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Developmental Cell Death in the Cerebral Cortex

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Abstract

In spite of the high metabolic cost of cellular production, the brain contains only a fraction of the neurons generated during embryonic development. In the rodent cerebral cortex, a first wave of programmed cell death surge at embryonic stages and affect primarily progenitor cells. A second, larger wave unfolds during early postnatal development that ultimately determine the final number of cortical neurons. Programmed cell death in the developing cortex is particularly dependent on neuronal activity and unfolds in a cell-specific manner with precise temporal control. Pyramidal cells and interneurons adjust their numbers in sync, which is likely crucial for the establishment of balanced networks of excitatory and inhibitory neurons. In contrast, several other neuronal populations are almost completely eliminated through apoptosis during the first two weeks of postnatal development, highlighting the importance of programmed cell death in sculpting the mature cerebral cortex.

Contents

Abundance, even of good things, prevents them from being valued

Don Quixote, Miguel de Cervantes

INTRODUCTION

The formation of the nervous system involves the generation of very diverse cellular elements through a precise sequence of developmental processes. Such modular approach provides autonomy for individual cell types to evolve their own developmental trajectories. Nonetheless, some level of coordination is required to ensure that the various cellular components of the nervous system arise during development in the correct proportions for normal function. To this end, one evolutionary-conserved strategy is based in the overproduction of cells during development and the subsequent elimination of those not incorporated into the system through the process of cell death (Hamburger & Levi-Montalcini 1949, Oppenheim 1991, Raff et al. 1993).

Three major types of cell death have been historically described based on their morphological manifestation: apoptosis, autophagy and necrosis (Galluzzi et al. 2018). This review will focus on apoptosis as this is the main mechanism of strictly physiological forms of regulated cell death – collectively known as programmed cell death – in the developing brain. The term “apoptosis” was first coined to describe a process of cell death that is characterized by unique morphological alternations such as chromatin condensation (pyknosis), nuclear fragmentation, and initial maintenance of the plasma membrane followed by cell fragmentation into small vesicles (Kerr et al. 1972). There are two main types of apoptosis – intrinsic and extrinsic – depending on whether apoptosis is primarily driven by intra- or extracellular microenvironment perturbations. Both types of apoptosis are precipitated by the activation of a family of cysteine-proteases known as caspases.

The critical step for intrinsic apoptosis is irreversible mitochondrial outer membrane permeabilization, through which proteins of the intermembrane space such as Smac/Diablo, a protein that binds and antagonize inhibitor of apoptosis proteins (IAPS), and cytochrome C are released into the cytosol (Kale et al. 2012, Saelens et al. 2004). The permeabilization of the mitochondrial outer membrane is regulated by pro-apoptotic and anti-apoptotic members of the Bcl2 apoptosis regulator protein family. Pro-apoptotic proteins include Bcl2-associated X protein (Bax) and Bcl2 antagonist/killer 1 (Bak), whereas anti-apoptotic factors include Bcl2 itself and Bcl2-like 1 (Bcl-X_L). The role of Bax and Bak in regulating this process is so critical that genetic deletion of these proteins can render cells insensitive to many lethal stimuli (Wei et al. 2001). In circumstances in which pro-apoptotic factors lead to the permeabilization of the mitochondrial outer membrane, Smac/Diablo interaction with IAPS favors the release of pro-caspase 9, which binds to cytochrome C and apoptotic peptidase activating factor 1 (Apaf1) to form a protein complex known as apoptosome, which is responsible for Casp9 activation (Li et al. 1997). The apoptosome subsequently catalyzes the activation of the so-called executioner caspases Casp3 and Casp7, which are ultimately responsible for the destruction of the cell in both types of apoptosis (Julien & Wells 2017).

External apoptosis is driven by the activation of two types of receptors, death and dependence receptors, due to perturbations in the extracellular microenvironment. Death receptors are triggered by specific ligands, whereas dependence receptors are only activated when the levels of the corresponding ligand(s) fall below a certain threshold. Dependence receptors are particularly abundant in the developing nervous system, and include neurotrophin receptors TrkA and TrkC, which bind to nerve growth factor (NGF) and neurotrophin-3 (NT-3), respectively (Dekkers et al. 2013), netrin 1 receptors (DCC, Unc5A, Unc5B, Unc5C and Unc5D), and the sonic hedgehog receptor Ptch1, among others

(Goldschneider & Mehlen 2010). The neurotrophin receptor p75^{NTR}, a member of the tumor necrosis factor receptor superfamily, interacts with TrkA and TrkC and function as a death receptor when directly activated by pro-neurotrophins (Dekkers et al. 2013, Nykjaer et al. 2005). The execution of extrinsic apoptosis involves several signaling cascades that are not well understood, but that generally involve the recruitment and activation of CASP8 and ultimately precipitated by executioner caspases such as Casp3 and Casp7. Some forms of extrinsic apoptosis seem to be independent of Bax and Bak activation.

The general mechanisms regulating programmed cell death in the nervous system has been extensively reviewed elsewhere (Dekkers et al. 2013, Pfisterer & Khodosevich 2017, Yamaguchi & Miura 2015). The purpose of this review is to summarize our current understanding of programmed cell death during the development of the cerebral cortex, using rodents as a model (Figure 1). Programmed cell death impacts the two main populations of cortical neuron, glutamatergic pyramidal cells and GABAergic interneurons, as well as other early-born neuronal populations that are only transiently present in the developing cortex, such as Cajal-Retzius cells and subplate neurons (Price et al. 1997). A comprehensive picture of this process is beginning to emerge, which indicates that programmed cell death shapes the organization of cortical circuits by decisively regulating their cellular composition.

EMBRYONIC CELL DEATH

There are two discrete developmental epochs where programmed cell death eliminates excess cells in the nervous system: in the embryo, at the level of progenitors and newborn neurons, and postnatally, as neurons mature and become incorporated into neuronal circuits. The earliest neural programmed cell death in mice occurs within the anterior distal epiblast, the presumptive neural plate at embryonic day (E) 6.5 (Yeo & Gautier 2004). This period is subsequently followed by extensive periods of cell death in the lateral edges of the hindbrain, which is key for neural tube closure, lamina terminalis and optic invagination. In the pallium, where the progenitor cells of pyramidal cells, Cajal-Retzius cells and subplate neurons reside, cell death has been reported to occur as early as E10.5 and concentrates primarily on cells residing on the germinal layers (Blaschke et al. 1996, Mihalas & Hevner 2018). In contrast, cell death seems to be relatively rare among progenitor cells in the subpallium (Hu et al. 2017), where cortical GABAergic interneurons are produced.

The extent of programmed cell death in the embryonic cerebral cortex remains unclear, with estimates ranging from 20% to nearly 70% (Blaschke et al. 1996, Thomaidou et al. 1997). Irrespectively, analysis of mouse mutants lacking important pro-apoptotic factors have demonstrated that embryonic cell death plays an important role in shaping the developing cerebral cortex. For example, mouse embryos lacking *Apaf-1* or *Casp9*, which are required to activate the executioner caspases Casp3 and Casp7, exhibit a prominent enlargement of the proliferative zones in the developing cortex (Cecconi et al. 1998, Kuida et al. 1998, Yoshida et al. 1998), a phenotype similar to that described in *Casp3* mutant mice (Kuida et al. 1996, Roth et al. 2000). Interestingly, programmed cell death in cortical progenitor cells is independent of the pro-apoptotic factors Bax and the anti-apoptotic protein Bcl-X_L (Motoyama et al. 1995, Nakamura et al. 2016, Roth et al. 2000, White et al. 1998), which suggests at least partially separate apoptotic mechanisms for progenitor cells and neurons.

Programmed cell death in cortical progenitor cells is likely linked to cell cycle checkpoints, which maintain the fidelity of DNA replication, repair, and division. Perhaps not surprisingly, alterations in mitotic progression – in particular, mitotic delay – or cell cycle checkpoint failure trigger progenitor cell death (Chen et al. 2014, Pilaz et al. 2016).

In addition to intrinsic factors, the interaction of progenitor cells with the local microenvironment also influences programmed cell death. For example, alterations in notch or ephrin signaling have been shown to increase progenitor cell death during brain development (Park et al. 2013, Yang et al. 2004), which suggest a possible feedback mechanism through which newborn neurons may regulate the number of progenitor cells (Dhumale et al. 2018).

POSTNATAL CELL DEATH

In rodents, the most extensive adjustment in the number of cortical cells occurs during the first and second postnatal weeks (Ferrer et al. 1990, Finlay & Slattery 1983, Heumann & Leuba 1983, Heumann et al. 1978, Miller 1995, Verney et al. 2000), following a temporal sequence that is only now beginning to be elucidated. Early estimates based on direct population counts and terminal transferase dUTP nick end-labeling (TUNEL) of apoptotic nuclei have suggested that cell death affects approximately 25-35% of cells in the early postnatal cortex (Ferrer et al. 1990, Finlay & Slattery 1983, Heumann & Leuba 1983, Heumann et al. 1978, Miller 1995, Verney et al. 2000). However, it is now clear that programmed cell death affects different populations of cortical cells unevenly: in some cases, it simply refines the final number of certain neuronal populations more or less extensively; in others, programmed cell death is responsible of the complete removal of entire populations of ‘transient’ cells. Glutamatergic pyramidal cells and GABAergic interneurons are among the cell populations that undergo significant but proportionally moderate programmed cell death. In contrast, for Cajal-Retzius cells and subplate neurons, as well as early-born oligodendrocytes, programmed cell death is a massive process of cellular extermination.

Pyramidal neurons

The developmental profile of cell death for excitatory neurons has not been precisely established until recently. Classical studies have shown that programmed cell death peaks in the cerebral cortex of rodents during the first week of postnatal development and estimated the amount of neuronal loss in the cerebral cortex in approximately 30% (Ferrer et al. 1990, Finlay & Slattery 1983, Heumann & Leuba 1983, Heumann et al. 1978, Miller 1995, Verney et al. 2000). Although it has been inferred that cell death would eliminate a comparable fraction of pyramidal cells (Nikolic et al. 2013), accurate predictions based on the identification of apoptotic cells are problematic because dying cells are rapidly eliminated by phagocytosis (Thomaidou et al. 1997). Indeed, a recent estimation – based on stereological quantifications – of the total number of excitatory neurons in the neocortex before and after the period of programmed cell death instead suggests that the fraction of cortical excitatory neurons that undergo programmed cell death is roughly 13% (Wong et al. 2018). Most neocortical pyramidal cells undergo cell death between P2 and P5 in the mouse, and cell death is Bax/Bak-dependent (Wong et al. 2018). Remarkably, the extent of programmed cell death among pyramidal cells is not homogeneous across functionally diverse neocortical areas and even across layers within a single cortical area (Blanquie et al. 2017b, Heumann & Leuba 1983, Verney et al. 2000). For instance, the motor cortex exhibits higher rates of apoptosis than the primary somatosensory cortex during the first week of postnatal development, and within each of these areas there are important differences in the rate of programmed cell death across layers (Blanquie et al. 2017b). These observations suggest that the mechanisms regulating the cell death of pyramidal cells in the cortex likely play an important role in shaping the cytoarchitectonic organization of neocortical areas.

Individual growth factors do not seem to play a prominent in regulating neuronal survival in the CNS (Dekkers et al. 2013). For instance, deletion of BDNF does not significantly alter the number of cortical neurons (Ernfors et al. 1994, Jones et al. 1994, Rauskolb et al. 2010). This is due to the fact that TrkB – the BDNF receptor and most highly expressed neurotrophin receptor in the CNS – does not function as a dependence receptor (Nikoletopoulou et al. 2010). In contrast, numerous studies have shown that neuronal activity prominently influences neuronal survival in the brain, most notably for cortical pyramidal cells.

The earliest evidence indicating that the survival of cortical excitatory neurons depends on neuronal activity derives from pharmacological manipulations in vitro. For example, blocking neuronal activity with Tetrodotoxin (TTX), an antagonist of voltage-dependent sodium channels, is sufficient to dramatically decrease the survival of cortical neurons in culture (Ruijter et al. 1991, Voigt et al. 1997). Conversely, elevating the concentration of extracellular K+ in culture (thereby increasing the frequency of spontaneous firing) reduces the rate of apoptosis of cortical neurons (Ghosh et al. 1994). These results are consistent with the observation that inactive neurons are much more likely to die than active neurons (Murase et al. 2011). Subsequent studies have added further support to the idea that activity plays a fundamental role in pyramidal cell survival through experiments in vivo. For instance, reducing neuronal activity in the early postnatal cortex through the injection of TTX or receptor antagonists elevates the rate of apoptosis (Ikonomidou et al. 1999, Murase et al. 2011), whereas intraperitoneal injection of kainate, which increases cortical activity, decreases the incidence of apoptosis (Blanquie et al. 2017b). In addition, unilateral whisker deafferentation significantly increase apoptosis in the contralateral barrel cortex, which indicates that the activity of thalamocortical neurons also influence the survival of pyramidal cells (Blanquie et al. 2017b). Altogether, these observations strongly indicate that the integration of pyramidal cells into synaptically-driven neuronal networks is critical for their survival.

How does neuronal activity increase survival? In the developing nervous system, the serine–threonine kinase AKT is a critical mediator of neuronal survival (Datta et al. 1997, Dudek et al. 1997). For instance, expression of a constitutively active form of AKT is sufficient to suppress pyramidal cell death in vitro (Murase et al. 2011). AKT is activated through phosphorylation by Phosphoinositide 3 kinase (PI3K), which is in turn recruited downstream of pro-survival signals such as trophic factors. Several lines of evidence suggest that neuronal activity increases the activity of AKT, probably through the intracellular Ca^{2+} elevation via ionotropic receptors and L-type calcium channels (Nicholson-Fish et al. 2016, Pezet et al. 2005, Vaillant et al. 1999). In addition, Ca^{2+} influx has been shown to promote the transcription and secretion of trophic factors (Flavell & Greenberg 2008), thereby further promoting neuronal survival.

Interneurons

Cell death estimates based on the quantification of Casp3+ neurons suggest that up to 40% of cortical GABAergic neurons undergo Bax/Bak-dependent cell death during the first two weeks of postnatal development in mice (Southwell et al. 2012). Quantification of the total number of interneurons in the neocortex before and after the period of programmed cell death indicates that cell death rates are probably lower, ranging from 20% to 30% for different classes of cortical interneurons (Priya et al. 2018, Wong et al. 2018). Consistent with these observations, preventing cell death in specific classes of interneurons through conditional genetic deletion of pro-apoptotic proteins leads to a 30% increase in the number of those interneurons (Priya et al. 2018, Wong et al. 2018). Interestingly, the period of programmed cell death for cortical interneurons is slightly shifted compared to pyramidal cells, with most apoptosis occurring between P5 and P10 in the mouse neocortex (Wong et al. 2018).

Heterochronic transplantation and in vitro experiments suggest that interneurons have an intrinsic timer that drives these cells to die when they reach a specific maturation stage (Southwell et al. 2012). Consistent with this notion, artificially preventing the maturation of cortical interneurons (via deletion of the calcium-dependent protein phosphatase Calcineurin) dramatically increases their survival beyond the programmed cell death period (Priya et al. 2018). The developmental trajectory of GABAergic interneurons is also consistent with the existence of a cell death timer in these cells. For instance, cell death peaks earlier for interneurons born in the MGE than in the CGE (Priya et al. 2018, Wong et al. 2018), which aligned with the corresponding birthdates of these neuronal populations (Miyoshi et al. 2010). Even within the MGE, early-born infragranular interneurons also die earlier than late-born supragranular interneurons (Wong et al. 2018). Thus, in contrast to the pattern of cell death observed in pyramidal cells, which seems unlinked from their neurogenic sequence (Blanquie et al. 2017b, Verney et al. 2000), the regulation of apoptosis in cortical interneurons is reminiscent of the lineage-specific programmed cell death mechanisms described in the nervous system of worms and flies (Yamaguchi & Miura 2015).

Neuronal activity plays a fundamental role in the survival of cortical interneurons during the critical cell death period. Calcium imaging experiments in vivo have shown that active interneurons are much more likely to survive than inactive interneurons (Wong et al. 2018). Moreover, cell-autonomously enhancing the excitability of cortical interneurons – by using designer exclusively activated by designer drugs (DREADDs) or by altering ion channel expression – increases their survival in vivo, whereas decreasing it exacerbates the proportion of interneurons undergoing cell death (Denaxa et al. 2018, Priya et al. 2018).

However, experiments in vitro suggest that interneurons alone are not capable to sustain the levels of activity required for survival. Indeed, cortical interneurons only survive in vitro when cultured over a “feeder” layer containing pyramidal cells and glia (Xu et al. 2004). Importantly, glial cells or conditioned media from neuronal cultures are not sufficient to prevent interneuron cell death in culture (Priya et al. 2018), which suggest that direct, physical interactions with pyramidal cells are required for the survival of cortical interneurons. In other words, GABAergic interneurons are fated to die in the absence of inputs from excitatory cells, which are already the main drivers of interneuron activity during the first week of postnatal development (Anastasiades et al. 2016). Consistent with this notion, increasing the activity of pyramidal cells with DREADDs during the period of interneuron cell death enhances the survival of interneurons, whereas reducing the excitability of pyramidal cells has the opposite effect (Wong et al. 2018).

Pyramidal cells non-cell autonomously influence the survival of cortical interneurons by regulating the levels of the phosphatase tensin homologue PTEN during the period of interneuron cell death (Wong et al. 2018). PTEN is a 3'-specific phosphatidylinositol (3,4,5) trisphosphate phosphatase that functions as an inhibitor of PI3K (Stambolic et al. 1998), thereby negatively regulating AKT-dependent survival. Interestingly, PTEN levels rise sharply in some cortical interneurons during the critical period of cell death, suggesting that PTEN activation is a component of the cell death timer that exists in these cells. Remarkably, increasing the activity of pyramidal cells is sufficient to decrease the levels of PTEN in cortical interneurons, thereby promoting their survival (Wong et al. 2018).

Cajal-Retzius cells

Cajal-Retzius cells are a population of early-born glutamatergic neurons that originate from progenitor cells at the margins of the pallium and populate cortical layer 1 during embryonic and early postnatal stages, where they play critical roles in controlling neuronal migration (Kirischuk et al. 2014, Marín & Rubenstein 2003, Soriano & Del Rio 2005). In contrast to pyramidal cells and GABAergic interneurons, whose numbers are adjusted through programmed cell death during the assembly of cortical circuits, Cajal-Retzius cells gradually disappear once the development of the cerebral cortex is completed (Chowdhury et al. 2010, Price et al. 1997).

Different regions of the developing pallium, including the cortical hem, the pallial-subpallial boundary (PSB) and the pallial septum, give rise to distinct subclasses of Cajal-Retzius cells with unique properties and developmental trajectories (Bielle et al. 2005, Takiguchi-Hayashi et al. 2004). Each of these populations of Cajal-Retzius exhibit different temporal dynamics and diverging mechanisms of cell death (Ledonne et al. 2016). Cajal-Retzius from the PSB die between P1 and P4, approximately about the same time than pyramidal cells undergo apoptosis, whereas the remaining populations of Cajal-Retzius gradually disappear between P4 and P10. It has been suggested that the delayed death of hem- and septum-derived Cajal-Retzius cells might be due to the expression of ΔNp73, a truncated version of p73 lacking direct transcriptional activity that has been shown to have an anti-apoptotic role during brain development (Ledonne et al. 2016, Tissir et al. 2009). Genetic studies have demonstrated that septum-derived but not hem-derived Cajal-Retzius cells undergo Bax-dependent apoptosis (Ledonne et al. 2016). The molecular mechanisms mediating the programmed cell death of Cajal-Retzius cells originating from the cortical hem remain unclear, but the relatively rare detection of activated Casp3 in Cajal-Retzius cells during the critical cell death period (Anstotz et al. 2014, Blanquie et al.

2017a, Chowdhury et al. 2010, Ledonne et al. 2016) suggests that most Cajal-Retzius cells may undergo cell death through a mechanism that does not require caspase executioners.

It has been suggested that programmed cell death of Cajal-Retzius is triggered by neural activity (Blanquie et al. 2017a, Del Rio et al. 1996, Mienville & Pesold 1999). However, in contrast to pyramidal cells (Heck et al. 2008, Ikonomidou et al. 1999, Murase et al. 2011, Ruijter et al. 1991, Voigt et al. 1997), blocking synaptic transmission with TTX *increases* the survival of Cajal-Retzius cells (Blanquie et al. 2017a, Del Rio et al. 1996). Several unique features of Cajal-Retzius cells likely contribute to this paradoxical effect. First, Cajal-Retzius cells exhibit a relatively low resting membrane potential (Mienville & Pesold 1999). Second, they persistently maintain high levels of the chloride inward transporter NKCC1 and low levels of the chloride outward transporter KCC2 during postnatal development (Achilles et al. 2007, Pozas et al. 2008). Consequently, GABA has an excitatory effect on Cajal-Retzius cells (Mienville 1998). Blocking glutamatergic receptors increases the survival of Cajal-Retzius cells (Del Rio et al. 1996, Mienville & Pesold 1999), whereas their activation during their period of cell death leads to a complete disappearance of the Cajal-Retzius cells (Supèr et al. 2000). However, it is presently unclear whether this is directly mediated by glutamatergic receptors on Cajal-Retzius cells or through the indirect modulation of GABAergic interneurons, which provide strong inputs to Cajal-Retzius cells (Kilb & Luhmann 2001). In any case, depolarizing $GABA_A$ receptor-mediated currents are directly linked to the cell death of Cajal-Retzius cells because their modulation alters the fate of these cells. For instance, the death of Cajal-Retzius cells is significantly delay in *Nkcc1* mutant mice (Blanquie et al. 2017a).

The mechanisms through which an excess of depolarizing currents may induce the death of Cajal-Retzius cells during the first week of postnatal development are not completely understood. It has been suggested that the low resting potential of Cajal-

Retzius cells may facilitate the excitotoxic effects Ca^{2+} influx through NMDA receptors (Mienville & Pesold 1999). Alternatively, depolarizing GABA responses have previously been shown to elicit cell death mediated by the neurotrophin receptor $p75^{NTR}$ (Shulga et al. 2012). Consistently, Cajal-Retzius cells express high levels of $p75^{NTR}$ and pharmacological inhibition of this receptor increases their survival in vitro (Allendoerfer et al. 1990, Blanquie et al. 2017a).

Subplate neurons

Subplate neurons are among the earliest born glutamatergic neurons of the cerebral cortex, where they constitute the deepest layer of grey matter (Hoerder-Suabedissen & Molnar 2015). They play a fundamental role in the establishment of thalamocortical connections by functioning as an transient relay station for axon ingrowth (Allendoerfer & Shatz 1994). It has been classically assumed that subplate neurons gradually disappear during early postnatal development (Price et al. 1997). However, subplate neurons are heterogeneous and recent studies have shown that a significant population of subplate neurons with distinctive molecular features survive beyond the critical period of cell death in the cerebral cortex (Hoerder-Suabedissen & Molnar 2013).

The mechanisms underlying the apparent elimination of subplate neurons have remain elusive. Analysis of cell death in the subplate during early postnatal development reveals comparable levels of apoptotic cells than in the other cortical regions, which would argue against the idea that subplate neurons are preferentially eliminated (Valverde et al. 1995). Consistently, other studies have suggested that subplate neurons do not die but rather they are passively dispersed into layer 6b or the adjacent white matter of the neocortex (Duque et al. 2016, Kostovic & Rakic 1980, Marx et al. 2017). It remains to be elucidated whether at least a fraction of subplate neurons undergo programmed cell death, and if they do, whether this process is similar to that described for pyramidal cells.

Glial cells

The cerebral cortex contains three main classes of glial cells: astrocytes, oligodendrocytes and microglial cells. Astrocytes and oligodendrocytes are initially generated from the same precursor cells than neurons, following a developmental switch that enable progenitors to become gliogenic once neurogenesis has been completed (Anthony et al. 2004, Kessaris et al. 2006). Microglial cells, in contrast, have an extra-embryonic origin. They derive from a wave of early hematopoiesis in the yolk sac, from where they invade the nervous system (Thion et al. 2018). While few cortical astrocytes seem to enter programmed cell death in the cerebral cortex (Ge et al. 2012), the number of both cortical oligodendrocytes and microglial cells are adjusted during the postnatal development.

Cortical oligodendrocytes are produced by three consecutives developmental waves (Kessaris et al. 2006). The earliest wave consists of oligodendrocytes generated in the most ventral regions of the subpallium (the medial ganglionic eminence MGE, and the preoptic area, POA), which migrate and colonize the cortex in parallel to coetaneous GABAergic interneurons produced from the same progenitor regions (Marín & Rubenstein 2001); subsequent waves originate in other subpallial regions and in the cortex. Approximately 30% of cortical oligodendrocytes generated embryonically undergo programmed cell death in the first week of postnatal development (Trapp et al. 1997). Intriguingly, most of the oligodendrocytes that are removed at this stage derived from the first wave of oligodendrogenesis (Kessaris et al. 2006). The precise mechanism leading to the death of MGE/POA-derived oligodendrocytes remains unknown, but cellular competition for resources has been shown to influence oligodendrocyte survival in other regions of the

CNS (Barres et al. 1992, Bergles & Richardson 2015). In addition, recent work has shown that neuronal activity impacts the proliferation and survival of oligodendrocytes. For example, reducing the activity of the barrel cortex through postnatal whisker deafferentation leads to a significant increase in the number of apoptotic oligodendrocyte precursor cells in this region of the somatosensory cortex (Hill et al. 2014).

Microglial cells proliferate and expand extensively in the cerebral cortex during the first weeks of postnatal development. However, microglia numbers are dramatically reduced by approximately 50% between the third and sixth postnatal week (Nikodemova et al. 2015). Although the exact mechanisms controlling the developmental regulation of microglia death are currently unknown, several factors secreted by neurons and astrocytes, such as CSF-1, IL34 and TGFβ, have been shown regulate microglia survival in other contexts (Bohlen et al. 2017).

PROGRAMMED CELL DEATH AS QUALITY CONTROL

The benefits of sustaining a sophisticated, cell-specific process of programmed cell death must convey important evolutionary advantages, especially considering the metabolic cost that involves the generation of an excessive number of neurons and glial cells in the CNS. From a classical perspective, the most obvious role of programmed cell death is to serve as a process through which anomalous, misplaced or potentially noxious cells are eliminated prior to the assembly of neural circuits (Figure 2). For instance, while neurons with mild forms of aneuploidy – the presence of an abnormal number of chromosomes in a cell – exists in the cerebral cortex, extreme aneuploid cells are very rare (Rehen et al. 2001, Yang et al. 2003). This suggests the existence of a mechanism that eliminates extremely aneuploid neurons during development. Consistent with this idea, mice in which programmed cell death has been attenuated exhibit a significant increase in extreme forms of aneuploidy in the cerebral cortex (Peterson et al. 2012). It has been suggested that aneuploidy reduces the fitness of cells to compete for resources (Zhu et al. 2018), which in the case of the developing cerebral cortex may translate into a disadvantage for establishing connections. Remarkably, mildly aneuploid neurons functionally integrate into cortical circuits (Kingsbury et al. 2005), which suggests that programmed cell death selects defective neurons for their inability to integrate into neuronal assemblies.

Analysis of mice mutants in which the lack of pro-apoptotic proteins prevents programmed cell death suggests that this process contributes to the elimination of misplaced and abnormally wired neuron. For example, the cerebellum of *Bax* mutants contains a small fraction of Purkinje cells located in the granular layer, a lamina typically devoid of these cells (Jung et al. 2008). However, mouse mutants such as *reeler* or *scrambler*, in which the majority of pyramidal cells and interneurons are mispositioned, do not exhibit outstanding rates of apoptosis in the cerebral cortex (Rice & Curran 2001). This suggests that programmed cell death might be particularly sensitive to connectivity defects and perhaps not so much about cellular location.

The idea that programmed cell death is responsible for correcting early wiring defects derives from classical studies in the chick visual system (Clarke 1992). Several studies in *Drosophila* support the notion that neurons that fail to acquire appropriate identities based on their location and end up projecting to the wrong target are eliminated through programmed cell death (Baek et al. 2013, Kuert et al. 2014). However, it has been shown that cortical pyramidal cells can correct initial mistargeted projections without causing apoptosis (De Carlos & O'Leary 1992), which suggests that abnormal targeting does not systematically elicit programmed cell death.

PROGRAMMED CELL DEATH IN CIRCUIT ASSEMBLY

It is perhaps not surprising that the most intensive period of programmed cell death in the cerebral cortex coexists with the assembly and maturation of neuronal circuits in this region of the brain. In rodents, the first two weeks of postnatal development in the cerebral cortex are characterized by profound changes in the patterns of neuronal activity (Allene & Cossart 2010, Khazipov & Luhmann 2006, Yuste et al. 1995), the remodeling of transient connections and structures established in the embryo (Allendoerfer & Shatz 1994, Butt et al. 2017), and the establishment of mature patterns of activity and connectivity (Luhmann & Khazipov 2018, Rochefort et al. 2009). It is increasingly clear that programmed cell death plays important roles in all these processes (Figure 3).

Adjusting cell numbers

The construction of tissues and organ with the appropriate number of cells requires a certain level of coordination between developmental programs. One notable example of such coordination is the regulation of cell numbers via quantitatively matching cells with reciprocal connections – also known as systems matching –, especially since the initial generation of the inputs and outputs temporally segregated. For example, there is a strong interdependency in neuronal numbers in the substantia nigra and the striatum (Granholm et al. 2000, Jackson-Lewis et al. 2000, Oo et al. 2003), two brain regions that are intimately interconnected.

In the cerebral cortex, it has been suggested that the proportion of cell death is likely determined locally for each area and even for each layer (Verney et al. 2000), and recent experiments indicate that this might be directly linked to the specific functional characteristics of each cortical area (Blanquie et al. 2017b). The discovery of the strong dependency of interneuron survival on the activity of pyramidal cells strongly suggests that the regulation of programmed cell death is indeed a critical mechanism for the precise sculping of the cytoarchitecture of distinct cortical areas (Wong et al. 2018). In this context, programmed cell death may have evolved as a mechanism responsible for adjusting ratios of excitatory and inhibitory neurons in the cerebral cortex – and perhaps other brain areas –, irrespectively of the size of the cerebral cortex. This would help to understand why the ratio of pyramidal cells and interneurons is relatively similar in species with dramatically different cortical volumes (DeFelipe et al. 2002).

It is worth noting that genetically modified mice in which the expression of pro-apoptotic genes has been altered exhibit relatively limited neuropathological and morphological alterations, especially in certain genetic backgrounds. For example, *Casp3* and *Bax* mutant mice, or mice in which neurons overexpress the anti-apoptotic gene *Bcl-2*, have relatively normal brain morphology (Krahe et al. 2015, Leonard et al. 2002, Rondi-Reig et al. 1999). On close examination, however, these mice have deficits in complex behaviors such as learning, which indicates that programmed cell death does indeed play an important role in refining developing neural circuits.

Removing scaffolds and signaling centers

Patterning of the developing brain relies on specific signaling centers, often referred to as organizers, that produce molecules responsible for regional identity and cell specification. The position, size and shape of brain organizers is critical for their function, as it determines the spread of signals under its influence (Kiecker & Lumsden 2012). Several studies have shown that programmed cell death is critically involved in the regulation of the size of brain organizers. For instance, apoptosis regulates the size of the anterior neural ridge (Nonomura et al. 2013), an organizing center that plays a fundamental role in patterning the telencephalon through the secretion of Fgf8 (Houart et al. 1998, Rubenstein et al. 1998). In the developing cerebral cortex, Cajal-Retzius cells also function as transient signaling units releasing diffusible molecules that modulate progenitor proliferation, neuronal differentiation and cell migration (Borello & Pierani 2010, Villar-Cerviño & Marín 2012). From that perspective, the programmed cell death of Cajal-Retzius cells serves the purpose of removing specific developmental signals once they are no longer required. In agreement with this notion, the persistence of Cajal-Retzius cells in the adult cortex has been linked to several neurodevelopment disorders such as polymicrogyria (Eriksson et al. 2001).

Programmed cell death might also be important for the removal of neurons that function as transient "placeholder" cells. Placeholder cells form temporary synaptic connections with other neurons, while these wait for the arrival of input from other neuronal populations (Chao et al. 2009). Both subplate neurons and Cajal-Retzius cells perform such a role during the development of the cerebral cortex. In particular, subplate neurons support the migration of pyramidal cells by establishing transient synapses with these cells during embryonic development, a process that is thought to facilitate their invasion of the cortical plate (Ohtaka-Maruyama et al. 2018). In addition, thalamocortical axons transiently contact subplate neurons and wait in this region before they invade the cortical plate to innervate their final targets (Allendoerfer & Shatz 1994, Ghosh et al. 1990). As pyramidal cells arrive to their final position within the cortical plate and thalamocortical axons reach layer 4, the placeholder role of subplate cells becomes superfluous and obsolete, which may explain why at least a fraction of these neurons may undergo programmed cell death (Price et al. 1997).

Cajal-Retzius cells also function as transient placeholders for entorhinal axons projecting into the hippocampus (Supèr et al. 1998). In the adult hippocampus, entorhinal axons form synapses with the distal dendrites of the pyramidal neurons, but in the embryo entorhinal axons form transient synapses with Cajal-Retzius cells before they transfer into their final targets. This transient scaffold is essential for the normal development of entorhinal projections into the hippocampus: embryonic ablation of Cajal-Retzius cells disrupt the layer specificity of entorhinal axons (Del Rio et al. 1997).

Making space

Cell death has also been shown to contribute to the normal spacing between neurons. In the retina, intrinsically photosensitive retinal ganglion cells (ipRGCs) undergo proximity-mediated and Bax-dependent cell death. In this process, ipRGCs that are in close proximity to other ipRGCs are more likely to enter apoptosis (Chen et al. 2013). Disruption of programmed cell death in *Bax* mutants alters ipRGCs spacing and, subsequently, disrupt the connectivity of these cells (Chen et al. 2013). It is been suggested that cell death in retina might be mediated by proteins encoded by the Down syndrome cell adhesion molecule *Dscam* (Keeley et al. 2012, Li et al. 2015), but the mechanism through which Dscam proteins may contribute to cell death and the regulation of cellular tiling in the retina remain unclear. In the developing cortex, the disappearance of Cajal-Retzius cells from layer 1 has also been hypothesized to free up additional space for the growth of the terminal dendrites of pyramidal cells (Causeret et al. 2018).

OUTLOOK

The mammalian neocortex has undergone a dramatic expansion during evolution. There are two main mechanisms under selective evolutionary pressure that can dramatically alter the number of cells present in the cortex: cell proliferation and cell death. It is undeniable that changes in the proliferative capacity of progenitor cells has contributed the expansion of the neocortex during evolution (Lui et al. 2011, Wilsch-Bräuninger et al. 2016).

However, the regulation of programmed cell death in progenitor cells may also have a very significant impact in the final number of cortical neurons (Kuida et al. 1998). Indeed, genes encoding for proteins involved in caspase-dependent apoptosis have undergo rapid positive selection in primates, which suggests that evolutionary changes in programmed cell death may have contributed to brain evolution in humans (Vallender & Lahn 2006). In addition, the survival of specific populations of neurons, such as subclasses of Cajal-Retzius cells and subplate neurons, may also contribute to brain evolution. For instance, a population of Cajal-Retzius cells that survive at the bottom of small sulci and ingrowing vasculature in the human cortex has been suggested to play a role in shaping the neocortical surface (Meyer & Gonzalez-Gomez 2018).

The regulation of programmed cell death and its impact in the final cellular organization of the cerebral cortex has also important implications in disease. For instance, mutations in the protein phosphatase PTEN are relatively common in a proportion of individuals with autism spectrum disorders (ASD) and macrocephaly (Butler et al. 2005, Buxbaum et al. 2007). While it has always been assumed that macrocephaly in these patients was due to defects in neurogenesis, the identification of PTEN as a critical regulator of apoptosis for cortical interneurons in mice suggests that abnormal apoptosis may also contribute to macrocephaly in patients carrying PTEN mutations (Wong et al. 2018).

Classical studies in nematodes originally illustrated the concept of cell-lineage dependent programmed cell death during development (Sulston & Horvitz 1977). In the mammalian cerebral cortex, however, it is presently unclear whether stereotyped patterns of cell death exist among specific lineages. Lineage studies in mice have suggested that postmitotic cell death may contribute to increase the diversity of pyramidal cell lineage configurations in the developing cortex (Llorca et al. 2018), but it seems likely that cell death appears stochastically depending on how neurons become incorporated into emerging neuronal networks.

Finally, it is becoming increasingly clear that neural activity impacts neuronal survival in a cell-specific manner, which it is somehow unexpected. It remains to be established how neuronal activity precisely signals downstream of neurotransmitter receptors in each neuronal class, and to what extent the regulation of calcium influx and signaling through trophic factors contributes to neuronal survival with cell specificity. It is expected that single-cell RNA sequencing studies may contribute to identify the specific pro-apoptotic and anti-apoptotic signaling pathways that are active in each neuronal subtype at the relevant developmental stages.

DISCLOSURE STATEMENT

O.M. serves on the Scientific Advisory Board of Neurona Therapeutics.

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FIGURE LEGENDS

Figure 1

Developmental timeline of programmed cell death in the mouse neocortex. During embryonic development programmed cell death is particularly abundant among progenitor cells in the ventricular zone (VZ) and subventricular (SVZ). Apoptotic progenitors are shown in red. During postnatal development, pyramidal cells (green) and subplate neurons (cyan) undergo programmed cell death starting from postnatal (P) day 2, followed by infragranular medial ganglionic eminence (MGE) interneurons (magenta) at P5, supragranular MGE interneurons (magenta) and Cajal-Retzius cell (yellow) at P7, and caudal ganglionic eminence (CGE) interneurons (blue) at P9.

Figure 2

The role of programmed cell death in removing developmental errors. Developmental errors (cells in red) such as (a) aneuploidy, (b) misplaced cells and (c) mistargeted cells are removed via programmed cell death to enable proper brain functioning.

Figure 3

The role of programmed cell death in sculpting the developing brain. (a) Pyramidal cells (green) regulate the survival of interneurons (magenta) to achieve appropriate balance between excitatory and inhibitory neurons in the cerebral cortex. (b) Sex hormones such as testosterone can alter the survival of certain cell types (apoptotic cells in red), leading to a difference in cell number between males and females in certain brain regions. (c) Subplate neurons (cyan) act as a transient place cell holder for the thalamocortical axons (yellow), while layer 4 pyramidal cells (green) reach their final position. (d) Cajal-Retzius cells (yellow) function as a signaling centers by secreting Reelin, which instructs pyramidal cells to migrate to the correct layer. (e) To ensure proper cellular maturation, excess cells are eliminated in order to make space for cells. (f) Apoptotic cells (red) can secrete proliferative signal to surrounding cells (green) in order to increase their proliferative capacity.

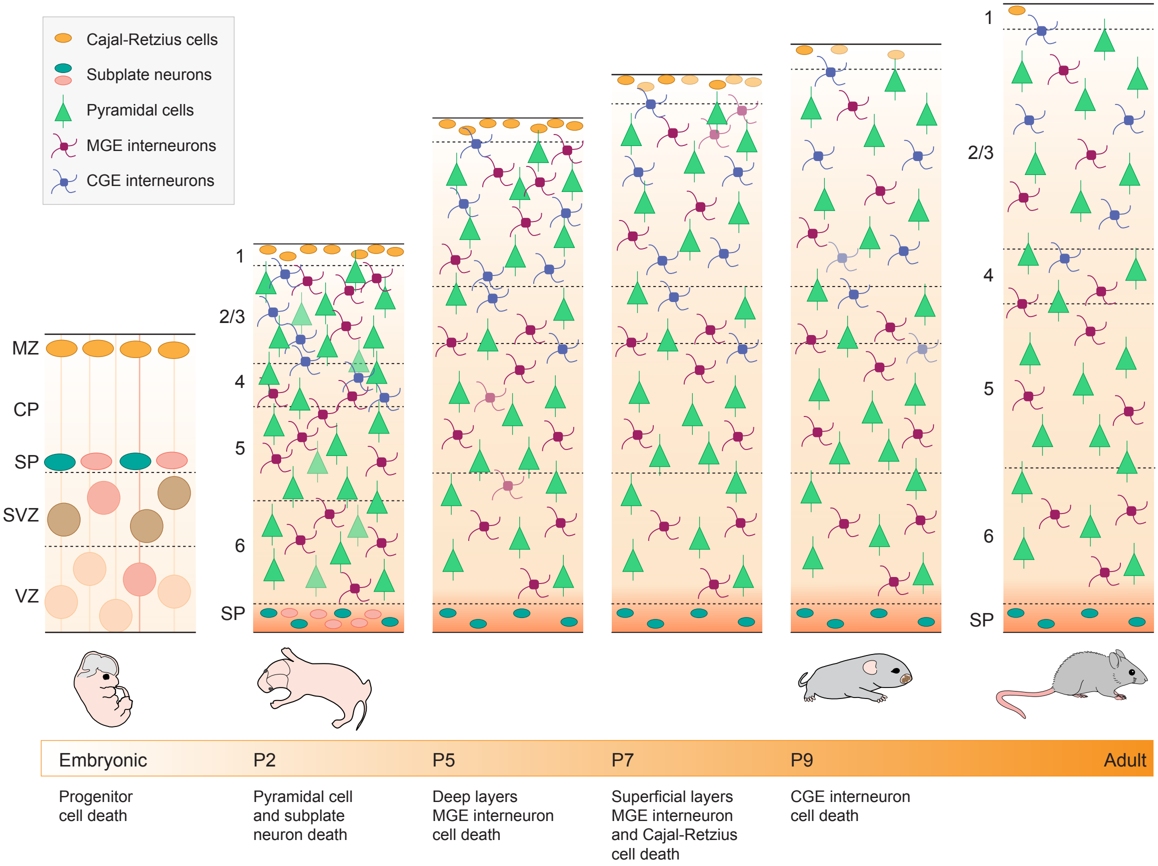

Figure 1
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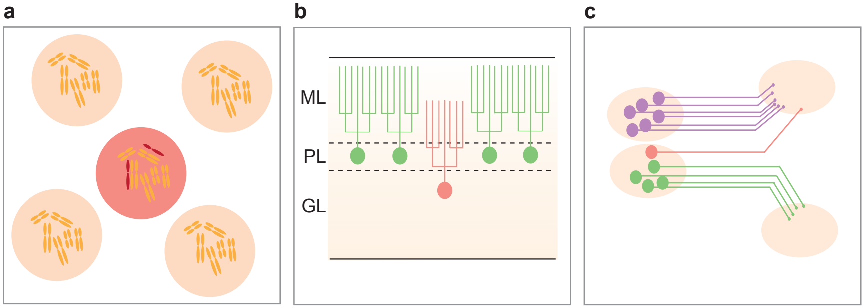

Figure 2
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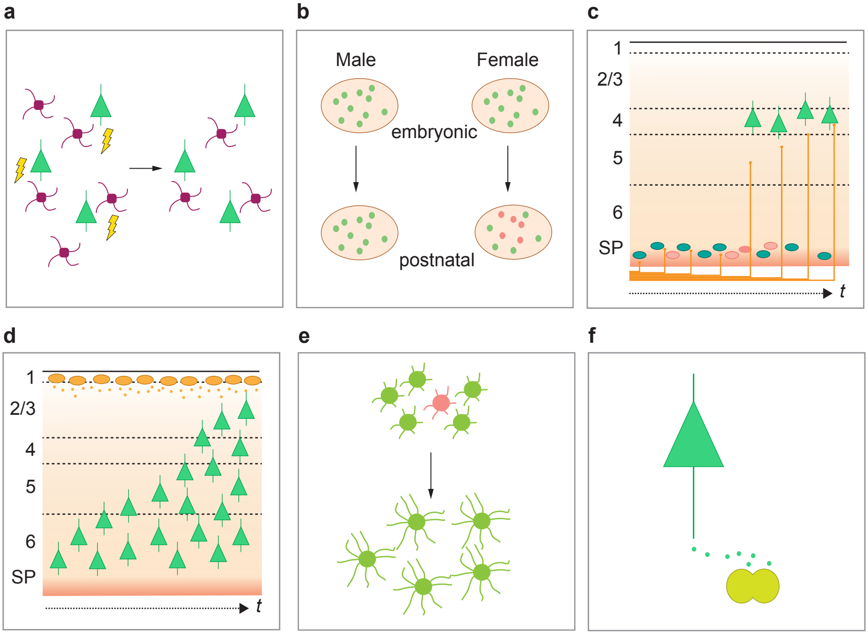

Figure 3
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