From early to late neurogenesis: Neural progenitors and the glial niche from a fly's point of view

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Abstract

Drosophila melanogaster is an important model organism used to study the brain development of organisms ranging from insects to mammals. The central nervous system in fruit flies is formed primarily in two waves of neurogenesis, one of which occurs in the embryo and one of which occurs during larval stages. In order to understand neurogenesis, it is important to research the behaviour of progenitor cells that give rise to the neural networks which make up the adult nervous system. This behaviour has been shown to be influenced by different factors including interactions with other cells within the progenitor niche, or local tissue microenvironment. Glial cells form a crucial part of this niche and play an active role in the development of the brain. Although in the early years of neuroscience it was believed that glia were simply scaffolding for neurons and passive components of the nervous system, their importance is nowadays recognized. Recent discoveries in progenitors and niche cells have led to new understandings of how the developing brain shapes its diverse regions. In this review, we attempt to summarize the distinct neural progenitors and glia in the *Drosophila melanogaster* central nervous system, from embryo to late larval stages, and make note of homologous features in mammals. We also outline the recent advances in this field in order to define the impact that glial cells have on progenitor cell niches, and we finally emphasize the importance of communication between glia and progenitor cells for proper brain formation.

KEYWORDS: Drosophila, Central nervous system, Development, Neural progenitors, Glia, Niche.

ABBREVIATIONS: CNS: central nervous system; VZ: ventricular zone; SVZ: subventricular zone; NSCs: neural stem cells; NBs: neuroblasts; NECs: neuroepithelial cells; INPs: intermediate neural progenitors; GMCs: ganglion mother cells; TFs: transcription factors; VNC: ventral nerve cord; CB: central brain; OL: optic lobe.

Introduction

Proper development of an organism's brain is crucial for successful communication between its organs and maintenance of their vital functions (Droujinine and Perrimon, 2016). *Drosophila melanogaster* has been instrumental in uncovering the molecular and cellular basis of fundamental developmental processes in metazoans, with research aided by an extensive range of genetic tools and reagents (Bellen et al., 2010). The fruit fly's transcriptome reveals that co-expression modules which are rich in developmental genes are shared across species (Gerstein et al., 2014), making it suitable for studying many biological principles also relevant for vertebrates (Ugur et al., 2016). Nowadays, studies of neurogenesis are carried out in *Drosophila* from the early embryo until the late larval stage.

Neurogenesis in *Drosophila melanogaster* takes place in two waves. The embryonic phase, when the first wave of neurogenesis takes place, has been the model system where historically the majority of in-depth studies of cell fate specification (e.g., NB5-6 generation of Apterous neurons; Baumgardt et al., 2007; Bivik et al., 2015) and neuronal wiring (e.g., generation of RP1, 2, 3, 4, 5 and their innervation of specific single muscle fibres; Landgraf and Thor, 2006) have been carried out at single-lineage and single-cell resolution. Although it is during embryogenesis that researchers have most effectively studied the development of neural circuits and their versatility, recent research on larval development, when the second wave of neurogenesis takes place, has allowed scientists to gather novel insights into generic lineages that give rise to specialised cell populations (Baek and Mann, 2009; Clark et al., 2016; Couton et al., 2015; Heckscher et al., 2015; Kim et al., 2009).

Neural progenitors are multipotent, and thus can proliferate and differentiate into varied cell types in response to both intrinsic and extrinsic cues (Doe, 2017; Knoblich, 2008; Kohwi and Doe, 2013; Morante et al., 2013; Speder et al., 2011; Syed et al., 2017a). Though more is currently known about intrinsic than extrinsic cues (Paridaen and Huttner, 2014; Tiberi et al., 2012), an understanding of both is extremely important for describing the modulation of brain development within different

stem cell niches. Niches are local tissue microenvironments that maintain and regulate stem cell capacity of cell division. Today, there is no doubt that the microenvironment of stem cells affects their development and behaviour (Lehtinen and Walsh, 2011; Lehtinen et al., 2011; Morrison and Spradling, 2008; Siegenthaler et al., 2009; Siegenthaler and Pleasure, 2011). It is clear that extrinsic cues received by stem cells need to be effectively interpreted to produce the correct intrinsic responses, but little is known about the specifics of these interactions.

In the nervous system, glial cells, like neurons, comprise a significant portion of the brain. However, in the early ages of brain science, glia were thought to serve only as scaffolding for neurons and to play a passive role in the nervous system. Nowadays, in contrast to what was initially believed, it is accepted that glia also play a relevant part in brain formation, and are important not only in structural development but also in controlling stem cell division (Awasaki et al., 2008; DeSalvo et al., 2014; Kanai et al., 2018; Kriegstein and Alvarez-Buylla, 2009; Okamoto and Nishimura, 2015; Ou et al., 2016). However, more knowledge of their interaction with precursor stem cells is needed. Since glia are so vital for brain growth and function, they have been thoroughly studied in organisms from *Drosophila* to vertebrates (Freeman, 2015; Freeman and Doherty, 2006; Hartenstein, 2011; Ou et al., 2014; Zuchero and Barres, 2015). In this review, we cover the interesting insights that the *Drosophila* central nervous system (CNS) has provided via studies carried out in stem cell niches (Banerjee et al., 2017; Berger et al., 2012) with vanguard systems of elementary genetic manipulation (Gratz et al., 2013; Manning and Doe, 2017; Sarov et al., 2016), and we attempt to contextualize the significance of these discoveries in the framework of vertebrates. To do so, we first provide an overview of the elements that constitute the stem cell niche, from embryonic (Figure 1) to larval (Figure 2 and Figure 3) progenitors and glia (Figure 4), and then explain their roles in the microenvironment they share. Finally, we end with the role that the glial niche may play in brain injuries and disease and emphasize the importance of future research on CNS development.

Drosophila neural stem cells

Drosophila neural stem cells (NSCs), known as neuroblasts (NBs), are a useful model for studying the complexity of brain development from youth to adulthood (Clark et al., 2018; Enriquez et al., 2018; Ito et al., 2013; Jefferis et al., 2001; Marin et al., 2002; Sato et al., 2013; Yang et al., 2013; Yu et al., 2013; Figure 1 and Figure 2). Thus, the widely-understood temporal transition in the NBs of the fly brain (Baumgardt et al., 2009; Bayraktar and Doe, 2013; Brody and Odenwald, 2000; Doe, 2017; Grosskortenhaus et al., 2005; Holguera and Desplan, 2018; Kambadur et al., 1998; Li et al., 2013; Maurange, 2012; Sato et al., 2013; Suzuki et al., 2013) has given insight into how similar sequential gene expression occurs in mammalian corticogenesis (Delaunay et al., 2017; Holguera and Desplan, 2018), for instance in the independent cell-cycle progression (Okamoto et al., 2016) or to confer early temporal competence to retinal progenitor cells (Elliott et al., 2008).

NBs undergo distinct modes of cell division to shape different areas of the brain (Figure 1 and Figure 3). The behaviour of progenitors depends on three parameters: first, each cell's identity; second, each cell's intrinsic timer status; and finally, each cell's location, or immediate environment. Regarding the first parameter, for example, the nature of two classical types of fly NBs (type I and type II) has been broadly studied (Doe, 2017). These two subtypes mainly differ on whether or not neurogenesis is mediated by the intermediate neural progenitors (INPs) prior to the production of ganglion mother cells (GMCs; Bello et al., 2008; Boone and Doe, 2008; Bowman et al., 2008; Figure 1A-2 and Figure 3A). In contrast, type 0 NBs produce progeny that directly differentiate into neurons (Ulvklo et al., 2012; Figure 1C-2 and Figure 3B). The second parameter consists of the sequential expression of transcription factors (TFs) in progenitors, a phenomenon known as temporal patterning (Baumgardt et al., 2009; Brody and Odenwald, 2000; Doe, 2017; Grosskortenhaus et al., 2005; Kambadur et al., 1998; Li et al., 2013; Maurange, 2012; Sato et al., 2013; Suzuki et al., 2013), which is important for the generation of the neural diversity required in adults. The final parameter that influences progenitor behaviour is the place, or environment, in which each progenitor resides, as each progenitor is exposed to a varying set of extrinsic cues depending on its location (Ferraro et al., 2010; Morrison and Spradling, 2008). The effect of each NB's environment is evidenced by the fact

that the same NB types may give rise to different neuronal populations depending on where they are located. For example, type 0 NBs have been described in late-embryonic ventral nerve cord (Baumgardt et al., 2014; Figure 1C-1) and larval optic lobe NB lineages (Bertet et al., 2014; Figure 2B-2). Some of the extrinsic cues involved in regulating cell division have been identified, as we will explain later. To make clear the progenitor types and their environments, we have divided them into the three main regions of the *Drosophila* CNS depending their origin: NBs of the central brain (CB; Figure 1A and Figure 2A), which delaminate from the embryonic procephalic neuroectoderm; NBs of the optic lobes (OLs; Figure 1B and Figure 2B), which delaminate from the neuroectoderm during embryogenesis and arise postembryonically via transformation from the OL proliferative neuroepithelium derived from an embryonic optic placode; and NBs of the ventral nerve cord (VNC; Figure 1C and Figure 2C), which delaminate from the embryonic VNC neuroectoderm.

Embryonic progenitors

Systematic studies of *Drosophila* embryonic NBs with single-lineage and single-cell resolution have elucidated the mechanisms controlling the generation of cellular diversity in the developing CNS, providing a detailed map of molecular markers of NBs in the early embryonic brain (Bossing et al., 1996; Doe, 2017; Schmidt et al., 1997; Urbach and Technau, 2004). Knowledge of the embryo also allows for the tracking of development from embryonic to larval stages and thus better contextualizes discoveries made in larvae. In this review, the terms VNC, CB and OL are used to refer to the parts of the embryonic CNS in order to maintain consistency with the terminology used in other stages of development.

The *Drosophila* VNC (Figure 1C), which is analogous to the spinal cord in vertebrates, is a region of the insect CNS in which temporal patterning of NBs was first observed (Baumgardt et al., 2009; Brody and Odenwald, 2000; Doe, 2017; Grosskortenhaus et al., 2005; Kambadur et al., 1998). In the VNC there are 30 NBs per bilateral hemisegment, arranged in rows and columns, which leads to their row/column naming scheme (e.g., NB5-6 is in row 5, column 6; Broadus et al., 1995; Hartenstein et

al., 1994; Figure 1C-1). Most, if not all, VNC NBs begin in type I mode: they undergo several rounds of Notch-dependent asymmetric cell division to produce a self-renewed NB and a series of smaller GMCs that typically differentiate into a pair of neurons (Buescher et al., 1998; Figure 1C-2). Subsequently, many of these type I NBs switch to type 0 mode, and each type 0 NB divides asymmetrically to self-renew and produce one neuron (Baumgardt et al., 2014; Baumgardt et al., 2009; Monedero Cobeta et al., 2017; Ulvklo et al., 2012; Figure 1C-2). This switch is controlled by a temporal cascade, a series of five TFs (Hunchback (Hb), Krüppel (Kr), Pdm1/Pdm2 (Pdm), Castor (Cas) and Grainy head (Grh)) that are expressed sequentially during lineage development. Hb, Kr and Pdm promote type I proliferation and Cas and Grh promote type 0 proliferation and NB cell cycle exit (Bahrampour et al., 2017). The orphan nuclear receptor Seven-up (Svp) and the Pipsqueak domain proteins Dan and Danr promote the Hb to Kr switch by working synergistically to repress Hb transcription (Kanai et al., 2005; Kohwi et al., 2011; Mettler et al., 2006). Temporal factors control the competence of the NB, as demonstrated by the generation of different types of neurons and glia at different time points (Baumgardt et al., 2009; Grosskortenhaus et al., 2005; Isshiki et al., 2001; Moris-Sanz et al., 2014).

As in many species, the *Drosophila* brain undergoes even greater expansion than the VNC throughout development (Yaghmaeian Salmani et al., 2018). Like in the VNC, type I NBs are present in the embryonic CB (Figure 1A-1), around 100 per hemisphere (Urbach et al., 2003; Urbach and Technau, 2004), although they do not switch to type 0 (Figure 1A-2). Recently, type II NBs have also been found to be present (Alvarez and Diaz-Benjumea, 2018; Walsh and Doe, 2017; Figure 1A-1 and Figure 1A-2). Mushroom body (MB) NBs are also found in the embryonic CB (Figure 1A-1), and divide in a mode similar to that of type I NBs, producing neurons via GMCs (Figure 1A-2); they eventually produce the Kenyon cells of the adult MB. Hence, there is a gradient of proliferation in the embryonic brain: there are a dozen hyperproliferative NBs (four MB NBs and eight type II NBs per brain lobe) while the rest of the brain's NBs exhibit exclusively type I behaviour (Alvarez and Diaz-Benjumea, 2018; Walsh and Doe, 2017; Yaghmaeian Salmani et al., 2018).

Although it was previously thought that OL development did not begin until larval stages, it was very recently discovered that in late embryogenesis, neuroepithelial cells (NECs) in the embryonic region that later gives rise to the OL delaminate into canonical NBs called embryonic optic neuroblasts (EONs; Hakes et al., 2018; Figure 1B-1). These EONs generate neurons and glia (Figure 1B-2).

At the end of embryogenesis, most of the remaining embryonic NBs in the entire CNS, except MB NBs (Kunz et al., 2012), enter into a quiescent state or are eliminated by apoptosis (Hakes et al., 2018; Truman and Bate, 1988; White et al., 1994). It is during the larval stage when they exit quiescence in a nutrition-dependent manner to start the second wave of neurogenesis (Chell and Brand, 2010; Lanet et al., 2013; Sousa-Nunes et al., 2011; Speder et al., 2011), generating the more complex CNS required for adult life.

Larval central brain and ventral nerve cord neuroblasts

Unlike in the embryo, the VNC in larval stages consists of only type I NBs (Figure 2C and Figure 3C) while the larval CB has type I, type II, MB and antennal lobe (AL) NBs (Das et al., 2013; Doe, 2017; Homem and Knoblich, 2012; Sousa-Nunes et al., 2010; Figure 2A and Figure 3A). In larvae, type I NB cells, which are reactivated following the embryonic stage, are Deadpan (Dpn)$^{+}$ Asense (Ase)$^{+}$ Pointed 1 (PntP1; Pnt)$^{-}$ and produce GMCs that are Dpn^{-} Prospero (Pros)$^{+}$ (Xie et al., 2016; Zhu et al., 2011). Unlike in the embryo, postembryonic type I NBs do not use Hb, Kr and Pdm to regulate temporal specification. In early larval stages, these NBs express Cas and give rise to a series of early-born neurons expressing the BTB transcription factor Chronologically inappropriate morphogenesis (Chinmo) and later-born neurons expressing Broad (Br; Maurange et al., 2008; Zhu et al., 2006). Svp is re-expressed in larval NBs to trigger this temporal transition from Chinmo to Br expression by terminating Cas expression (Maurange et al., 2008).

As in the embryo, there are eight type II NBs per brain lobe (Figure 2A): six dorsal medial (DM1–6) and two dorsal lateral (DL1 and DL2). Type II NBs have a distinct molecular profile, which is Dpn^{+} Ase^{-}

PntP1^{+} (Doe, 2017; Homem and Knoblich, 2012; Sousa-Nunes et al., 2010). These NBs also temporally express a series of factors including Dichaete (D), Cas and Svp (Bayraktar and Doe, 2013). As with neural progenitors in the human outer subventricular zone (SVZ; (Doe, 2017; Fernandez et al., 2016; Homem and Knoblich, 2012), type II NBs asymmetrically divide to generate INPs with the molecular profile Dpn^{+} Ase^{+}. These INPs also sequentially express the TFs D, Grh and Eyeless (Ey) in order to each generate approximately six GMCs that are Dpn^{-} Ase^{+}, which then differentiate into distinct neural cell types (Bayraktar and Doe, 2013; Bello et al., 2008; Boone and Doe, 2008; Bowman et al., 2008; Izergina et al., 2009; Zhu et al., 2011; Figure 3A). Together, these expression patterns in NBs and INPs increase the neural diversity in the adult fruit fly CNS. The aforementioned factors, along with novel candidate temporal factors expressed by larval NBs (Ren et al., 2017; Syed et al., 2017b) including the Ecdysone receptor (Syed et al., 2017b), were identified via the use of unbiased transcriptomic approaches. Further analysis will reveal the role of those potential novel temporal factors.

Also located in the larval CB are four MB NBs per hemisphere (Figure 2A), which emerge during the embryonic period and continuously divide during development until early adult stages without entering into quiescence (Kunz et al., 2012; Lee et al., 1999). Each give rise to three neuronal lineages, which together generate the adult centres for olfactory associative learning and cognitive functions (Lee et al., 1999). The cell division mode of MBs is similar to that of type I NBs: they produce MB neurons via GMCs (Figure 3A). Based on the age of the larva, two RNA-binding proteins, IGF-II mRNA-binding protein (Imp) and Syncrip (Syp), display opposing temporal gradients: young NBs have high Imp and low Syp expression, middle-aged NBs express both, and old NBs have low Imp and high Syp expression (Liu et al., 2015). The expression of these proteins thus controls mushroom body lineages by regulating translation of the temporal transcription factor Chinmo (Liu et al., 2015; Narbonne-Reveau et al., 2016).

The ALs are an important part of the olfactory system in *Drosophila*. The functioning olfactory system in larvae is generated in the embryo (Marin et al., 2005; Yu et al., 2010), although knowledge of which specific embryonic progenitor cells give rise to the olfactory system is limited. In larvae, the system consists of 21 olfactory sensory neurons located in the antenno-maxillary complex which then form 21 subunits called glomeruli consisting of 21 projections and 21 local interneurons (Ramaekers et al., 2005). In each of the two larval ALs there are five NBs (Figure 2A), which are defined as anterodorsal (adNB/BAmv3), lateral (lNB/BAlc), ventral (vNB/BAla1), ventrolateral (vlNB/BAla2) and BAlp4 (Das et al., 2013). These AL NBs found in larvae give rise to the adult olfactory system, which is much more complex. Larval AL NBs undergo proliferation and can generate approximately 40 neuron types from a single hemilineage (Yu et al., 2010). GMCs descended from these larval AL NBs (Figure 3A) make daughter cells with distinct neuronal A/B fates due to a Notch-mediated binary sister fate decision. Notably, many CNS lineages exist as a lone hemilineage because one entire hemilineage may undergo premature cell death as a result of this binary decision (Lin et al., 2012; Lin et al., 2010; Yu et al., 2010). Using this neurogenic strategy, during larval stages AL NBs generate approximately 200 adult olfactory projection neurons and 100 local interneurons (Das et al., 2013; Das et al., 2008; Jefferis et al., 2001; Lai et al., 2008; Yu et al., 2013). AL NBs end proliferation around pupation. Within each adult AL, approximately 1300 olfactory receptor neurons expressing the same receptors make connections to two types of interneurons, projection neurons and local interneurons, forming approximately 50 glomeruli (Vosshall et al., 2000). Projection neurons extend their axons to higher brain centres in the MB and lateral horn (Jefferis et al., 2001). The behaviour of developmental progenitors in the OL is more complex, and will be discussed next.

Larval optic lobe progenitors

The OL is the largest part of the *Drosophila* brain; it has four ganglia and is dedicated solely to vision, which reveals the importance of this sense for adult flies (Morante and Desplan, 2004). Neurons of

the mature OL are produced during the larval and early pupal periods (Hofbauer and Campos-Ortega, 1990).

Larval NECs delaminate into 800 OL NBs, which asymmetrically divide to self-renew and start neurogenesis and gliogenesis (Figure 2B and Figure 3B; Egger et al., 2007; Yasugi et al., 2008). This process resembles the behaviour of progenitors in the mammalian forebrain, which first divide symmetrically and then later divide asymmetrically to produce NSCs (Brand and Livesey, 2011; Gotz and Huttner, 2005; Kriegstein and Alvarez-Buylla, 2009). This region was thought to be unlike the rest of the fruit fly CNS, with most of the OL NBs transforming from NECs beginning at the second-instar larval stage (Ceron et al., 2001; Egger et al., 2007). However, as previously discussed in the section "Embryonic progenitors", a recent study has challenged this view (Hakes et al., 2018), showing that embryonic OL NECs start to produce EONs during embryonic development (Figure 1B).

Characterization by molecular markers and morphology indicates that the four fly OL ganglia originate from two distinct neuroepithelial regions (Figure 2): the outer proliferation centre (OPC; Figure 2B-1 and Figure 2B-2), which gives rise to the medulla and lamina neuropils, and the inner proliferation centre (IPC; Figure 2B-3), which produces the lobula and lobula plate neurons (Apitz and Salecker, 2015; Hasegawa et al., 2011; Hofbauer and Campos-Ortega, 1990; Li et al., 2013; Meinertzhagen and Hanson, 1993; Mora et al., 2018; Morante and Desplan, 2004, 2011; Morante et al., 2011; Pinto-Teixeira et al., 2018). Although they originate in different parts of the brain, these neuron types have been found to interact with one another in order to establish the various neuropil compartments (Suzuki et al., 2016).

The OPC neuroepithelium (Figure 2B-1 and Figure 2B-2) is patterned into spatial domains along the anterior-posterior axis by expression of Visual system homeobox 1 (Vsx1), Optix, Decapentaplegic (Dpp) and Wingless (Wg; Erclik et al., 2008; Erclik et al., 2017; Gold and Brand, 2014). This region can also be subdivided morphologically into the medial domain (m-OPC) and the lateral domain (l-OPC), which are separated by the lamina furrow (LF; Figure 2B-1). Differentiation of NBs from NECs in the

m-OPC and l-OPC is controlled at first by Notch, which maintains NECs, and then by JAK/STAT, which negatively regulates proneural wave progression; both Notch and JAK/STAT thus impede the switch from symmetric to asymmetric cell division (Egger et al., 2010; Ngo et al., 2010; Reddy et al., 2010; Yasugi et al., 2010; Yasugi et al., 2008). Later in development, Notch signalling remains active only in the m-OPC, which eventually gives rise to medulla neurons, while JAK/STAT signalling remains active only in the l-OPC, which gives rise to lamina neurons (Ngo et al., 2010). Apart from the ganglia they generate, these regions also differ in their distinct forms of neurogenesis, which we will discuss next.

In larval development in the m-OPC, dietary nutrients trigger insulin/PI3K/TOR signalling to regulate NEC proliferation (Lanet et al., 2013). The Fat-Hippo pathway also plays a role in regulation of growth through proliferation of NECs, and later arrests these cells in the G1 phase of the cell cycle before their conversion to NBs (Reddy et al., 2010). In late larval stages, proliferation becomes insensitive to dietary nutrients and the steroid hormone Ecdysone becomes active. Ecdysone acts via Delta/Notch signalling to initiate the shift from symmetric/proliferative cell division to asymmetric/neurogenerative cell division (Lanet et al., 2013). Expression of the proneural protein Lethal-of-scute (L(1)sc), which is induced by epidermal growth factor (EGF) receptor signalling, also promotes the conversion of NECs to NBs (Egger et al., 2010; Morante et al., 2013; Yasugi et al., 2010; Yasugi et al., 2008; Figure 2B-1). The microRNA miR-7, along with other factors, is important for buffering this transition (Caygill and Brand, 2017). In type I NBs, temporal expression of six different TFs (Homothorax (Hth), Klumpfuss (Klu), Ey, Sloppy-paired (Slp), D and Tailless (Tll)) acts in tandem with Notch-dependent binary fate choice to control differentiation of the neuronal progeny (Doe, 2017; Li et al., 2013; Suzuki et al., 2013; Figure 3B). Overall, the integration of this temporal signalling with spatial cues is essential for generating neural diversity (Erclik et al., 2017).

In the m-OPC, there is a particular area located in the tip of the OPC (tip-OPC; Figure 2B-2) in which NB behaviour depends on two temporal windows. First, when the TF Distalless (Dll) is expressed, NBs divide in type 0 mode; type 0 NBs were originally described in the embryo and are the only

postembryonic NBs which give rise to neuron progeny directly without further cell divisions (Bertet et al., 2014; Chen et al., 2016; Figure 3B). Later, when the TFs Ey, Slp and D are expressed, these NBs switch to type I division mode, though half of their progeny is eliminated via apoptosis. This temporal expression of TFs contributes to the generation of diverse neural subtypes in the tip-OPC that innervate the medulla, lobula and lobula plate in adult flies (Bertet et al., 2014); Figure 3B).

Another type of progenitor, common progenitor cells (CPCs), delaminate from NECs of the tip-OPC (Chen et al., 2016; Figure 2B-2). CPCs do not express the canonical NB marker Dpn but do express Pros and Ase, and therefore more closely resemble GMCs. CPCs divide asymmetrically to generate two distinct precursor cell populations: lamina wide-field precursor cells (Lawf PCs) and glial precursor cells (GPCs; Figure 2B-2 and Figure 3B). Each one of the latter two precursor types divide symmetrically and respectively generate Lawf neurons and lamina glia (Chen et al., 2016; Fischbach and Dittrich, 1989; Hasegawa et al., 2011; Winberg et al., 1992; Figure 3B). This neurogliogenesis fate choice mediated by the Notch pathway resembles in many ways the role that Notch signalling plays in vertebrates in establishing neurogenic or gliogenic phases during development (Gaiano and Fishell, 2002; Rowitch and Kriegstein, 2010; Taylor et al., 2007). Finally, after the precursor cells differentiate into glia and Lawf neurons, both these cell types migrate to their position in the medulla (Chen et al., 2016).

NECs in the l-OPC (Figure 2B-1) generate non-NB cells in the G2-phase, which divide symmetrically to produce lamina neurons (Figure 3B). These posterior LF progenitors, called lamina precursor cells (LPC; Figure 2B-1), proliferate and differentiate in a manner regulated by sequential release of secreted factors by photoreceptors (PRs). First, PRs release Hedgehog to promote terminal divisions of NECs, and second they release Spitz/TGF-α and Fibroblast growth factor (FGF) which respectively activate EGF receptor and FGF receptor in relay wrapping glia (Fernandes et al., 2017; Franzdottir et al., 2009; Huang and Kunes, 1996, 1998; Huang et al., 1998; Selleck and Steller, 1991). While EGF receptor activation is required for lamina neuron differentiation via insulin signalling (Fernandes et

al., 2017), FGF receptor signalling is required to induce glial cell differentiation such that glia wrap around photoreceptor axons and enter the lamina (Franzdottir et al., 2009).

The other proliferation centre, the IPC (Figure 2), consists of three domains, which are defined based on their location as surface, proximal and distal IPC (s-IPC, p-IPC and d-IPC, respectively; Apitz and Salecker, 2015). At the same time, the cells of the IPC belong to two proliferative zones: in the p-IPC region NECs divide symmetrically while the d-IPC is composed of NBs derived from the p-IPC cells (Figure 2B-3). Specifically, NECs in the p-IPC express either Dpp or Brinker (Brk), a negative regulator of Dpp target genes, which determines whether the NECs will give rise to either vertical or horizontal motion selective neurons (Apitz and Salecker, 2015, 2018; Mora et al., 2018; Pinto-Teixeira et al., 2018).

The IPC NECs then delaminate into migrating progenitors (MPs), which move from the p-IPC to the d-IPC (Apitz and Salecker, 2015; Figure 2B-3 and Figure 3B). This mechanism, which resembles the epithelial-mesenchymal transition, is required for facilitating spatio-temporal matching of neurogenesis and neuronal connectivity, and is similar to the migration of neurogenic radial glia-like cells from the meninges to the neonatal cortex of mice that then differentiate into functional integrated neurons (Bifari et al., 2017). Once MPs reach the d-IPC, they differentiate into NBs (Figure 2B-3 and Figure 3B) that sequentially express D and Ase and then Tll, Ato and Dac. These NBs first go through type I cell division orchestrated by Ase TFs to produce C- and T-neurons (Apitz and Salecker, 2015). When the NBs enter to the Ato temporal window they exclusively produce T4 and T5 neurons which respectively detect bright edge and dark edge motion (Apitz and Salecker, 2015; Oliva et al., 2014). Two alternative and likely coexisting models have been proposed for the production of T neurons in the second competence window. In the first model, Ato^{+} NBs generated by either a horizontal or vertical progenitor undergo two sequential Notch-dependent divisions to produce matching sets of two T4 and two T5 neurons (Apitz and Salecker, 2018; Pinto-Teixeira et al., 2018). In the second model, a transient amplification of d-IPC NBs by symmetric cell division ensures that the

correct number of T4 and T5 neurons is produced (Mora et al., 2018); the temporal transition in proneural protein expression from Ase to Ato regulates the timing of the onset of this NB transient amplification. Although symmetric cell division in NBs has been previously described in mammals (Obernier et al., 2018), this is the first time it has been seen in *Drosophila*. Thus, these symmetrically dividing cells are categorised as type III NBs due to their initial self-propagation and subsequent loss of multipotency (Mora et al., 2018; Figure 2B-3 and Figure 3B).

Glial cells in the *Drosophila* CNS

Glial cells in *Drosophila* are genetically similar to glia in mammals, and express many TF homologs such as the gene glial cells missing (gcm) which is responsible for glial cell fate (Freeman et al., 2003; Freeman and Doherty, 2006; Hosoya et al., 1995; Jones et al., 1995; Van De Bor and Giangrande, 2002). In the fruit fly, glial cells are derived from a small set of uniquely identifiable precursors (Figure 3). Glioblasts and neuroglioblasts, which are respectively named according to whether they give rise to only glia or both neurons and glia, delaminate from the neuroectoderm of the post-gastrulation embryo (Beckervordersandforth et al., 2008; Stork et al., 2012; von Hilchen et al., 2008). These cells increase in number at the third instar larval stage due to two mechanisms: division of differentiated glial cells (Colonques et al., 2007) and continued division of neuroglioblasts (Larsen et al., 2009; Omoto et al., 2015; Ren et al., 2017; Viktorin et al., 2013). Finally, the glial diversity in the adult CNS depends on embryonic and larval temporal patterning, and it is thus crucial to study glial development in order to fully understand the structure of the adult brain (Awasaki et al., 2008).

The embryonic *Drosophila* VNC contains around 25–30 glia per hemisegment. These glia can be divided into three main categories according to their location and morphology. The first category is surface glia (SG), made up of perineurial glia (PG) and subperineurial glia (SPG), which surround the CNS and peripheral nerves and contribute to the formation of the blood–brain barrier (BBB). The second category is cortex glia (CG), which ensheath neuronal cell bodies. The third category is neuropil glia (NG), which associate directly with the neuropil (Hartenstein et al., 1998; Ito et al.,

1995; Pereanu et al., 2005). Apart from these three categories, midline glia are a small set of glia also present in the embryonic CNS; they play an important role in axon pathfinding at the CNS midline and commissure formation in the VNC (Jacobs, 2000). Midline glia later differentiate as ensheathing glia (Jacobs, 2000).

Though in past years there has been a significant effort to characterise glial cells in the larval *Drosophila* CNS (Figure 4), it has proved difficult (Awasaki and Lee, 2011); this has resulted in varied and broad terminology used to describe the diverse population of glia. Here, we categorize larval glia into the same three main groups as embryonic glia; these classes are defined by location, characteristics and function (Freeman, 2015; Hartenstein, 2011; Ou et al., 2014; Stork et al., 2012).

The first group, located in the outer part of the brain, is the SG; this glial type wraps the entire CNS (Figure 4) and acts as a gatekeeper regulating entry into the brain for particles carried within the hemolymph (the fluid analogous to blood in insects). In larvae as in the embryo, this cell population can be divided into PG, the first physical and chemical brain barrier for large particles, and SPG, which communicate amongst themselves via septate junctions and function as an evolutionarily conserved BBB (DeSalvo et al., 2011; Stork et al., 2008). SPG are also in contact with other glial subtypes through adherents or gap junctions (DeSalvo et al., 2011). Hundreds of PG are found per hemisphere and they are smaller in size and have an elongated cell body, while SPG are large with flattened nuclei and are very low in number, around twenty cells per larval hemisphere (Pereanu et al., 2005; Figure 4A and Figure 4B). Ultimately, SG serve as an important mediator between the exterior environment and the interior of the brain (DeSalvo et al., 2011).

A second main glial type, CG, is located underneath the SPG in the larval OLs and is characterised by the expression of the conserved microRNA miR-8 (Morante et al., 2013). In each OL approximately 140 miR-8^{+} CG cells enwrap NECs, NBs, GMCs and neurons (Morante et al., 2013; Figure 4B). In the VNC and CB (Figure 4A and Figure 4C), CG also ensheathes NBs and their progeny and are required

for regulating NB proliferation as well as maintaining energy homeostasis (Chell and Brand, 2010; Sousa-Nunes et al., 2011; Speder et al., 2011; Volkenhoff et al., 2015).

A third main glial type, NG, is located between the cortex and the neuropil in the VNC and CB. This glial cell population is the most diverse due to its variety of structures and expression of molecular markers (Pereanu et al., 2005). Although these cells can be named in many different manners depending on their location, they are mainly divided into two subtypes: ensheathing glia (EG) and astrocyte-like glia (ALG; Figure 4A and Figure 4C). EG surround parts of the neuropil whilst ALG manage neural remodelling through axon pruning and synaptic formation (Freeman, 2015; Peco et al., 2016; Tasdemir-Yilmaz and Freeman, 2014; Wu et al., 2017).

In the complexity of the OL, it is possible to distinguish different glial subtypes (Chotard and Salecker, 2007; Huang and Kunes, 1996; Figure 4B). A CG subtype, satellite glia (sg), ensheathes lamina neuron cell bodies. Two subtypes of NG, epithelial glia (eg) and marginal glia (mg), originate in GPC regions and share lineage with lawf neurons (Chen et al., 2016). Later on, both of these subtypes migrate respectively to above (eg) and below (mg) the lamina plexus, into the area where axons of PRs project from the eye imaginal discs (Chotard and Salecker, 2007). Other subtypes of NG are the medulla glia (meg), which are found in the first optic chiasm though their origin is uncertain, and the medulla neuropil glia (mng), which are mainly generated from neuroglioblasts of the OPC and migrate to the anterior cortex-neuropil interface (Li et al., 2013; Richier et al., 2017). Therefore, the wide variety of glial functions, the presence of glia across the entire CNS, and the complexity of glial development strongly suggest that glial cells play an essential role in the brain's regulation and are evolutionarily conserved across species.

Glial-neural progenitor interactions in the developing *Drosophila* brain

In recent years, glial cells have been found to play important roles in microenvironments where they can act directly on different biological processes. In adult mammals, the SVZ, where neurogenesis is

maintained, receives external BBB inputs from diffusible morphogens, the blood or the cerebrospinal fluid (Dani and Lehtinen, 2016; Silva-Vargas et al., 2016). Similarly, in the larval insect brain, the BBB function of the SG layer highlights its importance as a member of a NSC microenvironment (Otsuki and Brand, 2017). SPG and CG cells are known to have large nuclei, as previously mentioned, and also are polyploid; this feature is required to maintain the integrity of the BBB as well as proper growth of SPG cells and the brain in general (Morante et al., 2013; Unhavaithaya and Orr-Weaver, 2012). Additionally, PG cells have been found to be involved in microenvironment signalling. Studies reveal that this SG subtype secretes the signal Dally-like (Dlp), a heparan sulfate proteoglycan, which interacts with NBs and acts as a key cue for brain development (Kanai et al., 2018). In return, NBs express Glass bottom boat (Gbb), a BMP homologue, which acts as a survival signal for PG cells as well as an autocrine signal for NBs (Kanai et al., 2018). These recent discoveries suggest that there is still more to be learned about the role of glia in the important processes of development.

Glial cells also act in the larval VNC and CB to reactivate quiescent embryological NBs, providing the source of *Drosophila* insulin-like peptides (dILPs) relevant for timely reactivation of NBs in response to nutrition (Chell and Brand, 2010; Lanet et al., 2013; Sousa-Nunes et al., 2011). As discovered recently, a VNC NB in the G2 cell cycle phase can enter into quiescence when induced by the evolutionarily conserved pseudokinase Tribbles (Otsuki and Brand, 2018), in contrast to the previously accepted belief that NBs could only arrest at G0. These arrested G2 cells can exit quiescence in the presence of dILP signalling originating from BBB glia, and these cells reactivate and regenerate neurons more quickly than arrested G0 NBs; this shows that the stage in which NSCs arrest affects the timing of their reactivation in response to external signals from the glial niche (Otsuki and Brand, 2018). Similarly, it has also been found that dILPs act in the *Drosophila* visual system as part of a signalling cascade involved in retinotopy. PRs communicate with lamina neurons by producing Spitz/TGF-α (Fernandes et al., 2017; Huang et al., 1998). PR Spitz/TGF-α then acts on wrapping glia, which are part of the peripheral nervous system and in turn produce dILPs which induce LPCs to differentiate into lamina neurons. The role of glia in this essential process

demonstrates their importance in regulating spatiotemporal differentiation patterns across distinct brain regions (Fernandes et al., 2017; Rossi and Fernandes, 2018). Altogether, these conclusions reinforce the idea that glia have a decisive role in the precursor niche.

NECs are another type of progenitor in which it has been shown that glial ligand expression can significantly influence the development of the OL in the larval brain. Some examples of this impact on the regulation of NEC proliferation are, for example, the non-autonomous effect that the transmembrane protein Serrate from SPG produces in the Notch pathway (Perez-Gomez et al., 2013) and the influence that the release of Spitz/TGF-α from CG has on the EGF receptor pathway in NECs (Morante et al., 2013).

In addition to glia, NSCs can also send retrograde signals to glia and these local and systemic signals induce the remodelling of CG and help adapt the niche to their needs (Speder and Brand, 2018). The first step of this signalling cascade is the activation of the PI3k/Akt pathway in NSCs via nutritional cues, and this activation causes cortex glia to expand their membrane processes and begin to encase the NSCs. Then, NSCs exit quiescence and resume proliferation, which sends the signal to glial cells to alter the niche according to what is required to restart neurogenesis (Speder and Brand, 2018). In all, recent discoveries have helped elucidate the specific impacts that glial cells may have on neural stem cell niches. However, taking into consideration that glia have an active role in NSC niches, both glia-to-progenitor and progenitor-to-glia signalling must be further studied.

The niche in brain injuries and future perspectives

As previously explained, temporal patterning is an essential mechanism for the development of the brain into such a complex organ. Dysregulation of the TFs important for the transition of neuronal precursors to neurons can lead to brain diseases including malignant tumour formation and loss of neural and glial identity (Maurange and Gould, 2005; Narbonne-Reveau et al., 2016). Additionally,

defects in the cell polarity of neuroepithelial progenitors may have a direct impact on their offspring and may be involved in tumorigenesis (McCaffrey and Macara, 2011).

Glial cells not only are required for regulating NB proliferation (Chell and Brand, 2010; Lanet et al., 2013; Sousa-Nunes et al., 2011) but also protect NB proliferation in conditions of hypoxia and oxidative stress (Bailey et al., 2015). Dysfunction in glia and their non-autonomous effect on stem cell niches can cause severe problems in the *Drosophila* brain. It has recently been shown that a glial lineage-specific WDR62 depletion, the second most commonly mutated gene in primary microcephaly, significantly decreased brain volume (Lim et al., 2017). These examples show the importance of relevant function of glia within the stem cell niche.

In conclusion, although a significant amount is already known about progenitor niches and the functions of glial cells in the regulation of neural development, there remains much that has yet to be discovered and more research is required in this field. Scientists should take the opportunity to investigate not only development of each individual element of the stem cell niche but also interactions between the elements, examining the effects that one dysfunctional cell type may have on the other and consequently on the brain as a whole. By combining recent discoveries in *Drosophila melanogaster*, a powerful model organism, with new studies in mammals, researchers may soon be able to more fully describe the roles of each progenitor type in neurogenesis.

FIGURE LEGENDS:

Figure 1. Embryonic Neural Progenitors, Cell Division Modes and Progeny in *Drosophila melanogaster* CNS.

Diagram of neural progenitors and cell division modes described in the CNS of the late embryonic fruit fly. Progenitors and cell division modes are organised by CNS region: **(A)** the central brain (CB), framed in red; **(B)** the optic lobe (OL), framed in blue; and **(C)** the ventral nerve cord (VNC), framed in green. **(A1)** NBs of the CB: type I, type II (eight cells per hemisphere), and mushroom body (MB) NBs (four cells per hemisphere). **(B1)**

Neuroepithelial cells (NECs) and embryonic optic neuroblasts (EONs) in the OL. **(C1)** NBs of the VNC: type I and type 0. **(A2), (B2)** and **(C2)** show cell division modes in each CNS region of the embryo. In **(B2)**, NECs divide to self-renew before transforming into EONs. GMC: ganglion mother cell, INP: intermediate neural progenitor, n: neuron cell, g: glia cell, NB: neuroblast. The axes indicate A-P (anterior-posterior) and D-V (dorsal-ventral).

Figure 2. Neural Progenitors in Larval CNS in *Drosophila melanogaster*.

Diagram of neural progenitors described in CNS of late larval fruit fly. **(A)** Circled in red, the four types of NBs located in the central brain: type I, type II (eight cells per hemisphere), mushroom body (MB) NBs (four cells per hemisphere), and antennal lobe (AL) NBs (five cells per hemisphere). **(B)** Circled in blue are the different types of progenitor cells which delaminate from neuroepithelial cells (NECs) of the optic lobe. **(B1)** shows the NECs of the outer proliferation centre (OPC), which delaminate into type I NBs and lamina precursor cells (LPCs). **(B2)** shows type 0 NBs of the tip-OPC and common progenitor cells (CPCs), which give rise to glial precursor cells (GPCs) and lamina wide-field precursor cells (Lawf PCs). This section of the figure is shown from a different angle. **(B3)** shows the NECs of the inner proliferation centre (IPC) that delaminate into migrating progenitors (MPs), which differentiate first into type I and finally into type III NBs. **(C)** Circled in green, the only NB type (type I) found in the ventral nerve cord. All views are frontal except for **(B2)**, which is seen from a dorsal perspective (for further points of view see figures in Ngo et al. 2017). Note that most of the NBs of the larval CNS originate during the first wave of neurogenesis in the embryonic stage. LF: lamina furrow, m-OPC: medial-OPC, l-OPC: lateral-OPC, p-IPC: proximal-IPC, d-IPC: distal-IPC. The axes indicate A-P (anterior-posterior), D-V (dorsal-ventral) and L-M (lateral-medial).

Figure 3. Neural Progenitor Cell Division Modes and Progeny in Larval CNS in *Drosophila melanogaster*.

Representation of all types of NBs (type 0, I, II and III) and cell progenitors described in the CNS of the late larval fruit fly. The illustration indicates the type of cell division taking place (asymmetric or symmetric) by showing the offspring that each division generates. The progenitors are organized by the CNS region in which they are located: **(A)** the central brain (CB), framed in red; **(B)** the optic lobe (OL), framed in blue; and **(C)** the ventral nerve cord (VNC), framed in green. In **(B)**, NECs divide to self-renew before transforming into progenitor cells. It is important to note that most NBs originate in the first wave of neurogenesis in the embryo. GMC: ganglion mother cell, INP: intermediate neural progenitor, n: neuron cell, g: glia cell, NB: neuroblast, MB:

mushroom body, AL: antennal lobe, MP: migrating progenitor, LPC: lamina precursor cell, CPC: common progenitor cell, GPC: glia precursor cell, Lawf PC: lamina wide-field precursor cell, NEC: neuroepithelial cell.

Figure 4. Glial Cell Types in Larval CNS of *Drosophila melanogaster*.

Diagram of distinct glial cell types found in the CNS of the late larval fruit fly. **(A)** The types of glia found in the central brain, circled in red. The outer surface glia layers are classified as perineural glia (PG), subperineural glia (SPG) and miR8^{+} surface-associated cortex glia (CG), and the neuropil glia (NG) are categorized as ensheathing glia (EG) and astrocyte-like glia (ALG). **(B)** The optic lobe, circled in blue. PG and SPG are the most external layers of glia, with the miR8^{+} CG beneath them. Underneath those layers are a CG subtype, satellite glia (sg), and various NG subtypes: epithelial glia (eg), marginal glia (mg), medulla glia (meg) and medulla neuropil glia (mng). **(C)** Glia in the ventral nerve cord, circled in green. From the outside inward: PG, SPG, CG, EG and ALG. The axes indicate A-P (anterior-posterior) and L-M (lateral-medial).

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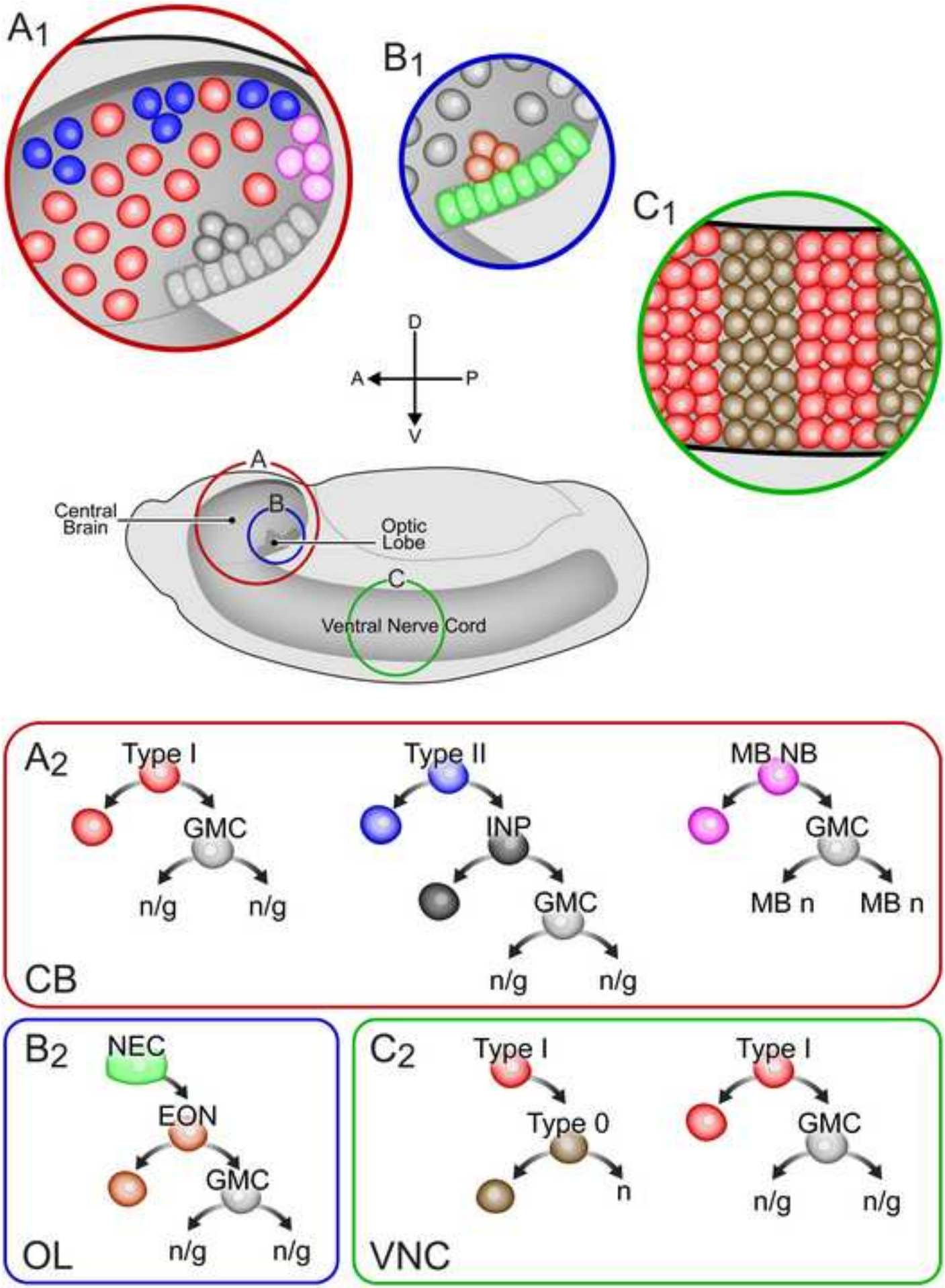

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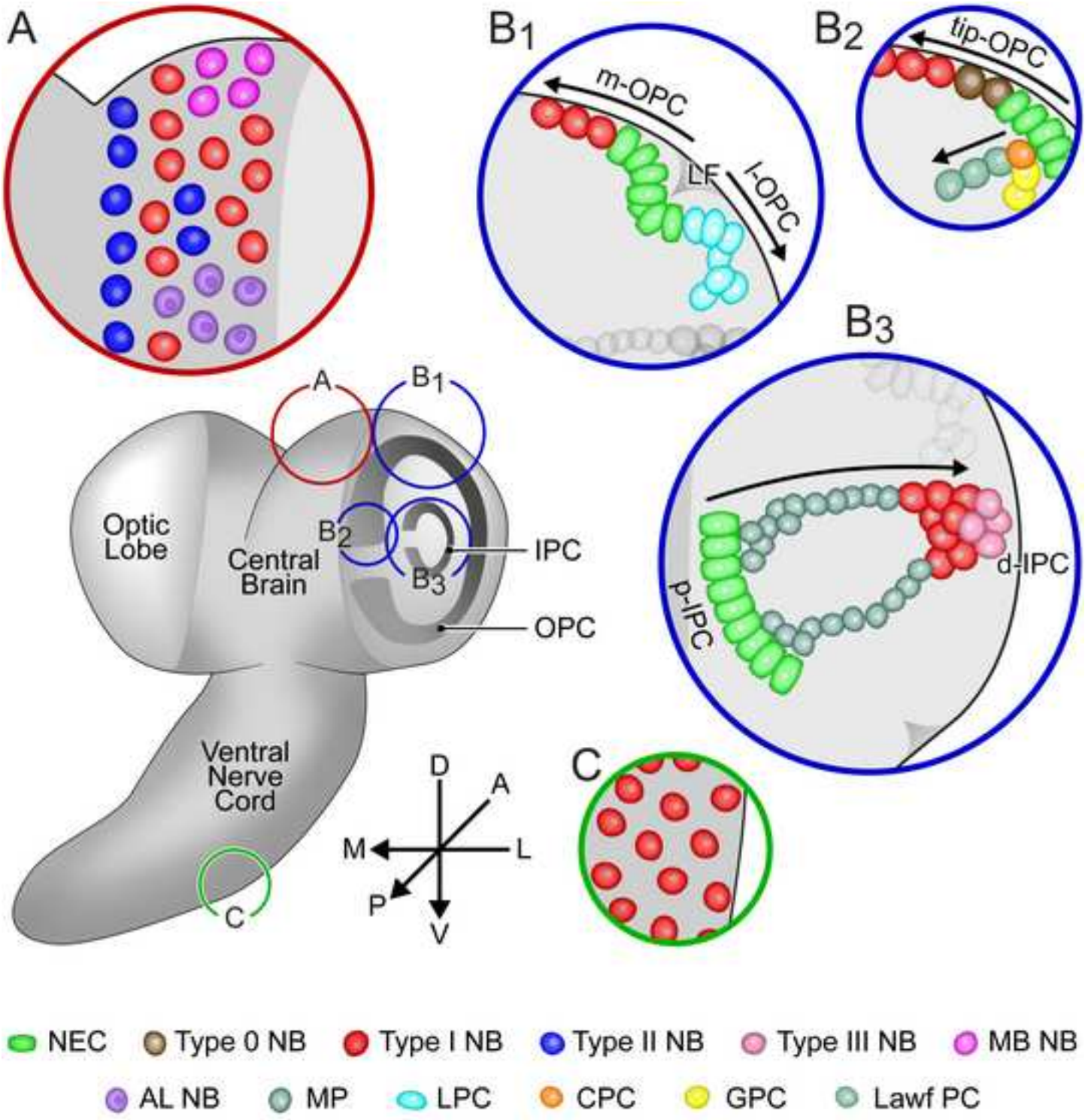

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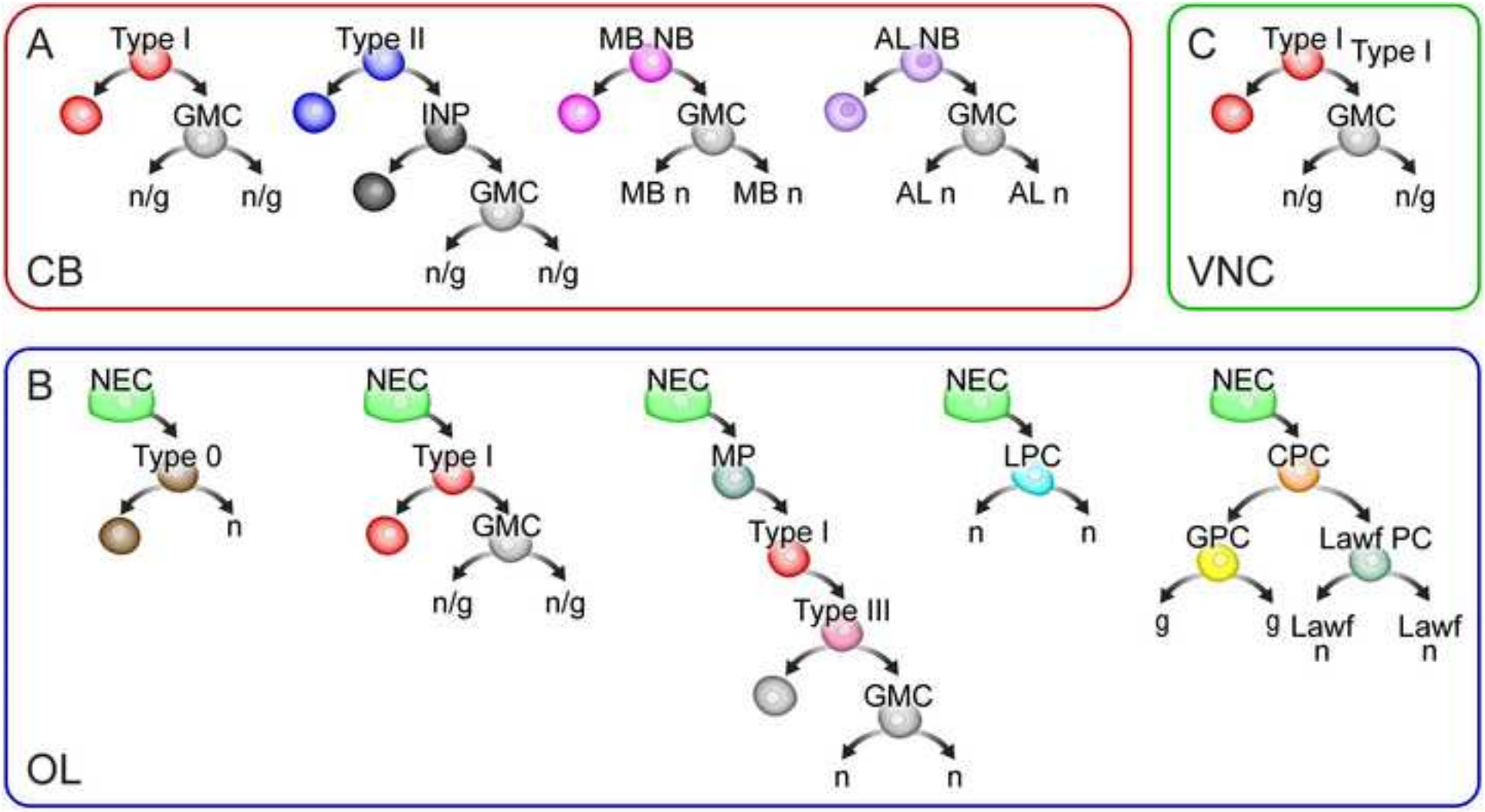

Figure 4

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