. https://doi.org/10.1016/j.cell.2012.10.042.

10. Shariatgorji, M., Nilsson, A., Goodwin, R.J.A., Källback, P., Schintu, N., Zhang, X., Crossman, A.R., Bezard, E., Svenningsson, P., and Andren, P.E. (2014). Direct targeted quantitative molecular imaging of neurotransmitters in brain tissue sections. Neuron *84*, 697–707. https://doi.org/10.1016/j.neuron.2014.10.011.

Fuse mitochondria to win peer competition

Lang Wang[1,2] and Yan Gu[3,4,*]
[1]Department of Neurology of the First Affiliated Hospital, Interdisciplinary Institute of Neuroscience and Technology, Zhejiang University School of Medicine, Hangzhou 310027, China
[2]School of Brain Science and Brain Medicine, Zhejiang University, Hangzhou 310058, China
[3]Center of Stem Cell and Regenerative Medicine, and Department of Neurology of the Second Affiliated Hospital, NHC and CAMS Key Laboratory of Medical Neurobiology, Zhejiang University School of Medicine, Hangzhou 310058, China
[4]MOE Frontier Science Center for Brain Science & Brain-Machine Integration, Zhejiang University, Hangzhou 310058, China
*Correspondence: guyan2015@zju.edu.cn
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In this issue of *Neuron*, Kochan et al.[1] report that enhanced mitochondrial fusion is essential for the heightened synaptic plasticity in adult-born neurons during the critical period, thus supporting their competition with neurons of similar age for survival.

New neurons are continuously generated from neural stem/progenitor cells (NSPCs) in the adult mammalian hippocampus. These adult-born granule cells (GCs) face challenges for survival right after their birth, with only the successfully integrated ones being recruited in hippocampal-related brain functions. Within the first 1–4 days, a large fraction of the newborn cells

