The notion that new neurons are added to the adult brain has been the subject of controversy ever since the mid-1980s, when Fernando Nottebohm's lab reported that adult neurogenesis occurs in the canary brain and is functionally linked to seasonal acquisition of new song. Early debates focused on whether or not this phenomenon was restricted to birds, but then came incontrovertible evidence of adult neurogenesis in rodents, specifically in the dentate gyrus of the hippocampus and the olfactory bulbs. Now, almost 30 years later, the debate is centered on the extent to which these findings in birds and rodents generalize to non-human primates and humans, and if they do, whether there are temporal and/or spatial (brain region) limits to postnatal neurogenesis in monkeys and humans? Nottebohm himself has stayed out of the fray, maintaining that the important question is how species solve problems that are relevant to them in nature. On the other hand, Pasko Rakic has taken the viewpoint that adult neurogenesis, particularly in the hippocampus and cortex, would in principle be evolutionarily disadvantageous to humans, because as a species, it would not be a good idea to sacrifice longevity of memory in the service of plasticity. Rakic was a long-time and staunch disbeliever of any type of adult neurogenesis in non-human primates and humans, but made small concessions every now and then, like the time when he himself found adult-born neurons in the hippocampus of macaque monkeys. Rakic has been particularly critical of work by Elizabeth Gould in rodents and non-human primates, and work by Fred Gage in humans has also figured prominently into the debate. A 2001 article from The New Yorker accompanies this question; it provides a human context for the debate as it stood some 10 years ago. Please address the following questions in this ongoing controversy.

1. What is the evidence for a gradual decrease in the prevalence of *adult* neurogenesis as one moves up the phylogenetic tree from birds to rodents, to monkeys, to humans?

2. To the extent that you find evidence for *postnatal* neurogenesis in rodents, non-human primates, and humans, does postnatal neurogenesis appear to be restricted to particular brain regions or to particular stages of development? In other words, evaluate the evidence for temporal or spatial constraints on postnatal neurogenesis in rodents, non-human primates, and humans.

3. To what extent do methodological considerations influence the debate? Do you think there are legitimate reasons for differences in interpretation of results based on methodology alone? If so, is it a matter of old vs new methods for identifying adult-born neurons, or is one method sufficiently flawed so as not to be trusted?

Controversy Question

Student 6

Pass, with high enthusiasm from all graders.

Student #6

Pass: I thought the author did a great job explaining how methods would affect the interpretation of specific studies. Very well-written and clear. Figures and table were helpful. Some typos which could have been caught with spell check and sometimes work was unreferenced.

Neurogenesis: defined

Neurogenesis refers to an intricate process culminating in the functional integration of new neurons to existing neural networks. This process is frequently separated into multiple stages including the proliferation, survival, migration, differentiation, maturation, and functional integration of neural precursors and neurons (Fig. 1). Historically, neural tissues were considered to be fully generated before birth. Within this framework, it was believed that an individual was born with all of the neurons he or she would ever develop and any plasticity was achieved through structural or chemical plasticity of existing cells. More recently, neurogenesis has been demonstrated in postnatal invertebrates and vertebrates—including birds and mammals, and even primates.

Comment [S1]: Need refs

Neurogenesis: common methods

While the field of adult neurogenesis is very young, it is growing rapidly. This growth has led to the development of many distinct methods with which to approach questions and hypotheses regarding adult neurogenesis. Most of the commonly used methods only identify certain steps along the process. Thus, the most convincing demonstrations often involve combining multiple methods to show converging evidence of complete neurogenesis. This section will provide a brief overview of commonly used methods, making note of each method's strengths and potential disadvantages. The methods used in specific experiments guide, and in certain cases limit, the interpretations one can draw from results, as will be discussed in the concluding remarks of this section.

Cell cycle and maturation

Student #6

Various methods for studying neurogenesis take advantage of the distinct steps involved in cell proliferation [1, 2]. During proliferation, cells undergo an orchestrated series of steps including Gap 1, Synthesis (S), Gap 2, and Mitosis. Cells can then either reenter the cell cycle to divide again or exit to differentiate and mature (Fig. 1).

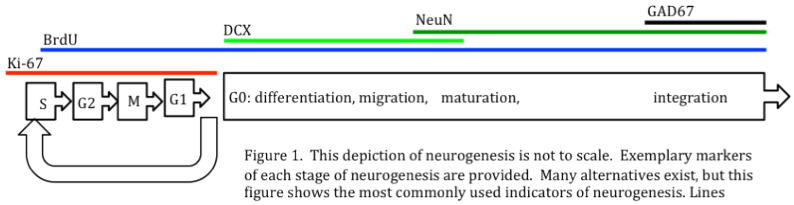

Figure 1. This depiction of neurogenesis is not to scale. Exemplary markers of each stage of neurogenesis are provided. Many alternatives exist, but this figure shows the most commonly used indicators of neurogenesis. Lines indicate times at which markers (named above) would be present.

Exogenous markers

During the S-phase of the cell cycle, cells replicate DNA in preparation for cell division. During DNA replication, bases (adenine, thymidine, guanine, and cytosine) are paired with the existing parental template to create a complementary strand. One of the earliest methods for labeling mitotic cells involved application of a radioactive version of thymidine: ^{3}H-thymidine [2, 3], which is readily incorporated into DNA in place of thymidine by cells in the S-phase at the time of injection. Because ^{3}H-thymidine is radioactive, it can be measured at any later time point using autoradiography [4]. The permanent incorporation of ^{3}H-thymidine's into DNA of proliferating cells allows retrospective birthdating of cells, but its use carries several inherent difficulties. Aside from the radioactivity of the label, the method by which the signal is detected, autoradiography, does not allow specification of neuronal phenotypes. Moreover, it is time consuming and limited to tissue depths of only a few microns.

To begin to address some of these methodological difficulties, thymidine analogues are now often used in place of ^{3}H-thymidine. Thymidine analogues (e.g. bromodeoxyuridine; BrdU) are so named because they are structurally very similar to thymidine [2]. Because of this similarity, cells readily incorporate exogenously applied thymidine analogues into DNA during synthesis [5]. In addition to avoiding radioactivity, BrdU detection through immunohistochemistry allows analysis of thicker brain sections and simultaneous labeling of endogenous proteins to differentiate neuronal

Comment [S2]: Figure 1 is really helpful (at least for me) in showing which markers are relevant to each stage.

Student #6

cells from other newly added cells (e.g. glia) [1]. It is also possible to provide injections of different types of thymidine analogues at different time points to label distinct cohorts of S-phase cells, which can be a powerful manipulation.

Comment [S3]: Why is this powerful?

BrdU, and other thymidine analogues, also have disadvantages. The use of thymidine analogues is very protocol sensitive. Dosage level, frequency and method of delivery, and post-injection survival time all have strong effects on the extent of labeling and the potential conclusions to be draw from data. First, there are several limits to the extent of labeling. While ^{3}H-thymidine and BrdU are only available for incorporation for 2 hours [3], the S-phase can last more than twice as long [6]. Moreover, while doses below 200-300 mg/kg bodyweight often label fewer cells [3] [7], saturating doses may confer greater adverse effects, such as cytotoxicity, especially when studying young or pregnant animals. Some reports suggest, however, that even saturating doses of BrdU do not confer adverse cytological effects [7]. Regardless, it is important to choose the level and frequency of dosage wisely, and to consider each of these parameters when comparing results across experiments.

Comment [S4]: interesting

Chief among the disadvantages of these exogenous markers is the fact that they label DNA synthesis, *not* cell proliferation [1]. This disadvantage is two pronged: 1) they do not label ALL proliferative cells, only those in S-phase within two hours of administration [3, 8], and 2) they do not label ONLY proliferative cells, as apoptotic cells sometimes undergo DNA synthesis as part of the abortive process. Fortunately, the latter issue is easily addressed through simultaneous immunohistochemical labeling: any cell coexpressing mature neuronal markers and BrdU at short post-injection survival time points e.g. [9] is likely an abortive cell. Although some skeptical critics also mention that thymidine analogues may label cells undergoing DNA repair, this claim has been discredited: 1) such repairs do not constitute addition of enough nucleotides for immunohistochemical detection of thymidine analogues, and 2) treatments that necessitate DNA repair while reducing cell proliferation cause dose-dependent reductions in BrdU labeling [10]. Other methodological concerns,

such as indiscriminate labeling of neuronal and nonneuronal cells are often addressed through simultaneous immunohistochemical labeling of endogenous markers.

Endogenous markers

The well-orchestrated expression of endogenous proteins during the cell cycle and neuronal differentiation and maturation (Fig. 1) makes immunohistochemistry a powerful tool in the study of adult neurogenesis. Cells naturally express many proteins during cell proliferation and maturation that can be targeted for analysis. Antibodies that target these proteins can be used to detect cells at respective points along the continuum of proliferation, differentiation, and maturation.

Proliferation

One antibody commonly used to detect proliferative cells is Ki-67, so named because it was discovered in the 67th well of a 96 well-plate in Kiel, Germany. Ki-67 labels all proliferative cells [11], and thus is useful when a more accurate measure of cell proliferation is the desired outcome. Ki-67 immunoreactivity consistently colabels BrdU+ cells [7] in the first day after BrdU injection, although it will label many more cells than BrdU because it is not limited to S-phase cells. If cells are Ki-67+/BrdU+ after longer post-injection survival times, it is likely that those cells have reentered the cellcycle to divide again after the time of BrdU administration. More than 20% of BrdU+ cells in the rodent dentate gyrus are also Ki-67+ on each of the first four days after BrdU application [12]. Given that the cell cycle in rodents is approximately 25 hours long [8] and BrdU is no longer detectable after several dilutions, this could contribute significantly to reductions in BrdU signal. Conversely, if cells divide once or twice then exit the cell cycle, they may amplify the signal.

Comment [S5]: I like how the author really spells out how each method could skew the results

It is important to note that there are currently several Ki-67 antibodies that recognize different epitopes on the same protein. Most antibodies label only proliferative cells [13] [11], but one antibody may label a very small subset of nonproliferative cells [13]. Interestingly, the protein that Ki-67 recognizes is in a different nuclear location in nonoproliferative cells, and is thought to be associated with synthesis of ribosomal RNA in these cells [13]. Cell cycle antibodies, like Ki-67, are very useful

Comment [S6]: What's an epitope?

when a more accurate measure of cell proliferation is the goal of the experiment, however these antibodies are not specific to neuronal precursors, and are not expressed at times when other phenotypic markers are likely to be present. Thus, if neuronal specificity is desired, other methods must be used.

Differentiation

When neuronal specification is deemed necessary, antibodies targeting endogenous proteins expressed in immature neuronal cells can be used—alone, or in conjunction with BrdU. When using such antibodies along with BrdU, the post-injection survival time must be considered carefully when choosing appropriate antibodies. Nestin is expressed by young neuroblasts and can be observed with BrdU from two to seven days after injections [14]. Doublecortin (DCX) is expressed next and can be seen with BrdU two to ten days after BrdU administration [15]. Of any phenotypic marker, DCX is most frequently used independently of BrdU to suggest ongoing neurogenesis. The selective expression of DCX in immature neuronal precursors suggests that this may be a sufficient indicator of proliferation and differentiation of neuronal precursors [15], but some suggest that DCX is also expressed in mature neurons undergoing physical plasticity changes and DCX-expression might be extended into adulthood in prenatally-derived cells[16]. Finally, the cell will begin to express proteins indicating a mature neuronal phenotype after at last seven days [17] to one month [10], and much longer in some species (e.g. 4 months in monkeys; [18]). These markers are very useful in indicating the neuronal phenotype of BrdU+ cells, but it this is by no means an exhaustive list of markers used to this end.

Functional integration

One of the major criticisms of adult neurogenesis is the scarcity of data supporting functional integration of neural precursors and neurons generated during adulthood. One way scientists have demonstrated functional integration is immunohistochemical detection of proteins expressed only by functioning neurons. Most of the adult-derived neurons seen thus far have been inhibitory

interneurons, and their functional integration has been shown through colocalization of BrdU with proteins such as the GAD67, the enzyme necessary for synthesis of GABA, an inhibitory neurotransmitter [19] [20]. Notably, uptake of exogenously applied retrograde tracers have also been used to definitively demonstrate functional integration of adult-derived cells [5]. Alternatively, electrophysiological measures can be used to demonstrate functional integration. Because functional integration, the last step in neurogenesis, is often not demonstrated in studies of postnatal neurogenesis, some skeptics have suggested that the process of adult neurogenesis is incomplete[REF].

Alternative methods

Viral manipulations

Some of the findings demonstrated in studies using BrdU have now been confirmed using recently developed technologies. For example, viral vectors can now be used to genetically label proliferative cells [21] [22]. In this way, reporter proteins can be inserted into the genome of proliferative cells so that they can be visualized after different time lengths to study migration, maturation and functional integration of adult-derived neurons. One disadvantage of the method is that viruses must be delivered stereotaxically into the area of interest, as they will not diffuse through tissue. Therefore, this method is not suited for exploratory research.

Carbon dating

Because administration of thymidine analogues may confer adverse effects including cytotoxicity, they are rarely administered to humans, and when they are, they are administered as a cancer prognostic tool, at levels much lower than those used in nonhuman animals. Recently, a method involving carbon dating cell nuclei to determine the age of the DNA, and thus the cell, has been developed. To date, this method has only been used in humans [23-25]. Although the retrospective method seems promising to investigate the age of human brain cells, the method operates under assumptions that may be violated. Namely, if cells recycle DNA, using nucleotides,

bases, or even only carbon from old, apoptotic cells, the method will provide a false negative. When cells containing BrdU are heat-killed and injected into the lateral ventricle of an animal, BrdU can be seen in proliferative cells of the subventricular zone thereafter [26], confirming that brain cells do recycle DNA products. Therefore, negative results, those that do not show evidence of adult neurogenesis, must be considered cautiously.

Comment [S7]: Interesting.

Methods: informing the interpretation of data

While each of the methods discussed above can be used to measure neurogenesis, it is important to correctly frame the definition of neurogenesis to encompass what is being measured, and no more. For example, the use of BrdU can show cell proliferation and survival, but it is important for new studies to employ double-, triple-, or quadruple- labeling in order to positively phenotype the cells as neural. Importantly, this is an important parameter for new studies now that such methods have become widely used and easily available, the remarkably high correlation [27] between phenotypic classification based on NeuN and morphological analysis (as was used before the advent of such markers) suggests that we need not discount data from previous studies.

Comment [S8]: Yay, an opinion!

Even after identifying a newly-derived, mature neuron, the process of demonstrating neurogenesis is not complete. Neurogenesis includes the functional integration of that neuron into the existing neural network. Thus, the most convincing data comes from studies employing several methods in conjunction. For example, using immunohistochemistry to label BrdU, NeuN, and GAD65 to demonstrate the survival, maturation, and functional integration of a new neuron would show complete neurogenesis. Much of the work in this field demonstrates only select parameters of neurogenesis (e.g. proliferation and survival of neuronal cells) without demonstrating the complete process. Several thorough demonstrations have produced fruitful results, however. Further, it is important to note that an absence of evidence is not evidence of absence, especially given the inherent likelihood of false negatives in many of these methods.

Comment [S9]: So it is important to use positive controls.

Student #6

In conclusion, while none of the methods currently under practice are sufficiently flawed as to discredit results based solely upon methodological choices, readers and scientists within this field should practice rigorous skepticism with room for appreciation of the technical difficulties and an open mind toward the possibility of constitutive neurogenesis in places previously considered nonneurogenic.

Adult neurogenesis through evolution

Replication is a very important part of the scientific process, and perhaps even more so when demonstrating novel findings that contradict previously held ideas, as is the case with adult neurogenesis. Unfortunately, many of the methods used to study adult neurogenesis are highly sensitive to protocol parameters and thus are difficult to replicate given even small deviations in methodology. BrdU, probably the most commonly used tool to study neurogenesis, is particularly sensitive to dosage, timing of delivery, and method of delivery. To illustrate this point, the half-life of BrdU in human plasma is between eight and eleven minutes [28]. Therefore the delivery of BrdU by intraperitoneal or intravenous injection rather than central infusion can have robust effects on the extent of labeling.

In comparing across species, even more parameters most be considered. Efforts to take circadian effects are important for neurogenesis, but matching the chronological time of injection between nocturnal and diurnal animals, as has been done previously [6], means reversing the levels of adrenal hormones and growth factors present at the time of injection, both associated with decreases in neurogenesis. To further complicate comparisons between species, differences in cell cycle dynamics and the time-span of neuronal maturation can differ greatly between species. For example, the cell cycle takes 2-5 times longer in monkeys than in rodents [6], and neuronal precursors of monkeys take more than 6 fold longer than those of rats to express NeuN [18], suggesting a slowed cell cycle and neuronal maturation in primates compared to rodents. These data provide a sampling of the species-specific differences that could affect results in studies of

Deleted: differentially

neurogenesis. In addition, they also demonstrate the nuances of interpreting data concerning species differences: although the lengthening of the cell cycle in primates compared to rodents may suggest a decrease in overall neurogenesis, it is important to note that primates also have longer gestation and postnatal lives, so the cells can enter the cell cycle many more times over. Indeed, despite the lengthened cell cycle, primates have many more cortical neurons than rodents.

Primary articles comparing across species or taxa are rare, and even those that do, often use different methodologies in the different species, including different survival times [29] or dosage [30], and sometimes failed to quantify results from each species [5, 19]. Despite difficulties in making direct comparisons between experiments and across species, statements as to the decline of adult neurogenesis as one climbs the phylogenetic tree are rampant in the literature. Some general patterns do suggest this progression. For example, adult neurogenesis is more common in brain regions with older ancestral origins (i.e. the olfactory bulb and hippocampus) [6]. In order to evaluate the premise that adult neurogenesis declines during the ascent of the phylogenetic tree, a small subset of existing data concerning adult neurogenesis from birds to rodents, non-human primates, and humans will be presented and compared within this section.

Spatial Specificity

The most common comparison between birds and mammals concerns the spatial specificity of neurogenesis. The prevalent theory is that while birds continue to add new neurons throughout the forebrain, postnatal mammalian neurogenesis is restricted to the olfactory bulb and the hippocampus, but an accumulating body of data suggests otherwise [REFS]. Therefore, levels of neurogenesis in these and other areas will be compared in birds and mammals, with specific examples from canaries, chickadees, mice, rats, monkeys, and humans.

Assessing the degree to which neurogenesis is restricted to confined spaces within the brain allows a comparison between species that is far less dependent upon methodological concerns, but this section will also contain quantitative comparisons while striving to normalize data to within

Comment [S10]: good

species parameters and to choose similar methodologies whenever possible. When discussing the location of neurogenesis, measures may be taken before or after migration. This section will be divided into an analysis of proliferative regions followed by neurogenic destinations.

Proliferative regions

Neurogenic proliferation within the subventricular zone (SVZ) lining the lateral ventricles is seen universally throughout the animal kingdom, including mammals [31] and birds [32]. Mammals have a second neurogenic niche in the subgranular zone (SGZ) of the dentate gyrus (DG) within the hippocampus [12, 20]. The hippocampal complex in the clade to which birds and reptiles belong, however, does not contain a neurogenic niche, instead all of the neuronal precursors divide along the walls of the lateral ventricles and migrate from there to multiple destinations (including the hippocampal complex) [33]. While this may suggest the opposite pattern—that adult neurogenesis increases as one climbs the phylogenetic tree—it is important to note that cells are continuously added to the hippocampi of mammals and birds alike, albeit from a different source. Further, fish and amphibians have many neurogenic niches, so the reptile- and bird- containing clade is distinct in having only one [34].

Comment [S11]: I'm not getting why this suggest the opposite pattern+
++

Neurogenic destination: hippocampus

Adult-derived hippocampal cells in mammals divide in the SGZ and migrate along radial glia a short distance to the granule cell layer. In birds, adult-derived hippocampal cells divide near the lateral ventricles and migrate into the hippocampal complex. It is likely that this difference reflects a difference in neuroanatomy rather than in adult neurogenesis.

Nonetheless, after careful analyses of empirical articles employing similar methods, a quantitative comparison in adult-derived hippocampal cells shows a trend toward reduction of adult neurogenesis as one climbs the phylogenetic tree from bird to primate. Approximately one month after 5-6 injections of a thymidine analogue, birds have produced enough cells to comprise 1/1000 neurons of the hippocampal complex [27], whereas rats have produced enough new cells to comprise

1/2000 [35], and monkeys have produced only 1000 neuronal granule cells, comprising approximately 1/48000 existing granule cells [6]. Further, whereas birds add between 240-650 cells/ mm^3 [4], humans add only between 2.2-48.4 [36] cells in the same volumetric unit. However, these data do not come from as closely related methodologies. While comparisons among these data are complicated by methodological and species-specific parameters, these data suggest a clear relationship between extent of adult neurogenesis within the hippocampus and place in the phylogenetic tree.

Neurogenic destination: olfactory bulb

In mammals, most neural precursors from the subventricular zone migrate together, in chains, along the rostral migrating stream culminating in the olfactory bulb [31]. While some studies suggest that the human SVZ and rostral migratory system are similar to those of other mammals [37], others suggest that cells migrate alone or in pairs, but not in chains [19]. Whereas rodents are thought to replace 50% of olfactory neurons each year [38], carbon dating methods suggest that less than one percent of cells in the human olfactory bulb are replaced after birth [24]. This finding may be tainted by the methodological concerns discussed earlier (recycling of DNA from apoptotic cells resulting in a false negative), as migrating neuroblasts have been seen in the human rostral migrating stream [39]. Interestingly, canaries generate, during the course of one day, enough cells to comprise 80 new cells/ mm^3 in olfactory bulb 38 days later, but they did not show a neuronal phenotype [32]. Taken together, while rodents seem to generate many new olfactory neurons during adulthood, data concerning adult olfactory neurogenesis in birds and humans is inconclusive, rendering phylogenetic comparisons difficult.

Comment [S12]: good

Neurogenic destination: parenchymal areas

Perhaps the most compelling argument for a decrease in adult neurogenesis as one climbs the phylogenetic tree from bird to mammal is that while it is widely accepted that adult neurogenesis occurs throughout the forebrain of avian species, adult neurogenesis outside of the hippocampus and olfactory system of mammals remains highly controversial. In fact the lack of proliferation outside of

these regions is so well accepted, that some companies suggest use of the cerebellum as a negative control for cell cycle markers (e.g. Leica-Novocastra), although adult neurogenesis has recently been characterized the cerebellum of rabbits [40].

Comment [S13]: interesting

Whereas neurogenesis within the SVZ/olfactory system is seen universally throughout the animal kingdom, and the SGZ/hippocampal system represents a mammalian neurogenic zone nearly without exception [41], evidence of parenchymal neurogenesis outside of these classic neurogenic zones is more heterogeneous both within and between species. Parenchymal neurogenesis is much more widely accepted in avian than mammalian species, but intraspecies differences can be driven by many external factors in both birds and mammals. One exemplary external factor that persists across species is the social environment. In birds [42] [4], as in rodents [43] the social environment affects neurogenesis in parenchymal forebrain structures. The common socially-stimulated region-specific recruitment of neuronal precursors suggests that social interactions are important for survival in both birds and mammals, but given that postnatal parenchymal neurogenesis is not nearly as well accepted as it is in birds, suggests a qualitative, or perhaps quantitative difference in widespread forebrain neurogenesis in birds and mammals.

It is well accepted that neural precursors in avian species migrate throughout the forebrain to differentiate and mature in distinct areas. Neurons seem to be recruited to particular areas, rather than dispersing throughout the forebrain at random. For example, in canaries the number of BrdU labeled cells increased over 37 days in some (HVC, Area X, and lMAN), but not all (RA) of the areas important for singing [32]. This region-specific recruitment is particularly surprising given that all of these areas are involved in the process of singing, suggesting that even within systems, parenchymal recruitment of SVZ cells in avian species is highly specific.

Student #6

Despite its lack of popularity, adult neurogenesis has been demonstrated in many parenchymal regions within the mammalian brain. Several well known examples are summarized in Table1, and I will go over one strong case briefly here. Adult neurogenesis is often seen in the hypothalamus [22, 46]. Moreover, adult-derived hypothalamic neurons have been shown to form synapses and express hypocretin [22], suggesting functional integration and thus complete neurogenesis. DiI and viral GFP labeling indicate that cells proliferate in the lining of the third ventricle, and migrate only locally into the hypothalamus [22]. This finding led to the hypothesis that cerebrospinal fluid supports constitutive neurogenesis [48] within the hypothalamus itself [22, 46] and provides evidence for a third neurogenic niche in the mammalian brain.

Table 1. Parenchymal neurogenesis in mammals (nonexhaustive list)

Region	Species	Evidence
Cortex	Rat Monkey Human + Human -	BrdU /NeuN, BrdU/GAD67 [44] BrdU /NeuN [29]; BrdU with uptake of a retrograde tracer [5] DCX [19] BrdU not colocalized with NeuN, carbon dating [25]
Amygdala	Mouse Vole Monkey	BrdU/DCX, BrdU/NeuN [45] BrdU/DCX, BrdU/NeuN [43] BrdU/NeuN [29]
Hypothalamus	Mouse Mouse	BrdU/Ki-67, BrdU/DCX, BrdU/NeuN [46] Viral GFP, dil, BrdU/DCX [22]
Brainstem	Rat	BrdU/DCX, BrdU/NeuN [47]

Comment [S14]: I was hoping someone would make a table

Therefore, while data from within the hippocampus support they premise that adult neurogenesis is reduced as one ascends the phylogenetic tree from bird to rodent, monkey, and human, evidence in the SVZ/olfactory pathway is inconclusive, and evidence from parenchymal regions are too heterogeneous to compare between species.

Postnatal neurogenesis through development

It has been suggested that postnatal neurogenesis, within species, is restricted by age. In this section, I will present and analyze data concerning age-related changes in postnatal neurogenesis in mammals, with a focus on the hippocampus, SVZ/olfactory, and cortical neurogenesis.

Hippocampus

Rats add seven times more cells to the DG during development than during adulthood, but survival of newly-derived cells in the granule cell layer is substantially lower in developing rats than

adult rats [12]. Therefore, although cell proliferation is higher during development, the increased rate of survival during adulthood may ameliorate the effects of aging on long-term addition of hippocampal granule cells [12]. Addition of new neuronal cells declines even further during aging, however. As rats age from six months to one year, the number of BrdU+ cells one month after injections shows a five-fold decrease [17], suggesting that survival also declines after aging beyond adulthood, perhaps indicating senscence.

Developing neurons (DCX+) are present in the human hippocampus in infants and centenarians, although the number of DCX+ cells decreases exponentially with age [20]. The number of DCX+ cells colabled with mature neuronal markers including NeuN and βIII-tubulin also decreases with age, but remains present through adulthood and into senescence [20]. The number of DCX+ cells colabeled with Ki-67 also decreases through aging [20], but this is just as likely to indicate a slowed neuronal maturation as a decrease in proliferating neuronal cells, because of the brevity of overlap between expression of Ki-67 and DCX (Fig. 1). These data suggest a clear and robust reduction in neurogenesis with aging. They do not indicate a restriction of neurogenesis, however, as cell proliferation and neuronal differentiation persists through adulthood and aging, but rather indicate a steady reduction in neurogenesis as a function of aging.

SVZ/Olfactory bulb

SVZ and olfactory neurogenesis are also characterized by age-related changes. Juvenile neurogenesis results in a substantial increase in olfactory and hippocampal neurons (contributing to 40%, and 25% increases, respectively [49]. In contrast, neither early postnatal nor adult neurogenesis cause a significant increase in neurons in either site [49]. A similar juvenile addition to sexually dimorphic regions of the hypothalamus is seen in rodents, suggesting a role for pubertal hormones in increasing neurogenesis [50]. In humans, SVZ proliferation may decline even earlier, before adolesecence. Evidence from surgical excisions and postmortem tissue suggests that the SVZ is

radically changed between infancy and adulthood, with a rapid decline in both proliferation and migration between six months and 18 months of age. These data corroborate the results substantiated by carbon dating which suggested that no substantial addition to cortex [23, 25] or olfactory bulb [24] occur after birth. Taken together, these data suggest that unlike the steady decline in hippocampal neurogenesis, SVZ/olfactory neurogenesis is characterized by more sudden age-related changes.

Cortex

There is substantial evidence for mammalian adult neurogenesis in several cortical areas. Immature neuronal (DCX+) cells are visible throughout cortical areas of the frontal lobe in cats, monkeys and humans [19]. This finding was confirmed in the temporal and prefrontal cortex of monkeys using BrdU and phenotypic neuronal markers [30]. In adult monkeys, immature cortical neurons are outnumbered by immature hippocampal neurons by a factor of 20-40, which could contribute to the widespread skepticism and several negative findings in humans [25]. The most compelling evidence, however, shows that adult-derived cortical neurons are labeled by retrograde tracers [5] in primates and contain GAD67 in rats [44]. These finding suggests that adult-derived cortical neurons are not only present, but functionally integrated to local circuits.

Although neurogenesis may continue through adulthood, there is no doubt that it is reduced in comparison to embryonic and infant neurogenesis. For example, DCX expression within the dorsolateral prefrontal cortex is present in humans from infancy through senescence, but it decreases substantially over time, with a 94% reduction from infancy to adulthood [51]. A similar, but less robust effect of age is seen in monkeys, with a 77% reduction in adult compared to neonate macaques [51].

Conclusions

The above data clearly demonstrate that postnatal development and aging are associated with marked reductions in neurogenesis, possibly with a rebound during adolescence. Despite some negative results [23-25], there is a wealth of data supporting ongoing neurogenesis in adulthood both

within and outside of the two classic neurogenic niches. Despite rather global, age-related reductions in neurogenesis, neurogenesis does not seem to be restricted to the classic neurogenic niches, even in adulthood.

Although neurogenesis is commonly defined as a developmental phenomenon, recent work has demonstrated that it is not restricted to prenatal or even early postnatal development. Instead, it continues throughout adulthood in many regions in birds, mammals and even primates, indicating a general reduction, rather than restriction, over the course of both development and evolution.

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This is an outstanding answer! Great job!

Neurogenesis: defined

Neurogenesis refers to an intricate process culminating in the functional integration of new neurons to existing neural networks. This process is frequently separated into multiple stages including the proliferation, survival, migration, differentiation, maturation, and functional integration of neural precursors and neurons (Fig. 1). Historically, neural tissues were considered to be fully generated before birth. Within this framework, it was believed that an individual was born with all of the neurons he or she would ever develop and any plasticity was achieved through structural or chemical plasticity of existing cells. More recently, neurogenesis has been demonstrated in postnatal invertebrates and vertebrates—including birds and mammals, and even primates.

Neurogenesis: common methods

While the field of adult neurogenesis is very young, it is growing rapidly. This growth has led to the development of many distinct methods with which to approach questions and hypotheses regarding adult neurogenesis. Most of the commonly used methods only identify certain steps along the process. Thus, the most convincing demonstrations often involve combining multiple methods to show converging evidence of complete neurogenesis. This section will provide a brief overview of commonly used methods, making note of each method's strengths and potential disadvantages. The methods used in specific experiments guide, and in certain cases limit, the interpretations one can draw from results, as will be discussed in the concluding remarks of this section.

Cell cycle and maturation

Various methods for studying neurogenesis take advantage of the distinct steps involved in cell proliferation [1, 2]. During proliferation, cells

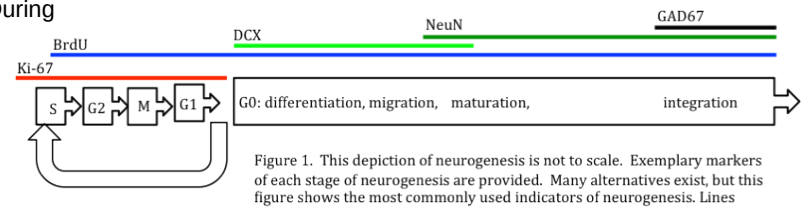

Figure 1. This depiction of neurogenesis is not to scale. Exemplary markers of each stage of neurogenesis are provided. Many alternatives exist, but this figure shows the most commonly used indicators of neurogenesis. Lines indicate times at which markers (named above) would be present.

undergo an orchestrated series of steps including Gap 1, Synthesis (S), Gap 2, and Mitosis. Cells can then either reenter the cell cycle to divide again or exit to differentiate and mature (Fig. 1).

Exogenous markers

During the S-phase of the cell cycle, cells replicate DNA in preparation for cell division. During DNA replication, bases (adenosine, thymidine, guanine, and cytosine) are paired with the existing parental template to create a complementary strand. One of the earliest methods for labeling mitotic cells involved application of a radioactive version of thymidine: ^{3}H-thymidine [2, 3], which is readily incorporated into DNA in place of thymidine by cells in the S-phase at the time of injection. Because ^{3}H-thymidine is radioactive, it can be measured at any later time point using autoradiography [4]. The permanent incorporation of ^{3}H-thymidines into DNA of proliferating cells allows retrospective birthdating of cells, but its use carries several inherent difficulties. Aside from the radioactivity of the label, the method by which the signal is detected, autoradiography, does not allow specification of neuronal phenotypes. Moreover, it is time consuming and limited to tissue depths of only a few microns.

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To begin to address some of these methodological difficulties, thymidine analogues are now often used in place of ^{3}H-thymidine. Thymidine analogues (e.g. bromodeoxyuridine; BrdU) are so named because they are structurally very similar to thymidine [2]. Because of this similarity, cells readily incorporate exogenously applied thymidine analogues into DNA during synthesis [5]. In addition to avoiding radioactivity, BrdU detection through immunohistochemistry allows analysis of thicker brain sections and simultaneous labeling of endogenous proteins to differentiate neuronal cells from other newly added cells (e.g. glia) [1]. It is also possible to provide injections of different types of thymidine analogues at different time points to label distinct cohorts of S-phase cells, which can be a powerful manipulation.

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BrdU, and other thymidine analogues, also have disadvantages. The use of thymidine analogues is very protocol sensitive. Dosage level, frequency and method of delivery, and post-injection survival time all have strong effects on the extent of labeling and the potential conclusions to be draw from data. First, there are several limits to the extent of labeling. While ^{3}H-thymidine and BrdU are only available for incorporation for 2 hours [3], the S-phase can last more than twice as long [6]. Moreover, while doses below 200-300 mg/kg bodyweight often label fewer cells [3] [7], saturating doses may confer greater adverse effects, such as cytotoxicity, especially when studying young or pregnant animals. Some reports suggest, however, that even saturating doses of BrdU do not confer adverse cytological effects [7]. Regardless, it is important to choose the level and frequency of dosage wisely, and to consider each of these parameters when comparing results across experiments.

Chief among the disadvantages of these exogenous markers is the fact that they label DNA synthesis, *not* cell proliferation [1]. This disadvantage is two pronged: 1) they do not label ALL proliferative cells, only those in S-phase within two hours of administration [3, 8], and 2) they do not label ONLY proliferative cells, as apoptotic cells sometimes undergo DNA synthesis as part of the abortive process. Fortunately, the latter issue is easily addressed through simultaneous immunohistochemical labeling: any cell coexpressing mature neuronal markers and BrdU at short post-injection survival time points e.g. [9] is likely an abortive cell. Although some skeptical critics also mention that thymidine analogues may label cells undergoing DNA repair, this claim has been discredited: 1) such repairs do not constitute addition of enough nucleotides for immunohistochemical detection of thymidine analogues, and 2) treatments that necessitate DNA repair while reducing cell proliferation cause dose-dependent reductions in BrdU labeling [10]. Other methodological concerns, such as indiscriminate labeling of neuronal and nonneuronal cells are often addressed through simultaneous immunohistochemical labeling of endogenous markers.

Endogenous markers

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The well-orchestrated expression of endogenous proteins during the cell cycle and neuronal differentiation and maturation (Fig. 1) makes immunohistochemistry a powerful tool in the study of adult neurogenesis. Cells naturally express many proteins during cell proliferation and maturation that can be targeted for analysis. Antibodies that target these proteins can be used to detect cells at respective points along the continuum of proliferation, differentiation, and maturation.

Proliferation

One antibody commonly used to detect proliferative cells is Ki-67, so named because it was discovered in the 67th well of a 96 well-plate in Kiel, Germany. Ki-67 labels all proliferative cells [11], and thus is useful when a more accurate measure of cell proliferation is the desired outcome. Ki-67 immunoreactivity consistently colabels BrdU+ cells [7] in the first day after BrdU injection, although it will label many more cells than BrdU because it is not limited to S-phase cells. If cells are Ki-67+/BrdU+ after longer post-injection survival times, it is likely that those cells have reentered the cell cycle to divide again after the time of BrdU administration. More than 20% of BrdU+ cells in the rodent dentate gyrus are also Ki-67+ on each of the first four days after BrdU application [12]. Given that the cell cycle in rodents is approximately 25 hours long [8] and BrdU is no longer detectable after several dilutions, this could contribute significantly to reductions in BrdU signal. Conversely, if cells divide once or twice then exit the cell cycle, they may amplify the signal.

It is important to note that there are currently several Ki-67 antibodies that recognize different epitopes on the same protein. Most antibodies label only proliferative cells [13] [11], but one antibody may label a very small subset of nonproliferative cells [13]. Interestingly, the protein Ki-67 recognizes is in a different nuclear location in nonoproliferative cells, and is thought to be associated with synthesis of ribosomal RNA in these cells [13]. Cell cycle antibodies, like Ki-67, are very useful when a more accurate measure of cell proliferation is the goal of the experiment, however these antibodies are not specific to neuronal precursors, and are not expressed at times when other phenotypic markers are likely to be present. Thus, if neuronal specificity is desired, other methods must be used.

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Differentiation

When neuronal specification is deemed necessary, antibodies targeting endogenous proteins expressed in immature neuronal cells can be used—alone, or in conjunction with BrdU. When using such antibodies along with BrdU, the post-injection survival time must be considered carefully when choosing appropriate antibodies. Nestin is expressed by young neuroblasts and can be observed with BrdU from two to seven days after injections [14]. Doublecortin (DCX) is expressed next and can be seen with BrdU two to ten days after BrdU administration [15]. Of any phenotypic marker, DCX is most frequently used independently of BrdU to suggest ongoing neurogenesis. The selective expression of DCX in immature neuronal precursors suggests that this may be a sufficient indicator of proliferation and differentiation of neuronal precursors [15], but some suggest that DCX is also expressed in mature neurons undergoing physical plasticity changes and DCX-expression might be extended into adulthood in prenatally-derived cells[16]. Finally, the cell will begin to express proteins indicating a mature neuronal phenotype after at last seven days [17] to one month [10], and much longer in some species (e.g. 4 months in monkeys; [18]). These markers are very useful in indicating the neuronal phenotype of BrdU+ cells, but it this is by no means an exhaustive list of markers used to this end.

Functional integration

One of the major criticisms of adult neurogenesis is the scarcity of data supporting functional integration of neural precursors and neurons generated during adulthood. One way scientists have demonstrated functional integration is immunohistochemical detection of proteins expressed only by functioning neurons. Most of the adult-derived neurons seen thus far have been inhibitory interneurons, and their functional integration has been shown through colocalization of BrdU with proteins such as the GAD67, the enzyme necessary for synthesis of GABA, an inhibitory neurotransmitter [19] [20]. Notably, uptake of exogenously applied retrograde tracers have also been used to definitively demonstrate functional integration of adult-derived cells [5]. Alternatively,

electrophysiological measures can be used to demonstrate functional integration. Because functional integration, the last step in neurogenesis, is often not demonstrated in studies of postnatal neurogenesis, some skeptics have suggested that the process of adult neurogenesis is incomplete.

Alternative methods

Viral manipulations

Some of the findings demonstrated in studies using BrdU have now been confirmed using recently developed technologies. For example, viral vectors can now be used to genetically label proliferative cells [21] [22]. In this way, reporter proteins can be inserted into the genome of proliferative cells so that they can be visualized after different time lengths to study migration, maturation and functional integration of adult-derived neurons. One disadvantage of the method is that viruses must be delivered stereotaxically into the area of interest, as they will not diffuse through tissue. Therefore, this method is not suited for exploratory research.

Carbon dating

Because administration of thymidine analogues may confer adverse effects including cytotoxicity, they are rarely administered to humans, and when they are, they are administered as a cancer prognostic tool, at levels much lower than those used in nonhuman animals. Recently, a method involving carbon dating cell nuclei to determine the age of the DNA, and thus the cell, has been developed. To date, this method has only been used in humans [23-25]. Although the retrospective method seems promising to investigate the age of human brain cells, the method operates under assumptions that may be violated. Namely, if cells recycle DNA, using nucleotides, bases, or even only carbon from old, apoptotic cells, the method will provide a false negative. When cells containing BrdU are heat-killed and injected into the lateral ventricle of an animal, BrdU can be seen in proliferative cells of the subventricular zone thereafter [26], confirming that brain cells do recycle DNA products. Therefore, negative results, those that do not show evidence of adult neurogenesis, must be considered cautiously.

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Methods: informing the interpretation of data

While each of the methods discussed above can be used to measure neurogenesis, it is important to correctly frame the definition of neurogenesis to encompass what is being measured, and no more. For example, the use of BrdU can show cell proliferation and survival, but it is important for new studies to employ double-, triple-, or quadruple- labeling in order to positively phenotype the cells as neural. Importantly, this is an important parameter for new studies now that such methods have become widely used and easily available, the remarkably high correlation [27] between phenotypic classification based on NeuN and morphological analysis (as was used before the advent of such markers) suggests that we need not discount data from previous studies.

Even after identifying a newly-derived, mature neuron, the process of demonstrating neurogenesis is not complete. Neurogenesis includes the functional integration of that neuron into the existing neural network. Thus, the most convincing data comes from studies employing several methods in conjunction. For example, using immunohistochemistry to label BrdU, NeuN, and GAD65 to demonstrate the survival, maturation, and functional integration of a new neuron would show complete neurogenesis. Much of the work in this field demonstrates only select parameters of neurogenesis (e.g. proliferation and survival of neuronal cells) without demonstrating the complete process. Several thorough demonstrations have produced fruitful results, however. Further, it is important to note that an absence of evidence is not evidence of absence, especially given the inherent likelihood of false negatives in many of these methods.

In conclusion, while none of the methods currently under practice are sufficiently flawed as to discredit results based solely upon methodological choices, readers and scientists within this field should practice rigorous skepticism with room for appreciation of the technical difficulties and an open mind toward the possibility of constitutive neurogenesis in places previously considered nonneurogenic.

Adult neurogenesis through evolution

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Replication is a very important part of the scientific process, and perhaps even more so when demonstrating novel findings that contradict previously held ideas, as is the case with adult neurogenesis. Unfortunately, many of the methods used to study adult neurogenesis are highly sensitive to protocol parameters and thus are difficult to replicate given even small deviations in methodology. BrdU, probably the most commonly used tool to study neurogenesis, is particularly sensitive to dosage, timing of delivery, and method of delivery. To illustrate this point, the half-life of BrdU in human plasma is between eight and eleven minutes [28]. Therefore the delivery of BrdU by intraperitoneal or intravenous injection rather than central infusion can have robust effects on the extent of labeling.

In comparing across species, even more parameters most be considered. Efforts to take circadian effects are important for neurogenesis, but matching the chronological time of injection between nocturnal and diurnal animals, as has been done previously [6], means reversing the levels of adrenal hormones and growth factors present at the time of injection, both associated with decreases in neurogenesis. To further complicate comparisons between species, differences in cell cycle dynamics and the time-span of neuronal maturation can differ greatly between species. For example, the cell cycle takes 2-5 times longer in monkeys than in rodents [6], and neuronal precursors of monkeys take more than 6 fold longer than those of rats to express NeuN [18], suggesting a slowed cell cycle and neuronal maturation in primates compared to rodents. These data provide a sampling of the species-specific differences that could differentially affect results in studies of neurogenesis. In addition, they also demonstrate the nuances of interpreting data concerning species differences: although the lengthening of the cell cycle in primates compared to rodents may suggest a decrease in overall neurogenesis, it is important to note that primates also have longer gestation and postnatal lives, so the cells can enter the cell cycle many more times over. Indeed, despite the lengthened cell cycle, primates have many more cortical neurons than rodents.

Primary articles comparing across species or taxa are rare, and even those that do, often use

different methodologies in the different species, including different survival times [29] or dosage [30], and sometimes failed to quantify results from each species [5, 19]. Despite difficulties in making direct comparisons between experiments and across species, statements as to the decline of adult neurogenesis as one climbs the phylogenetic tree are rampant in the literature. Some general patterns do suggest this progression. For example, adult neurogenesis is more common in brain regions with older ancestral origins (i.e. the olfactory bulb and hippocampus) [6]. In order to evaluate the premise that adult neurogenesis declines during the ascent of the phylogenetic tree, a small subset of existing data concerning adult neurogenesis from birds to rodents, non-human primates, and humans will be presented and compared within this section.

Spatial Specificity

The most common comparison between birds and mammals concerns the spatial specificity of neurogenesis. The prevalent theory is that while birds continue to add new neurons throughout the forebrain, postnatal mammalian neurogenesis is restricted to the olfactory bulb and the hippocampus, but an accumulating body of data suggests otherwise. Therefore, levels of neurogenesis in these and other areas will be compared in birds and mammals, with specific examples from canaries, chickadees, mice, rats, monkeys, and humans.

Assessing the degree to which neurogenesis is restricted to confined spaces within the brain allows a comparison between species that is far less dependent upon methodological concerns, but this section will also contain quantitative comparisons while striving to normalize data to within species parameters and to choose similar methodologies whenever possible. When discussing the location of neurogenesis, measures may be taken before or after migration. This section will be divided into an analysis of proliferative regions followed by neurogenic destinations.

Proliferative regions

Neurogenic proliferation within the subventricular zone (SVZ) lining the lateral ventricles is seen universally throughout the animal kingdom, including mammals [31] and birds [32]. Mammals

have a second neurogenic niche in the subgranular zone (SGZ) of the dentate gyrus (DG) within the hippocampus [12, 20]. The hippocampal complex in the clade to which birds and reptiles belong, however, does not contain a neurogenic niche, instead all of the neuronal precursors divide along the walls of the lateral ventricles and migrate from there to multiple destinations (including the hippocampal complex) [33]. While this may suggest the opposite pattern—that adult neurogenesis increases as one climbs the phylogenetic tree—it is important to note that cells are continuously added to the hippocampi of mammals and birds alike, albeit from a different source. Further, fish and amphibians have many neurogenic niches, so the reptile- and bird- containing clade is distinct in having only one [34].

Neurogenic destination: hippocampus

Adult-derived hippocampal cells in mammals divide in the SGZ and migrate along radial glia a short distance to the granule cell layer. In birds, adult-derived hippocampal cells divide near the lateral ventricles and migrate into the hippocampal complex. It is likely that this difference reflects a difference in neuroanatomy rather than in adult neurogenesis.

Nonetheless, after careful analyses of empirical articles employing similar methods, a quantitative comparison in adult-derived hippocampal cells shows a trend toward reduction of adult neurogenesis as one climbs the phylogenetic tree from bird to primate. Approximately one month after 5-6 injections of a thymidine analogue, birds have produced enough cells to comprise 1/1000 neurons of the hippocampal complex [27], whereas rats have produced enough new cells to comprise 1/2000 [35], and monkeys have produced only 1000 neuronal granule cells, comprising approximately 1/48000 existing granule cells [6]. Further, whereas birds add between 240-650 cells/ mm^3 [4], humans add only between 2.2-48.4 [36] cells in the same volumetric unit. However, these data do not come from as closely related methodologies. While comparisons among these data are complicated by methodological and species-specific parameters, these data suggest a clear relationship between extent of adult neurogenesis within the hippocampus and place in the phylogenetic tree.

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Neurogenic destination: olfactory bulb

In mammals, most neural precursors from the subventricular zone migrate together, in chains, along the rostral migrating stream culminating in the olfactory bulb [31]. While some studies suggest that the human SVZ and rostral migratory system are similar to those of other mammals [37], others suggest that cells migrate alone or in pairs, but not in chains [19]. Whereas rodents are thought to replace 50% of olfactory neurons each year [38], carbon dating methods suggest that less than one percent of cells in the human olfactory bulb are replaced after birth [24]. This finding may be tainted by the methodological concerns discussed earlier (recycling of DNA from apoptotic cells resulting in a false negative), as migrating neuroblasts have been seen in the human rostral migrating stream [39]. Interestingly, canaries generate, during the course of one day, enough cells to comprise 80 new cells/ mm^3 in olfactory bulb 38 days later, but they did not show a neuronal phenotype [32]. Taken together, while rodents seem to generate many new olfactory neurons during adulthood, data concerning adult olfactory neurogenesis in birds and humans is inconclusive, rendering phylogenetic comparisons difficult.

Neurogenic destination: parenchymal areas

Perhaps the most compelling argument for a decrease in adult neurogenesis as one climbs the phylogenetic tree from bird to mammal is that while it is widely accepted that adult neurogenesis occurs throughout the forebrain of avian species, adult neurogenesis outside of the hippocampus and olfactory system of mammals remains highly controversial. In fact the lack of proliferation outside of these regions is so well accepted, that some companies suggest use of the cerebellum as a negative control for cell cycle markers (e.g. Leica-Novocastra), although adult neurogenesis has recently been characterized the cerebellum of rabbits [40].

Whereas neurogenesis within the SVZ/olfactory system is seen universally throughout the animal kingdom, and the SGZ/hippocampal system represents a mammalian neurogenic zone nearly without exception [41], evidence of parenchymal neurogenesis outside of these classic neurogenic

zones is more heterogeneous both within and between species. Parenchymal neurogenesis is much more widely accepted in avian than mammalian species, but intraspecies differences can be driven by many external factors in both birds and mammals. One exemplary external factor that persists across species is the social environment. In birds [42] [4], as in rodents [43] the social environment affects neurogenesis in parenchymal forebrain structures. The common socially-stimulated region-specific recruitment of neuronal precursors suggests that social interactions are important for survival in both birds and mammals, but given that postnatal parenchymal neurogenesis is not nearly as well accepted as it is in birds, suggests a qualitative, or perhaps quantitative difference in widespread forebrain neurogenesis in birds and mammals.

It is well accepted that neural precursors in avian species migrate throughout the forebrain to differentiate and mature in distinct areas. Neurons seem to be recruited to particular areas, rather than dispersing throughout the forebrain at random. For example, in canaries the number of BrdU labeled cells increased over 37 days in some (HVC, Area X, and lMAN), but not all (RA) of the areas important for singing [32]. This region-specific recruitment is particularly surprising given that all of these areas are involved in the process of singing, suggesting that even within systems, parenchymal recruitment of SVZ cells in avian species is highly specific.

Table 1. Parenchymal neurogenesis in mammals (nonexhaustive list)

Region	Species	Evidence
Cortex	Rat	BrdU /NeuN, BrdU/GAD67 [44]
	Monkey	BrdU /NeuN [29]; BrdU with uptake of a retrograde tracer
	Human +	[5]
	Human -	DCX [19]

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		BrdU not colocalized with NeuN, carbon dating [25]
Amygdala	Mouse Vole Monkey	BrdU/DCX, BrdU/NeuN [45] BrdU/DCX, BrdU/NeuN [43] BrdU/NeuN [29]
Hypothalamus	Mouse Mouse	BrdU/Ki-67, BrdU/DCX, BrdU/NeuN [46] Viral GFP, dil, BrdU/DCX [22]
Brainstem	Rat	BrdU/DCX, BrdU/NeuN [47]

Despite its lack of popularity, adult neurogenesis has been demonstrated in many parenchymal regions within the mammalian brain. Several well known examples are summarized in Table1, and I will go over one strong case briefly here. Adult neurogenesis is often seen in the hypothalamus [22, 46]. Moreover, adult-derived hypothalamic neurons have been shown to form synapses and express hypocretin [22], suggesting functional integration and thus complete neurogenesis. DiI and viral GFP labeling indicate that cells proliferate in the lining of the third ventricle, and migrate only locally into the hypothalamus [22]. This finding led to the hypothesis that cerebrospinal fluid supports constitutive neurogenesis [48] within the hypothalamus itself [22, 46] and provides evidence for a third neurogenic niche in the mammalian brain.

Therefore, while data from within the hippocampus support they premise that adult neurogenesis is reduced as one ascends the phylogenetic tree from bird to rodent, monkey, and human, evidence in the SVZ/olfactory pathway is inconclusive, and evidence from parenchymal regions are too heterogeneous to compare between species.

Postnatal neurogenesis through development

It has been suggested that postnatal neurogenesis, within species, is restricted by age. In this section, I will present and analyze data concerning age-related changes in postnatal neurogenesis in mammals, with a focus on the hippocampus, SVZ/olfactory, and cortical neurogenesis.

Hippocampus

Rats add seven times more cells to the DG during development than during adulthood, but survival of newly-derived cells in the granule cell layer is substantially lower in developing rats than adult rats [12]. Therefore, although cell proliferation is higher during development, the increased rate

of survival during adulthood may ameliorate the effects of aging on long-term addition of hippocampal granule cells [12]. Addition of new neuronal cells declines even further during aging, however. As rats age from six months to one year, the number of BrdU+ cells one month after injections shows a five-fold decrease [17], suggesting that survival also declines after aging beyond adulthood, perhaps indicating senscence.

Developing neurons (DCX+) are present in the human hippocampus in infants and centenarians, although the number of DCX+ cells decreases exponentially with age [20]. The number of DCX+ cells colabled with mature neuronal markers including NeuN and βIII-tubulin also decreases with age, but remains present through adulthood and into senescence [20]. The number of DCX+ cells colabeled with Ki-67 also decreases through aging [20], but this is just as likely to indicate a slowed neuronal maturation as a decrease in proliferating neuronal cells, because of the brevity of overlap between expression of Ki-67 and DCX (Fig. 1). These data suggest a clear and robust reduction in neurogenesis with aging. They do not indicate a restriction of neurogenesis, however, as cell proliferation and neuronal differentiation persists through adulthood and aging, but rather indicate a steady reduction in neurogenesis as a function of aging.

SVZ/Olfactory bulb

SVZ and olfactory neurogenesis are also characterized by age-related changes. Juvenile neurogenesis results in a substantial increase in olfactory and hippocampal neurons (contributing to 40%, and 25% increases, respectively [49]. In contrast, neither early postnatal nor adult neurogenesis cause a significant increase in neurons in either site [49]. A similar juvenile addition to sexually dimorphic regions of the hypothalamus is seen in rodents, suggesting a role for pubertal hormones in increasing neurogenesis [50]. In humans, SVZ proliferation may decline even earlier, before adolesecence. Evidence from surgical excisions and postmortem tissue suggests that the SVZ is radically changed between infancy and adulthood, with a rapid decline in both proliferation and

migration between six months and 18 months of age. These data corroborate the results substantiated by carbon dating which suggested that no substantial addition to cortex [23, 25] or olfactory bulb [24] occur after birth. Taken together, these data suggest that unlike the steady decline in hippocampal neurogenesis, SVZ/olfactory neurogenesis is characterized by more sudden age-related changes.

Cortex

There is substantial evidence for mammalian adult neurogenesis in several cortical areas. Immature neuronal (DCX+) cells are visible throughout cortical areas of the frontal lobe in cats, monkeys and humans [19]. This finding was confirmed in the temporal and prefrontal cortex of monkeys using BrdU and phenotypic neuronal markers [30]. In adult monkeys, immature cortical neurons are outnumbered by immature hippocampal neurons by a factor of 20-40, which could contribute to the widespread skepticism and several negative findings in humans [25]. The most compelling evidence, however, shows that adult-derived cortical neurons are labeled by retrograde tracers [5] in primates and contain GAD67 in rats [44]. These finding suggests that adult-derived cortical neurons are not only present, but functionally integrated to local circuits.

Although neurogenesis may continue through adulthood, there is no doubt that it is reduced in comparison to embryonic and infant neurogenesis. For example, DCX expression within the dorsolateral prefrontal cortex is present in humans from infancy through senescence, but it decreases substantially over time, with a 94% reduction from infancy to adulthood [51]. A similar, but less robust effect of age is seen in monkeys, with a 77% reduction in adult compared to neonate macaques [51].

Conclusions

The above data clearly demonstrate that postnatal development and aging are associated with marked reductions in neurogenesis, possibly with a rebound during adolescence. Despite some negative results [23-25], there is a wealth of data supporting ongoing neurogenesis in adulthood both within and outside of the two classic neurogenic niches. Despite rather global, age-related reductions

in neurogenesis, neurogenesis does not seem to be restricted to the classic neurogenic niches, even in adulthood.

Although neurogenesis is commonly defined as a developmental phenomenon, recent work has demonstrated that it is not restricted to prenatal or even early postnatal development. Instead, it continues throughout adulthood in many regions in birds, mammals and even primates, indicating a general reduction, rather than restriction, over the course of both development and evolution.

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HIGH PASS Coherent, organized, well-informed and a display of mature judgment (e.g., how the idea of a change across phylogeny is widespread despite very little data).

Neurogenesis: defined

Neurogenesis refers to an intricate process culminating in the functional integration of new neurons to existing neural networks. This process is frequently separated into multiple stages including the proliferation, survival, migration, differentiation, maturation, and functional integration of neural precursors and neurons (Fig. 1). Historically, neural tissues were considered to be fully generated before birth. Within this framework, it was believed that an individual was born with all of the neurons he or she would ever develop and any plasticity was achieved through structural or chemical plasticity of existing cells. More recently, neurogenesis has been demonstrated in postnatal invertebrates and vertebrates—including birds and mammals, and even primates.

Neurogenesis: common methods

While the field of adult neurogenesis is very young, it is growing rapidly. This growth has led to the development of many distinct methods with which to approach questions and hypotheses regarding adult neurogenesis. Most of the commonly used methods only identify certain steps along the process. Thus, the most convincing demonstrations often involve combining multiple methods to show converging evidence of complete neurogenesis. This section will provide a brief overview of commonly used methods, making note of each method's strengths and potential disadvantages. The methods used in specific experiments guide, and in certain cases limit, the interpretations one can draw from results, as will be discussed in the concluding remarks of this section.

Cell cycle and maturation

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Various methods for studying neurogenesis take advantage of the distinct steps involved in cell proliferation [1, 2]. During proliferation, cells undergo an orchestrated series of steps including Gap 1, Synthesis (S), Gap 2, and Mitosis. Cells can then either reenter the cell cycle to divide again or exit to differentiate and mature (Fig. 1).

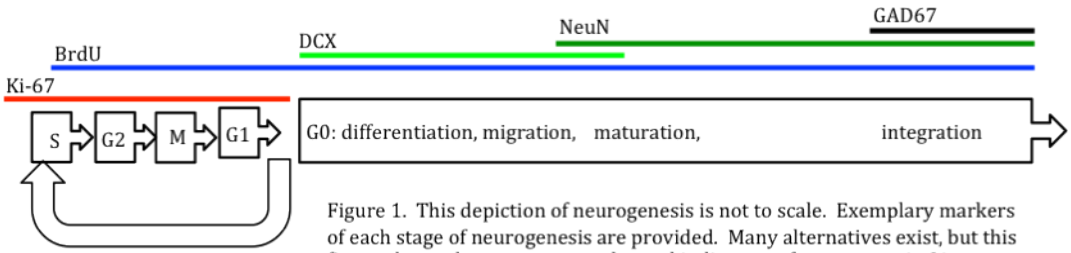

Figure 1. This depiction of neurogenesis is not to scale. Exemplary markers of each stage of neurogenesis are provided. Many alternatives exist, but this figure shows the most commonly used indicators of neurogenesis. Lines indicate times at which markers (named above) would be present.

Exogenous markers

During the S-phase of the cell cycle, cells replicate DNA in preparation for cell division. During DNA replication, bases (adenine, thymidine, guanine, and cytosine) are paired with the existing parental template to create a complementary strand. One of the earliest methods for labeling mitotic cells involved application of a radioactive version of thymidine: ^{3}H-thymidine [2, 3], which is readily incorporated into DNA in place of thymidine by cells in the S-phase at the time of injection. Because ^{3}H-thymidine is radioactive, it can be measured at any later time point using autoradiography [4]. The permanent incorporation of ^{3}H-thymidine's into DNA of proliferating cells allows retrospective birthdating of cells, but its use carries several inherent difficulties. Aside from the radioactivity of the label, the method by which the signal is detected, autoradiography, does not allow specfication of neuronal phenotypes. Moreover, it is time consuming and limited to tissue depths of only a few microns.

To begin to address some of these methodological difficulties, thymidine analogues are now often used in place of ^{3}H-thymidine. Thymidine analogues (e.g. bromodeoxyuridine; BrdU) are so named because they are structurally very similar to thymidine [2]. Because of this similarity, cells readily incorporate exogenously applied thymidine analogues into DNA during synthesis [5]. In addition to avoiding radioactivity, BrdU detection through immunohistochemistry allows analysis of thicker brain sections and simultaneous labeling of endogenous proteins to differentiate neuronal

cells from other newly added cells (e.g. glia) [1]. It is also possible to provide injections of different types of thymidine analogues at different time points to label distinct cohorts of S-phase cells, which can be a powerful manipulation.

BrdU, and other thymidine analogues, also have disadvantages. The use of thymidine analogues is very protocol sensitive. Dosage level, frequency and method of delivery, and post-injection survival time all have strong effects on the extent of labeling and the potential conclusions to be draw from data. First, there are several limits to the extent of labeling. While ^{3}H-thymidine and BrdU are only available for incorporation for 2 hours [3], the S-phase can last more than twice as long [6]. Moreover, while doses below 200-300 mg/kg bodyweight often label fewer cells [3] [7], saturating doses may confer greater adverse effects, such as cytotoxicity, especially when studying young or pregnant animals. Some reports suggest, however, that even saturating doses of BrdU do not confer adverse cytological effects [7]. Regardless, it is important to choose the level and frequency of dosage wisely, and to consider each of these parameters when comparing results across experiments.

Chief among the disadvantages of these exogenous markers is the fact that they label DNA synthesis, *not* cell proliferation [1]. This disadvantage is two pronged: 1) they do not label ALL proliferative cells, only those in S-phase within two hours of administration [3, 8], and 2) they do not label ONLY proliferative cells, as apoptotic cells sometimes undergo DNA synthesis as part of the abortive process. Fortunately, the latter issue is easily addressed through simultaneous immunohistochemical labeling: any cell coexpressing mature neuronal markers and BrdU at short post-injection survival time points e.g. [9] is likely an abortive cell. Although some skeptical critics also mention that thymidine analogues may label cells undergoing DNA repair, this claim has been discredited: 1) such repairs do not constitute addition of enough nucleotides for immunohistochemical detection of thymidine analogues, and 2) treatments that necessitate DNA repair while reducing cell proliferation cause dose-dependent reductions in BrdU labeling [10]. Other methodological concerns,

such as indiscriminate labeling of neuronal and nonneuronal cells are often addressed through simultaneous immunohistochemical labeling of endogenous markers.

Endogenous markers

The well-orchestrated expression of endogenous proteins during the cell cycle and neuronal differentiation and maturation (Fig. 1) makes immunohistochemistry a powerful tool in the study of adult neurogenesis. Cells naturally express many proteins during cell proliferation and maturation that can be targeted for analysis. Antibodies that target these proteins can be used to detect cells at respective points along the continuum of proliferation, differentiation, and maturation.

Proliferation

One antibody commonly used to detect proliferative cells is Ki-67, so named because it was discovered in the 67th well of a 96 well-plate in Kiel, Germany. Ki-67 labels all proliferative cells [11], and thus is useful when a more accurate measure of cell proliferation is the desired outcome. Ki-67 immunoreactivity consistently colabels BrdU+ cells [7] in the first day after BrdU injection, although it will label many more cells than BrdU because it is not limited to S-phase cells. If cells are Ki-67+/BrdU+ after longer post-injection survival times, it is likely that those cells have reentered the cellcycle to divide again after the time of BrdU administration. More than 20% of BrdU+ cells in the rodent dentate gyrus are also Ki-67+ on each of the first four days after BrdU application [12]. Given that the cell cycle in rodents is approximately 25 hours long [8] and BrdU is no longer detectable after several dilutions, this could contribute significantly to reductions in BrdU signal. Conversely, if cells divide once or twice then exit the cell cycle, they may amplify the signal.

It is important to note that there are currently several Ki-67 antibodies that recognize different epitopes on the same protein. Most antibodies label only proliferative cells [13] [11], but one antibody may label a very small subset of nonproliferative cells [13]. Interestingly, the protein Ki-67 recognizes is in a different nuclear location in nonoproliferative cells, and is thought to be associated with synthesis of ribosomal RNA in these cells [13]. Cell cycle antibodies, like Ki-67, are very useful when

a more accurate measure of cell proliferation is the goal of the experiment, however these antibodies are not specific to neuronal precursors, and are not expressed at times when other phenotypic markers are likely to be present. Thus, if neuronal specificity is desired, other methods must be used.

Differentiation

When neuronal specification is deemed necessary, antibodies targeting endogenous proteins expressed in immature neuronal cells can be used—alone, or in conjunction with BrdU. When using such antibodies along with BrdU, the post-injection survival time must be considered carefully when choosing appropriate antibodies. Nestin is expressed by young neuroblasts and can be observed with BrdU from two to seven days after injections [14]. Doublecortin (DCX) is expressed next and can be seen with BrdU two to ten days after BrdU administration [15]. Of any phenotypic marker, DCX is most frequently used independently of BrdU to suggest ongoing neurogenesis. The selective expression of DCX in immature neuronal precursors suggests that this may be a sufficient indicator of proliferation and differentiation of neuronal precursors [15], but some suggest that DCX is also expressed in mature neurons undergoing physical plasticity changes and DCX-expression might be extended into adulthood in prenatally-derived cells[16]. Finally, the cell will begin to express proteins indicating a mature neuronal phenotype after at last seven days [17] to one month [10], and much longer in some species (e.g. 4 months in monkeys; [18]). These markers are very useful in indicating the neuronal phenotype of BrdU+ cells, but it this is by no means an exhaustive list of markers used to this end.

Functional integration

One of the major criticisms of adult neurogenesis is the scarcity of data supporting functional integration of neural precursors and neurons generated during adulthood. One way scientists have demonstrated functional integration is immunohistochemical detection of proteins expressed only by functioning neurons. Most of the adult-derived neurons seen thus far have been inhibitory interneurons, and their functional integration has been shown through colocalization of BrdU with

proteins such as the GAD67, the enzyme necessary for synthesis of GABA, an inhibitory neurotransmitter [19] [20]. Notably, uptake of exogenously applied retrograde tracers have also been used to definitively demonstrate functional integration of adult-derived cells [5]. Alternatively, electrophysiological measures can be used to demonstrate functional integration. Because functional integration, the last step in neurogenesis, is often not demonstrated in studies of postnatal neurogenesis, some skeptics have suggested that the process of adult neurogenesis is incomplete.

Alternative methods

Viral manipulations

Some of the findings demonstrated in studies using BrdU have now been confirmed using recently developed technologies. For example, viral vectors can now be used to genetically label proliferative cells [21] [22]. In this way, reporter proteins can be inserted into the genome of proliferative cells so that they can be visualized after different time lengths to study migration, maturation and functional integration of adult-derived neurons. One disadvantage of the method is that viruses must be delivered stereotaxically into the area of interest, as they will not diffuse through tissue. Therefore, this method is not suited for exploratory research.

Carbon dating

Because administration of thymidine analogues may confer adverse effects including cytotoxicity, they are rarely administered to humans, and when they are, they are administered as a cancer prognostic tool, at levels much lower than those used in nonhuman animals. Recently, a method involving carbon dating cell nuclei to determine the age of the DNA, and thus the cell, has been developed. To date, this method has only been used in humans [23-25]. Although the retrospective method seems promising to investigate the age of human brain cells, the method operates under assumptions that may be violated. Namely, if cells recycle DNA, using nucleotides, bases, or even only carbon from old, apoptotic cells, the method will provide a false negative. When cells containing BrdU are heat-killed and injected into the lateral ventricle of an animal, BrdU can be

seen in proliferative cells of the subventricular zone thereafter [26], confirming that brain cells do recycle DNA products. Therefore, negative results, those that do not show evidence of adult neurogenesis, must be considered cautiously.

Methods: informing the interpretation of data

While each of the methods discussed above can be used to measure neurogenesis, it is important to correctly frame the definition of neurogenesis to encompass what is being measured, and no more. For example, the use of BrdU can show cell proliferation and survival, but it is important for new studies to employ double-, triple-, or quadruple- labeling in order to positively phenotype the cells as neural. Importantly, this is an important parameter for new studies now that such methods have become widely used and easily available, the remarkably high correlation [27] between phenotypic classification based on NeuN and morphological analysis (as was used before the advent of such markers) suggests that we need not discount data from previous studies.

Even after identifying a newly-derived, mature neuron, the process of demonstrating neurogenesis is not complete. Neurogenesis includes the functional integration of that neuron into the existing neural network. Thus, the most convincing data comes from studies employing several methods in conjunction. For example, using immunohistochemistry to label BrdU, NeuN, and GAD65 to demonstrate the survival, maturation, and functional integration of a new neuron would show complete neurogenesis. Much of the work in this field demonstrates only select parameters of neurogenesis (e.g. proliferation and survival of neuronal cells) without demonstrating the complete process. Several thorough demonstrations have produced fruitful results, however. Further, it is important to note that an absence of evidence is not evidence of absence, especially given the inherent likelihood of false negatives in many of these methods.

In conclusion, while none of the methods currenty under practice are sufficiently flawed as to discredit results based solely upon methodological choices, readers and scientists within this field should practice rigorous skepticism with room for appreciation of the technical difficulties and an open

mind toward the possibility of constitutive neurogenesis in places previously considered nonneurogenic.

Adult neurogenesis through evolution

Replication is a very important part of the scientific process, and perhaps even more so when demonstrating novel findings that contradict previously held ideas, as is the case with adult neurogenesis. Unfortunately, many of the methods used to study adult neurogenesis are highly sensitive to protocol parameters and thus are difficult to replicate given even small deviations in methodology. BrdU, probably the most commonly used tool to study neurogenesis, is particularly sensitive to dosage, timing of delivery, and method of delivery. To illustrate this point, the half-life of BrdU in human plasma is between eight and eleven minutes [28]. Therefore the delivery of BrdU by intraperitoneal or intravenous injection rather than central infusion can have robust effects on the extent of labeling.

In comparing across species, even more parameters most be considered. Efforts to take circadian effects are important for neurogenesis, but matching the chronological time of injection between nocturnal and diurnal animals, as has been done previously [6], means reversing the levels of adrenal hormones and growth factors present at the time of injection, both associated with decreases in neurogenesis. To further complicate comparisons between species, differences in cell cycle dynamics and the time-span of neuronal maturation can differ greatly between species. For example, the cell cycle takes 2-5 times longer in monkeys than in rodents [6], and neuronal precursors of monkeys take more than 6 fold longer than those of rats to express NeuN [18], suggesting a slowed cell cycle and neuronal maturation in primates compared to rodents. These data provide a sampling of the species-specific differeces that could differentially affect results in studies of neurogenesis. In addition, they also demonstrate the nuances of interpreting data concerning species differences: although the lengthening of the cell cycle in primates compared to rodents may suggest a decrease in overall neurogenesis, it is important to note that primates also have longer

gestation and postnatal lives, so the cells can enter the cell cycle many more times over. Indeed, despite the lengthened cell cycle, primates have many more cortical neurons than rodents.

Primary articles comparing across species or taxa are rare, and even those that do, often use different methodologies in the different species, including different survival times [29] or dosage [30], and sometimes failed to quantify results from each species [5, 19]. Despite difficulties in making direct comparisons between experiments and across species, statements as to the decline of adult neurogenesis as one climbs the phylogenetic tree are rampant in the literature. Some general patterns do suggest this progression. For example, adult neurogenesis is more common in brain regions with older ancestral origins (i.e. the olfactory bulb and hippocampus) [6]. In order to evaluate the premise that adult neurogenesis declines during the ascent of the phylogenetic tree, a small subset of existing data concerning adult neurogenesis from birds to rodents, non-human primates, and humans will be presented and compared within this section.

Spatial Specificity

The most common comparison between birds and mammals concerns the spatial specificity of neurogenesis. The prevalent theory is that while birds continue to add new neurons throughout the forebrain, postnatal mammalian neurogenesis is restricted to the olfactory bulb and the hippocampus, but an accumulating body of data suggests otherwise. Therefore, levels of neurogenesis in these and other areas will be compared in birds and mammals, with specific examples from canaries, chickadees, mice, rats, monkeys, and humans.

Assessing the degree to which neurogenesis is restricted to confined spaces within the brain allows a comparison between species that is far less dependent upon methodological concerns, but this section will also contain quantitative comparisons while striving to normalize data to within species parameters and to choose similar methodologies whenever possible. When discussing the location of neurogenesis, measures may be taken before or after migration. This section will be divided into an analysis of proliferative regions followed by neurogenic destinations.

Proliferative regions

Neurogenic proliferation within the subventricular zone (SVZ) lining the lateral ventricles is seen universally throughout the animal kingdom, including mammals [31] and birds [32]. Mammals have a second neurogenic niche in the subgranular zone (SGZ) of the dentate gyrus (DG) within the hippocampus [12, 20]. The hippocampal complex in the clade to which birds and reptiles belong, however, does not contain a neurogenic niche, instead all of the neuronal precursors divide along the walls of the lateral ventricles and migrate from there to multiple destinations (including the hippocampal complex) [33]. While this may suggest the opposite pattern—that adult neurogenesis increases as one climbs the phylogenetic tree—it is important to note that cells are continuously added to the hippocampi of mammals and birds alike, albeit from a different source. Further, fish and amphibians have many neurogenic niches, so the reptile- and bird- containing clade is distinct in having only one [34].

Neurogenic destination: hippocampus

Adult-derived hippocampal cells in mammals divide in the SGZ and migrate along radial glia a short distance to the granule cell layer. In birds, adult-derived hippocampal cells divide near the lateral ventricles and migrate into the hippocampal complex. It is likely that this difference reflects a difference in neuroanatomy rather than in adult neurogenesis.

Nonetheless, after careful analyses of empirical articles employing similar methods, a quantitative comparison in adult-derived hippocampal cells shows a trend toward reduction of adult neurogenesis as one climbs the phylogenetic tree from bird to primate. Approximately one month after 5-6 injections of a thymidine analogue, birds have produced enough cells to comprise 1/1000 neurons of the hippocampal complex [27], whereas rats have produced enough new cells to comprise 1/2000 [35], and monkeys have produced only 1000 neuronal granule cells, comprising approximately 1/48000 existing granule cells [6]. Further, whereas birds add between 240-650 cells/ mm^3 [4], humans add only between 2.2-48.4 [36] cells in the same volumetric unit. However, these data do not

come from as closely related methodologies. While comparisons among these data are complicated by methodological and species-specific parameters, these data suggest a clear relationship between extent of adult neurogenesis within the hippocampus and place in the phylogenetic tree.

Neurogenic destination: olfactory bulb

In mammals, most neural precursors from the subventricular zone migrate together, in chains, along the rostral migrating stream culminating in the olfactory bulb [31]. While some studies suggest that the human SVZ and rostral migratory system are similar to those of other mammals [37], others suggest that cells migrate alone or in pairs, but not in chains [19]. Whereas rodents are thought to replace 50% of olfactory neurons each year [38], carbon dating methods suggest that less than one percent of cells in the human olfactory bulb are replaced after birth [24]. This finding may be tainted by the methodological concerns discussed earlier (recycling of DNA from apoptotic cells resulting in a false negative), as migrating neuroblasts have been seen in the human rostral migrating stream [39]. Interestingly, canaries generate, during the course of one day, enough cells to comprise 80 new cells/ mm^3 in olfactory bulb 38 days later, but they did not show a neuronal phenotype [32]. Taken together, while rodents seem to generate many new olfactory neurons during adulthood, data concerning adult olfactory neurogenesis in birds and humans is inconclusive, rendering phylogenetic comparisons difficult.

Neurogenic destination: parenchymal areas

Perhaps the most compelling argument for a decrease in adult neurogenesis as one climbs the phylogenetic tree from bird to mammal is that while it is widely accepted that adult neurogenesis occurs throughout the forebrain of avian species, adult neurogenesis outside of the hippocampus and olfactory system of mammals remains highly controversial. In fact the lack of proliferation outside of these regions is so well accepted, that some companies suggest use of the cerebellum as a negative control for cell cycle markers (e.g. Leica-Novocastra), although adult neurogenesis has recently been characterized the cerebellum of rabbits [40].

Whereas neurogenesis within the SVZ/olfactory system is seen universally throughout the animal kingdom, and the SGZ/hippocampal system represents a mammalian neurogenic zone nearly without exception [41], evidence of parenchymal neurogenesis outside of these classic neurogenic zones is more heterogeneous both within and between species. Parenchymal neurogenesis is much more widely accepted in avian than mammalian species, but intraspecies differences can be driven by many external factors in both birds and mammals. One exemplary external factor that persists across species is the social environment. In birds [42] [4], as in rodents [43] the social environment affects neurogenesis in parenchymal forebrain structures. The common socially-stimulated region-specific recruitment of neuronal precursors suggests that social interactions are important for survival in both birds and mammals, but given that postnatal parenchymal neurogenesis is not nearly as well accepted as it is in birds, suggests a qualitative, or perhaps quantitative difference in widespread forebrain neurogenesis in birds and mammals.

It is well accepted that neural precursors in avian species migrate throughout the forebrain to differentiate and mature in distinct areas. Neurons seem to be recruited to particular areas, rather than dispersing throughout the forebrain at random. For example, in canaries the number of BrdU labeled cells increased over 37 days in some (HVC, Area X, and lMAN), but not all (RA) of the areas important for singing [32]. This region-specific recruitment is particularly surprising given that all of these areas are involved in the process of singing, suggesting that even within systems, parenchymal recruitment of SVZ cells in avian species is highly specific.

Table 1. Parenchymal neurogenesis in mammals (nonexhaustive list)

Region	Species	Evidence

Cortex	Rat Monkey Human + Human -	BrdU /NeuN, BrdU/GAD67 [44] BrdU /NeuN [29]; BrdU with uptake of a retrograde tracer [5] DCX [19] BrdU not colocalized with NeuN, carbon dating [25]
Amygdala	Mouse Vole Monkey	BrdU/DCX, BrdU/NeuN [45] BrdU/DCX, BrdU/NeuN [43] BrdU/NeuN [29]
Hypothalamus	Mouse Mouse	BrdU/Ki-67, BrdU/DCX, BrdU/NeuN [46] Viral GFP, dil, BrdU/DCX [22]
Brainstem	Rat	BrdU/DCX, BrdU/NeuN [47]

Despite its lack of popularity, adult neurogenesis has been demonstrated in many parenchymal regions within the mammalian brain. Several well known examples are summarized in Table1, and I will go over one strong case briefly here. Adult neurogenesis is often seen in the hypothalamus [22, 46]. Moreover, adult-derived hypothalamic neurons have been shown to form synapses and express hypocretin [22], suggesting functional integration and thus complete neurogenesis. DiI and viral GFP labeling indicate that cells proliferate in the lining of the third ventricle, and migrate only locally into the hypothalamus [22]. This finding led to the hypothesis that cerebrospinal fluid supports constitutive neurogenesis [48] within the hypothalamus itself [22, 46] and provides evidence for a third neurogenic niche in the mammalian brain.

Therefore, while data from within the hippocampus support they premise that adult neurogenesis is reduced as one ascends the phylogenetic tree from bird to rodent, monkey, and human, evidence in the SVZ/olfactory pathway is inconclusive, and evidence from parenchymal regions are too heterogeneous to compare between species.

Postnatal neurogenesis through development

It has been suggested that postnatal neurogenesis, within species, is restricted by age. In this section, I will present and analyze data concerning age-related changes in postnatal neurogenesis in mammals, with a focus on the hippocampus, SVZ/olfactory, and cortical neurogenesis.

Hippocampus

Rats add seven times more cells to the DG during development than during adulthood, but survival of newly-derived cells in the granule cell layer is substantially lower in developing rats than adult rats [12]. Therefore, although cell proliferation is higher during development, the increased rate

of survival during adulthood may ameliorate the effects of aging on long-term addition of hippocampal granule cells [12]. Addition of new neuronal cells declines even further during aging, however. As rats age from six months to one year, the number of BrdU+ cells one month after injections shows a five-fold decrease [17], suggesting that survival also declines after aging beyond adulthood, perhaps indicating senscence.

Developing neurons (DCX+) are present in the human hippocampus in infants and centenarians, although the number of DCX+ cells decreases exponentially with age [20]. The number of DCX+ cells colabled with mature neuronal markers including NeuN and βIII-tubulin also decreases with age, but remains present through adulthood and into senescence [20]. The number of DCX+ cells colabeled with Ki-67 also decreases through aging [20], but this is just as likely to indicate a slowed neuronal maturation as a decrease in proliferating neuronal cells, because of the brevity of overlap between expression of Ki-67 and DCX (Fig. 1). These data suggest a clear and robust reduction in neurogenesis with aging. They do not indicate a restriction of neurogenesis, however, as cell proliferation and neuronal differentiation persists through adulthood and aging, but rather indicate a steady reduction in neurogenesis as a function of aging.

SVZ/Olfactory bulb

SVZ and olfactory neurogenesis are also characterized by age-related changes. Juvenile neurogenesis results in a substantial increase in olfactory and hippocampal neurons (contributing to 40%, and 25% increases, respectively [49]. In contrast, neither early postnatal nor adult neurogenesis cause a significant increase in neurons in either site [49]. A similar juvenile addition to sexually dimorphic regions of the hypothalamus is seen in rodents, suggesting a role for pubertal hormones in increasing neurogenesis [50]. In humans, SVZ proliferation may decline even earlier, before adolesecence. Evidence from surgical excisions and postmortem tissue suggests that the SVZ is radically changed between infancy and adulthood, with a rapid decline in both proliferation and

migration between six months and 18 months of age. These data corroborate the results substantiated by carbon dating which suggested that no substantial addition to cortex [23, 25] or olfactory bulb [24] occur after birth. Taken together, these data suggest that unlike the steady decline in hippocampal neurogenesis, SVZ/olfactory neurogenesis is characterized by more sudden age-related changes.

Cortex

There is substantial evidence for mammalian adult neurogenesis in several cortical areas. Immature neuronal (DCX+) cells are visible throughout cortical areas of the frontal lobe in cats, monkeys and humans [19]. This finding was confirmed in the temporal and prefrontal cortex of monkeys using BrdU and phenotypic neuronal markers [30]. In adult monkeys, immature cortical neurons are outnumbered by immature hippocampal neurons by a factor of 20-40, which could contribute to the widespread skepticism and several negative findings in humans [25]. The most compelling evidence, however, shows that adult-derived cortical neurons are labeled by retrograde tracers [5] in primates and contain GAD67 in rats [44]. These finding suggests that adult-derived cortical neurons are not only present, but functionally integrated to local circuits.

Although neurogenesis may continue through adulthood, there is no doubt that it is reduced in comparison to embryonic and infant neurogenesis. For example, DCX expression within the dorsolateral prefrontal cortex is present in humans from infancy through senescence, but it decreases substantially over time, with a 94% reduction from infancy to adulthood [51]. A similar, but less robust effect of age is seen in monkeys, with a 77% reduction in adult compared to neonate macaques [51].

Conclusions

The above data clearly demonstrate that postnatal development and aging are associated with marked reductions in neurogenesis, possibly with a rebound during adolescence. Despite some negative results [23-25], there is a wealth of data supporting ongoing neurogenesis in adulthood both within and outside of the two classic neurogenic niches. Despite rather global, age-related reductions

in neurogenesis, neurogenesis does not seem to be restricted to the classic neurogenic niches, even in adulthood.

Although neurogenesis is commonly defined as a developmental phenomenon, recent work has demonstrated that it is not restricted to prenatal or even early postnatal development. Instead, it continues throughout adulthood in many regions in birds, mammals and even primates, indicating a general reduction, rather than restriction, over the course of both development and evolution.

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