Review

Newborn cortical neurons: only for neonates?

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Despite a century of debate over the existence of adult cortical neurogenesis, a consensus has not yet been reached. Here, we review evidence of the existence, origin, migration, and integration of neurons into the adult and neonatal cerebral cortex. We find that the lack of consensus likely stems from the low rate of postnatal cortical neurogenesis that has been observed, the fact that neurogenesis may be limited to subtypes of interneurons, and variability in other conditions, both physiological and environmental. We emphasize that neurogenesis occurs in the neonatal cortex and that neural stem cells are present into adulthood; it is possible that these progenitors are dormant, but they may be reactivated, for example, following injury.

Introduction

The rapid and robust expansion of the cerebral cortex relative to other brain areas has been proposed as the crowning achievement of human evolution. Indeed, the cerebral cortex plays a critical role in all perceptions, thoughts, and behaviors that distinguish humans from other animals. Do these complex cognitive phenomena require a stable brain structure? Early experimental evidence suggested that cortical neurogenesis (see Glossary) in primates occurred only during embryonic development [1]. These findings, coupled with the complexity of primate behavior, led to the development of a 'central dogma': that the postnatal (and thus, adult) cerebral cortex possesses a stable number of neurons, all generated prior to birth, and the lack of new neurons or other regenerative capacity was a trade-off for the complexity and diversity of cognitive functions performed by the cerebral cortex. However, a substantial number of recent studies have challenged these central concepts.

The central dogma was first called into serious question in 1999 by a study showing evidence for adult-born neurons in the neocortex of macaques [2]. This work was quickly followed by several other studies in different mammalian classes reporting conflicting data on adult neurogenesis in part due to the limitations of the labeling techniques. Work in adults has also called attention to the neonatal period, which was recently shown to display protracted neurogenesis in different cortical regions, including the prefrontal cortex (PFC) in infant humans [3].

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The occurrence of newborn neurons in the postnatal cortex implies the existence of neural progenitor cells (NPCs) or neural stem cells (NSCs). The brain contains two well-accepted postnatal NSC niches – the subventricular zone (SVZ) and hippocampal subgranular zone (SGZ) – that contribute to persistent neurogenesis in the olfactory bulb and hippocampus [4]. Additional niches close to the SVZ in the neonatal and adult brain have been reported, but evidence for a neurogenic niche in the cortex is lacking. Nevertheless, several studies have provided evidence of dormant bipotential progenitor cells in the cortical parenchyma. These different pools of progenitor cells are amenable to manipulation for the generation of neurons during brain repair.

Many studies have found postnatal neurogenesis in pathological conditions [5] that arise from NPCs in the SVZ and/or the parenchyma. Two conditions, hypoxic insult and mTOR hyperactivity as observed in tuberous sclerosis complex (TSC), will be discussed.

Neonatal and adult cortical neurogenesis has been the subject of several thorough reviews [1,4,6–8]. Here, we focus mainly on recent work in adult and neonates related to neurogenesis and the sites of NPCs. In the interest of

Glossary

Bipotential/neural progenitor cells: cells generated from NSCs that are bipotential (i.e., generate neurons and glia), and have self-renewal capacity, but which is more limited than for NSCs.
Neonatal electroporation: this technique allows cDNA to enter cells by applying voltage; this creates small pores in the cell membranes and pushes charged cDNA to enter cells. In neonatal mice, following pressure ejection of a cDNA plasmid into the LV, voltages are applied across the head of anesthetized pups, allowing plasmid entry into NSCs lining the ventricle.
Neural stem cells (NSCs): these can generate both neurons and glia, and can self-renew indefinitely. In the adult, NSCs have a more restricted fate than embryonic NSCs and generate only specific types of neurons.
Neurogenesis: a process leading to the generation of neurons through several stages: rare NSC asymmetric division and generation of bipotential progenitor cells; proliferation of progenitor cells leading to pool amplification and generation of neuroblasts; neuroblast proliferation, post-mitotic differentiation, migration, maturation, and synaptic integration.
Transgenic floxed, inducible Cre (CreERT2), and Rosa26R reporter mice: the term 'floxed' is used to describe the positioning of a DNA sequence between two LoxP sites and is abbreviated 'flanked by LoxP.' Cre recombinase expression in cells expressing a floxed gene leads to gene sequence excision and deletion. In reporter Rosa26R mice, a stop sequence is inserted in the Rosa26 locus between two LoxP sites preceding a reporter gene (e.g., YFP). Upon Cre expression, the stop sequence is excised, leading to reporter expression. CreERT2 mice express the CreERT2 fusion protein that requires tamoxifen to be active. Upon tamoxifen injection, Cre is translocated to the nucleus and excises any floxed gene in CreERT2 mice crossed with floxed mice. Using CreERT2-Rosa26R mice allows the labeling of cells that express a specific promoter driving CreERT2 and the tracking of their progeny.

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Table 1. Advantages and disadvantages of the different approaches used to identify newborn neurons

Technique	Specific approach	Advantages	Disadvantages
Anatomical	Cellular morphometry, Nissl or Golgi staining, and electron microscopy	• Can be performed on tissues of any species, including humans	• Unable to determine the birth date
	Cell count	• *In vivo* labeling of cells is unnecessary	• Often limited to a few regions because it is difficult to count all regions • Stereology is used to extrapolate to other sections, but extrapolations do not account for regional differences
Birthdating		• Labels cells in S phase	• Labels only a fraction of cells • Incorporation during DNA repair and non-productive mitosis • Presence of BrdU labeling in $NeuN^+$ cells following transplantation of $BrdU^+$ dead cells
	Tritiated thymidine	• Linear kinetics for integration and emission	• Radioactive • Induces DNA damage • Indirect visualization
	Halogenated thymidine analogs (i.e., BrdU)	• Multiplexing possible	• Toxic • Can induce neuronal differentiation or fate changes • Rapid dilution during proliferation • Indirect visualization by fluorescent antibodies
	^{14}C	• Applicable to human tissues	• Use based on population analysis with resolution limited to <1% of total cell population
Fluorescent tracing		• Live imaging possible	• Injection can lead to tissue damage
	Lentiviral Adenoviral	• Widespread integration	• Labels mitotic and postmitotic cells • Large genomic insertions
	Retroviral	• Integrate into proliferating cells	• Not specific for progenitor cells (e.g., glia, microglia) • Can induce microglial fusion • Site of genomic integration can lead to pathological defects
	Postnatal electroporation	• Amenable for use with most plasmids	• Labels mitotic and possibly postmitotic cells • Rapid dilution if not used with genomic recombination • Precise birthdating not possible • Application of large amounts of voltage with unknown outcome
Transgenic mice		• Live imaging possible • Restricted expression possible • Appropriate with inducible systems	• Transgene may not distinguish birth date • May be expressed in many cell types • Transcriptional activity may be altered with injury

brevity, we have essentially limited our assessments to studies of neurogenesis in the neocortex and piriform cortex (Pir).

Evidence for and against adult-born cortical neurons

A widespread method for labeling dividing cells is the use of exogenous nucleotide analogs that are incorporated into DNA during its synthesis and thus into cells in the S-phase of the cell cycle. These nucleotide analogs include tritiated thymidine and the thymidine analog bromodeoxyuridine (BrdU) [9]. Use of BrdU, which can be visualized immunohistochemically, allows costaining for neuronal markers. Advantages and limitations of the labeling methods are summarized in Table 1. We discuss studies reporting adult neurogenesis in the neocortex and Pir, as well as several studies that did not detect it. The neocortex, which is the most evolutionary recent part of the cerebral cortex, is organized as six layers whereas the Pir is phylogenetically old and is organized as three layers.

The use of tritiated thymidine aided the identification of the two brain regions with the most prominent postnatal neurogenesis: the SVZ–olfactory bulb and the SGZ–hippocampal granule cell layer [10,11]. Tritiated thymidine also labeled a sparse population of proliferative cells in the neocortex of adult rats [10,12], of presumed neuronal identity based on electron microscopy, but at exceedingly low rates (0.011%) [12].

More recently, supporting evidence for neocortical neurogenesis, albeit at an extremely low rate, has been published using BrdU labeling and coimmunostaining for the neuronal marker NeuN (Table 2, Figure 1a) [13–18]. Although the use of BrdU is widespread, the field lacks standard experimental paradigms and very few studies quantified the number of adult-born $BrdU/NeuN^+$ cells [13,17]. Quantification revealed that the incidence of adult-born neurons was extremely low (between 0.0026% and 0.012% in monkeys; 3 newborn neurons/mm^3 in rats [17]). From these studies, two important characteristics of adult-born neurons emerged. First, their existence is likely transient [13]. Second, they are small, GABAergic interneurons, and not large, glutamatergic projection neurons [17,19].

Some studies found no evidence for adult-born neurons in the neocortex [20–24] (Table S1 in the supplementary material online). One study, performed in humans, used a clever approach: the use of radioactive ^{14}C taken up following nuclear weapons testing [20]. The biggest caveat to this study is the relatively low detection sensitivity of 1%, which is below the reported rate of adult cortical neurogenesis. The authors also calculated that they would miss