closer to comprehending how cortical stem and progenitor cells build the most complex organ in the natural world.

REFERENCES

Betizeau, M., Cortay, V., Patti, D., Pfister, S., Gautier, E., Bellemin-Ménard, A., Afanassieff, M., Huissoud, C., Douglas, R.J., Kennedy, H., et al. (2013). Neuron 80, this issue, 442–457.

Borrell, V., and Reillo, I. (2012). Dev. Neurobiol. 72, 955–971.

Charvet, C.J., Striedter, G.F., and Finlay, B.L. (2011). Brain Behav. Evol. 78, 248–257.

Dehay, C., and Kennedy, H. (2007). Nat. Rev. Neurosci. 8, 438–450.

Fietz, S.A., and Huttner, W.B. (2011). Curr. Opin. Neurobiol. 21, 23–35.

Fietz, S.A., Kelava, I., Vogt, J., Wilsch-Bräuninger, M., Stenzel, D., Fish, J.L., Corbeil, D., Riehn, A., Distler, W., Nitsch, R., and Huttner, W.B. (2010). Nat. Neurosci. 13, 690–699.

Götz, M., and Huttner, W.B. (2005). Nat. Rev. Mol. Cell Biol. 6, 777–788.

Hansen, D.V., Lui, J.H., Parker, P.R., and Kriegstein, A.R. (2010). Nature 464, 554–561.

Lui, J.H., Hansen, D.V., and Kriegstein, A.R. (2011). Cell 146, 18–36.

Lukaszewicz, A., Savatier, P., Cortay, V., Giroud, P., Huissoud, C., Berland, M., Kennedy, H., and Dehay, C. (2005). Neuron 47, 353–364.

Rakic, P. (2009). Nat. Rev. Neurosci. 10, 724–735.

Reillo, I., de Juan Romero, C., García-Cabezas, M.A., and Borrell, V. (2011). Cereb. Cortex 21, 1674–1694.

Shitamukai, A., Konno, D., and Matsuzaki, F. (2011). J. Neurosci. 31, 3683–3695.

Smart, I.H., Dehay, C., Giroud, P., Berland, M., and Kennedy, H. (2002). Cereb. Cortex 12, 37–53.

Wang, X., Tsai, J.W., LaMonica, B., and Kriegstein, A.R. (2011). Nat. Neurosci. 14, 555–561.

The Declaration of Independence of the Neurovascular Intimacy

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http://dx.doi.org/10.1016/j.neuron.2013.09.040

In this issue of *Neuron*, Oh and Gu (2013) present a model in which intimately related embryonic nerves and blood vessels are patterned independently in response to different guidance cues from a central organizer: the whisker.

Nerves and blood vessels form highly branched, ramified networks extending into nearly every part of our body. The intimate association of some blood vessels and nerves in peripheral tissues reflects the functional interdependence relationship between the two systems: the nervous system requires vascularization to ensure nutrient and oxygen supply, and nerve cells in turn provide precise control of vascular caliber and blood flow. Dysfunction in this crosstalk contributes to various neurological and vascular disorders. The high degree of similarity and spatial congruency between the nervous and vascular networks has raised the question of whether the two systems are built through collaborative interactions or independently of each other. Previous studies have provided evidence for reciprocal guidance events, with vessel-derived signals directing the extension of nerves along the vasculature, and vice versa (James and Mukouyama, 2011; Glebova and Ginty, 2005). In contrast, in this issue of *Neuron*, Oh and Gu (2013) propose a model in which nerves and vessels use independent mechanisms to coinnervate the same specific target.

During early embryonic development, endothelial cell precursors differentiate from the mesoderm and coalesce into tubes to form a network of uniformly sized primitive blood vessels, called the primary capillary plexus. With the onset of blood circulation, the primary capillary plexus is remodeled into more complex branching networks of arteries, veins, and capillaries. Nervous innervation of peripheral tissues and organs occurs when the primary capillary network is already formed. Then, two different scenarios are observed. In the first scenario, in the embryonic limbs, ingrowth of spinal-motor and dorsal-root-ganglion sensory axons precedes vascular remodeling. The arteries then align with nerves and follow their branching pattern (Mukouyama et al., 2002). In the second scenario, axons from several sympathetic ganglia extend along remodeled arteries and veins to reach their final targets (Glebova and Ginty, 2005; Nam et al., 2013). This sequence of events suggests that each system can potentially influence the patterning of the other. The use of genetic models with selective ablation or modification of nerves and/or vasculature has indeed provided evidence for this "one-patterns-the-other" model. Moreover, the molecular factors that direct neurovascular association have begun to be identified. Congruence in the limb skin is established through the nerve-derived chemokine CXCL12 that exerts a chemotactic effect on endothelial cells (Li et al., 2013), whereas vessel-derived guidance cues such as artemin, endothelin, or nerve growth factor (NGF) are

responsible for the close association of sympathetic fibers with blood vessels (Honma et al., 2002; Makita et al., 2008; Nam et al., 2013).

In their present study, Oh and Gu (2013) investigate the mechanistic basis of neurovascular congruence in the rodent whisker (mystacial vibrissae) system. Whiskers are sophisticated tactile sense organs, patterned in discrete rows around the muzzle, which are used to locate and discriminate nearby objects. They differentiate from ordinary hairs in that they are implanted in a large follicle, heavily vascularized and innervated, called the follicle-sinus complex (FSC) (Bosman et al., 2011). Most nerve supply of the whisker follicle arises from sensory neurons that have their cell bodies in the trigeminal ganglion. Trigeminal ganglion cells extend an axon that bifurcates into a peripheral and a central branch. The nerve endings in the whisker follicles are terminals of the peripheral branches, which associate with several types of mechanoreceptors. A contact between vibrissae and objects in the environment activates mechanoreceptors, initiating afferent signals that spread to the brainstem trigeminal nuclei via the central trigeminal branch and then continue to the barrel field of the somatosensory cortex. Each whisker follicle is encased in a blood-filled capsule, called the blood sinus, organized around nerve bundles. The blood sinus essentially rigidifies the whisker follicle. However, changes in blood pressure may also contribute to some extent to vibrissae movement and have also been suggested to modulate the sensitivity ranges of the vibrissal mechanoreceptors.

The neurovascular organization of the FSC is established in a stepwise pattern of developmental events. Oh and Gu (2013) reported that the trigeminal axons reach the base of the embryonic whisker after a primary capillary network is established, and ascend along the developing vibrissa follicle. When viewed in cross-section, nerve terminals form a "ring structure" encircling the hair shaft. At this early stage, there is no obvious association between trigeminal nerves and the random meshwork of disorganized blood vessels. Vascular remodeling occurs at a later step, when vessels are recruited to the whisker follicle and

reproducibly organized concentrically around the nerve shaft. This "double ring" structure, with nerves inside and vessels outside, prefigures the organization of the adult FSC.

Because sensory innervation precedes vascular remodeling, the authors examined whether the nerves control vascular patterning in the whisker system, as reported in embryonic limb skin. To address this question they used *neurogenin 1* knockout mouse embryos, which completely lack sensory innervation of the whisker pad. They observed a normal pattern of vascular remodeling around the whisker follicles. Reciprocally, in embryos with conditional deletion of *neuropilin 1* in endothelial cells, in which vascular development is reduced and disorganized, the nerve-ring structure appeared to form normally. Thus, neither peripheral nerves nor blood vessels serve as a template that guides the formation of the double neurovascular ring. Rather, each system is patterned independently of one another.

A question raised by these observations is what guidance mechanisms operate to regulate the formation of the double ring structure around whisker follicles? One possible model is that neurovascular congruency arises through shared patterning mechanisms orchestrated by the target structure itself. Indeed, it is now well established that axons and vessels use common signaling cues to regulate their guidance. For example, several members of the ephrin, netrin, semaphorin, and slit families of neural guidance molecules regulate endothelial tip cell behaviors, whereas the vascular endothelial growth factor (VEGF) has been shown to control axonal growth cone pathfinding (Chauvet et al., 2013). Oh and Gu (2013) found that the secreted Semaphorin 3E (Sema3E) is expressed at the developing whisker follicle. Sema3E is an interesting candidate for patterning the double-ring structure because it has been shown in independent studies to shape vascular and neuronal networks. In the developing whisker of *Sema3e* mutant embryos or embryos lacking its receptor Plexin D1, the stereotypical "nerve inside – vessel outside" pattern was severely disrupted. Both nerves and vessels targeted and remodeled around whisker follicles, but

the two ring structures appeared intermingled. Further analyses revealed that this phenotype was the effect of the inward displacement of the vascular ring, whereas the nerve ring remained essentially unaffected. Thus, expression of Sema3E at the whisker follicle provides a repulsive signal for Plexin D1-expressing endothelial cells that is required to maintain the vascular ring in its outer position. The observed lack of effect of Sema3E/Plexin D1 signaling on the sensory innervation of the developing whisker was surprising, given the expression of the Plexin D1 receptor in trigeminal ganglion cells and the repulsive effect exerted by the Sema3E ligand on these same cells in vitro. Here, the authors describe a mechanism leading to neutralization of Sema3E inhibition in vivo. Using a tagged Sema3E ligand as a probe to detect Plexin D1 expression, they showed that the receptor is heterogeneously distributed along the trigeminal axon pathway and is completely absent from the distal-most segments of the peripheral trigeminal branches. Not only may this local downregulation of Plexin D1 explain why nerve patterning occurs normally in the absence of Sema3E/Plexin D1 signaling in vivo, but in a wild-type context it may also allow the nerve ring to maintain its inner position close to the source of the Sema3E repellent.

If Sema3E does not directly affect nerve patterning, then how are trigeminal axons initially directed to innervate the whisker follicle? The NGF/TrkA signaling system is a probable candidate for this innervation, given that NGF is expressed around the whisker follicle and its TrkA receptor is present all along innervating trigeminal axons. Previous research reported that peripheral sensory axons fail to properly innervate the whisker pads in mutants lacking *trkA* (Patel et al., 2000). In this study, Oh and Gu (2013) further show that sensory axons extend normally along the trigeminal nerve in the absence of NGF, but that they fail to innervate the whisker pads and to form a well-organized nerve-ring structure. Together, the presented results support a model in which copatterning of nerves and blood vessels proceeds independently from each other through the coordinated activities of attractive (NGF and VEGF) and repulsive (Sema3E) guidance cues
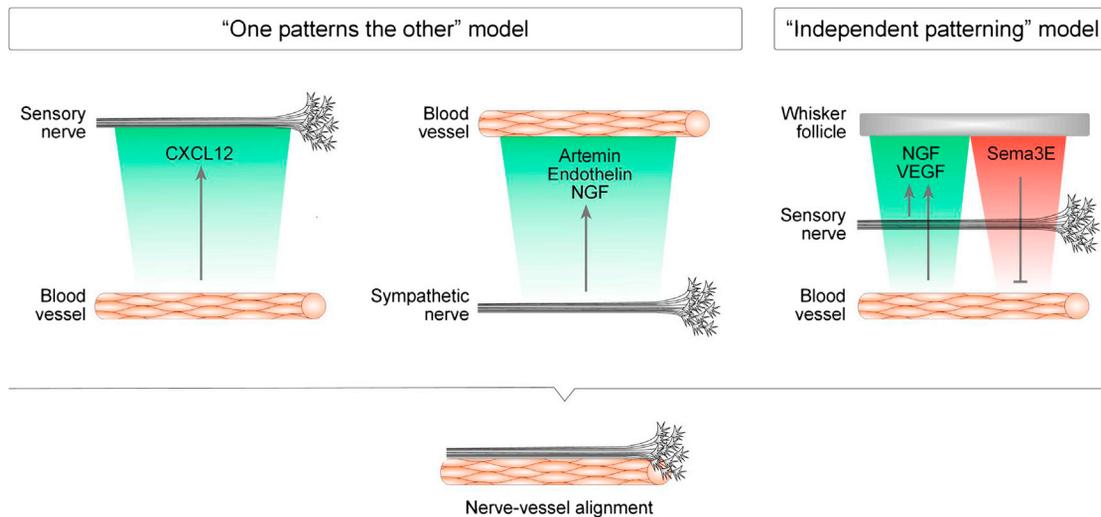
Figure 1. Two Models of Neurovascular Congruency
(Left) "One-patterns-the-other" model in which either the nervous or vascular system instructs the other system to form using its established architecture as a template. This model allows for the parallel trajectories of nerves and vessels, independent of their position relative to their surroundings.
(Right) "Independent-patterning" model in which balanced attractive and repulsive signals originating from the central organizer—the target tissue—pattern neurovascular congruency. When nerves and vessels reach their target tissues, the precise architecture of the trio of nerves, vessels, and target tissues becomes functionally relevant according to the unique requirement of the target tissue.

emanating from a "central organizer," the whisker follicle. This model provides an alternative to previous observations that signals derived from either the nerves or the vessels determine anatomical neurovascular congruence (Figure 1).

The present study opens the door to several more questions. It has been clearly established that regulated expression of guidance receptors to specific axonal segments plays an important role in the formation of neuronal connectivity. This is particularly well described for embryonic spinal commissural axons, which express distinct receptors on their pre- and postcrossing axon segments. A receptor switch regulates axonal sensitivity to midline guidance cues that instruct axons to enter and later exit their intermediate target (Nawabi and Castellani, 2011). Here the turn-off of Plexin D1 expression may allow axons to innervate the target expressing a repulsive ligand. Yet little is known about how intra-axonal patterns of receptor expression are elaborated and how they are maintained without diffusing into a uniform distribution. This may result from the localized activities of extracellular proteolytic enzymes or involve intrinsic mechanisms such as spatially controlled protein synthesis, endocytosis, or vesicular trafficking. Another unanswered

question is what role, if any, Plexin D1 expression plays in developing trigeminal ganglion cells. In this regard, important insights could come from the analysis of their central projections. Future studies may also ask how the early neurovascular defects caused by the absence of Sema3E/Plexin D1 signaling may affect the anatomical structure of an adult whisker follicle and its functional properties.

Although the present study shows that initial patterning of nerve and vessel rings occurs independently, it is not excluded that both systems interdependently control later aspects of their development, as for example in the limb skin where nerves are required to induce the differentiation of vessels into arteries (Mukouyama et al., 2002). The vascular organization of the adult FSC is complex. In most species, it is composed of two compartments (ring and cavernous sinuses), with varying densities and types of blood capillaries. Additional studies are needed to determine whether the sensory trigeminal nerves exert control on the development of the blood sinuses.

Finally, it is interesting to note that the independent versus cooperative developmental logics used for neurovascular congruency apply to distinct situations.

The "one-patterns-the-other" model provides an economical mean to coordinate the development of complex branched networks of nerves and blood vessels. This is particularly obvious in the limb skin, where sensory cutaneous axons display divergent, highly variable branching patterns. The reproducibility of this branching profile is achieved by alignment of vessels along the nerves. In contrast, the "independent patterning" model regulates the convergence of axons and vessels onto a defined target structure, which provides sufficient guidance for precise neurovascular organization. It will be interesting to identify other neurovascular structures in which this model applies, both in peripheral tissues and also in the brain, where neurons are also intimately associated with blood vessels but the mechanism underlying it is completely unknown.

REFERENCES

Bosman, L.W., Houweling, A.R., Owens, C.B., Tanke, N., Shevchouk, O.T., Rahmati, N., Teunissen, W.H., Ju, C., Gong, W., Koekkoek, S.K., and De Zeeuw, C.I. (2011). Front. Integr. Neurosci. 5, 53.

Chauvet, S., Burk, K., and Mann, F. (2013). Cell. Mol. Life Sci. 70, 1685–1703.

Glebova, N.O., and Ginty, D.D. (2005). Annu. Rev. Neurosci. 28, 191–222.

Honma, Y., Araki, T., Gianino, S., Bruce, A., Heuckeroth, R., Johnson, E., and Milbrandt, J. (2002). Neuron 35, 267–282.

James, J.M., and Mukouyama, Y.S. (2011). Semin. Cell Dev. Biol. 22, 1019–1027.

Li, W., Kohara, H., Uchida, Y., James, J.M., Soneji, K., Cronshaw, D.G., Zou, Y.R., Nagasawa, T., and Mukouyama, Y.S. (2013). Dev. Cell 24, 359–371.

Makita, T., Sucov, H.M., Gariepy, C.E., Yanagisawa, M., and Ginty, D.D. (2008). Nature 452, 759–763.

Mukouyama, Y.S., Shin, D., Britsch, S., Taniguchi, M., and Anderson, D.J. (2002). Cell 109, 693–705.

Nam, J., Onitsuka, I., Hatch, J., Uchida, Y., Ray, S., Huang, S., Li, W., Zang, H., Ruiz-Lozano, P., and Mukouyama, Y.S. (2013). Development 140, 1475–1485.

Nawabi, H., and Castellani, V. (2011). Cell. Mol. Life Sci. 68, 2539–2553.

Oh, W.-J., and Gu, C. (2013). Neuron 80, this issue, 458–469.

Patel, T.D., Jackman, A., Rice, F.L., Kucera, J., and Snider, W.D. (2000). Neuron 25, 345–357.

Of Mice and Men: What Physiological Correlates of Cognitive Deficits in a Mouse Model of Schizophrenia Tell Us about Psychiatric Disease

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http://dx.doi.org/10.1016/j.neuron.2013.10.012

Hippocampal neurons "replay" activity related to previous events during rest. In this issue of *Neuron*, Suh et al. (2013) show that this physiological correlate of learning is impaired in mice lacking calcineurin, establishing a link between synaptic anomalies and cognitive deficits observed in this schizophrenia model.

Replay of exploration-associated hippocampal activity during rest is an important aspect of spatial learning (Davidson et al., 2009; Karlsson and Frank, 2009; Skaggs and McNaughton, 1996). The hippocampus contains neurons that are active at specific spots in a maze animals are trained to navigate, and these neurons have been termed "place cells" (O'Keefe and Dostrovsky, 1971). Place cells are thought to encode spatial location and the overall pattern of hippocampal neural ensembles may therefore be encoding cues used to navigate. Several groups have provided evidence that replay of neural ensemble activity during sleep or quiet awake states is critical for memory consolidation and allows navigating using spatial cues (Euston et al., 2007). Reactivation of neural activity associated with behavioral sequences has been shown to be more than simple recall of recent experience. Neural replay includes patterns of activity associated with all possible trajectories during the learned navigation task (Gupta et al., 2010), suggesting that replay is a critical physio-logical element in high-order cognitive processes. This is perhaps one of the highest-order cognitive physiological mechanisms unveiled in rodents, as it relates to more than memory but to pondering of different scenarios evaluated in the learning process. The composition of active and replayed neural ensembles can take a large number of possible combinations, conferring a relatively small circuit such as the hippocampus with the necessary flexibility to learn in a changing environment, a feat virtually impossible with hardwired connections. The selection and reactivation of neural ensembles is perhaps the simplest solution for such a complex behavioral need. One could speculate that ensemble coding, with the large number of combinations of neural activity and their replay after experience, is a common mechanism for many, if not all, learning processes in the brain and not necessarily limited to spatial learning. If this is the case, replay could be an ideal measure to identify altered function in brains with manipulations intended to model disorders with cognitive impairment, such as schizophrenia.

To better understand the neural underpinnings of altered cognition it is critical to explore the impact of manipulations of schizophrenia-related genes in rodent models. In this issue of *Neuron*, Suh et al. (2013) show enhanced firing and increased ripple activity during replay in the hippocampus of calcineurin knockout (KO) mice. These mice target a gene associated with risk for schizophrenia (Gerber et al., 2003) and show altered synaptic plasticity and deficits in working memory (Zeng et al., 2001) as well as a number of cognitive and behavioral abnormalities reminiscent of symptoms in schizophrenia. This is therefore an interesting model to test specifically the neurophysiological correlates of altered cognition that may be associated with risk for the disease. The observation of enhanced firing in KO mice is consistent with convergent reports of disinhibited cortical circuits in other animal models and in patients. The critical new observation here is that awake reactivation is