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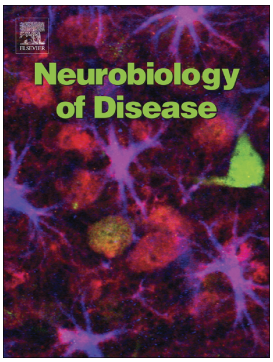

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The vasculature as a neural stem cell niche

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Abstract

Neural stem cells (NSCs) are multipotent, self-renewing progenitors that generate progeny that differentiate into neurons and glia. NSCs in the adult mammalian brain are generally quiescent. Environmental stimuli such as learning or exercise can activate quiescent NSCs, inducing them to proliferate and produce new neurons and glia. How are these behaviours coordinated? The neurovasculature, the circulatory system of the brain, is a key component of the NSC microenvironment, or 'niche'. Instructive signals from the neurovasculature direct NSC quiescence, proliferation, self-renewal and differentiation. During ageing, a breakdown in the niche accompanies NSC dysfunction and cognitive decline. There is much interest in reversing these changes and enhancing NSC activity by targeting the neurovasculature therapeutically. Here we discuss principles of neurovasculature-NSC crosstalk, and the implications for the design of NSC-based therapies. We also consider the emerging contributions to this field of the model organism *Drosophila melanogaster*.

Introduction

Neural stem cells (NSCs) are multipotent progenitors that self-renew and give rise to cells that differentiate into neurons and glia. NSCs proliferate rapidly during embryogenesis, when they generate the functional nervous system. In adult mammals, in contrast, NSCs are relatively quiescent and are restricted to two regions of the brain – the ventricular-subventricular zone (V-SVZ) of the lateral ventricles and the subgranular zone (SGZ) of the dentate gyrus in the hippocampus (Doetsch et al., 1999a; Morshead et al., 1994; Seri et al., 2001). Physiological or pathological processes such as learning, exercise or injury stimulate proliferation and neurogenesis (Gould et al., 1999; Gould and Tanapat, 1997; Kempermann et al., 1997; Rochefort et

al., 2002; van Praag et al., 1999a; 2005; Zhang et al., 2001). New neurons integrate functionally into existing circuitry in the V-SVZ and SGZ and allow adaptive responses to environmental changes, for example by increasing odour discrimination (Carleton et al., 2003; Livneh et al., 2014; van Praag et al., 2002) or spatial pattern separation (Clelland et al., 2009; Sahay et al., 2011; Tronel et al., 2012). NSCs therefore play essential roles in development, homeostasis and behavioural plasticity in the nervous system.

Each of the NSC behaviours – quiescence, proliferation, self-renewal and differentiation – must be precisely balanced to avoid pathology. Excessive self-renewal at the expense of differentiation, for example, can result in brain tumour formation (Palm and Schwamborn, 2010; Sanai et al., 2009). In contrast, premature differentiation can deplete the stem cell pool.

NSCs reside in specialised microenvironments, or 'niches', in the V-SVZ and SGZ, which tightly regulate their behaviour (Bjornsson et al., 2015; Bond et al., 2015; Fuentealba et al., 2012; Silva-Vargas et al., 2013). The vascular system is a prominent feature of both niches (Gómez-Gaviro et al., 2012a; Licht and Keshet, 2015). Blood vessels distribute oxygen, hormones and metabolites around the body, performing essential support and signalling functions. Pericytes and niche astrocyte end feet associate closely with the walls of blood vessels and control their development and functional properties. NSCs and their progeny frequently divide in the vicinity of blood vessels (Mirzadeh et al., 2008; Palmer et al., 2000; Shen et al., 2008; Tavazoie et al., 2008). In the V-SVZ, NSCs further away from blood vessels extend long processes to contact the vasculature (Mirzadeh et al., 2008; Shen et al., 2008; Tavazoie et al., 2008). It is now clear that the vasculature not only supports but directly controls NSC behaviours. Several diffusible and non-diffusible vascular signals control NSCs and their progeny. Importantly, blood vessels are intimately associated with stem cells in many other organs (Gómez-Gaviro et al., 2012a). It is likely that the principles of NSC regulation discovered in the brain also apply to other stem cell compartments, such as muscle and bone marrow, and *vice versa*.

Here we review our understanding of the vasculature as a NSC niche, focussing on the V-SVZ, the largest germinal zone in the adult mammalian brain. NSC number and

activity decline during ageing, accompanied by drastic reductions in the neurovascular network (Ahlenius et al., 2009; Farkas and Luiten, 2001; Katsimpardi et al., 2014; Maslov et al., 2004; Riddle et al., 2003; Tropepe et al., 1997). What factors cause these changes and, importantly, are they reversible? Simpler organisms, such as *Drosophila melanogaster*, are emerging as powerful models to study the neurovascular niche. We consider how the principles learnt from rodents and *Drosophila* could shape future therapies targeting human NSCs.

1) NSCs: a lifetime of neurons and glia

NSCs in the adult V-SVZ, also known as Type B cells, are a subpopulation of astrocyte glia residing in the walls of the lateral ventricles (Doetsch et al., 1999a). Rodent models have allowed detailed characterisation of adult NSCs and their progeny. Unlike embryonic NSCs, adult NSCs are generally quiescent. They can become activated to divide and produce new neurons and glia, and have attracted attention as potential targets for stem cell-based regenerative therapies (Cave et al., 2014; Taupin, 2007). Quiescent NSCs are sufficient to regenerate the V-SVZ following ablation of activated progenitors (Doetsch et al., 1999b). This demonstrates that quiescent NSCs are *bona fide* stem cells with lifelong neurogenic and gliogenic potential.

Several stimuli activate quiescent NSCs in the V-SVZ and SGZ, including learning (Gould et al., 1999; Kempermann et al., 1997; Rochefort et al., 2002), exercise (van Praag et al., 2005; 1999b) and injury (Gould and Tanapat, 1997; Zhang et al., 2001). Activated V-SVZ NSCs give rise to progeny with characteristic molecular profiles (**Fig. 1A**). NSCs generate rapidly dividing transit-amplifying progenitors (TAPs, also known as Type C cells) that, in turn, divide symmetrically around three times before differentiating into neuroblasts (NBs, also known as Type A cells) (Doetsch et al., 1999b). In rodents, NBs migrate as long chains in the rostral migratory stream towards the olfactory bulb (Doetsch and Alvarez-Buylla, 1996) where they differentiate into post-mitotic interneurons and integrate into the local circuitry (Carleton et al., 2003). V-SVZ NSCs also produce some corpus callosum oligodendrocyte glia, which function in axon myelination.

The V-SVZ niche

Stem cells in many adult tissues reside in a dedicated microenvironment, or niche. V-SVZ NSCs and their progeny are effectively sandwiched between two major niches in the walls of the lateral ventricles (**Fig. 1B**). On the apical side is a layer of ependymal cells, arranged in pinwheel structures (Mirzadeh et al., 2008). Ependymal cells line the walls of the ventricles, which are filled with cerebrospinal fluid (CSF) - a growth factor and cytokine-rich fluid demonstrated recently to control adult NSC proliferation (Silva-Vargas et al., 2016). The choroid plexuses, heavily vascularised tissues suspended in the ventricles, are responsible for secreting the CSF (Lehtinen et al., 2013). NSCs can intercalate or penetrate the ependymal cell layer (Conover et al., 2000; Doetsch et al., 1999b; Mirzadeh et al., 2008; Shen et al., 2008) and extend a primary cilium into the ventricle (Conover et al., 2000; Doetsch et al., 1999b) to communicate with the ependymal cell/CSF niche. Since the choroid plexuses are heavily vascularised, the CSF represents one access route for blood-borne or vascular endothelial cell signals to reach V-SVZ NSCs (Silva-Vargas et al., 2016).

The neurovasculature is located basally, deeper in the ventricle wall. Some NSCs located in the ependymal cell layer extend a basal process to contact blood vessels (Mirzadeh et al., 2008; Shen et al., 2008). These NSCs are in a unique environment, able to sense signals both from the vasculature and the ependymal cell/CSF niches. Just beneath these NSCs lies a second group of 'tangential' NSCs, with one or more cellular processes that run along or between blood vessels (Shen et al., 2008). The vasculature is therefore a prominent feature of the NSC niche, forming multiple contacts with NSCs. Apart from the apical (ependymal cell/CSF) and basal (neurovascular) niches, NSCs also contact their TAP and NB progeny that may be considered a third, 'intermediate' niche (Fuentealba et al., 2012; Silva-Vargas et al., 2013).

2) The neurovasculature: more than a support line

The neurovasculature acts as the blood-brain barrier (BBB), which permits transport of oxygen, nutrients and hormones to the brain, whilst removing harmful xenobiotics and waste metabolites. The formation and physical features of the BBB during health and disease have been reviewed excellently elsewhere (Banks, 2016; Weiss et al., 2009; Zhao et al., 2015), and also in this issue (Stefanie Limmer, this issue), and shall not be covered here. We will consider the emerging roles of the neurovasculature

specifically in the context of stem cell regulation. The BBB and vasculature exhibit unique features specifically in neurogenic regions of the adult mammalian brain, and it is tempting to think that they are adaptations to permit dialogue with NSCs.

General features of the neurovasculature

The neurovasculature refers to the cellular and acellular components associated with blood vessels, primarily capillaries, in the brain. Endothelial cells are the structural elements of capillary vessel walls and form the conduits for blood transport. Tight junctions between endothelial cells prevent paracellular leakage. A basal lamina rich in extracellular matrix (ECM) molecules such as laminin, collagen IV and fibronectin (Kazanis et al., 2010; Mercier et al., 2002; Tavazoie et al., 2008) encases endothelial cells. Pericytes and niche astrocytes enwrap and contact blood vessels respectively, and modulate BBB permeability, neurovascular signalling and angiogenesis (Abbott et al., 2006; Armulik et al., 2010). Together, the neurovascular niche is ideally placed to receive systemic signals from the circulation and instruct local responses in the brain. Systemic signals that affect NSC behaviour include changes in diet, age, exercise or stress (Katsimpardi et al., 2014; Rafalski and Brunet, 2011).

Specialisations in the V-SVZ neurovasculature

Whole mounts and 3D reconstructions have revealed unique morphological and functional specialisations of the V-SVZ vasculature that are absent from non-neurogenic regions of the adult brain (Culver et al., 2013; Mirzadeh et al., 2008; Shen et al., 2008; Tavazoie et al., 2008). V-SVZ blood vessels form a relatively shallow, but extensive, planar network running parallel to the plane of the ventricle, with perpendicular branches invading the underlying striatum (Shen et al., 2008; Tavazoie et al., 2008). This is dissimilar to the tortuous organisation of blood vessels in non-neurogenic regions such as the cortex (Culver et al., 2013; Tavazoie et al., 2008). The V-SVZ is more densely vascularised than neighbouring brain regions (Culver et al., 2013; Kazanis et al., 2010). Interestingly, the V-SVZ vasculature is largely quiescent (Tavazoie et al., 2008). This contrasts with the SGZ, the other major neurogenic region of the adult mammalian brain, in which angiogenesis (sprouting of new blood vessels) proceeds in parallel with neurogenesis (Palmer et al., 2000; Tavazoie et al., 2008).

A particularly conspicuous feature of the V-SVZ is the presence of fractones - thin, highly branched rods of ECM composed of heparan sulphate proteoglycans, perlecan, nidogen and collagens (Kerever et al., 2007; Mercier et al., 2002). Fractones are continuous with the blood vessel basal lamina, contact all cell types of the V-SVZ and terminate at bulb-like structures just beneath the ependymal cell layer (Mercier et al., 2002; Tavazoie et al., 2008). Fractones therefore connect NSCs with both the neurovascular and ependymal cell/CSF niches. Recent studies implicate fractones in capturing CSF-borne growth factors and distributing them throughout the ventricle wall (Douet et al., 2012; 2013; Kerever et al., 2007; Mercier and Douet, 2014). These growth factors also bind blood vessel ECM (Douet et al., 2013; 2012; Mercier and Douet, 2014), suggesting an intimate relationship with the neurovasculature that is yet to be resolved.

In addition to a unique structure, the V-SVZ vasculature exhibits functional differences in its BBB properties and transport of blood. V-SVZ blood vessels are more permeable, due to a local reduction in endothelial cell tight junctions and sparser pericyte and astrocyte end feet coverage (Tavazoie et al., 2008). This selective leakiness indicates that blood-borne factors have easier access to the brain parenchyma in the vicinity of NSCs, although the importance of this *in vivo* remains to be determined. Blood flow is slower in the V-SVZ (Culver et al., 2013). Although this was expected to cause a hypoxic environment around blood vessels, chemical tracers showed that this was not the case (Culver et al., 2013; Roitbak et al., 2011). Nevertheless, fluid dynamics and shear stress can influence endothelial cell gene expression (Chien, 2007; Tzima et al., 2005) and a role of blow flow rate in stem cell regulation cannot be excluded. Indeed, when NSCs are induced to proliferate by infusion of growth factors, blood flow increases locally (Lacar et al., 2012). Fluid flow plays an important role in the CSF niche (Sawamoto et al., 2006). Further studies will illuminate the contributions of fluid dynamics and BBB leakiness to NSC regulation.

3) Partners in crime: NSC-neurovasculature crosstalk

Endothelial cells secrete soluble factors that promote V-SVZ progenitor self-renewal and differentiation *in vitro* (Shen et al., 2004). This important observation opened up the possibility of a similar interaction occurring in the adult brain *in vivo*. Indeed,

dividing NSCs and TAPs are associated with blood vessels both in the V-SVZ (Shen et al., 2008; Tavazoie et al., 2008) and the SGZ (Palmer et al., 2000). Quantitative analyses have confirmed that proliferating cells are, on average, significantly closer to blood vessels than other V-SVZ cell types (Shen et al., 2008; Tavazoie et al., 2008). Remarkably, dissociated and transplanted adult progenitor cells (NSCs and TAPs) migrate and associate preferentially with the vasculature (Kokovay et al., 2010), demonstrating that this partnership is actively established. Vascular association is dependent on chemokine receptor 4 (CXCR4) and stromal-derived factor 1 (SDF1, also known as CXCL12), which allow NSCs to bind laminin in the basal lamina (Kokovay et al., 2010; Shen et al., 2008).

The neurovasculature can signal to NSCs through three major routes (**Fig. 1C**). The first is direct contact between NSCs and blood vessels. Dividing NSCs associate with the vasculature, and more distant NSCs have basal processes terminating on blood vessels in areas lacking pericyte and astrocyte coverage (Tavazoie et al., 2008). Secondly, neurovascular cells can synthesise and secrete diffusible cues into the V-SVZ. Diffusible cues can also arrive from the ependymal cell/CSF niche, for capture and distribution by fractones and blood vessel-associated ECM. Notably, several ligands controlling NSC behaviour are present both in the CSF (some originating from the vasculature in the choroid plexus) and the local neurovasculature. Thirdly, blood-borne cues such as hormones, synthesised outside of the V-SVZ, can diffuse into the NSC niche, likely aided by the locally permeable BBB. We consider each of these access routes in turn.

Direct contact maintains quiescence

Despite the intimate association between V-SVZ NSCs and the neurovasculature (Mirzadeh et al., 2008; Shen et al., 2008; Tavazoie et al., 2008), only one study has demonstrated contact-mediated NSC regulation *in vivo*. Endothelial cells express ephrinB2 (Efnb2) and Jagged1 (Jag1), transmembrane ligands for Eph and Notch receptors respectively (Ottone et al., 2014). Conditional knockout of either ligand specifically in endothelial cells *in vivo* results in an increase in dividing NSCs, suggesting loss of signals inhibitory to proliferation. The increased division is followed by a significant, long-term depletion of NSCs (Ottone et al., 2014). This depletion phenotype is reminiscent of that observed upon mutation of FoxO genes in

adult NSCs (Paik et al., 2009), which abrogates quiescence. It is thought that excessively dividing NSCs eventually lose the capacity to self-renew. These data are consistent with Efnb2 and Jag1 normally promoting quiescence. Convincingly, Jag1 localises to sites between NSCs and the vasculature (Ottone et al., 2014). Mice mutant for both Efnb2 and Jag1 exhibit stronger phenotypes than each of the single mutants, suggesting that these ligands act in a cooperative but independent manner to maintain NSC quiescence.

Diffusible signals: a cocktail of factors with opposing roles

In the embryo, the nervous and vascular systems develop in parallel and share expression of patterning transcription factors (Vasudevan et al., 2008). In an analogous manner, classical signals acting on the vasculature can also influence adult NSCs. Important angiogenic signals, sometimes secreted from the vasculature itself, are vascular endothelial growth factors (VEGFs), which bind VEGF receptors (VEGFRs) (Keck et al., 1989; Leung et al., 1989), and angiopoietin-1 (Ang1) and angiopoietin-2 (Ang2), which bind the Tie2 receptor (Maisonpierre et al., 1997; Suri et al., 1996). NSCs and TAPs also express VEGFRs (Calvo et al., 2011; Jin et al., 2002) and Tie2 (Androutsellis-Theotokis et al., 2009; Rosa et al., 2010). Introducing VEGFA, VEGFB or Ang2 into the V-SVZ stimulates neural progenitor cell proliferation *in vivo* (Androutsellis-Theotokis et al., 2009; Jin et al., 2002; Sun et al., 2006), while Ang1 has a similar effect on V-SVZ-derived cell cultures (Rosa et al., 2010). It is possible that at least some of these responses are indirect consequences of building a larger vasculature. Indeed, in the adult songbird brain, VEGF stimulates endothelial cells to secrete brain-derived neurotrophic factor (BDNF) that, in turn, promotes neurogenesis (Louissaint et al., 2002). Notably, VEGFC and its receptor VEGFR3 stimulate adult neurogenesis but not angiogenesis (Calvo et al., 2011), providing the first evidence for a direct effect of VEGF upon NSCs. Numerous V-SVZ cell types express VEGFC (Calvo et al., 2011), and it will be important to identify the sources relevant to NSCs.

Endothelial cells secrete additional pro-proliferative signals. Pigment epithelium-derived factor (PEDF, also known as Serpin-F1), produced by endothelial cells and ependymal cells, specifically increases NSC self-renewal *in vivo* (Ramírez-Castillejo et al., 2006) through influencing Notch-dependent transcription (Andreu-Agulló et al.,

2009). It remains unclear whether PEDF recruits more quiescent NSCs to an activated state and/or increases self-renewal of previously activated NSCs. Intriguingly, PEDF protein contains sites for interaction with the ECM, in particular collagen I (Meyer et al., 2002), suggesting that its levels or activity may be modulated by the basal lamina or fractones. Endothelial cells also secrete the epidermal growth factor (EGF) family member betacellulin (BTC) (Gómez-Gaviro et al., 2012b). BTC binds epidermal growth factor receptor (EGFR) on NSCs, and ErbB4 receptors on NBs, and promotes proliferation through the MEK/Erk and Akt pathways (Gómez-Gaviro et al., 2012b). Since the choroid plexus also produces BTC (Gómez-Gaviro et al., 2012b), it can presumably also reach NSCs through the ependymal cell/CSF niche.

In contrast to PEDF and BTC, blood vessel-derived Neurotrophin-3 (NT-3) reduces NSC proliferation (Delgado et al., 2014), demonstrating conflicting roles for diffusible ligands. Conditional NT-3 knockout in endothelial cells increases NSC proliferation *in vivo* (Delgado et al., 2014) and, in a similar manner to FoxO, Efnb2 and Jag1 mutant mice (Ottone et al., 2014; Paik et al., 2009), heterozygous NT-3 mutants exhibit a short-term increase in activated NSCs, which become depleted over time (Delgado et al., 2014). NT-3 promotes the activity of the endothelial isoform of the enzyme nitric oxide synthase (eNOS) in NSCs. eNOS produces nitric oxide (NO), a gaseous signalling molecule that acts in an autocrine manner to reduce proliferation. Although the authors conclude that NT-3 promotes NSC quiescence, it also remains possible that NT-3 decreases the proliferation of activated NSCs. How the NO produced by NSCs is distinguished from other sources of NO, such as the nitrergic neurons adjacent to the V-SVZ (Moreno-López et al., 2000), remains unclear.

In addition to synthesising its own factors, the vasculature can capture and concentrate diffusible signals from other sources. It is thought that many growth factors, such as fibroblast growth factor 2 (FGF2), bone morphogenetic protein 4 (BMP4) and BMP7, are produced in the choroid plexus and transported to the V-SVZ *via* the CSF. FGF2 promotes NSC/TAP proliferation (Kuhn et al., 1997), while BMP4 and BMP7 inhibit proliferation (Lim et al., 2000; Mercier and Douet, 2014). All of these factors bind to N-sulphated heparan sulphate proteoglycans in fractones and in the basal lamina of the vasculature (Douet et al., 2013; 2012; Kerever et al., 2007; Mercier and Douet, 2014). N-sulphated heparan sulphate proteoglycans are abundant

in neurogenic regions of the brain (Mercier and Arikawa Hirasawa, 2012). Abrogating FGF2 or BMP7 binding to the ECM eliminates their mitogenic effects on NSCs (Douet et al., 2013; 2012; Kerever et al., 2007), suggesting that the ECM stimulates growth factor signalling. Conversely, disrupting the ECM association of BMP4 strengthens its inhibitory effect on NSC proliferation (Mercier and Douet, 2014), demonstrating that the ECM can also sequester and antagonise growth factor activity. The branched networks of fractones and the basal lamina are an ideal substrate for growth factor delivery to NSCs throughout the V-SVZ. It will be interesting to see whether blood vessel ECM and fractones act as a functional continuum, or whether they play distinct roles in growth factor binding.

Circulating factors couple systemic metabolism to NSC activity

Metabolites and nutrients in the bloodstream fulfil the energy demands of adult NSCs. However, more context-specific roles have been shown for several circulating factors. For example, the hormone prolactin promotes neurogenesis during pregnancy in female mice (Shingo et al., 2003). A fascinating insight into blood-borne signals came from experiments in which the circulatory systems of young and aged mice were joined experimentally (heterochronic parabiosis). 'Young' blood in aged brains can rejuvenate the neurovasculature and increase NSC/TAP proliferation and neuron production in the V-SVZ (Katsimpardi et al., 2014) (see also section: *4) NSCs and the neurovasculature during ageing*). Thus the contents of the blood change during ageing and, importantly, the infusion of a 'youthful' factor can restore neurogenesis.

Oxygen concentration fluctuates around 2% in the adult brain (Silver and Erecińska, 1998; L.-L. Zhu et al., 2005), compared to the 20% oxygen levels used to culture NSCs *in vitro*. Lowering the oxygen concentration to physiological levels enhances embryonic NSC proliferation in culture (Studer et al., 2000). Remarkably, a complete lack of oxygen (anoxia), for example during transient ischaemic stroke, can trigger NSC proliferation and neurogenesis in the adult V-SVZ (Arvidsson et al., 2002). These experiments demonstrate the abilities of NSCs to sense and respond to oxygen. Oxygen sensing is likely to occur through hypoxia-inducible factor 1α (HIF-1α), as shown for embryonic NSCs *in vivo* (Tomita et al., 2003). Indeed HIF-1α supports NSC maintenance in the adult V-SVZ (L. Li et al., 2014). Interestingly, HIF-1α mutation causes first a deterioration of the vascular network followed by NSC

depletion (L. Li et al., 2014), suggesting a survival effect dependent on the neurovascular niche.

Recent results also implicate circulating platelets in NSC control. Platelets contain a cocktail of bioactive molecules, such as platelet derived growth factor (PDGF), FGF, EGF and VEGF (for a review, see (Mazzucco et al., 2010)), many of which affect NSCs. Injection of platelet lysate significantly increases proliferation of vascular and V-SVZ progenitor cells following stroke (Hayon et al., 2013). In a corpus callosum injury model, platelets migrate out of the vasculature in the V-SVZ in proximity to NSCs and are associated with increased survival of NSC lineages and production of oligodendrocyte glia precursors (Kazanis et al., 2015). These data suggest that, in addition to their well-known role in blood clotting, platelets also promote neurogenesis and gliogenesis following injury and may act as an injury-sensitive niche. However the signalling molecules involved, and the target progenitor cell population (NSC or TAP) remain unclear.

An array of diffusible, non-diffusible and circulating cues influences NSC quiescence, proliferation, self-renewal and differentiation. A key question is how NSCs integrate all of these signals, particularly since they can have overlapping or antagonistic effects. Some ligands, such as PEDF and BTC, have multiple sources (Gómez-Gaviro et al., 2012b; Ramírez-Castillejo et al., 2006) – does the source influence the effect on NSCs? The majority of neurovascular studies have focussed on endothelial cells. The difficulty in identifying pericytes *in vivo* means that their contributions to NSC behaviour are only just emerging (Crouch et al., 2015). How do pericytes and astrocytes interact with the endothelial cell-NSC crosstalk? Advances in single cell transcriptional profiling, as well as cell type-specific tools for manipulating cells *in vivo*, will no doubt shed light on these questions.

4) V-SVZ NSCs and the neurovasculature during ageing

Neurogenesis in the V-SVZ declines during ageing, concomitant with a reduction in NSC activity and number (Ahlenius et al., 2009; Enwere et al., 2004; Maslov et al., 2004; Tropepe et al., 1997). The neurovascular niche also deteriorates, with reductions in blood vessel density and blood flow (Farkas and Luiten, 2001; Katsimpardi et al., 2014; Riddle et al., 2003). These changes are thought to contribute

to the decline in cognitive ability commonly associated with ageing. Despite these drastic changes, NSCs isolated from aged mice have an almost similar capacity to proliferate and differentiate *in vitro* as those from younger mice (Ahlenius et al., 2009; Tropepe et al., 1997). This important observation demonstrates that changes in the NSC niche, not the NSCs themselves, can drive reductions in neurogenesis. Developing methods to restore endogenous NSC activity would be beneficial not only for ageing individuals, but also for sufferers of neurodegenerative diseases such as Parkinson's disease or Alzheimer's disease. Emerging data indicate that rejuvenating the neurovascular niche is an efficient way to achieve this.

The ageing circulation is associated with accumulation of detrimental factors and loss of 'youthful' factors (Katsimpardi et al., 2014). Joining the bloodstreams of young and aged mice results in significant restoration of blood vessel volume and branching and blood flow levels in the aged partner. This increases NSC/TAP proliferation and neurogenesis, and enhances the performance of aged mice in behavioural assays. Remarkably, infusion of GDF11 (also known as BMP11) alone recapitulates many of these improvements (Katsimpardi et al., 2014). GDF11 is a circulating factor whose levels decline in the bloodstream during ageing (Loffredo et al., 2013). Thus GDF11 is a 'youthful' factor, with rejuvenating properties. In contrast, vascular endothelial cells increasingly express transforming growth factor β1 (TGFβ1) with ageing (Pineda et al., 2013), which is therefore an 'ageing' factor. TGFβ1 signalling induces NSC quiescence and apoptosis of TAPs (Pineda et al., 2013; Wachs et al., 2006). Blocking TGFβ signalling pharmacologically or by using antibodies restores NSC proliferation and neurogenesis in aged mice (Pineda et al., 2013). Thus GDF11 and TGFβ are age-regulated factors associated with the neurovasculature, and are promising targets for rejuvenating blood vessel networks to restore NSC function.

In an interesting parallel, a recent study demonstrated that changes in the choroid plexus secretome during ageing also alter NSC proliferation (Silva-Vargas et al., 2016). Conditioned medium harvested from the choroid plexuses of young mice increased NSC proliferation when infused into the lateral ventricles of aged mice. Reciprocally, 'aged' conditioned medium decreased NSC proliferation. Since many neurovascular signals are also present in the CSF, the interactions between these two niche compartments during ageing will be of great future interest.

5) *Drosophila:* an invertebrate model to understand the neurovascular niche

Drosophila melanogaster is emerging as a simpler model to study the neurovascular niche. The ease of cell type-specific genetic manipulation and opportunities for live imaging make *Drosophila* an attractive system to dissect neurovascular regulation in manners that are difficult or not currently possible in the mammalian brain.

As in mammals, *Drosophila* NSCs generate the nervous system during embryonic development, become quiescent and must be reactivated post-embryonically (**Fig. 2A**) (Datta, 1995; Hartenstein et al., 1987; Ito and Hotta, 1992; Truman and Bate, 1988). *Drosophila* NSCs are well studied in the context of niche regulation *in vivo* (Chell and Brand, 2010; Ding et al., 2016; Poon et al., 2016; Sousa-Nunes et al., 2011). The brain is suspended in an open solute- and metabolite-filled fluid known as the haemolymph, which is circulated around the body cavity by a heart-like organ and is analogous to the blood circulation (**Fig. 2B**). Two layers of glial cells, the perineurial and subperineurial glia, encase the brain and together form the BBB (reviewed in (Limmer et al., 2014)). In particular, subperineurial glia form septate junctions with one another, which function similarly to endothelial cell tight junctions in the mammalian brain by blocking paracellular flow. The BBB glia equate to the neurovascular niche for *Drosophila* NSCs. Remarkably, despite the difference in structure of the BBB between *Drosophila* and mammals, parallels can be found in the principles of NSC regulation. Interestingly, the BBBs of primitive vertebrates such as elasmobranch fish (sharks and skates) are also composed of glia (Bundgaard and Cserr, 1981), not endothelial cells as found in mammals. The *Drosophila* BBB may be closely related to an evolutionarily ancestral structure from which the mammalian BBB also derives (Bundgaard and Abbott, 2008).

Drosophila NSC behaviours

Drosophila NSCs divide in a self-renewing, asymmetric manner to generate intermediate progenitor cells known as ganglion mother cells (GMCs). GMCs divide once, differentiating into neurons or glia. This ‘Type I’ division mode closely resembles that of NSCs in the adult mammalian V-SVZ, although lacking a transit-amplifying cell equivalent. *Drosophila* NSCs proliferate in two waves during development. The first occurs during embryogenesis, giving rise to the larval nervous

system, and the second during larval life, resulting in the complete adult nervous system (**Fig. 2A**) (Datta, 1995; Hartenstein et al., 1987; Ito and Hotta, 1992; Truman and Bate, 1988). NSCs become quiescent between these two waves of proliferation, and must 'reactivate' post-embryonically to contribute to the second proliferative wave. Reactivation occurs in a synchronous manner across the brain. *Drosophila* NSCs therefore exhibit the self-renewal, differentiation, quiescence and proliferation characteristics of mammalian NSCs.

The Drosophila neurovascular niche

Drosophila NSCs are located at the surface of the brain, in close proximity to the BBB glia (**Fig. 2C**). The BBB is, in turn, well placed to sense and respond to changes in the circulation. Feeding, for instance, alters haemolymph metabolite composition (Handke et al., 2013; Rovenko et al., 2015) and acts as a readout for environmental changes.

The BBB communicates organismal nutrition to NSCs

Dietary nutrition underpins NSC reactivation from quiescence. NSCs fail to reactivate when larvae are starved of essential amino acids (Britton and Edgar, 1998). The sensing of amino acids in the circulation occurs in the fat body - the liver and adipose tissue equivalent. The fat body secretes unidentified signal(s), originally called the fat body-derived mitogen (FBDM), to reactivate NSCs (Britton and Edgar, 1998).

The *Drosophila* neurovasculature plays a central role in the nutritional relay between fat body and NSCs (**Fig. 2D**). BBB glia on the surface of the brain sense the fat body signal(s) in the circulation. In response, the BBB glia secrete the growth-promoting *Drosophila* insulin/IGF-like peptides (dILPs) directly onto quiescent NSCs (Chell and Brand, 2010; Spéder and Brand, 2014) located just beneath them. dILPs activate PI3K and TOR signalling in NSCs to induce reactivation. Remarkably, activating the PI3K or TOR pathways in NSCs is sufficient for reactivation in the absence of amino acids, uncoupling NSCs from systemic metabolic signals (Chell and Brand, 2010; Sousa-Nunes et al., 2011). The NSCs only respond to dILPs synthesised locally by the BBB niche and are physically isolated from systemic dILPs circulating in the haemolymph, which instead control overall body size (Chell and Brand, 2010; Sousa-Nunes et al.,

2011). This example demonstrates the function of the BBB in communicating the nutritional status of the animal to NSCs.

Live imaging of a calcium sensor (gCAMP) (Tian et al., 2009) expressed specifically in the subperineurial glia revealed that they exhibit synchronised calcium pulses in response to dietary amino acids (Spéder and Brand, 2014). These calcium waves induce both dILP expression and secretion from the BBB, and are dependent on functional gap junctions (Spéder and Brand, 2014). Thus BBB coupling is required to coordinate glia and induce synchronous NSC reactivation. Remarkably, this is reminiscent of insulin secretion by β-cells in the mammalian pancreas, which is also dependent upon gap junctions and coordinated calcium oscillations (MacDonald and Rorsman, 2006; Rocheleau et al., 2006).

Drosophila: future contributions

The *Drosophila* BBB is only recently attracting attention as a model for the neurovascular niche (Hindle and Bainton, 2014). One of the strengths of *Drosophila* is the powerful genetic toolkit available, which allows unambiguous labelling and manipulation of specific cell types in isolation from one another – a feat not always possible in mammalian models. Side-by-side comparisons have highlighted striking similarities between the transcriptional signatures of BBB glia and the mammalian BBB (Daneman et al., 2010; DeSalvo et al., 2014; Zlokovic, 2008). Indeed, insulin/IGF signalling also promotes NSC proliferation in the adult mammalian SGZ (Bracko et al., 2012). Cell type-specific transcriptional profiling *in vivo* (Gay et al., 2013; Southall et al., 2013) will identify new molecular signals between the BBB and NSCs.

6) Future perspectives

Recent advances in cell isolation and transcriptional profiling have highlighted unique gene signatures for V-SVZ NSCs (Codega et al., 2014; Llorens-Bobadilla et al., 2015; Mich et al., 2014) and the various cells of the neurovascular niche (Crouch et al., 2015; Daneman et al., 2010; Lee et al., 2012; Nolan et al., 2013). Cell sorting and co-culture experiments recently demonstrated that placental growth factor 2 (PlGF2), secreted by endothelial cells, activates quiescent NSCs and promotes proliferation of active NSCs and TAPs *in vitro* (Crouch et al., 2015). Specific isolation of poorly

understood cell types, such as pericytes, will allow assessment of their contribution to NSC behaviour. Co-culturing primary V-SVZ pericytes with NSCs revealed that pericytes increase NSC proliferation and differentiation of their progeny into neurons (rather than glia), although not as significantly as endothelial cells (Crouch et al., 2015). In the future, it will be possible to identify novel pericyte- and astrocyte-derived signals and their effects on NSCs.

A better understanding of niche signalling will enable several complex questions to be dissected. It is now appreciated that adult NSCs are a molecularly and functionally heterogeneous population (Chaker et al., 2016; Llorens-Bobadilla et al., 2015; Shin et al., 2015). It will be interesting to discover how heterogeneous the components of the neurovasculature are, both within the V-SVZ and in comparison to the SGZ, and whether regional specialisations of the neurovasculature impact upon NSC heterogeneity. Blood vessel endothelial cells isolated from the cortex, a non-neurogenic region of the adult brain, can induce NSC proliferation more potently than V-SVZ endothelial cells (Crouch et al., 2015). This surprising result also indicates that further study of the regional properties of the vasculature will be fruitful.

The myriad effects of the neurovasculature have important implications for the design of NSC-based therapies. The deterioration of the neurovasculature during ageing, and consequent reduction in NSC proliferation, demonstrate that blood vessels should be considered in rejuvenation and tissue repair strategies. Transplanted NSCs are a potential treatment for neurodegenerative conditions such as Parkinson's disease, as they may be able to self-renew and replenish lost neurons (reviewed in (Chou et al., 2015)). However, neural progenitors often exhibit poor survival upon transplantation. Co-transplantation of neural progenitors with vascular progenitor cells or endothelial cells leads to enhanced survival, proliferation, neurogenesis and functional recovery after stroke (J. Li et al., 2014; Nakagomi et al., 2009). In a similar manner, neural cells engineered to express angiogenic factors such as VEGF survive better upon engraftment to diseased and non-diseased brains (Casper et al., 2002; Maurer et al., 2008; W. Zhu et al., 2005). Reconstituting a local vascular niche is clearly beneficial for NSC transplantation-based therapies. An alternative strategy to stimulate neurogenesis is to enhance the activity of endogenous NSCs in the adult brain. Exciting studies have demonstrated that rejuvenating the neurovascular niche is an

efficient way to increase NSC proliferation and neurogenesis (Katsimpardi et al., 2014; Pineda et al., 2013).

With the potential for therapeutic applications, it is important to verify that the NSC-neurovasculature interactions identified in rodents are conserved in humans. Neurogenesis occurs both in the V-SVZ and SGZ of adult humans (Eriksson et al., 1998). One striking difference is that NBs produced in the V-SVZ migrate to the medial prefrontal cortex in humans (Sanai et al., 2011), rather than the olfactory bulb. V-SVZ NSCs may also contribute neurons to the striatum in adult humans (Ernst et al., 2014), although these results are contested (Wang et al., 2014). Clearly, there are some differences between rodent models and humans.

A key limitation in studying humans remains, of course, access to living material. A recent study has co-cultured human adult endothelial cells with human embryonic NSCs to emulate human neurovascular interactions *in vitro* (Chou et al., 2014). Although promising, this system does not recapitulate the 3D environment or the full cellular complement present *in vivo*. Current ambitious projects aim to fully recapitulate 3D human neuron/neurovasculature/CSF interactions 'on a chip' *in vitro* (see, for example, (Alcendor et al., 2013; Brown et al., 2015; Walter et al., 2016), reviewed in (van der Helm et al., 2016)). These models reconstitute some, or all, of the cell types of the neurovascular unit and will allow pharmacological drug testing on cells derived from patients of different ages, metabolic conditions or pathologies. The inclusion of NSCs would allow direct testing of the neurovascular niche in a human context. Such projects, combined with *in vivo* data on signalling molecules from rodents and *Drosophila*, will surely allow us to harness the neurovasculature for NSC-based treatments in human patients.

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References

Abbott, N.J., Rönnbäck, L., Hansson, E., 2006. Astrocyte-endothelial interactions at the blood-brain barrier. Nature Reviews Neuroscience 7, 41–53. doi:10.1038/nrn1824

Ahlenius, H., Visan, V., Kokaia, M., Lindvall, O., Kokaia, Z., 2009. Neural stem and progenitor cells retain their potential for proliferation and differentiation into functional neurons despite lower number in aged brain. Journal of Neuroscience 29, 4408–4419. doi:10.1523/JNEUROSCI.6003-08.2009

Alcendor, D.J., Block, F.E., Cliffel, D.E., Daniels, J.S., Ellacott, K.L.J., Goodwin, C.R., Hofmeister, L.H., Li, D., Markov, D.A., May, J.C., McCawley, L.J., McLaughlin, B., McLean, J.A., Niswender, K.D., Pensabene, V., Seale, K.T., Sherrod, S.D., Sung, H.-J., Tabb, D.L., Webb, D.J., Wikswo, J.P., 2013. Neurovascular unit on a chip: implications for translational applications. Stem Cell Res Ther 4 Suppl 1, S18. doi:10.1186/scrt379

Andreu-Agulló, C., Morante-Redolat, J.M., Delgado, A.C., Fariñas, I., 2009. Vascular niche factor PEDF modulates Notch-dependent stemness in the adult subependymal zone. Nat. Neurosci. 12, 1514–1523. doi:10.1038/nn.2437

Androutsellis-Theotokis, A., Rueger, M.A., Park, D.M., Mkhikian, H., Korb, E., Poser, S.W., Walbridge, S., Munasinghe, J., Koretsky, A.P., Lonser, R.R., McKay, R.D., 2009. Targeting neural precursors in the adult brain rescues injured dopamine neurons. PNAS 106, 13570–13575. doi:10.1073/pnas.0905125106

Armulik, A., Genové, G., Mäe, M., Nisancioglu, M.H., Wallgard, E., Niaudet, C., He, L., Norlin, J., Lindblom, P., Strittmatter, K., Johansson, B.R., Betsholtz, C., 2010. Pericytes regulate the blood-brain barrier. Nature 468, 557–561. doi:10.1038/nature09522

Arvidsson, A., Collin, T., Kirik, D., Kokaia, Z., Lindvall, O., 2002. Neuronal replacement from endogenous precursors in the adult brain after stroke. Nature Medicine 8, 963–970. doi:10.1038/nm747

Banks, W.A., 2016. From blood-brain barrier to blood-brain interface: new opportunities for CNS drug delivery. Nat Rev Drug Discov 15, 275–292. doi:10.1038/nrd.2015.21

Bjornsson, C.S., Apostolopoulou, M., Tian, Y., Temple, S., 2015. It Takes a Village: Constructing the Neurogenic Niche. Developmental Cell 32, 435–446. doi:10.1016/j.devcel.2015.01.010

Bond, A.M., Ming, G.-L., Song, H., 2015. Adult Mammalian Neural Stem Cells and Neurogenesis: Five Decades Later. Cell Stem Cell 17, 385–395. doi:10.1016/j.stem.2015.09.003

Bracko, O., Singer, T., Aigner, S., Knobloch, M., Winner, B., Ray, J., Clemenson, G.D., Suh, H., Couillard-Despres, S., Aigner, L., Gage, F.H., Jessberger, S., 2012. Gene expression profiling of neural stem cells and their neuronal progeny reveals IGF2 as a regulator of adult hippocampal neurogenesis. Journal of Neuroscience 32, 3376–3387. doi:10.1523/JNEUROSCI.4248-11.2012

Britton, J.S., Edgar, B.A., 1998. Environmental control of the cell cycle in Drosophila: nutrition activates mitotic and endoreplicative cells by distinct mechanisms. Development 125, 2149–2158.

Brown, J.A., Pensabene, V., Markov, D.A., Allwardt, V., Neely, M.D., Shi, M., Britt, C.M., Hoilett, O.S., Yang, Q., Brewer, B.M., Samson, P.C., McCawley, L.J., May, J.M., Webb, D.J., Li, D., Bowman, A.B., Reiserer, R.S., Wikswo, J.P., 2015. Recreating blood-brain barrier physiology and structure on chip: A novel neurovascular microfluidic bioreactor. Biomicrofluidics 9, 054124. doi:10.1063/1.4934713

Bundgaard, M., Abbott, N.J., 2008. All vertebrates started out with a glial blood-brain barrier 4-500 million years ago. Glia 56, 699–708. doi:10.1002/glia.20642

Bundgaard, M., Cserr, H., 1981. A glial blood-brain barrier in elasmobranchs. Brain Research 226, 61–73.

Calvo, C.-F., Fontaine, R.H., Soueid, J., Tammela, T., Makinen, T., Alfaro-Cervello, C., Bonnaud, F., Miguez, A., Benhaim, L., Xu, Y., Barallobre, M.-J., Moutkine, I., Lyytikkä, J., Tatlisumak, T., Pytowski, B., Zalc, B., Richardson, W., Kessaris, N., García-Verdugo, J.M., Alitalo, K., Eichmann, A., Thomas, J.-L., 2011. Vascular endothelial growth factor receptor 3 directly regulates murine neurogenesis. Genes & Development 25, 831–844. doi:10.1101/gad.615311

Carleton, A., Petreanu, L.T., Lansford, R., Alvarez-Buylla, A., Lledo, P.-M., 2003. Becoming a new neuron in the adult olfactory bulb. Nat. Neurosci. 6, 507–518. doi:10.1038/nn1048

Casper, D., Engstrom, S.J., Mirchandani, G.R., Pidel, A., Palencia, D., Cho, P.H., Brownlee, M., Edelstein, D., Federoff, H.J., Sonstein, W.J., 2002. Enhanced vascularization and survival of neural transplants with ex vivo angiogenic gene transfer. ct 11, 331–349.

Cave, J.W., Wang, M., Baker, H., 2014. Adult subventricular zone neural stem cells as a potential source of dopaminergic replacement neurons. Front Neurosci 8, 16. doi:10.3389/fnins.2014.00016

Chaker, Z., Codega, P., Doetsch, F., 2016. A mosaic world: puzzles revealed by adult neural stem cell heterogeneity. WIREs Dev Biol 5, 640–658. doi:10.1002/wdev.248

Chell, J.M., Brand, A.H., 2010. Nutrition-Responsive Glia Control Exit of Neural Stem Cells from Quiescence. Cell 143, 1161–1173. doi:10.1016/j.cell.2010.12.007

Chien, S., 2007. Mechanotransduction and endothelial cell homeostasis: the wisdom of the cell. Am. J. Physiol. Heart Circ. Physiol. 292, H1209–24. doi:10.1152/ajpheart.01047.2006

Chou, C.-H., Fan, H.-C., Hueng, D.-Y., 2015. Potential of Neural Stem Cell-Based Therapy for Parkinson's Disease. Parkinsons Dis 2015, 571475–9. doi:10.1155/2015/571475

Chou, C.-H., Sinden, J.D., Couraud, P.-O., Modo, M., 2014. In Vitro Modeling of the Neurovascular Environment by Coculturing Adult Human Brain Endothelial Cells with Human Neural Stem Cells. PLoS ONE 9, e106346. doi:10.1371/journal.pone.0106346

Clelland, C.D., Choi, M., Romberg, C., Clemenson, G.D., Fragniere, A., Tyers, P., Jessberger, S., Saksida, L.M., Barker, R.A., Gage, F.H., Bussey, T.J., 2009. A functional role for adult hippocampal neurogenesis in spatial pattern separation. Science 325, 210–213. doi:10.1126/science.1173215

Codega, P., Silva-Vargas, V., Paul, A., Maldonado-Soto, A.R., Deleo, A.M., Pastrana, E., Doetsch, F., 2014. Prospective identification and purification of quiescent adult neural stem cells from their in vivo niche. Neuron 82, 545–559. doi:10.1016/j.neuron.2014.02.039

Conover, J.C., Doetsch, F., García-Verdugo, J.M., Gale, N.W., Yancopoulos, G.D., Alvarez-Buylla, A., 2000. Disruption of Eph/ephrin signaling affects migration and proliferation in the adult subventricular zone. Nat. Neurosci. 3, 1091–1097. doi:10.1038/80606

Crouch, E.E., Liu, C., Silva-Vargas, V., Doetsch, F., 2015. Regional and stage-specific effects of prospectively purified vascular cells on the adult V-SVZ neural

stem cell lineage. Journal of Neuroscience 35, 4528–4539. doi:10.1523/JNEUROSCI.1188-14.2015

Culver, J.C., Vadakkan, T.J., Dickinson, M.E., 2013. A specialized microvascular domain in the mouse neural stem cell niche. PLoS ONE 8, e53546. doi:10.1371/journal.pone.0053546

Daneman, R., Zhou, L., Agalliu, D., Cahoy, J.D., Kaushal, A., Barres, B.A., Ben A Barres, 2010. The mouse blood-brain barrier transcriptome: a new resource for understanding the development and function of brain endothelial cells. PLoS ONE 5, e13741. doi:10.1371/journal.pone.0013741

Datta, S., 1995. Control of proliferation activation in quiescent neuroblasts of the Drosophila central nervous system. Development 121, 1173–1182.

Delgado, A.C., Ferrón, S.R., Vicente, D., Porlan, E., Perez-Villalba, A., Trujillo, C.M., D'Ocón, P., Fariñas, I., 2014. Endothelial NT-3 delivered by vasculature and CSF promotes quiescence of subependymal neural stem cells through nitric oxide induction. Neuron 83, 572–585. doi:10.1016/j.neuron.2014.06.015

DeSalvo, M.K., Hindle, S.J., Rusan, Z.M., Orng, S., Eddison, M., Halliwill, K., Bainton, R.J., 2014. The Drosophila surface glia transcriptome: evolutionary conserved blood-brain barrier processes. Front Neurosci 8, 346. doi:10.3389/fnins.2014.00346

Ding, R., Weynans, K., Bossing, T., Barros, C.S., Berger, C., 2016. The Hippo signalling pathway maintains quiescence in Drosophila neural stem cells. Nature Communications 7, 10510. doi:10.1038/ncomms10510

Doetsch, F., Alvarez-Buylla, A., 1996. Network of tangential pathways for neuronal migration in adult mammalian brain. Proc. Natl. Acad. Sci. U.S.A. 93, 14895–14900.

Doetsch, F., Caillé, I., Lim, D.A., García-Verdugo, J.M., Alvarez-Buylla, A., 1999a. Subventricular Zone Astrocytes Are Neural Stem Cells in the Adult Mammalian Brain. Cell 97, 703–716.

Doetsch, F., García-Verdugo, J.M., Alvarez-Buylla, A., 1999b. Regeneration of a germinal layer in the adult mammalian brain. Proc. Natl. Acad. Sci. U.S.A. 96, 11619–11624. doi:10.1073/pnas.96.20.11619

Douet, V., Arikawa Hirasawa, E., Mercier, F., 2012. Fractone-heparan sulfates mediate BMP-7 inhibition of cell proliferation in the adult subventricular zone. Neurosci. Lett. 528, 120–125. doi:10.1016/j.neulet.2012.08.077

Douet, V., Kerever, A., Arikawa-Hirasawa, E., Mercier, F., 2013. Fractone-heparan sulphates mediate FGF-2 stimulation of cell proliferation in the adult subventricular zone. Cell Prolif. 46, 137–145. doi:10.1111/cpr.12023

Enwere, E., Shingo, T., Gregg, C., Fujikawa, H., Ohta, S., Weiss, S., 2004. Aging results in reduced epidermal growth factor receptor signaling, diminished olfactory neurogenesis, and deficits in fine olfactory discrimination. Journal of Neuroscience 24, 8354–8365. doi:10.1523/JNEUROSCI.2751-04.2004

Eriksson, P.S., Perfilieva, E., Björk-Eriksson, T., Alborn, A.-M., Nordborg, C., Peterson, D.A., Gage, F.H., 1998. Neurogenesis in the adult human hippocampus. Nature Medicine 4, 1313–1317. doi:10.1038/3305

Ernst, A., Alkass, K., Bernard, S., Salehpour, M., Perl, S., Tisdale, J., Possnert, G., Druid, H., Frisén, J., 2014. Neurogenesis in the striatum of the adult human brain. Cell 156, 1072–1083. doi:10.1016/j.cell.2014.01.044

Farkas, E., Luiten, P.G.M., 2001. Cerebral microvascular pathology in aging and Alzheimer's disease. Progress in Neurobiology 64, 575–611. doi:10.1016/S0301-0082(00)00068-X

Fuentealba, L.C., Obernier, K., Alvarez-Buylla, A., 2012. Adult Neural Stem Cells Bridge Their Niche. Cell Stem Cell 10, 698–708. doi:10.1016/j.stem.2012.05.012

Gay, L., Miller, M.R., Ventura, P.B., Devasthali, V., Vue, Z., Thompson, H.L., Temple, S., Zong, H., Cleary, M.D., Stankunas, K., Doe, C.Q., 2013. Mouse TU tagging: a chemical/genetic intersectional method for purifying cell type-specific nascent RNA. Genes & Development 27, 98–115. doi:10.1101/gad.205278.112

Gould, E., Beylin, A., Tanapat, P., Reeves, A., Shors, T.J., 1999. Learning enhances adult neurogenesis in the hippocampal formation. Nat. Neurosci. 2, 260–265. doi:10.1038/6365

Gould, E., Tanapat, P., 1997. Lesion-induced proliferation of neuronal progenitors in the dentate gyrus of the adult rat. Neuroscience 80, 427–436. doi:10.1016/S0306-4522(97)00127-9

Gómez-Gaviro, M.V., Lovell-Badge, R., Fernández-Avilés, F., Lara-Pezzi, E., 2012a. The Vascular Stem Cell Niche. J. of Cardiovasc. Trans. Res. 5, 618–630. doi:10.1007/s12265-012-9371-x

Gómez-Gaviro, M.V., Scott, C.E., Sesay, A.K., Matheu, A., Booth, S., Galichet, C., Lovell-Badge, R., 2012b. Betacellulin promotes cell proliferation in the neural stem cell niche and stimulates neurogenesis. PNAS 109, 1317–1322. doi:10.1073/pnas.1016199109

Handke, B., Poernbacher, I., Goetze, S., Ahrens, C.H., Omasits, U., Marty, F., Simigdala, N., Meyer, I., Wollscheid, B., Brunner, E., Hafen, E., Lehner, C.F., 2013. The hemolymph proteome of fed and starved Drosophila larvae. PLoS ONE 8, e67208. doi:10.1371/journal.pone.0067208

Hartenstein, V., Rudloff, E., Ortega, J.A.C., 1987. The pattern of proliferation of the neuroblasts in the wild-type embryo of Drosophila melanogaster. Roux's Arch Dev Biol 196, 473–485. doi:10.1007/BF00399871

Hayon, Y., Dashevsky, O., Shai, E., Varon, D., Leker, R.R., 2013. Platelet lysates stimulate angiogenesis, neurogenesis and neuroprotection after stroke. Thromb. Haemost. 110, 323–330. doi:10.1160/TH12-11-0875

Hindle, S.J., Bainton, R.J., 2014. Barrier mechanisms in the Drosophila blood-brain barrier. Front Neurosci 8, 414. doi:10.3389/fnins.2014.00414

Ito, K., Hotta, Y., 1992. Proliferation pattern of postembryonic neuroblasts in the brain of Drosophila melanogaster. Developmental Biology 149, 134–148.

Jin, K., Zhu, Y., Sun, Y., Mao, X.O., Xie, L., Greenberg, D.A., 2002. Vascular endothelial growth factor (VEGF) stimulates neurogenesis in vitro and in vivo. Proc. Natl. Acad. Sci. U.S.A. 99, 11946–11950. doi:10.1073/pnas.182296499

Katsimpardi, L., Litterman, N.K., Schein, P.A., Miller, C.M., Loffredo, F.S., Wojtkiewicz, G.R., Chen, J.W., Lee, R.T., Wagers, A.J., Rubin, L.L., 2014. Vascular and Neurogenic Rejuvenation of the Aging Mouse Brain by Young Systemic Factors. Science 344, 630–634. doi:10.1126/science.1251141

Kazanis, I., Feichtner, M., Lange, S., Rotheneichner, P., Hainzl, S., Öller, M., Schallmoser, K., Rohde, E., Reitsamer, H.A., Couillard-Despres, S., Bauer, H.-C., Franklin, R.J.M., Aigner, L., Rivera, F.J., 2015. Lesion-induced accumulation of platelets promotes survival of adult neural stem / progenitor cells. Exp. Neurol. 269, 75–89. doi:10.1016/j.expneurol.2015.03.018

Kazanis, I., Lathia, J.D., Vadakkan, T.J., Raborn, E., Wan, R., Mughal, M.R., Eckley, D.M., Sasaki, T., Patton, B., Mattson, M.P., Hirschi, K.K., Dickinson, M.E., ffrench-Constant, C., 2010. Quiescence and activation of stem and precursor cell populations in the subependymal zone of the mammalian brain are associated with distinct cellular and extracellular matrix signals. Journal of Neuroscience 30,

9771–9781. doi:10.1523/JNEUROSCI.0700-10.2010
Keck, P.J., Hauser, S.D., Krivi, G., Sanzo, K., Warren, T., Feder, J., Connolly, D.T., 1989. Vascular permeability factor, an endothelial cell mitogen related to PDGF. Science 246, 1309–1312.
Kempermann, G., Kuhn, H.G., Gage, F.H., 1997. More hippocampal neurons in adult mice living in an enriched environment. Nature 386, 493–495. doi:10.1038/386493a0
Kerever, A., Schnack, J., Vellinga, D., Ichikawa, N., Moon, C., Arikawa Hirasawa, E., Efird, J.T., Mercier, F., 2007. Novel Extracellular Matrix Structures in the Neural Stem Cell Niche Capture the Neurogenic Factor Fibroblast Growth Factor 2 from the Extracellular Milieu. Stem Cells 25, 2146–2157. doi:10.1634/stemcells.2007-0082
Kokovay, E., Goderie, S., Wang, Y., Lotz, S., Lin, G., Sun, Y., Roysam, B., Shen, Q., Temple, S., 2010. Adult SVZ lineage cells home to and leave the vascular niche via differential responses to SDF1/CXCR4 signaling. Cell Stem Cell 7, 163–173. doi:10.1016/j.stem.2010.05.019
Kuhn, H.G., Winkler, J., Kempermann, G., Thal, L.J., Gage, F.H., 1997. Epidermal growth factor and fibroblast growth factor-2 have different effects on neural progenitors in the adult rat brain. J. Neurosci. 17, 5820–5829. doi:10.1002/cne.901240303
Lacar, B., Herman, P., Hartman, N.W., Hyder, F., Bordey, A., 2012. S phase entry of neural progenitor cells correlates with increased blood flow in the young subventricular zone. PLoS ONE 7, e31960. doi:10.1371/journal.pone.0031960
Lee, C., Hu, J., Ralls, S., Kitamura, T., Loh, Y.P., Yang, Y., Mukouyama, Y.-S., Ahn, S., 2012. The molecular profiles of neural stem cell niche in the adult subventricular zone. PLoS ONE 7, e50501. doi:10.1371/journal.pone.0050501
Lehtinen, M.K., Bjornsson, C.S., Dymecki, S.M., Gilbertson, R.J., Holtzman, D.M., Monuki, E.S., 2013. The choroid plexus and cerebrospinal fluid: emerging roles in development, disease, and therapy. Journal of Neuroscience 33, 17553–17559. doi:10.1523/JNEUROSCI.3258-13.2013
Leung, D.W., Cachianes, G., Kuang, W.J., Goeddel, D.V., Ferrara, N., 1989. Vascular endothelial growth factor is a secreted angiogenic mitogen. Science 246, 1306–1309. doi:10.1126/science.2479986
Li, J., Tang, Y., Wang, Y., Tang, R., Jiang, W., Yang, G.-Y., Gao, W.-Q., 2014. Neurovascular Recovery via Cotransplanted Neural and Vascular Progenitors Leads to Improved Functional Restoration after Ischemic Stroke in Rats. Stem Cell Reports 3, 101–114. doi:10.1016/j.stemcr.2014.05.012
Li, L., Candelario, K.M., Thomas, K., Wang, R., Wright, K., Messier, A., Cunningham, L.A., 2014. Hypoxia inducible factor-1α (HIF-1α) is required for neural stem cell maintenance and vascular stability in the adult mouse SVZ. Journal of Neuroscience 34, 16713–16719. doi:10.1523/JNEUROSCI.4590-13.2014
Licht, T., Keshet, E., 2015. The vascular niche in adult neurogenesis. Mech. Dev. 138, 56–62. doi:10.1016/j.mod.2015.06.001
Lim, D.A., Tramontin, A.D., Trevejo, J.M., Herrera, D.G., García-Verdugo, J.M., Alvarez-Buylla, A., 2000. Noggin Antagonizes BMP Signaling to Create a Niche for Adult Neurogenesis. Neuron 28, 713–726. doi:10.1016/S0896-6273(00)00148-3
Limmer, S., Weiler, A., Volkenhoff, A., Babatz, F., Klämbt, C., 2014. The Drosophila blood-brain barrier: development and function of a glial endothelium. Front

Neurosci 8, 365. doi:10.3389/fnins.2014.00365
Livneh, Y., Adam, Y., Mizrahi, A., 2014. Odor processing by adult-born neurons. Neuron 81, 1097–1110. doi:10.1016/j.neuron.2014.01.007
Llorens-Bobadilla, E., Zhao, S., Baser, A., Saiz-Castro, G., Zwadlo, K., Martin-Villalba, A., 2015. Single-Cell Transcriptomics Reveals a Population of Dormant Neural Stem Cells that Become Activated upon Brain Injury. Cell Stem Cell 17, 329–340. doi:10.1016/j.stem.2015.07.002
Loffredo, F.S., Steinhauser, M.L., Jay, S.M., Gannon, J., Pancoast, J.R., Yalamanchi, P., Sinha, M., Dall'Osso, C., Khong, D., Shadrach, J.L., Miller, C.M., Singer, B.S., Stewart, A., Psychogios, N., Gerszten, R.E., Hartigan, A.J., Kim, M.-J., Serwold, T., Wagers, A.J., Lee, R.T., 2013. Growth Differentiation Factor 11 Is a Circulating Factor that Reverses Age-Related Cardiac Hypertrophy. Cell 153, 828–839. doi:10.1016/j.cell.2013.04.015
Louissaint, A., Rao, S., Leventhal, C., Goldman, S.A., 2002. Coordinated interaction of neurogenesis and angiogenesis in the adult songbird brain. Neuron 34, 945–960.
MacDonald, P.E., Rorsman, P., 2006. Oscillations, intercellular coupling, and insulin secretion in pancreatic beta cells. PLOS Biol 4, e49. doi:10.1371/journal.pbio.0040049
Maisonpierre, P.C., Suri, C., Jones, P.F., Bartunkova, S., Wiegand, S.J., Radziejewski, C., Compton, D., McClain, J., Aldrich, T.H., Papadopoulos, N., Daly, T.J., Davis, S., Sato, T.N., Yancopoulos, G.D., 1997. Angiopoietin-2, a Natural Antagonist for Tie2 That Disrupts in vivo Angiogenesis. Science 277, 55–60. doi:10.1126/science.277.5322.55
Maslov, A.Y., Barone, T.A., Plunkett, R.J., Pruitt, S.C., 2004. Neural stem cell detection, characterization, and age-related changes in the subventricular zone of mice. Journal of Neuroscience 24, 1726–1733. doi:10.1523/JNEUROSCI.4608-03.2004
Maurer, M.H., Thomas, C., Bürgers, H.F., Kuschinsky, W., 2008. Transplantation of adult neural progenitor cells transfected with vascular endothelial growth factor rescues grafted cells in the rat brain. Int. J. Biol. Sci. 4, 1–7.
Mazzucco, L., Borzini, P., Gope, R., 2010. Platelet-derived factors involved in tissue repair-from signal to function. Transfus Med Rev 24, 218–234. doi:10.1016/j.tmrv.2010.03.004
Mercier, F., Arikawa Hirasawa, E., 2012. Heparan sulfate niche for cell proliferation in the adult brain. Neurosci. Lett. 510, 67–72. doi:10.1016/j.neulet.2011.12.046
Mercier, F., Douet, V., 2014. Bone morphogenetic protein-4 inhibits adult neurogenesis and is regulated by fractone-associated heparan sulfates in the subventricular zone. Journal of Chemical Neuroanatomy 57-58, 54–61. doi:10.1016/j.jchemneu.2014.03.005
Mercier, F., Kitasako, J.T., Hatton, G.I., 2002. Anatomy of the brain neurogenic zones revisited: Fractones and the fibroblast/macrophage network. Journal of Comparative Neurology 451, 170–188. doi:10.1002/cne.10342
Meyer, C., Notari, L., Becerra, S.P., 2002. Mapping the type I collagen-binding site on pigment epithelium-derived factor Implications for its antiangiogenic activity. Journal of Biological Chemistry.
Mich, J.K., Signer, R.A., Nakada, D., Pineda, A., Burgess, R.J., Vue, T.Y., Johnson, J.E., Morrison, S.J., 2014. Prospective identification of functionally distinct stem cells and neurosphere-initiating cells in adult mouse forebrain. eLife 3, e02669. doi:10.7554/eLife.02669

Mirzadeh, Z., Merkle, F.T., Soriano-Navarro, M., García-Verdugo, J.M., Alvarez-Buylla, A., 2008. Neural Stem Cells Confer Unique Pinwheel Architecture to the Ventricular Surface in Neurogenic Regions of the Adult Brain. Cell Stem Cell 3, 265–278. doi:10.1016/j.stem.2008.07.004

Moreno-López, B., Noval, J.A., González-Bonet, L.G., Estrada, C., 2000. Morphological bases for a role of nitric oxide in adult neurogenesis11Published on the World Wide Web on 25 May 2000. Brain Research 869, 244–250. doi:10.1016/S0006-8993(00)02474-4

Morshead, C.M., Reynolds, B.A., Craig, C.G., McBurney, M.W., Staines, W.A., Morassutti, D., Weiss, S., van der Kooy, D., 1994. Neural stem cells in the adult mammalian forebrain: a relatively quiescent subpopulation of subependymal cells. Neuron 13, 1071–1082.

Nakagomi, N., Nakagomi, T., Kubo, S., Nakano Doi, A., Saino, O., Takata, M., Yoshikawa, H., Stern, D.M., Matsuyama, T., Taguchi, A., 2009. Endothelial Cells Support Survival, Proliferation, and Neuronal Differentiation of Transplanted Adult Ischemia - Induced Neural Stem/Progenitor Cells After Cerebral Infarction. Stem Cells 27, 2185–2195. doi:10.1002/stem.161

Nolan, D.J., Ginsberg, M., Israely, E., Palikuqi, B., Poulos, M.G., James, D., Ding, B.-S., Schachterle, W., Liu, Y., Rosenwaks, Z., Butler, J.M., Xiang, J., Rafii, A., Shido, K., Rabbany, S.Y., Elemento, O., Rafii, S., 2013. Molecular signatures of tissue-specific microvascular endothelial cell heterogeneity in organ maintenance and regeneration. Developmental Cell 26, 204–219. doi:10.1016/j.devcel.2013.06.017

Ottone, C., Krusche, B., Whitby, A., Clements, M., Quadrato, G., Pitulescu, M.E., Adams, R.H., Parrinello, S., 2014. Direct cell-cell contact with the vascular niche maintains quiescent neural stem cells. Nat. Cell Biol. 16, 1045–1056. doi:10.1038/ncb3045

Paik, J.-H., Ding, Z., Narurkar, R., Ramkissoon, S., Muller, F., Kamoun, W.S., Chae, S.-S., Zheng, H., Ying, H., Mahoney, J., Hiller, D., Jiang, S., Protopopov, A., Wong, W.H., Chin, L., Ligon, K.L., DePinho, R.A., 2009. FoxOs cooperatively regulate diverse pathways governing neural stem cell homeostasis. Cell Stem Cell 5, 540–553. doi:10.1016/j.stem.2009.09.013

Palm, T., Schwamborn, J.C., 2010. Brain tumor stem cells. Biological Chemistry 391, 607–617. doi:10.1515/bc.2010.059

Palmer, T.D., Willhoite, A.R., Gage, F.H., 2000. Vascular niche for adult hippocampal neurogenesis. J. Comp. Neurol. 425, 479–494. doi:10.1002/1096-9861(20001002)425:4<479::AID-CNE2>3.0.CO;2-3

Pineda, J.R., Daynac, M., Chicheportiche, A., Cebrian-Silla, A., Sii-Felice, K., García-Verdugo, J.M., Boussin, F.D., Mouthon, M.-A., 2013. Vascular-derived TGF-β increases in the stem cell niche and perturbs neurogenesis during aging and following irradiation in the adult mouse brain. EMBO Mol Med 5, 548–562. doi:10.1002/emmm.201202197

Poon, C.L.C., Mitchell, K.A., Kondo, S., Cheng, L.Y., Harvey, K.F., 2016. The Hippo Pathway Regulates Neuroblasts and Brain Size in Drosophila melanogaster. Current Biology 0. doi:10.1016/j.cub.2016.02.009

Rafalski, V.A., Brunet, A., 2011. Energy metabolism in adult neural stem cell fate. Progress in Neurobiology 93, 182–203. doi:10.1016/j.pneurobio.2010.10.007

Ramírez-Castillejo, C., Sánchez-Sánchez, F., Andreu-Agulló, C., Ferrón, S.R., Aroca-Aguilar, J.D., Sánchez, P., Mira, H., Escribano, J., Fariñas, I., 2006. Pigment epithelium-derived factor is a niche signal for neural stem cell renewal. Nat.

Neurosci. 9, 331–339. doi:10.1038/nn1657
Riddle, D.R., Sonntag, W.E., Lichtenwalner, R.J., 2003. Microvascular plasticity in aging. Ageing Research Reviews 2, 149–168. doi:10.1016/S1568-1637(02)00064-8
Rochefort, C., Gheusi, G., Vincent, J.-D., Lledo, P.-M., 2002. Enriched odor exposure increases the number of newborn neurons in the adult olfactory bulb and improves odor memory. Journal of Neuroscience 22, 2679–2689. doi:10.1002/cne.901240303
Rocheleau, J.V., Remedi, M.S., Granada, B., Head, W.S., Koster, J.C., Nichols, C.G., Piston, D.W., 2006. Critical role of gap junction coupled KATP channel activity for regulated insulin secretion. PLOS Biol 4, e26. doi:10.1371/journal.pbio.0040026
Roitbak, T., Surviladze, Z., Cunningham, L.A., 2011. Continuous expression of HIF-1α in neural stem/progenitor cells. Cell. Mol. Neurobiol. 31, 119–133. doi:10.1007/s10571-010-9561-5
Rosa, A.I., Gonçalves, J., Cortes, L., Bernardino, L., Malva, J.O., Agasse, F., 2010. The angiogenic factor angiopoietin-1 is a proneurogenic peptide on subventricular zone stem/progenitor cells. Journal of Neuroscience 30, 4573–4584. doi:10.1523/JNEUROSCI.5597-09.2010
Rovenko, B.M., Perkhulyn, N.V., Gospodaryov, D.V., Sanz, A., Lushchak, O.V., Lushchak, V.I., 2015. High consumption of fructose rather than glucose promotes a diet-induced obese phenotype in Drosophila melanogaster. Comp. Biochem. Physiol., Part A Mol. Integr. Physiol. 180, 75–85. doi:10.1016/j.cbpa.2014.11.008
Sahay, A., Scobie, K.N., Hill, A.S., O'Carroll, C.M., Kheirbek, M.A., Burghardt, N.S., Fenton, A.A., Dranovsky, A., Hen, R., 2011. Increasing adult hippocampal neurogenesis is sufficient to improve pattern separation. Nature 472, 466–470. doi:10.1038/nature09817
Sanai, N., Alvarez-Buylla, A., Berger, M.S., 2009. Neural Stem Cells and the Origin of Gliomas. http://dx.doi.org/10.1056/NEJMra043666 353, 811–822. doi:10.1056/NEJMra043666
Sanai, N., Nguyen, T., Ihrie, R.A., Mirzadeh, Z., Tsai, H.-H., Wong, M., Gupta, N., Berger, M.S., Huang, E., García-Verdugo, J.M., Rowitch, D.H., Alvarez-Buylla, A., 2011. Corridors of migrating neurons in the human brain and their decline during infancy. Nature 478, 382–386. doi:10.1038/nature10487
Sawamoto, K., Wichterle, H., Gonzalez-Perez, O., Cholfin, J.A., Yamada, M., Spassky, N., Murcia, N.S., García-Verdugo, J.M., Marin, O., Rubenstein, J.L.R., Tessier-Lavigne, M., Okano, H., Alvarez-Buylla, A., 2006. New neurons follow the flow of cerebrospinal fluid in the adult brain. Science 311, 629–632. doi:10.1126/science.1119133
Seri, B., García-Verdugo, J.M., McEwen, B.S., Alvarez-Buylla, A., 2001. Astrocytes give rise to new neurons in the adult mammalian hippocampus. Journal of Neuroscience 21, 7153–7160.
Shen, Q., Goderie, S.K., Jin, L., Karanth, N., Sun, Y., Abramova, N., Vincent, P., Pumiglia, K., Temple, S., 2004. Endothelial Cells Stimulate Self-Renewal and Expand Neurogenesis of Neural Stem Cells. Science 304, 1338–1340. doi:10.1126/science.1095505
Shen, Q., Wang, Y., Kokovay, E., Lin, G., Chuang, S.-M., Goderie, S.K., Roysam, B., Temple, S., 2008. Adult SVZ Stem Cells Lie in a Vascular Niche: A Quantitative Analysis of Niche Cell-Cell Interactions. Cell Stem Cell 3, 289–300. doi:10.1016/j.stem.2008.07.026

Shin, J., Berg, D.A., Zhu, Y., Shin, J.Y., Song, J., Bonaguidi, M.A., Enikolopov, G., Nauen, D.W., Christian, K.M., Ming, G.-L., Song, H., 2015. Single-Cell RNA-Seq with Waterfall Reveals Molecular Cascades underlying Adult Neurogenesis. Cell Stem Cell 17, 360–372. doi:10.1016/j.stem.2015.07.013

Shingo, T., Gregg, C., Enwere, E., Fujikawa, H., Hassam, R., Geary, C., Cross, J.C., Weiss, S., 2003. Pregnancy-stimulated neurogenesis in the adult female forebrain mediated by prolactin. Science 299, 117–120. doi:10.1126/science.1076647

Silva-Vargas, V., Crouch, E.E., Doetsch, F., 2013. Adult neural stem cells and their niche: a dynamic duo during homeostasis, regeneration, and aging. Curr. Opin. Neurobiol. 23, 935–942. doi:10.1016/j.conb.2013.09.004

Silva-Vargas, V., Maldonado-Soto, A.R., Mizrak, D., Codega, P., Doetsch, F., 2016. Age-Dependent Niche Signals from the Choroid Plexus Regulate Adult Neural Stem Cells. Cell Stem Cell. doi:10.1016/j.stem.2016.06.013

Silver, I., Erecińska, M., 1998. Oxygen and Ion Concentrations in Normoxic and Hypoxic Brain Cells, in: Oxygen Transport to Tissue XX, Advances in Experimental Medicine and Biology. Springer US, Boston, MA, pp. 7–16. doi:10.1007/978-1-4615-4863-8_2

Sousa-Nunes, R., Yee, L.L., Gould, A.P., 2011. Fat cells reactivate quiescent neuroblasts via TOR and glial insulin relays in Drosophila. Nature 471, 508–512. doi:10.1038/nature09867

Southall, T.D., Gold, K.S., Egger, B., Davidson, C.M., Caygill, E.E., Marshall, O.J., Brand, A.H., 2013. Cell-type-specific profiling of gene expression and chromatin binding without cell isolation: assaying RNA Pol II occupancy in neural stem cells. Developmental Cell 26, 101–112. doi:10.1016/j.devcel.2013.05.020

Spéder, P., Brand, A.H., 2014. Gap Junction Proteins in the Blood-Brain Barrier Control Nutrient-Dependent Reactivation of Drosophila Neural Stem Cells. Developmental Cell 30, 309–321. doi:10.1016/j.devcel.2014.05.021

Studer, L., Csete, M., Lee, S.H., Kabbani, N., Walikonis, J., Wold, B., McKay, R., 2000. Enhanced proliferation, survival, and dopaminergic differentiation of CNS precursors in lowered oxygen. Journal of Neuroscience 20, 7377–7383.

Sun, Y., Jin, K., Childs, J.T., Xie, L., Mao, X.O., Greenberg, D.A., 2006. Vascular endothelial growth factor-B (VEGFB) stimulates neurogenesis: Evidence from knockout mice and growth factor administration. Developmental Biology 289, 329–335. doi:10.1016/j.ydbio.2005.10.016

Suri, C., Jones, P.F., Patan, S., Bartunkova, S., Maisonpierre, P.C., Davis, S., Sato, T.N., Yancopoulos, G.D., 1996. Requisite role of angiopoietin-1, a ligand for the TIE2 receptor, during embryonic angiogenesis. Cell 87, 1171–1180. doi:10.1016/S0092-8674(00)81813-9

Taupin, P., 2007. Potential of adult neural stem cells for cellular therapy. Biologics.

Tavazoie, M., Van der Veken, L., Silva-Vargas, V., Louissaint, M., Colonna, L., Zaidi, B., García-Verdugo, J.M., Doetsch, F., 2008. A Specialized Vascular Niche for Adult Neural Stem Cells. Cell Stem Cell 3, 279–288. doi:10.1016/j.stem.2008.07.025

Tian, L., Hires, S.A., Mao, T., Huber, D., Chiappe, M.E., Chalasani, S.H., Petreanu, L., Akerboom, J., McKinney, S.A., Schreiter, E.R., Bargmann, C.I., Jayaraman, V., Svoboda, K., Looger, L.L., 2009. Imaging neural activity in worms, flies and mice with improved GCaMP calcium indicators. Nature Publishing Group 6, 875–881. doi:10.1038/nmeth.1398

Tomita, S., Ueno, M., Sakamoto, M., Kitahama, Y., Ueki, M., Maekawa, N., Sakamoto, H., Gassmann, M., Kageyama, R., Ueda, N., Gonzalez, F.J.,

Takahama, Y., 2003. Defective brain development in mice lacking the Hif-1alpha gene in neural cells. Molecular and Cellular Biology 23, 6739–6749. doi:10.1128/MCB.23.19.6739-6749.2003

Tronel, S., Belnoue, L., Grosjean, N., Revest, J.-M., Piazza, P.-V., Koehl, M., Abrous, D.N., 2012. Adult-born neurons are necessary for extended contextual discrimination. Hippocampus 22, 292–298. doi:10.1002/hipo.20895

Tropepe, V., Craig, C.G., Morshead, C.M., van der Kooy, D., 1997. Transforming growth factor-alpha null and senescent mice show decreased neural progenitor cell proliferation in the forebrain subependyma. J. Neurosci. 17, 7850–7859.

Truman, J.W., Bate, M., 1988. Spatial and temporal patterns of neurogenesis in the central nervous system of Drosophila melanogaster. Developmental Biology 125, 145–157. doi:10.1016/0012-1606(88)90067-X

Tzima, E., Irani-Tehrani, M., Kiosses, W.B., Dejana, E., Schultz, D.A., Engelhardt, B., Cao, G., DeLisser, H., Schwartz, M.A., 2005. A mechanosensory complex that mediates the endothelial cell response to fluid shear stress. Nature 437, 426–431. doi:10.1038/nature03952

van der Helm, M.W., van der Meer, A.D., Eijkel, J.C.T., van den Berg, A., Segerink, L.I., 2016. Microfluidic organ-on-chip technology for blood-brain barrier research. Tissue Barriers 4, e1142493. doi:10.1080/21688370.2016.1142493

van Praag, H., Christie, B.R., Sejnowski, T.J., Gage, F.H., 1999a. Running enhances neurogenesis, learning, and long-term potentiation in mice. Proc. Natl. Acad. Sci. U.S.A. 96, 13427–13431. doi:10.1073/pnas.96.23.13427

van Praag, H., Kempermann, G., Gage, F.H., 1999b. Running increases cell proliferation and neurogenesis in the adult mouse dentate gyrus. Nat. Neurosci. 2, 266–270. doi:10.1038/6368

van Praag, H., Schinder, A.F., Christie, B.R., Toni, N., Palmer, T.D., Gage, F.H., 2002. Functional neurogenesis in the adult hippocampus. Nature 415, 1030–1034. doi:10.1038/4151030a

van Praag, H., Shubert, T., Zhao, C., Gage, F.H., 2005. Exercise enhances learning and hippocampal neurogenesis in aged mice. Journal of Neuroscience 25, 8680–8685. doi:10.1523/JNEUROSCI.1731-05.2005

Vasudevan, A., Long, J.E., Crandall, J.E., Rubenstein, J.L.R., Bhide, P.G., 2008. Compartment-specific transcription factors orchestrate angiogenesis gradients in the embryonic brain. Nat. Neurosci. 11, 429–439. doi:10.1038/nn2074

Wachs, F.-P., Winner, B., Couillard-Despres, S., Schiller, T., Aigner, R., Winkler, J., Bogdahn, U., Aigner, L., 2006. Transforming growth factor-beta1 is a negative modulator of adult neurogenesis. J. Neuropathol. Exp. Neurol. 65, 358–370. doi:10.1097/01.jnen.0000218444.53405.f0

Walter, F.R., Valkai, S., Kincses, A., Petneházi, A., Czeller, T., Veszelka, S., Ormos, P., Deli, M.A., Dér, A., 2016. A versatile lab-on-a-chip tool for modeling biological barriers. Sensors and Actuators B: Chemical 222, 1209–1219. doi:10.1016/j.snb.2015.07.110

Wang, C., You, Y., Qi, D., Zhou, X., Wang, L., Wei, S., Zhang, Z., Huang, W., Liu, Z., Liu, F., Ma, L., Yang, Z., 2014. Human and monkey striatal interneurons are derived from the medial ganglionic eminence but not from the adult subventricular zone. Journal of Neuroscience 34, 10906–10923. doi:10.1523/JNEUROSCI.1758-14.2014

Weiss, N., Miller, F., Cazaubon, S., Couraud, P.-O., 2009. The blood-brain barrier in brain homeostasis and neurological diseases. Biochimica et Biophysica Acta (BBA) - Biomembranes 1788, 842–857. doi:10.1016/j.bbamem.2008.10.022

Zhang, R.L., Zhang, Z.G., Zhang, L., Chopp, M., 2001. Proliferation and differentiation of progenitor cells in the cortex and the subventricular zone in the adult rat after focal cerebral ischemia. Neuroscience 105, 33–41. doi:10.1016/S0306-4522(01)00117-8

Zhao, Z., Nelson, A.R., Betsholtz, C., Zlokovic, B.V., 2015. Establishment and Dysfunction of the Blood-Brain Barrier. Cell 163, 1064–1078. doi:10.1016/j.cell.2015.10.067

Zhu, L.-L., Wu, L.-Y., Yew, D.T., Fan, M., 2005. Effects of hypoxia on the proliferation and differentiation of NSCs. Mol Neurobiol 31, 231–242. doi:10.1385/MN:31:1-3:231

Zhu, W., Mao, Y., Zhao, Y., Zhou, L.-F., Wang, Y., Zhu, J.-H., Zhu, Y., Yang, G.-Y., 2005. Transplantation of vascular endothelial growth factor-transfected neural stem cells into the rat brain provides neuroprotection after transient focal cerebral ischemia. Neurosurgery 57, 325–33– discussion 325–33.

Zlokovic, B.V., 2008. The blood-brain barrier in health and chronic neurodegenerative disorders. Neuron 57, 178–201. doi:10.1016/j.neuron.2008.01.003

Figure Legends

Figure 1. The adult mammalian V-SVZ niche

A: The lineage of adult V-SVZ neural stem cells (NSCs). Quiescent NSCs become activated and generate, in sequence, transit amplifying progenitors (TAPs), migratory neuroblasts (NBs) and neurons (Neu). It is unclear whether activated NSCs can return to a quiescent state. **B:** The anatomy of the V-SVZ, illustrating the neurovascular and CSF niches. Abbreviations – Epend: ependymal cell, Ast: niche astrocyte, Peri: pericyte, Endo: endothelial cell. **C:** Three routes for neurovascular signalling to NSCs.

Figure 2. *Drosophila* as a model for the BBB niche

A: *Drosophila* NSC behaviour during embryonic and post-embryonic development. NSCs proliferate in two waves, separated by a quiescent period. Proliferating NSCs generate ganglion mother cells (GMCs), which divide to produce neurons and glia. **B:** Simplified anatomy of a *Drosophila* larva. The brain and fat body (liver and adipose tissue equivalent) are suspended in an open circulatory system (haemolymph). **C:** The BBB niche for *Drosophila* NSCs. Quiescent NSCs are in close contact with blood-brain barrier (BBB) glia (inner, subperineurial glia (SPG) and outer, perineurial glia (PG)). Quiescent NSCs also contact the neuropil, the synapse-dense region of the brain. **D:** BBB signalling reactivates quiescent NSCs. Amino acids in the diet cause the fat body to secrete unknown signal(s) that induce BBB glia to secrete *Drosophila* insulin/IGF-like peptides. This activates insulin signalling in quiescent NSCs. Insulin secretion is dependent on gap junctions and calcium signalling.

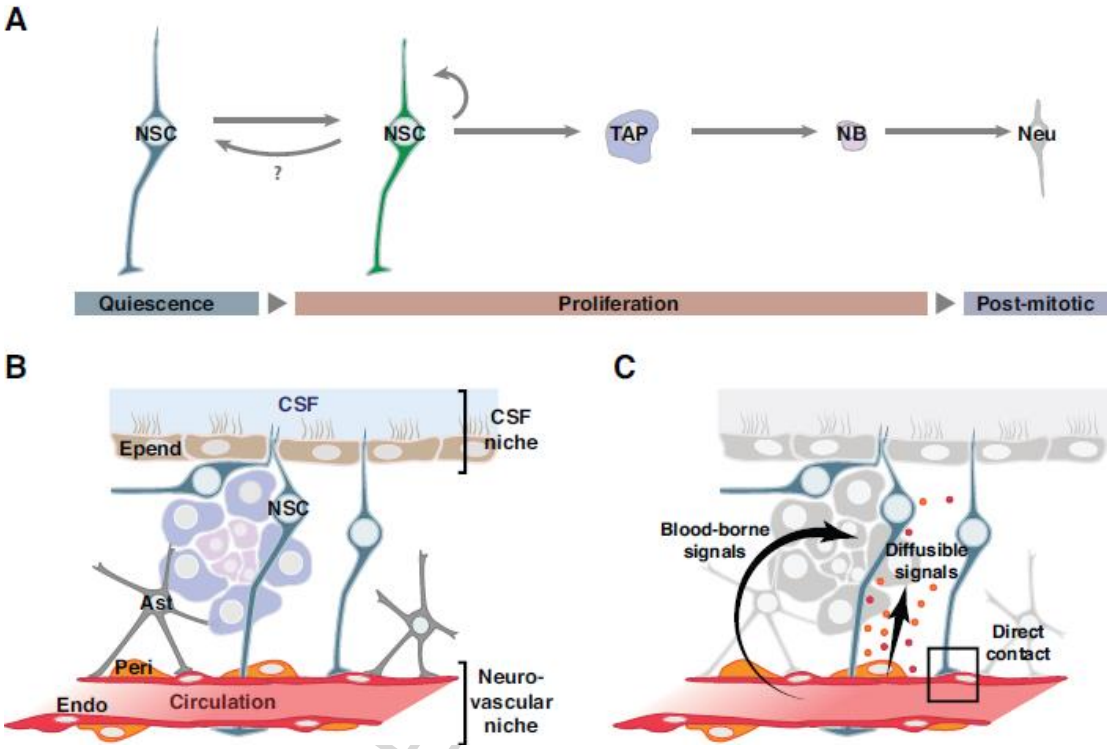

Fig. 1

Figure 2

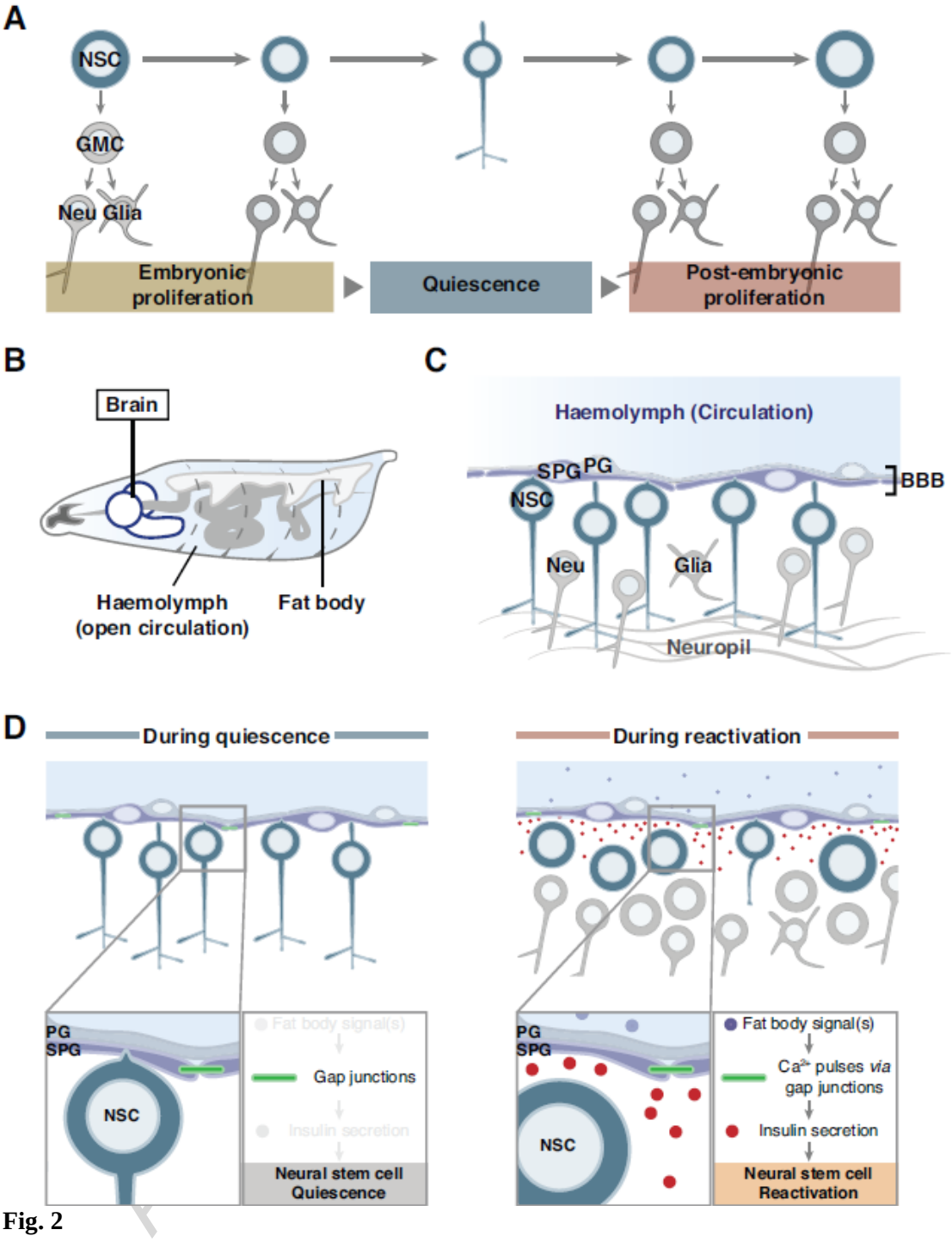

Fig. 2