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Trends in Neurosciences

Review

Quiescent Neural Stem Cells for Brain Repair and Regeneration: Lessons from Model Systems

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Neural stem cells (NSCs) are multipotent progenitors that are responsible for producing all of the neurons and macroglia in the nervous system. In adult mammals, NSCs reside predominantly in a mitotically dormant, quiescent state, but they can proliferate in response to environmental inputs such as feeding or exercise. It is hoped that quiescent NSCs could be activated therapeutically to contribute towards repair in humans. This will require an understanding of quiescent NSC heterogeneities and regulation during normal physiology and following brain injury. Non-mammalian vertebrates (zebrafish and salamanders) and invertebrates (*Drosophila*) offer insights into brain repair and quiescence regulation that are difficult to obtain using rodent models alone. We review conceptual progress from these various models, a first step towards harnessing quiescent NSCs for therapeutic purposes.

A Range of Animal Models To Study NSC Quiescence

Neurons and glia are produced throughout life in adult mice (*Mus musculus*) by NSCs residing in the ventricular/subventricular zone (V/SVZ) of the walls of the lateral ventricles and the subgranular zone (SGZ) of the hippocampus (Figure 1A) [1,2]. However, only a minority of NSCs (<9%) proliferates at any one time [3,4]. Instead, most adult NSCs are quiescent, dividing rarely and activating sporadically (once every few weeks in the mouse V/SVZ [5]) to sustain the pool of shorter-lived active NSCs [1,2,4]. Several environmental stimuli, such as feeding or exercise, can increase the proportion of proliferating NSCs in adult mice [6–8]. Brain injury can trigger limited neuron and glia production in mice, although it remains unclear to what extent brain function is restored. By contrast, quiescent NSCs in some vertebrate species, including zebrafish (*Danio rerio*) and salamanders, can replenish neurons and glia after injury, resulting in behavioural recovery. Thus, these vertebrates offer unique insights into brain repair that are difficult to achieve in rodents. Quiescent NSCs might also be present in adult humans. From the translational perspective, a key open question is whether quiescent NSCs in humans could be stimulated therapeutically to generate neurons and glia for brain repair.

The development of NSC-based therapies will require answers to several questions. What is quiescence? Are quiescent NSCs heterogeneous? How do environmental stimuli activate quiescent NSCs? We review here progress towards answering these questions, highlighting the conceptual synergies arising from studies in rodent and non-rodent models. We also discuss concepts emerging from salamanders and zebrafish that might guide future brain regeneration research in mammals. A significant challenge has been the difficulty in distinguishing between quiescent and active NSCs in vertebrates. A genetically tractable model organism in which quiescent and active NSCs can be identified unambiguously *in vivo* is the invertebrate *Drosophila melanogaster* (Figure 1B). *Drosophila* NSCs (called neuroblasts) divide every 40–50 minutes

Highlights

The adult mammalian brain harbours quiescent neural stem cells (NSCs), which possess a latent capacity to generate neurons and glia.

Mechanistic insights into the origins and functional properties of quiescent NSCs are starting to arise in rodents, *Drosophila*, and regenerative vertebrates.

It is becoming apparent that NSCs undergo different types of quiescence, such as G_0 and G_2 quiescence, or resting and dormant quiescence.

Environmental signals, such as exercise or feeding, might increase the activation of quiescent NSCs. Putative trajectories from quiescence to activation have been reconstructed bioinformatically from single-cell transcriptome data.

In general, quiescent NSCs are restricted to producing specific neuron subtypes after activation *in vivo*. It is possible to modify these outputs experimentally in some instances. The ability to control the outputs of quiescent NSCs will be an essential step in harnessing them for brain repair.

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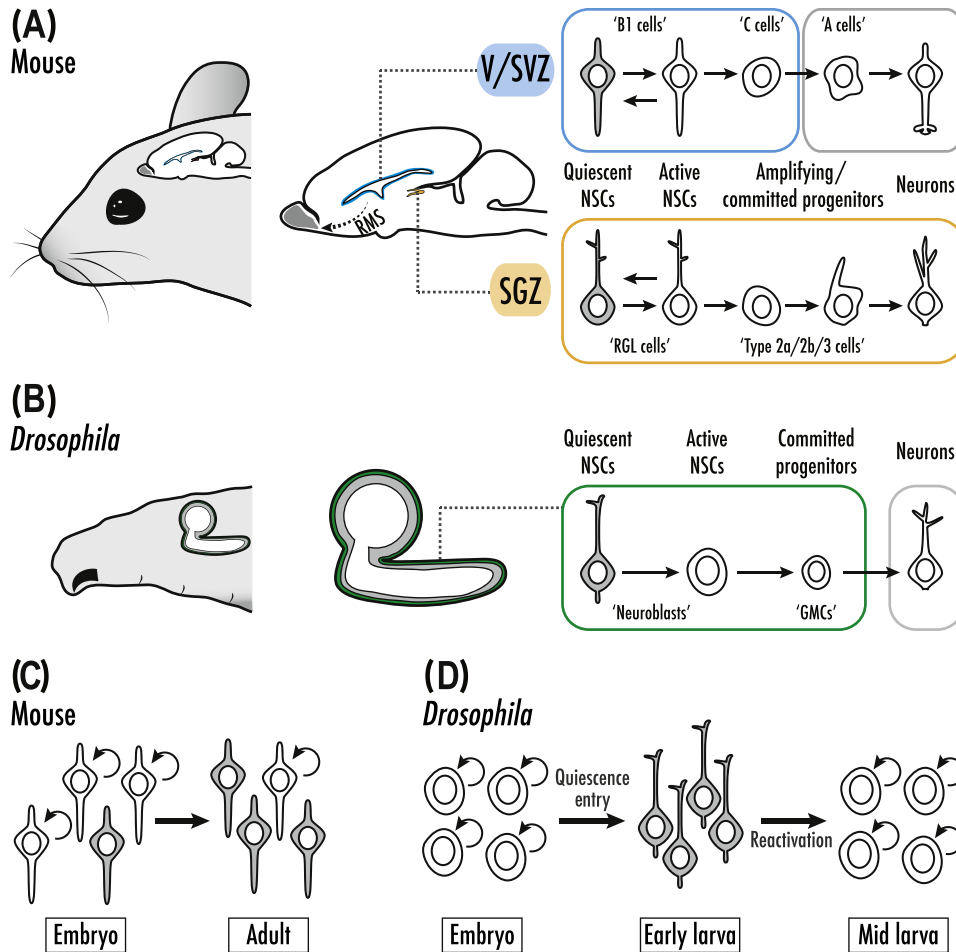a.brand@gurdon.cam.ac.uk (A.H. Brand).

Figure 1. Quiescent Neural Stem Cells (NSCs) in the Adult Mouse and Larval *Drosophila* Brain. (A) In the adult mouse brain, quiescent NSCs reside in two major neurogenic regions. The first is the ventricular/subventricular zone (V/SVZ) in the lateral ventricle walls (blue). V/SVZ NSCs (also called B1 cells) activate to generate transit-amplifying C cells. C cells divide several times to generate A cells (neuroblasts) that converge from throughout the V/SVZ and migrate in the rostral migratory stream (RMS) towards the olfactory bulb (grey), where they differentiate into local interneurons. The second neurogenic zone is the subgranular zone (SGZ) of the hippocampus (orange). SGZ NSCs (also called RGL cells) activate to generate intermediate progenitor cells (type 2a/2b/3 cells) that ultimately produce granule neurons in the local granule cell layer. Recently, adult NSCs have also been described in the hypothalamus (not shown) [113–115]. The quiescence and activation dynamics of hypothalamic NSCs are not well understood. (B) Quiescent NSCs are present throughout the early larval *Drosophila* brain (green). Once activated at later larval stages, NSCs self-renew and produce ganglion mother cells (GMCs) that divide to generate neurons and/or glia. (C) Quiescent and active NSCs coexist in the mouse brain and are difficult to distinguish *in vivo*. (D) *Drosophila* NSCs transit between quiescence and proliferation relatively synchronously, making them easy to distinguish. Quiescent NSCs have a smaller cell body and a distinct morphology compared to active NSCs.

throughout embryogenesis, then cease to proliferate in the late embryo and become quiescent for ~24 h (a time-period approximately equal to embryogenesis in its entirety) [9–11]. Quiescent *Drosophila* NSCs can resume proliferation (reactivate) postembryonically in response to dietary amino acid intake [12] (Figure 1C,D). The ability to reliably distinguish between quiescent and active NSCs has made it possible to discover molecular mechanisms that have been difficult to reveal in vertebrates.

Glossary

Blood–brain barrier: a border that separates the brain from the systemic circulation that enables oxygen, nutrients, and hormones to pass into the brain while restricting the entry of pathogens. In mammals, endothelial cells, astrocyte endfeet and pericytes contribute to barrier function. In *Drosophila* and basal vertebrates such as sharks the barrier is composed of glia.

Enriched environment: in rodent studies, enriched environment paradigms often refer to greater cage space and introduction of objects that promote exploration and interaction, including toys, ladders, platforms, tunnels, and a wider variety of food. These animals have a heightened social experience relative to rodents reared under standard laboratory conditions, but are still relatively deprived compared to those in the wild.

Fate-mapping: characterization of progeny cells through clonal labelling and lineage analysis.

Heterotypic grafting: transplantation of cells or tissues from their normal location to an ectopic one.

Homology-directed DNA repair: a high-fidelity pathway to repair double-stranded DNA lesions that can only operate during S/G_2 phases because it requires a homologous repair template. Outside S/G_2, DNA lesions are repaired by the lower fidelity non-homologous end-joining pathway, which can introduce nucleotide insertions and/or deletions.

Hypothalamus: a subdivision of the vertebrate brain that has important functions in homeostasis, feeding, growth, and general metabolism; the hypothalamus was recently discovered to be a third site of adult neurogenesis in rodents.

Label retention: an assay to identify non-dividing or rarely dividing cells. Cells are pulse labelled (e.g., through brief exposure to BrdU or transient expression of a fluorescent protein) and are then subjected to a long chase period, often of several weeks to months. Rapidly dividing cells dilute their label at each cell division, whereas non-dividing and rarely dividing cells maintain high levels of labelling. Label-retaining cells include quiescent cells but also, for example, cells that underwent terminal differentiation after incorporating the label.

What Is Quiescence: Stop or G_0?

For about 20 years, adult NSCs in rodents have been described to be 'quiescent', but it remains unclear whether they are cell cycle-arrested, progress slowly through the cell cycle, or have reversibly exited the cell cycle [1,2,13]. Quiescent NSCs are characterised by the lack of expression of cell-cycle progression factors (PCNA, MCM2, or Ki67) and failure to incorporate **thymidine analogues** (see Glossary) after long-term incubation (>2 weeks), assays that readily label active NSCs [14–17]. However, these assays do not completely exclude active NSCs because they only label specific cell-cycle phases. **Label retention** is another classical assay for slowly dividing or non-dividing cells. However, label retention does not accurately distinguish cell-cycle arrest from slow progression. Many investigators equate 'quiescence' with 'G_0 phase' by analogy to a nutrient withdrawal-induced arrest state in yeast and cultured cells [18,19]. G_0 cells have a $2n$ DNA content and take longer to enter S phase than G_1 phase cells when nutrients become available [18,19]. However, there are no diagnostic markers for G_0 and, in addition to quiescent cells, both differentiated and senescent cells are described to reside in G_0 despite (generally) being unable to resume cell-cycle progression.

The cell-cycle properties of quiescent NSCs were addressed recently in *Drosophila*, revealing an unexpected heterogeneity. All NSCs are quiescent in the **ventral nerve cord** of the early *Drosophila* larva [10,12,20–22]. Quiescent *Drosophila* NSCs are likely to be cell cycle-arrested, rather than slowly proliferating, because they never enter S phase even when quiescence is prolonged for 1 week by rearing larvae on a diet lacking amino acids (by contrast, proliferating embryonic NSCs divide every 40–50 minutes) [9,12]. Surprisingly, it was found that *Drosophila* NSCs arrest heterogeneously during quiescence. 75% of quiescent NSCs reside in G_2, indicated by cyclin A and cyclin B protein expression and a $4n$ DNA content [22]. The remaining 25% of quiescent NSCs resides in a G_0-like state, lacking cyclin expression and having a $2n$ DNA content and smaller nuclei than G_2 quiescent NSCs (Figure 2A) [22]. Thus, quiescent NSCs arrest in either G_0 or G_2 of the cell cycle in *Drosophila* (Figure 2A). G_0 quiescence is regulated by dacapo, a member of the p21/p27/p57 cyclin-dependent kinase inhibitor family that antagonises progression from G_0/G_1 to S [23–25]. G_2 quiescence is regulated by tribbles pseudokinase, which targets the mitosis-inducing factor Cdc25 for degradation [22,26–28]. G_2 quiescent NSCs reactivate more quickly than G_0 quiescent NSCs in response to dietary amino acid intake (Figure 2B) [22,25]. Understanding the function of this asynchrony in reactivation timing will be an important direction for future research.

Owing to the discovery of G_2 quiescent NSCs, quiescence can no longer be equated solely to G_0. G_2 quiescent NSCs transcribe genes that are often considered to be 'proliferation markers', such as those encoding cyclin proteins. Thus, G_2 quiescent NSCs could be difficult to distinguish from proliferating NSCs in vertebrates. Nevertheless, G_2-arrested muscle stem cells transcribing *cyclin A* and *cyclin B* were reported recently in zebrafish [29]. Interestingly, several studies have linked G_2 phase to regenerative ability. For example, adult stem cells in the regenerative polyp *Hydra*, and fibroblasts isolated from the highly regenerative **Murphy Roths large (MRL) mouse** strain, spend most of their time in G_2 [30,31]. Because G_2 cells can perform high-fidelity **homology-directed DNA repair** and enter mitosis rapidly compared to G_0 cells, G_2 quiescence could be beneficial for injury-responsive tissue stem cells.
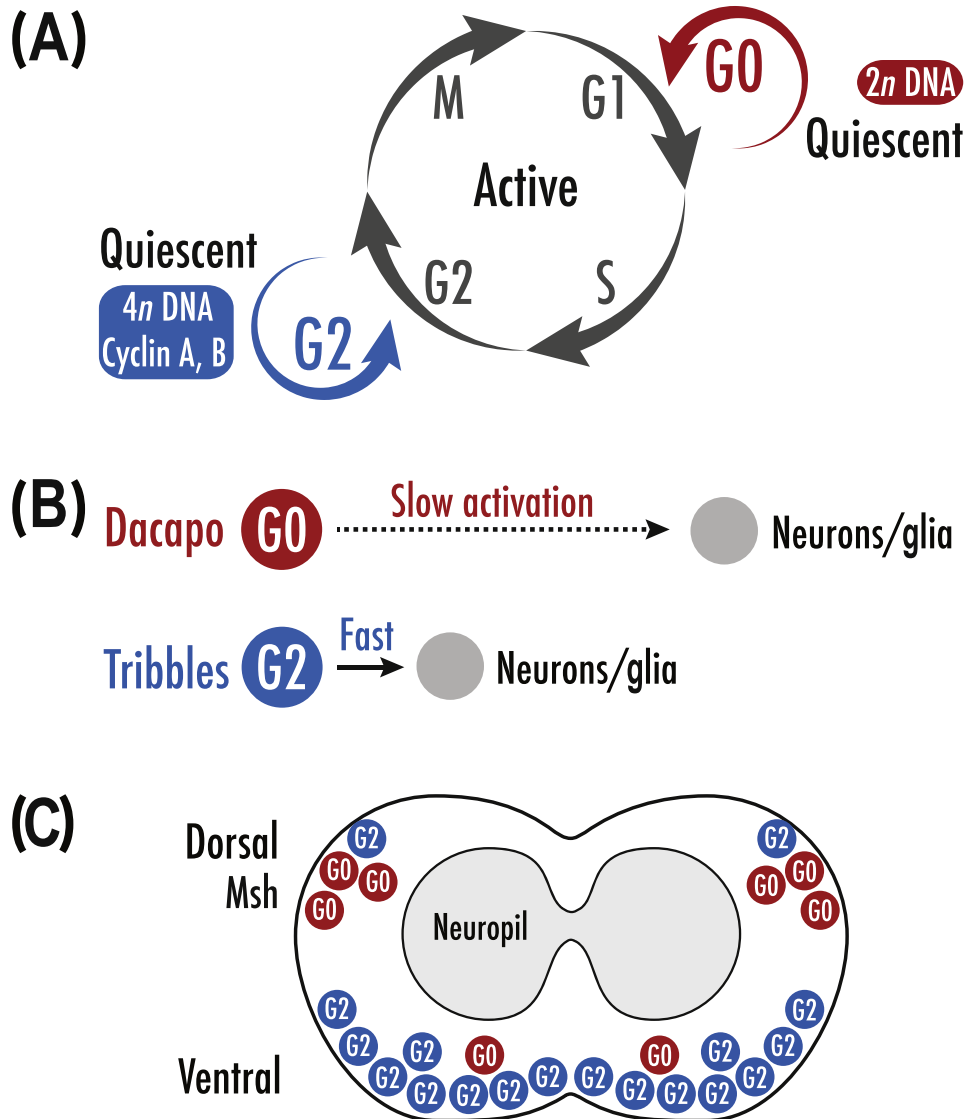
Where Do Quiescent NSCs Come From?

Identifying the developmental origins of quiescent NSCs might help in understanding their neurogenic properties. Quiescent NSCs in rodents and *Drosophila* arise from embryonic NSC populations through distinct mechanisms. SGZ NSCs in mice and rats originate from embryonic dentate neuroepithelial cells, whose descendants migrate along the dentate migratory stream to

Figure 2. Two Types of Neural Stem Cell (NSC) Quiescence in *Drosophila*. (A) Quiescent NSCs in *Drosophila* are arrested in G_0 phase quiescence (red) or G_2 (blue). G_0 NSCs have a $2n$ DNA content and do not express cyclin proteins. G_2 NSCs have a $4n$ DNA content and express cyclin A and cyclin B [22]. (B) The p21/p27/p57 orthologue dacapo is necessary for NSCs to enter G_0 quiescence in *Drosophila*. Tribbles pseudokinase regulates quiescence entry in G_2 NSCs. In response to dietary amino acid intake, G_2 quiescent NSCs reactivate first and generate neurons and glia more rapidly than G_0 quiescent NSCs [22,25]. (C) The dorsal patterning factor Msh is one of the upstream regulators that induces dacapo expression in *Drosophila* NSCs. Thus, more dorsal NSCs undergo G_0 quiescence and more ventral NSCs undergo G_2 quiescence in the ventral nerve cord [25].

establish the primitive dentate structure [32,33]. These cells proliferate throughout embryogenesis and perinatal stages before becoming quiescent in the second postnatal week (i.e., postembryonically) [33,34]. An additional source of adult SGZ NSCs is the ventral hippocampus, but here the timing of quiescence entry is not known [35]. In contrast to SGZ NSCs, the precursors to V/SVZ NSCs enter quiescence in the embryo between embryonic (E) days E13.5 and E15.5 [36,37]. The cyclin-dependent kinase inhibitor p57[Kip2] (*p57*, *Cdkn1c*) becomes highly

expressed in a subset of NSCs in the ganglionic eminences, and induces these NSCs to reduce cell division and persist into the adult V/SVZ as quiescent cells, while the remaining NSCs continue to proliferate and become depleted [36,37].

In *Drosophila*, NSCs proliferate throughout embryogenesis, then either enter quiescence or undergo apoptosis in the late embryo (Figure 1D) [9,11,38]. Each NSC in the ventral nerve cord can be distinguished molecularly and has been **fate-mapped**, making it possible to profile the timing with which individual NSCs become quiescent. For example, the NSC NB3-3T becomes quiescent at stage 15 and NB3-5A8 at stage 17 [39,40]. One mechanism that directs NSCs to enter G_0 versus G_2 quiescence in the thoracic segments of the late embryo was recently revealed [25,41]. The homeobox transcription factor muscle segment homeobox (Msh), which is expressed in NSCs originating from the dorsal neuroectoderm, binds directly to the *dacapo* locus and promotes its expression, leading these cells to enter G_0 quiescence (Figure 2C) [25,41,42]. NSCs originating from the ventral neuroectoderm do not generally express Msh or dacapo and instead enter G_2 quiescence. In *msh* mutants, the defect in G_0 quiescence is less severe than in *dacapo* mutants (in which G_0 quiescence is almost completely abrogated), suggesting that additional transcription factors control *dacapo* expression and G_0 quiescence in NSCs [25]. Indeed *dacapo* expression is also regulated in NSCs by transcription factors expressed at defined axial positions (such as *Hox* genes) or with precise timing in the late embryo (such as the zinc finger transcription factor castor) [39,43].

The expression of dacapo (p21/p27/p57 orthologue) in a subset of *Drosophila* NSCs during mid-embryogenesis, and subsequent induction of G_0 quiescence, are remarkable parallels with p57 function in the mouse V/SVZ [25,36]. In the developing mouse V/SVZ, the cyclin-dependent kinase inhibitor (CKI) domain of p57 is necessary for its pro-quiescence function [36]. However, in addition to their roles as CKIs, p21/p27/p57 are also known to function as transcriptional regulators in NSCs [44–46]. It will be interesting to assess if dacapo induces NSCs to enter G_0 quiescence rather than G_2 quiescence by directly affecting gene transcription. In *Drosophila*, it has also been shown that expression of dacapo alters the lineage structure of embryonic NSCs [43]. *Drosophila* NSCs usually generate one ganglion mother cell (GMC) at each cell division that, in turn, divides once to produce two differentiated progeny. By contrast, dacapo-expressing NSCs generate GMCs that differentiate directly without division. It will be interesting to assess whether p57-expressing mouse NSCs also alter their lineage structure before becoming quiescent in the presumptive V/SVZ.

The developmental origins of quiescent NSCs could be important in understanding the range of progeny that they can produce. In both the mouse SGZ and *Drosophila* central nervous system, NSCs are thought to proliferate during embryogenesis, enter quiescence, then reactivate postnatally, giving rise to similar neuron classes before and after quiescence [33,34,47]. For example, SGZ NSC precursors in the mouse give rise to dentate neurons during embryogenesis, enter quiescence postnatally, and continue to produce dentate neurons upon activation in the adult [33,34]. By contrast, mouse V/SVZ NSCs can give rise to cortical, striatal, or septal neurons in the embryo, but are largely restricted to producing olfactory bulb interneurons in the adult. This striking difference in neuronal output might reflect the fact that, although embryonic and adult V/SVZ NSCs originate in the same brain regions, they are distinct NSC populations. The precursors to adult V/SVZ NSCs are 'set aside' and quiescent from E13.5, and are thought to produce few neurons during embryogenesis, whereas other NSCs – presumably those that produce cortical, striatal, and septal neurons – generate neurons throughout embryogenesis before disappearing in the late embryo [36,37].

Are There Specific Markers for Quiescent NSCs?

To date, no marker is diagnostic of quiescence in rodents, although several genes exhibit preferential transcription in quiescent NSCs compared to active NSCs. Populations of putatively quiescent or active NSCs have been isolated for gene expression profiling using transgenic mice and/or cell-surface epitopes (Box 1). Genes whose expression is enriched in quiescent mouse V/SVZ NSCs include those encoding the transcription factors Sox9 and Id2 [17,48], as well as several quiescence-promoting genes such as *Id3* and the adhesion molecule genes *Vcam1* and *Cdh2* [15,17,48–53]. Some genes whose expression is enriched in quiescent NSCs are also expressed in quiescent stem cells in other tissues, for example, *Klf9* (muscle stem cells) and *Lrig1* (skin and intestinal stem cells), suggesting conserved regulation [15,17]. Quiescence-enriched genes common to V/SVZ and SGZ NSCs include *Id3*, *Id4*, and *Sox9* [48,54–56], whereas others are unique, such as *Hopx* in SGZ NSCs [55,57]. All the above genes require characterisation *in vivo* before they can be used as quiescence markers. For example, the protein products of some genes, including *Id3*, *Vcam1*, and *Hopx*, have been observed in proliferating adult V/SVZ and SGZ NSCs, precluding their use as quiescence markers [50,57,58].

In *Drosophila*, the gene encoding *tribbles* pseudokinase is expressed in G_2 quiescent NSCs and to a lesser extent in G_0 quiescent NSCs, but is not expressed in active NSCs [22]. There might be no pan-quiescent NSC marker, a possibility supported by heterogeneity in the transcriptional profiles and functions of single quiescent NSCs in mice (reviewed in [13]; see also the following section). Recent studies have highlighted important post-translational regulation of NSC quiescence and activation, underscoring the need to investigate beyond the transcriptome [59,60].

Are There Different Types of NSC Quiescence?

An important discovery has been that NSCs undergo different types of quiescence. For example, G_0 and G_2 quiescent NSCs in *Drosophila* have distinct underlying genetic regulation and exhibit asynchronous reactivation timings (Figure 2A,B) [22,25]. In mice, two types of quiescent NSC have been proposed: 'resting' NSCs that have proliferated previously, and 'dormant' NSCs that have not yet proliferated. NSCs alternate between quiescence and activation in the mouse V/SVZ and SGZ [15,60,61]. In the SGZ, the proneural transcription factor Ascl1 (also known as Mash1) is expressed in active, but not quiescent, NSCs, and its expression is necessary for NSCs to proliferate in response to neurogenic stimuli [62]. The E3-ubiquitin ligase Huwe1 targets Ascl1 protein for degradation, thereby putting active SGZ NSCs into quiescence (Figure 3A) [60]. Conditional knockout of *Huwe1* depletes active NSCs by preventing them from becoming quiescent, which leads to their exhaustion over time (Figure 3B) [60]. Interestingly, *Huwe1* knockout does not induce activation (or exhaustion) of already quiescent NSCs. Thus, it is proposed that Huwe1 activity defines two pools of quiescent NSC in the mouse SGZ. Huwe1 enables active NSCs to enter 'resting' quiescence while, by contrast, it is not required to maintain quiescence in 'dormant' NSCs. The extent to which resting and dormant quiescent NSCs differ is not yet known.
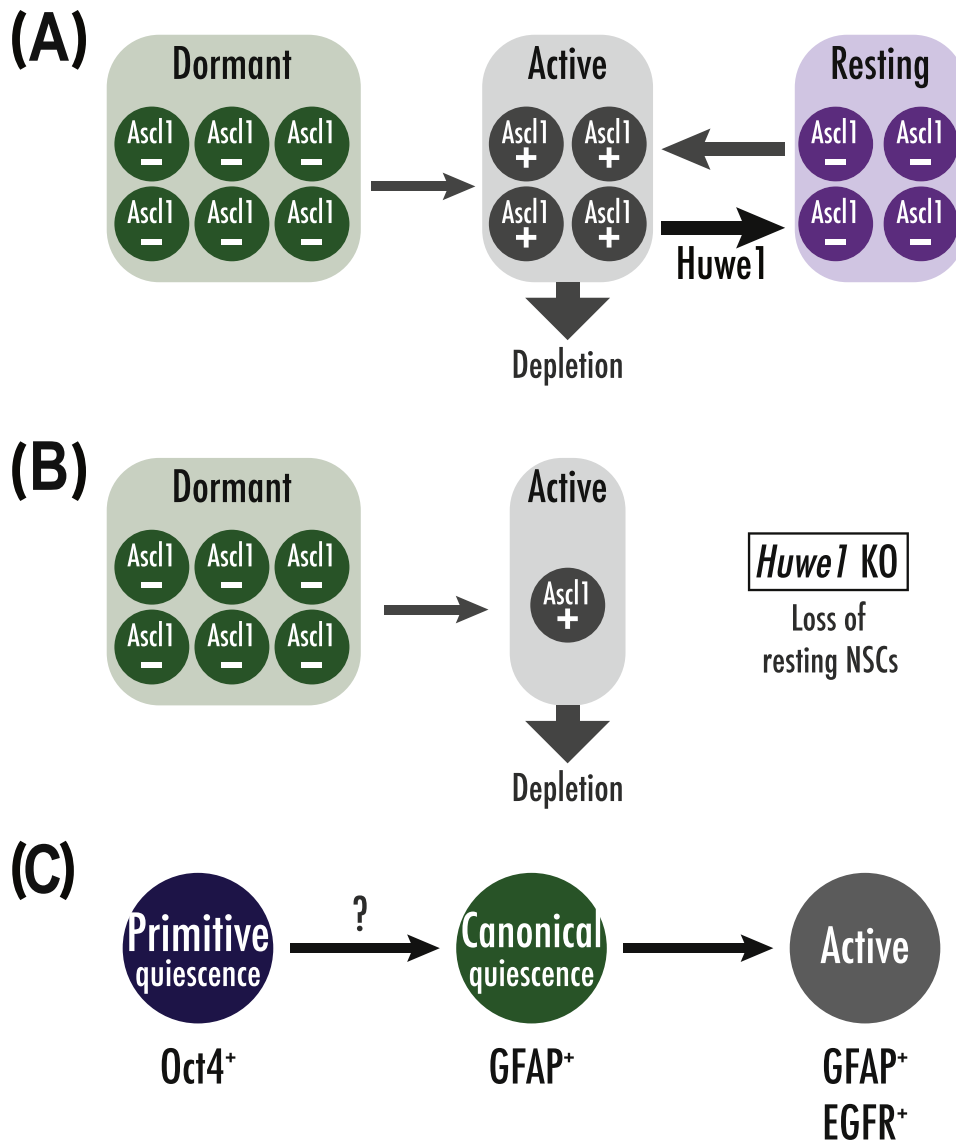
Figure 3. Different Types of Neural Stem Cell (NSC) Quiescence in the Adult Mouse Brain. (A) Two types of quiescent NSCs have been proposed in the adult mouse subgranular zone (SGZ) – 'dormant' and 'resting' [60]. Both dormant and resting NSCs become activated by upregulating Ascl1 protein. Active NSCs have limited self-renewal capacity and deplete over time, whereas quiescent NSCs are longer-lived cells. The E3-ubiquitin ligase Huwe1 can return active NSCs to resting quiescence by targeting Ascl1 protein for degradation [60]. (B) Upon conditional knockout (KO) of *Huwe1*, active NSCs no longer return to resting quiescence and the active NSC pool becomes depleted. Huwe1 is not necessary for dormant NSCs to remain in quiescence [60]. (C) 'Primitive' quiescent NSCs have been proposed in the ventricular/subventricular zone (V/SVZ) [5,63]. Primitive quiescent NSCs do not express GFAP, unlike other known adult NSCs, and instead express low levels of Oct4. After GFAP+ NSCs are ablated, primitive quiescent NSCs are suggested to replenish them *in vivo*, in an Oct4-dependent manner. The lineage relationship and mechanisms connecting primitive quiescent NSCs and 'canonical' quiescent NSCs must be confirmed by clonal analysis.

An additional type of quiescent NSC in the mouse V/SVZ has been proposed – the primitive NSC. Unlike 'canonical' NSCs, primitive NSCs do not express GFAP, express low levels of the pluripotency factor Oct4, and are able to replenish GFAP-expressing NSCs which have been

Box 2. Inferring Quiescence-to-Activation Trajectories from scRNA-Seq Data

The number of single cells sequenced from the adult mouse V/SVZ and SGZ has increased from ~100 cells in initial reports to over 41 000 cells in 2019, facilitated by advances in technology [48,55,65]. Single-cell transcriptomes of quiescent or active NSCs have been obtained through both prospective labelling [51,61] and retrospective identification [34,48,54,55,65,78] (also Box 1). Based on the assumption that NSCs transition from quiescence to activation through relatively gradual transcriptional changes, it is possible to connect scRNA-seq data into a linear trajectory from quiescence to activation, so-called pseudotime reconstruction. Pseudotime relationships can be reconstructed bioinformatically from single-timepoint 'snapshot' data. However, bioinformatic reconstructions cannot substitute for clonal and functional analyses, and their predictions should be carefully confirmed *in vivo*.

ablated *in vivo* (Figure 3C) [5,63]. Based on *in vitro* assays, primitive NSCs divide five to eight times during the lifetime of a mouse, similar to the frequency reported for dormant haematopoietic stem cells [5,64]. The self-renewal and multipotency of primitive NSCs have been demonstrated almost exclusively *in vitro*, and will need to be confirmed *in vivo* using molecular markers and clonal analysis [5,63].

Single-cell RNA-sequencing (scRNA-seq) data have been used to infer that some quiescent NSCs are 'primed' and closer to activation than are other quiescent NSCs. Independent pseudotime reconstructions (Box 2) suggest that quiescent NSCs activate through a stereotyped sequence of transcriptional changes [48,51,55]. First, quiescent NSCs increase transcription of ribosomal subunit genes such as *Rpl32* [48,51,55]. Then, V/SVZ NSCs upregulate a class of genes including *Ascl1* and *Egfr* [65]. Subsequently, cell cycle-promoting genes, such as *Cdk1*, *Ccna2*, and *Mki67*, become transcribed [51,55]. It is suggested that NSCs that express ribosomal subunit genes, but not cell cycle-promoting genes are in a primed quiescent state closer to activation [48]. However, the term 'primed' may be misleading because it implies a functional distinction from other quiescent NSCs that has not yet been demonstrated. In an earlier study, NSCs isolated from the mouse V/SVZ re-entered the cell cycle before expressing EGFR, thus deviating from the activation trajectory described above [66]. It is important to note that several assumptions underlie pseudotime reconstructions. The studies discussed here, with the exception of [34], isolate cells from a single timepoint, and assume that all intermediates from quiescent to activated NSC are present at the same time. A second assumption is that NSCs activate through a gradual and unidirectional series of transcriptional changes. Experimental validation is necessary to test if these assumptions are valid for adult NSCs.

What Are the Stimuli That Activate Quiescent NSCs?

Several environmental stimuli increase adult neurogenesis in the rodent V/SVZ and SGZ. In the SGZ, such stimuli include exercise, exposure to an **enriched environment**, fear conditioning, and kainic acid-induced seizures [67–70]. Stimuli for adult V/SVZ neurogenesis include prolactin (highly expressed during pregnancy and lactation), exercise, and feeding [8,71,72]. Increased neurogenesis could result not only from increased activation of quiescent NSCs but also from other mechanisms, including increased proliferation of already active NSCs and increased survival of newly born neurons. Feeding, exercise, and seizures act by increasing NSC activity at some level, although there are conflicting reports on the underlying mechanisms in the case of exercise [6,7,14,73,74].

How do environmental stimuli trigger NSC proliferation? NSCs extend radial processes and are well placed to interact with their niche – the cellular and acellular stem cell environment. As well as local neurons, glia, and other NSCs, quiescent NSCs receive inputs from systemic blood circulation and, in the case of V/SVZ NSCs, from the cerebrospinal fluid in the lateral ventricles (reviewed in [75]). The mechanisms connecting environmental stimuli, the NSC niche, and increased NSC proliferation are only now beginning to emerge. Nkx2.1-expressing NSCs in the

anterior-ventral V/SVZ are contacted by long-range projections from proopiomelanocortin-expressing (POMC$^+$) neurons in the **hypothalamus** [8]. Feeding increases POMC$^+$ neuron activity, which triggers Nkx2.1-expressing NSCs to proliferate through an undetermined mechanism [8]. NSCs in the SGZ are contacted by granule neurons expressing ephrin B3 and secreted frizzled-like protein 3 (Sfrp3), ligands that maintain NSC quiescence [76,77]. Exercise increases the activity of granule neurons, which downregulate ephrin B3 and Sfrp3 expression and release NSCs from quiescence [77]. A future challenge will be to discriminate environmental effects that activate quiescent NSCs from those that increase the proliferation of already active NSCs. Several investigators have performed scRNA-seq on large numbers of niche cells from the V/SVZ and SGZ, and this could yield insights into how environmental signals are transduced into NSC activation [34,54,65,78].

In *Drosophila*, many of the links between the environment, the NSC niche, and quiescence have been defined. Dietary amino acids are the environmental signal that triggers quiescent NSCs to reactivate [12]. Amino acids are a reactivation-specific signal, and are not merely a basal nutritional requirement because, once activated, NSCs continue to proliferate if amino acids are removed from the diet [12]. Dietary amino acids are sensed by the fat body (an organ performing many of the functions of the mammalian liver and adipose tissue), which sends an unidentified signal to glial cells enwrapping the brain [20]. Upon feeding, these glia, which constitute the **blood–brain barrier**, initiate synchronised calcium oscillations and secrete *Drosophila* insulin/IGF-like peptides (Dilps, specifically Dilp6) that are received by quiescent NSCs residing directly beneath the glia [20,79,80]. Dilps activate the evolutionarily conserved insulin receptor (PI3K/Akt) pathway in quiescent NSCs, and this is necessary and sufficient for reactivation [20,21]. Thus, the blood–brain barrier glia are the key niche for reactivation of quiescent NSCs. Remarkably, genetic activation of PI3K/Akt signalling in quiescent *Drosophila* NSCs is sufficient to induce reactivation in the absence of dietary amino acids (i.e., in the absence of the environmental stimulus) [20,21].

How does PI3K/Akt signalling induce reactivation? Activated Akt downregulates the transcription of *tribbles*, the G_2 quiescence-promoting factor [22]. Also downstream of PI3K/Akt signalling are the spindle matrix protein chromator, members of the STRIPAK complex, and the E3-ubiquitin ligase complex CRL4^{Mahj} [81–83]. The STRIPAK complex and CRL4^{Mahj} inhibit Hippo signalling, a pathway that promotes quiescence by dephosphorylating Hippo kinase and targeting its downstream effector, Wts, for degradation [82–85].

Can Quiescent NSCs Contribute to Brain Repair?

Several injury paradigms, such as stroke and seizures, can induce NSCs to proliferate and generate progeny in adult mice and rats [86,87]. However, the capacity to repair brain function in mammals is poorly understood and modest at best. This is in contrast to regenerative animals that can clearly repair brain function using endogenous NSCs (discussed in the following section).

A key step in designing regenerative therapies will be to induce quiescent NSCs to generate a variety of neurons and glia. In the uninjured adult mouse V/SVZ, quiescent NSCs give rise to progeny in a region-specific manner. For example, NSCs in dorsal regions of the lateral wall give rise primarily to superficial granule interneurons and tyrosine hydroxylase-expressing periglomular cells, whereas those in ventral regions produce deep granule interneurons and calbindin-expressing periglomular cells [88,89]. Location-based fate restrictions arise at least as early as E11.5, persist in NSCs after **heterotypic grafting**, and probably depend on transcription factors expressed in regionally restricted manners, such as *Emx1* (dorsal), *Gsx2* (lateral), and *Nkx2.1* (ventral) [37,88–90]. ScRNA-seq data indicate heterogeneous expression of these transcription

factors in quiescent NSCs, suggesting that fate restrictions are already in place during quiescence, and are not only implemented after activation [48,55].

Importantly, these fate restrictions can, to some extent, be rewritten. Ectopic activation of sonic hedgehog signalling in dorsal V/SVZ NSCs can alter their lineages from producing dorsal progeny (superficial granule neurons) to generating ventral progeny (deep granule neurons) [91]. Knockdown of the E-protein E2-2 in dorsal V/SVZ NSCs increases the production of glutamatergic progenitors at the expense of GABAergic and oligodendrocyte progenitors [90]. SGZ NSCs do not normally generate oligodendrocytes but can be induced to do so through Ascl1 overexpression [92]. Id4 overexpression can redirect SGZ NSCs from producing granule neurons to generating astrocytes [56]. The ability to control the fates of NSC progeny will be an essential step in the development of therapies.

NSC Responses to Injury in Regenerative Vertebrates
Regenerative vertebrates, such as zebrafish and salamanders, restore brain function efficiently following injury and could provide inspiration for strategies to harness quiescent NSCs for brain repair. Salamanders have the highest regenerative capacities among tetrapods and perform remarkable brain repair – for example, the axolotl (*Ambyostoma mexicanum*) telencephalon can regenerate structurally after surgical removal of one third of the tissue [93,94]. As in mammals, quiescent NSCs/progenitors have been defined in adult zebrafish and salamanders by label retention and the rare incorporation of proliferation markers [95–99]. The transcriptional features of quiescence and proliferation have not been mapped as extensively as in the adult rodent brain. Nevertheless, evolutionarily conserved signalling pathways underlie proliferation decisions in adult NSCs in mammals and regenerative vertebrates [97,100].

In the adult zebrafish brain, two NSC/progenitor populations have been described: radial glia and neuroepithelial cells. Almost all regions of the adult zebrafish brain exhibit high levels of proliferation and neurogenesis; however, subsets of radial glia in the pallium, optic tectum, and cerebellum of the adult zebrafish brain are relatively quiescent (rarely dividing) [95–97,101]. Stab-injury paradigms have revealed heterogeneous repair potential among radial glia and neuroepithelial cells. In the pallium, radial glia activate and proliferate to replace lost neurons [102]. Stab injury also activates radial glia in the optic tectum and cerebellum but, in a fascinating twist, they generate no (or few) neurons. In the optic tectum, activated radial glia only generate new radial glia: neuroepithelial cells replace neurons [95]. In the cerebellum, radial glia divide rarely after injury and produce only inhibitory neurons [103]. Neuroepithelial cells in the cerebellum can fully restore granule neurons; however, they cannot replace other neurons such as Purkinje cells [103]. Interestingly, cerebellar radial glia in juvenile zebrafish (<6 months old), but not adult zebrafish, can regenerate Purkinje cells [103]. Age-related changes in progeny production are also seen in adult rodents. The differential responses of radial glia and neuroepithelial cells serve as a reminder that regenerative capacity in mammals, if present, is likely to be restricted and heterogeneous.

The red spotted newt (*Notophthalmus viridescens*), a salamander, has been a valuable model to understand how adult NSCs regenerate specific types of neuron. Adult salamander NSCs are Gfap-expressing ependymoglia lining the brain ventricles [98,99]. Ependymoglia in the adult *Notophthalmus* midbrain are mitotically dormant at steady state, and fewer than three proliferating cells are observed at any time [98]. However, neurotoxin-mediated ablation of midbrain dopaminergic neurons induces quiescent ependymoglia to activate and give rise to dopaminergic neurons, resulting in behavioural recovery within 30 days [98]. Cholinergic neuron ablation instead leads to regeneration of cholinergic neurons [104]. Ependymoglia might restore the correct type

of neuron by responding to feedback regulation. Similarly to adult mouse NSCs, ependymoglia are contacted by neurons. Dopaminergic neurons normally maintain ependymoglia in quiescence through D2 dopamine receptor-mediated signalling. Neuron ablation abolishes this feedback, leading to ependymoglial activation and the production of dopaminergic neurons [104]. Experiments demonstrating neurotransmitter-specific effects on ependymoglia suggest that salamander NSCs are primed towards generating specific neuronal subtypes during regeneration [98,104]. This mirrors the fate restrictions of NSCs in the adult mouse V/SVZ and the zebrafish cerebellum. Understanding the fate restrictions of quiescent NSCs will be crucial in identifying the repertoire of neurons and glia that can be replaced following injury.

What Insights Are Emerging from the Regenerative Vertebrates?

One important question is whether, after injury, a normal complement of cell types can be restored in the brain. After mechanical injury to the dorsal pallium, the axolotl is able to restore a range of neuron subtypes that mature and exhibit electrical activity [105]. However, the regenerated neurons do not perfectly recapitulate the spatial layout of the neurons before injury, and long-range contacts with the olfactory bulb are not fully restored [105]. The impact of these imprecisions on behavioural recovery – the ultimate goal of brain regeneration research – remains to be assessed, but the existing data reveal obstacles to tissue repair even in a highly regenerative animal. Meanwhile, *Notophthalmus* is able to regenerate dopaminergic neurons after chemical ablation to a level supporting recovery of locomotor activity [106]. In a remarkable parallel, the goldfish telencephalon is able to restore dopaminergic neurons (and motor function) after chemical ablation, but cannot repair large physical lesions to the telencephalon [107,108]. This could suggest an injury type-specific response within the same brain region, which must be considered when designing therapies [109]. Profiling genes whose expression increases in salamander NSCs after different types of injury could identify candidates to test for mitogenic or regenerative functions in rodent models. The recent availability of genome sequences and gene-editing technologies in several salamander models will enable much deeper characterisation of quiescent NSCs in these species [110–112].

Concluding Remarks

The adult mammalian brain harbours populations of quiescent NSCs that have significant neurogenic and gliogenic capacities. Several challenges must be overcome before quiescent NSCs can be targeted therapeutically to contribute to brain repair in humans (see Outstanding Questions), hand in hand with practical considerations such as the therapeutic delivery method and interface with the immune system, which we have not discussed here. A first challenge is to identify markers for different types of NSC quiescence, which will help to define the relevant target populations in the human brain. A second is to unravel the mechanisms connecting environmental stimuli to the activation of quiescent NSCs. In *Drosophila*, it is possible to reactivate quiescent NSCs genetically in the absence of an environmental stimulus, a first step towards therapeutic design. A third challenge is that quiescent NSCs, once activated, appear to be transient and exhaust over time. How do regenerative vertebrates maintain sufficient numbers of NSCs to support brain repair throughout life? A fourth is that quiescent NSCs are fate-primed, both during normal physiology in mammals and following brain injury in zebrafish and *Notophthalmus*. Altering the outputs of quiescent NSCs will be essential for replacing the appropriate neurons and glia following injury or disease in human patients.

Outstanding Questions

Transcriptional profiling and clonal analysis suggest that quiescent NSCs in adult rodents are fate-restricted. Similarly, quiescent NSCs in adult zebrafish and salamanders exhibit heterogeneities and restrictions in their contributions to brain repair. What are the epigenetic mechanisms that underlie these properties? Could these properties be altered through transgenesis or gene delivery?

scRNA-seq and pseudotime reconstruction have not been extensively applied to quiescent NSCs except in rodents. How do the activation trajectories of G_0 and G_2 quiescent NSCs compare in *Drosophila*? How similar are the mechanisms underlying steady-state neurogenesis and regenerative neurogenesis in zebrafish and salamanders?

Transcriptional profiling has been instrumental in interrogating quiescent NSCs, but cannot account for post-transcriptional regulation. What are the features of quiescent and proliferating NSCs at the protein level? Conversely, what are the contributions of non-coding genes to quiescence regulation?

What are the distributions and functions of quiescent NSCs in the adult human brain?

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