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 7

Pass, with high enthusiasm from all graders.

Student #7

Pass: Very well organized and well-written essay. Answered all parts of the question and gave an explicit opinion.

We are born with all the neurons we're going to have. For many years, this statement was considered a well-known fact in the field of neuroscience. However, within the last 30 years the field has changed drastically with the finding that neurogenesis can occur in specific regions of the brain throughout adulthood. Here I will consider current ideas and controversies in the field of adult neurogenesis. In this review, I will highlight the methods used for identifying new neurons in the brain, evidence for a decreased prevalence of neurogenesis when comparing birds versus rodents and primates, the temporal and spatial constraints of postnatal neurogenesis, and methodological issues that may contribute to controversies in the field.

Adult neurogenesis: Methodology

Two common techniques used to identify newly generated neurons in the brain are ^{3}H-thymidine autoradiography and 5-bromo-2-deoxyuridine (BrdU) labeling. ^{3}H-thymidine autoradiography is an older technique. With this method, isotopically labeled thymidine (^{3}H-thymidine) is injected into an animal and ^{3}H-thymidine is incorporated into the DNA of cells going through the S-phase (synthesis phase) of cell cycle [1]. Thus, labeled cells will be those that were in the S-phase of mitosis when ^{3}H-thymidine was injected. Labeled cells are then visualized using autoradiography. Morphological characteristics of the cells must be examined to determine if the newly formed cells are neurons [2]. A newer technique for labeling proliferating cells in the brain is through the use of BrdU labeling. BrdU is a thymidine analog that is also incorporated into the DNA of cells going through the S-phase of the cell cycle [3]. Similar to ^{3}H-thymidine autoradiography, BrdU is injected into animals, animals are sacrificed

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after a period of time (known as the survival time) and BrdU labeling can be detected through the use of antibodies [2]. The benefit of using BrdU is that cells can be co-labeled with neuronal markers to determine the identity of BrdU labeled cells [3]. Both of these techniques have been used in determining whether adult neurogenesis occurs in various parts of the brain.

Adult neurogenesis: Gradual decrease from birds to rodents to primates

Adult neurogenesis occurs in many animals with the distribution and amount of neurogenesis varying by taxa. Comparatively, birds are believed to have a higher prevalence of adult neurogenesis than rodents and primates.[REFs] Below I will highlight the discovery of adult neurogenesis in songbirds and provide evidence as to why rodents and primates are believed to have a reduced levels of adult neurogenesis compared to birds. Note that further details on the discovery of adult neurogenesis in rodents and primates will be discussed in "Mammalian postnatal neurogenesis: Spatial constraints".

Adult neurogenesis in birds, specifically songbirds, was first noted by Nottebohm and colleagues in the mid-1980s. They discovered in adult canaries that newly divided cells in the brain were generated in the ventricular zone and then migrated to areas of the telencephalon, notably the higher vocal center (HVC)[4, 5]; these cells then differentiated into neurons and some of the new neurons produced action potentials indicating that they were potentially functional within the neuronal circuitry [5, 6]. This finding was intriguing, since the HVC is a brain structure important in song acquisition and production in songbirds; specifically, the HVC projection to the RA is essential in song production [7]. Thus, adult neurogenesis in the HVC may be essential for the production of a new songs in canaries, since these birds produce a new song repertoire each year [8, 9]. In fact, neurogenesis in the HVC is highest during the times of

Comment [s1]: Need to spell out

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year in which the bird's song is modified [10] and many of the newly generated neurons project to the RA [11] further illustrating that neurogenesis in the HVC may be important in the production of new songs each year. Additionally, these new neurons are believed to replace existing neurons in the HVC, since neuronal death proceeds neuronal production in the HVC and the total number of neurons in the HVC does not change across the year [10, 12]. Notably, this high level of neurogenesis and neuronal turnover in songbirds is hypothesized to be important for establishing new neuronal circuits, so new learning can occur each year [7]. One basis for this hypothesis is that canaries have a relatively long lifespan compared to similarly sized mammals (about 5x longer than that of a mouse) and due to space limitations in the brain, higher neurogenesis may have evolved to allow new memories to be formed each year [13, 14]. Although I highlighted the importance of neurogenesis in the HVC of the canary, adult neurogenesis in the hippocampus has also been implicated in the formation of new memories each year in chickadees [15]. Thus, in birds, higher levels of adult neurogenesis may serve as a form of plasticity within the brain to enable new learning to occur each year.

Comment [s2]: interesting

Compared to songbirds and other non-mammalian vertebrates, the prevalence of adult neurogenesis in the rodent is believed to be somewhat reduced. While adult neurogenesis in birds can occur across the telencephalon, in rodents, adult neurogenesis is for the most part restricted to the hippocampal dentate gyrus and the subventricular zone (SVZ); new cells are produced in the SVZ and migrate to the olfactory bulb where the differentiate into neurons [16-18]. A reason why neurogenesis may have been retained and restricted to these brain sites over the course of evolution, is that it may have been beneficial to conserve plasticity mechanisms in areas of the brain important for detecting and encoding complex environments and novel stimuli [17]. In fact, neurogenesis is enhanced in the hippocampus of rodents maintained in an enriched

Comment [s3]: is there a cost to having high plasticity across the brain? If so, what is it? Seems like birds are good cachers too yet they have plasticity in multiple regions…

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environment [19] and novel stimuli elicit greater cellular responsiveness, as measured by c-Fos, in new adult born olfactory neurons compared to preexisting olfactory neurons [20]. Thus, both of these studies suggest that neurogenesis in the hippocampus and olfactory bulb in rodents may aid in novelty learning and detection. In comparison to birds, adult neurogenesis in rodents may still serve as mechanism of plasticity, but to lesser extent.

Comment [s4]: this sentence is too vague. Does the author mean that even in the hippocampus, neurogenesis in the rat is less than in birds? Does lesser extent mean lesser spatial extent?

Compared to rodents and birds, adult neurogenesis in primates is considered to be significantly decreased. In non-human primates, adult neurogenesis occurs both in the hippocampal dentate gyrus and the SVZ [21-23], however, the overall levels of neurogenesis that occur in the dentate gyrus, for example, in non-human primates are dramatically reduced compared to rodents; it is estimated that new neurons contribute to .004% of the granule cell layer of the hippocampus per day in macaques, compared to 0.1% per day in rodents [3, 21]. Although these numbers are not completely reliable, since methodological issues can contribute discrepancies in the total number of new neurons counted (see "Methodological considerations for adult neurogenesis"), it does suggest that neurogenesis in primates is decreased compared to rodents. Adult neurogenesis has also been observed in the hippocampus of humans, but the overall levels compared to rodents are believed to be reduced [24]. Thus, these studies suggest that reduced levels of adult neurogenesis may have been selected for in mammalian evolution, particularly in primates [17]. In fact Rakic has postulated that in primates, such as humans, neurogenesis may not be as advantageous, since adding or replacing neurons in a stable neuronal circuitry may disrupt already stored experiences and memories important for long-lived species, such as humans [25, 26]. Thus, although we do not currently know the functional implications of adult neurogenesis in humans, its presence suggests that adult neurogenesis has been conserved across evolution and perhaps may sub-serve some role in plasticity.

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Mammalian postnatal neurogenesis: Temporal constraints

As noted above, adult neurogenesis occurs across the lifetime of many animals. However, the concept of *postnatal* neurogenesis is applied to neurogenesis that occurs after birth and in some cases across the lifespan of an animal. Thus, there are two defined periods of postnatal neurogenesis; "protracted" neurogenesis occurs for a limited time after birth and is considered as an extension of embryonic neurogenesis, while "persistent" neurogenesis occurs across the lifespan of the animal [18]. Both protracted and persistent neurogenesis occur in discrete brain areas. For example, protracted neurogenesis occurs in the cerebellum of most mammals, while persistent neurogenesis mainly occurs in the hippocampal dentate gyrus and SVZ [18]. Below I will highlight evidence for temporal constraints of both protracted and persistent postnatal neurogenesis.

Protracted postnatal neurogenesis occurs in the cerebellum of most mammals. Cerebellar postnatal neurogenesis occurs via cell proliferation in the external granular layer (EGL), a part of the cerebellum present for a short time after birth; the dividing cells that eventually differentiate into neurons reside in the EGL [18]. Cell proliferation in the cerebellum can occur from weeks to 1 year postnatally, depending on the species [27]. In mice, cell proliferation in the cerebellum, as measured by ^{3}H-thymidine labeling, is high up to postnatal day 10 with no cell proliferation observed after postnatal day 20 [28]. The last cells to develop and differentiate from these proliferating cells are small granule neurons in the cerebellum [29]. In humans, postnatal neurogenesis in the cerebellum can extend for a year after birth [27]. Thus, it is possible that the delayed production of granule neurons after birth may play an important role in motor learning during the early stages of a mammal's life, as adding new neurons and creating new neuronal

connections during this period may aid in the development of fundamental motor movements, such as walking [18].

Contrary to protracted neurogenesis, persistent neurogenesis occurs across the lifetime of an animal, but the amount of neurogenesis may change in an age-dependent fashion. In rats, cell proliferation is reduced in the hippocampal dentate gyrus in old (21 month) versus young (6 month) rats [30], indicating that although cell proliferation and neurogenesis may persist in old age, the overall levels are reduced. Levels of cell proliferation and neurogenesis are also reduced with age in the hippocampal dentate gyrus and SVZ in non-human primates [31, 32]. Notably, neurogenesis in the hippocampal dentate gyrus is significantly decreased after 3 months of age and after 1 year, the levels of neurogenesis are relatively low [31]. Thus, these studies suggest that although neurogenesis may occur across an animal's lifetime in specific brain areas, temporal constraints may be limiting the overall levels of neurogenesis during different stages of life.

Comment [s5]: What about birds?

Mammalian postnatal neurogenesis: Spatial constraints

The first reports of postnatal neurogenesis in rodents were noted by Altman and colleagues in the mid-1960's [29, 33], but at that time little attention was given to such findings. Other reports then came from Kaplan and colleagues in late-1970's and mid-1980's; these studies used ^{3}H-thymidine autoradiography and electron microscopy to indicated postnatal neurogenesis in hippocampal dentate gyrus of rodents [34, 35]. Again, these findings were not initially recognized by the scientific community. It wasn't until Nottebohm's discovery of adult neurogenesis in songbirds [4-6, 13] that considerable attention was given to the notion of postnatal neurogenesis in rodents as well. In fact, many studies have now confirmed that

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postnatal neurogenesis exists in two brain sites of rodents under normal physiological conditions, the hippocampal dentate gyrus and SVZ; newly divided cells in the SVZ migrate to the olfactory bulb via the rostral migratory stream (RMS) where they differentiate into neurons [2, 16, 36]. Both the hippocampal dentate gyrus and SVZ are known as "neurogenic" zones, since neurogenesis is recognized to occur there in adult rodents; all other regions of the brain are known as "non-neurogenic", since adult neurogenesis is not widely recognized to occur in these other brain regions [2].

Although postnatal neurogenesis in rodents became recognized in the field after the mid-1980's the idea of neurogenesis in adult primates remained a controversial issue. In 1985, Rakic used ^{3}H-thymindine autoradiographic labeling in adult rhesus monkeys to determine that adult neurogenesis does not occur in primates [25]. He further speculated that in long-lived primates, neurogenesis during adulthood may be disadvantageous, since new neurons would need to form synaptic connections and integrate into neuronal networks established by prior experiences [25]. Contrary to this notion, Eriksson et al. used BrdU and neuronal makers to establish that newly generated neurons were produced in the SVZ and hippocampal dentate gyrus of deceased adult human cancer patients; cells expressing BrdU in the dentate gyrus were co-labeled with neuronal markers indicating that these cells did in fact differentiate into neurons [24]. This study brought new insight to the field suggesting that humans do have the capability of generating new neurons in adulthood. Reevaluation of neurogenesis in adult non-human primates was performed by both Kornack and Rakic and Gould et al. in 1999; both labs found levels of adult neurogenesis in the hippocampal dentate gryus [21, 23]. Furthermore, existence a RMS contributing to new neurons in the olfactory bulb in adult non-human primates [22] and potentially in humans [37], further

illustrates that neurogenesis in primates, similar to rodents, occurs in the aforementioned neurogenic sites of the brain.

Although it is well-established that adult neurogenesis occurs in neurogenic zones, neurogenesis in non-neurogenic zones is a topic of much debate. Areas of the brain that have controversial evidence for and against neurogenesis are the neocortex, striatum, hypothalamus, amygdala, and substantia nigra [2, 18, 38]. Here, I will limit my discussion to conflicting evidence of adult neurogenesis in the neocortex.

Adult neurogenesis in the neocortex of rodents is still a controversial topic. Kaplan was one of the first to report adult neurogenesis in the neocortex using ^{3}H-thymidine autoradiography and electron microscopy [39]. However, subsequent studies failed to find the existence of adult neurogenesis in the neocortex under normal physiological conditions; these studies found cell proliferation in the neocortex, but noted that such cells were either glial or undifferentiated cells, not neurons [40, 41]. Contrary to these results, a more recent study found adult neurogenesis in many areas of the necortex [42]. The investigators noted that many of the newly formed neurons were very small in size and since neurogenesis in the neocortex may occur at low levels, it may have made it difficult for prior investigators to find these neurons [42, 43]. In fact, these new neurons expressed GAD-67, a GABA synthesizing enzyme, indicating that new neurons generated in the neocortex may be GABA-ergic interneurons [42]. Thus, further studies in rodents may be warranted to confirm if low levels of neurogenesis in the neocortex may be contributing to the interneuron population in adulthood.

Deleted: following

Similar to evidence in rats, adult neurogenesis in the cortex is very controversial in primates. Gould et al. reported new neurons in the prefrontal, parietal, and temporal cortex of adult macaques; some of the new proliferating cells were suggested to have divided in the

neocortex, while others were believed to have originated from newly divided cells in SVZ that migrated to the neocortex where they differentiated into neurons [44, 45]. While other studies have found proliferating cells in the neocortex of non-human primates, it was noted that many of cells failed to also express neuronal markers; those cells that appeared to be newly divided neurons were in fact two separate cells (one a newly divided cell and the other a neuron) in close approximation to one another [46, 47].

Further evidence against adult neurogenesis in the cortex came from a study using human brain tissue. Bhardwaj et al. used carbon-14 (^{14}C) dating to identify newly generated cells in human brain tissue [48]. The basis for this technique is that during the atomic bomb testing in the Cold War, atmospheric ^{14}C levels were dramatically increased; ^{14}C would have been incorporated into the human body, specifically DNA of newly dividing cells at that time. Individuals born before or after the atomic bomb testing were used to determine via ^{14}C dating that no new neurons were detected in human cortex [48]. Further, labeling for BrdU and neuronal markers in the human cortex indicated no adult neurogenesis [48]. Overall, these studies have lead some scientists to believe that adult neurogenesis does not occur in the cortex [26]. While this conclusion may be true, differences in methodological procedures may have contributed to some of the discrepancies between different studies. These methodological considerations will now be reviewed.

Methodological considerations for adult neurogenesis

Both ^{3}H-thymidine autoradiography and BrdU labeling have been widely used in studies of adult neurogenesis. ^{3}H-thymidine autoradiography is an older technique that labels cells in the S-phase of mitosis and requires that morphological features of the cells be characterized in

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order to determine if the newly generated cells are in fact neurons. This technique is very time consuming and it can be difficult to perform reliably [49]. However, many of the hallmark studies establishing the existence of adult neurogenesis have used ^{3}H-thymidine autoradiography and such conclusions have been confirmed with other labeling techniques, such as double labeling with BrdU and neuronal markers [2, 19, 29, 33, 34]. Thus, I do not think that controversies in the field are based primarily on whether an "old" versus a "new" technique was used. This is not to say that one technique, such as BrdU double labeled with neuronal markers, might provide more compelling evidence of neurogenesis than another, but rather other methodological issues may be contributing to discrepancies between studies. I think there may be many issues contributing to whether a "false negative" (saying there is no effect when there is one) versus a "false positive" (saying there is an effect when there isn't one) is occurring. Below I will give examples of methodological issues that could be contributing to conflicting conclusions about adult neurogenesis.

Comment [s6]: Why?

Comment [s7]: Yeah, an opinion!!

Methodological issues could contribute to some researchers concluding prematurely that adult neurogenesis does not occur in specific brain areas. One factor that could contribute to a false negative is the amount of survival time given after the ^{3}H-thymidine or BrdU injection. Survival time issues can especially come into play when looking at a brain area that might already have low levels of neurogenesis; if the survival time is too long, the labeled neurons may have already died, while if the survival times are too short, the newly divided cells may not have had enough time to differentiate into neurons leading to the false conclusion that neurogenesis does not occur in that brain area [7]. The length of survival time may have come into play when adult neurogenesis was examined in non-human primates, since the first study claimed that adult neurogenesis does not occur (survival times ranged from 3 months to 6 years) [25], while

following studies did find adult neurogenesis in non-human primates (survival times ranged from 2hrs to 97 days) [21-23]. An additional, methodological consideration is the age of the animals used for the study. Some studies looking at neurogenesis in non-neurogenic sites use animals of varying ages [23, 25]. As stated before, levels of neurogenesis reduce with age [31, 32], so using an old subject base to determine that neurogenesis does not occur in a specific brain site may bias the results.

Another methodological issue that could contribute to a false negative result is the quality of staining and quantitative measures used for determining what is considered a new labeled neuron. If the quality of staining is poor, brain areas that may be producing new neurons, but at lower levels, may not be considered as neurogenic brain sites. Gould has indicated the importance of using a positive control, such as verifying that cells are co-labeled with BrdU and neuronal markers in the dentate gyrus, whenever looking at brain sites that are considered non-neurogenic [2]. This consideration is important in ensuring that the tissue is adequately stained to label new neurons in areas where they may be produced at low levels. Another important consideration is using a consistent standard for counting new neurons in the different brain sites. For example, many of the cells considered to be new neurons in the cortex are believed to be GABA-ergic interneurons, which have a smaller soma than surrounding cortical pyramidal neurons [42, 43]. Thus, it may not only be harder to find such newly generated neurons, but if new neurons labeled with BrdU and neuronal markers are not counted consistently across different brain sites (for example, the cortex versus the hippocampal dentate gyrus), then it may be prematurely concluded that adult neurogenesis does not exist in such areas. Thus, it is important that standards of counting double labeled cells be kept across all brain sites that are analyzed.

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In addition to false negatives, false positives may occur depending on how the tissue is analyzed. It is possible that some cells stained with BrdU appear to be co-labeled with neuronal markers, but are in fact separate cells in close proximity to labeled neurons. By using confocal z-series analysis, which basically takes 3D images of stained tissue, Koketsu et al. determined that many BrdU cells that appeared to be co-labeled with neuronal markers were in fact separately labeled cells [47]. Thus, studies that do not use this technique may be inaccurately counting newly divided cells as neurons when they may not be.

Overall, a systematic method of determining whether adult neurogenesis occurs in particular areas of the brain, especially in non-neurogenic areas, should be used. The fact that different standards are used for identifying newly generated neurons in non-neurogenic zones most likely contributes conflicting results across studies. To get around this issues, multiple measures should be used to validate claims, such as double labeling cells with neuronal markers, using z-series analysis to confirm that cells are double labeled, using positive controls to validate quality of tissue staining, and using consistent standards in evaluating neurogenesis across brain areas.

1. Sanes, D.H., T.A. Reh, and W.A. Harris, *Development of the nervous system*. 3 ed. 2012: Elsevier Inc.
2. Gould, E., *Opinion - How widespread is adult neurogenesis in mammals?* Nature Reviews Neuroscience, 2007. **8**(6): p. 481-488.
3. Taupin, P., *BrdU immunohistochemistry for studying adult neurogenesis: Paradigms, pitfalls, limitations, and validation.* Brain Research Reviews, 2007. **53**(1): p. 198-214.
4. Goldman, S.A. and F. Nottebohm, *Neuronal production, migration, and differentiation in a vocal control nucleus of the adult female canary brain.* Proceedings of the National Academy of Sciences of the United States of America-Biological Sciences, 1983. **80**(8): p. 2390-2394.
5. Alvarezbuylla, A. and F. Nottebohm, *Migration of young neurons in adult avian brain.* Nature, 1988. **335**(6188): p. 353-354.

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6. Paton, J.A. and F. Nottebohm, *Neurons generated in adult brain are recruited into functional circuits.* Science, 1984. **225**(4666): p. 1046-1048.
7. Nottebohm, F., *Why are some neurons replaced in adult brain?* Journal of Neuroscience, 2002. **22**(3): p. 624-628.
8. Nottebohm, F., *From bird song to neurogenesis.* Scientific American, 1989. **260**(2): p. 74-79.
9. Nottebohm, F., *Neuronal replacement in adult brain.* Brain Research Bulletin, 2002. **57**(6): p. 737-749.
10. Kirn, J., et al., *Cell death and neuronal recruitment in the high vocal center of adult male canaries are temporally related to changes in song.* Proceedings of the National Academy of Sciences of the United States of America, 1994. **91**(17): p. 7844-7848.
11. Alvarezbuylla, A., J.R. Kirn, and F. Nottebohm, *Birth of projection neurons in adult avian brain may be related to perceptual or motor learning.* Science, 1990. **249**(4975): p. 1444-1446.
12. Alvarezbuylla, A., C.Y. Ling, and F. Nottebohm, *High vocal center growth and its relation to neurogenesis, neuronal replacement and song acquisition in juvenile canaries.* Journal of Neurobiology, 1992. **23**(4): p. 396-406.
13. Nottebohm, F., *Neuronal replacement in adulthood.* Annals of the New York Academy of Sciences, 1985. **457**: p. 143-161.
14. Kirn, J.R. and F. Nottebohm, *Direct evidence for loss and replacement of projection neurons in adult canary brain.* Journal of Neuroscience, 1993. **13**(4): p. 1654-1663.
15. Barnea, A. and F. Nottebohm, *Seasonal recruitment of hippocampal neurons in adult free ranging black capped chickadees.* Proceedings of the National Academy of Sciences of the United States of America, 1994. **91**(23): p. 11217-11221.
16. Lois, C. and A. Alvarezbuylla, *Long-distance neuronal migration in the adult mammalian brain.* Science, 1994. **264**(5162): p. 1145-1148.
17. Kaslin, J., J. Ganz, and M. Brand, *Proliferation, neurogenesis and regeneration in the non-mammalian vertebrate brain.* Philosophical Transactions of the Royal Society B-Biological Sciences, 2008. **363**(1489): p. 101-122.
18. Bonfanti, L. and P. Peretto, *Adult neurogenesis in mammals - a theme with many variations.* European Journal of Neuroscience, 2011. **34**(6): p. 930-950.
19. Kempermann, G., H.G. Kuhn, and F.H. Gage, *More hippocampal neurons in adult mice living in an enriched environment.* Nature, 1997. **386**(6624): p. 493-495.
20. Magavi, S.S.P., et al., *Adult-born and preexisting olfactory granule neurons undergo distinct experience-dependent modifications of their olfactory responses in vivo.* Journal of Neuroscience, 2005. **25**(46): p. 10729-10739.
21. Kornack, D.R. and P. Rakic, *Continuation of neurogenesis in the hippocampus of the adult macaque monkey.* Proceedings of the National Academy of Sciences of the United States of America, 1999. **96**(10): p. 5768-5773.
22. Kornack, D.R. and P. Rakic, *The generation, migration, and differentiation of olfactory neurons in the adult primate brain.* Proceedings of the National Academy of Sciences of the United States of America, 2001. **98**(8): p. 4752-4757.
23. Gould, E., et al., *Hippocampal neurogenesis in adult Old World primates.* Proceedings of the National Academy of Sciences of the United States of America, 1999. **96**(9): p. 5263-5267.

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24. Eriksson, P.S., et al., *Neurogenesis in the adult human hippocampus.* Nature Medicine, 1998. **4**(11): p. 1313-1317.
25. Rakic, P., *Limits of neurogenesis in primates.* Science, 1985. **227**(4690): p. 1054-1056.
26. Rakic, P., *No more cortical neurons for you.* Science, 2006. **313**(5789): p. 928-929.
27. Walton, R.M., *Postnatal Neurogenesis: Of Mice, Men, and Macaques.* Veterinary Pathology, 2012. **49**(1): p. 155-165.
28. Fujita, S., M. Shimada, and T. Nakamura, *H3-Thymindine autoradiographic studies on cell proliferation and differentiation in external and internal granular layers of mouse cerebellum.* Journal of Comparative Neurology, 1966. **128**(2): p. 191-&.
29. Altman, J., *Autoradiographic and histological studies of postnatal neurogenesis. 2. A longitudinal investigation of kinetics migration and transfromation of cells incorporating tritiated thymidine in infant rats with special reference to postnatal neurogenesis in some brain regions.* Journal of Comparative Neurology, 1966. **128**(4): p. 431-&.
30. Kuhn, H.G., H. DickinsonAnson, and F.H. Gage, *Neurogenesis in the dentate gyrus of the adult rat: Age-related decrease of neuronal progenitor proliferation.* Journal of Neuroscience, 1996. **16**(6): p. 2027-2033.
31. Jabes, A., et al., *Quantitative analysis of postnatal neurogenesis and neuron number in the macaque monkey dentate gyrus.* European Journal of Neuroscience, 2010. **31**(2): p. 273-285.
32. Leuner, B., et al., *Diminished adult neurogenesis in the marmoset brain precedes old age.* Proceedings of the National Academy of Sciences of the United States of America, 2007. **104**(43): p. 17169-17173.
33. Altman, J. and G.D. Das, *Autoradiographic and histological evidence of postnatal hippocampal neurogenesis in rats.* Journal of Comparative Neurology, 1965. **124**(3): p. 319-&.
34. Kaplan, M.S. and D.H. Bell, *Mitotic neuroblasts in the 9 day and 11 month old rodent hippocampus.* Journal of Neuroscience, 1984. **4**(6): p. 1429-1441.
35. Kaplan, M.S. and J.W. Hinds, *Neurogenesis in the adult ratL electron microscopic analysis of light autoradiographs.* Science, 1977. **197**(4308): p. 1092-1094.
36. Luskin, M.B., *Restricted proliferation and migration of postnatally generated neurons derived from the forebrain subventricular zone.* Neuron, 1993. **11**(1): p. 173-189.
37. Bedard, A. and A. Parent, *Evidence of newly generated neurons in the human olfactory bulb.* Developmental Brain Research, 2004. **151**(1-2): p. 159-168.
38. Migaud, M., et al., *Emerging new sites for adult neurogenesis in the mammalian brain: a comparative study between the hypothalamus and the classical neurogenic zones.* European Journal of Neuroscience, 2010. **32**(12): p. 2042-2052.
39. Kaplan, M.S., *Neurogenesis in the 3 month old rat visual cortex.* Journal of Comparative Neurology, 1981. **195**(2): p. 323-338.
40. Ehninger, D. and G. Kempermann, *Regional effects of wheel running and environmental enrichment on cell genesis and microglia proliferation in the adult murine neocortex.* Cerebral Cortex, 2003. **13**(8): p. 845-851.
41. Magavi, S.S., B.R. Leavitt, and J.D. Macklis, *Induction of neurogenesis in the neocortex of adult mice.* Nature, 2000. **405**(6789): p. 951-955.
42. Dayer, A.G., et al., *New GABAergic interneurons in the adult neocortex and striatum are generated from different precursors.* Journal of Cell Biology, 2005. **168**(3): p. 415-427.

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43. Cameron, H.A. and A.G. Dayer, *New Interneurons in the adult neocortex: Small, sparse, but significant?* Biological Psychiatry, 2008. **63**(7): p. 650-655.
44. Gould, E., et al., *Neurogenesis in the neocortex of adult primates.* Science, 1999. **286**(5439): p. 548-552.
45. Gould, E., et al., *Adult-generated hippocampal and neocortical neurons in macaques have a transient existence.* Proceedings of the National Academy of Sciences of the United States of America, 2001. **98**(19): p. 10910-10917.
46. Kornack, D.R. and P. Rakic, *Cell proliferation without neurogenesis in adult primate neocortex.* Science, 2001. **294**(5549): p. 2127-2130.
47. Koketsu, D., et al., *Nonrenewal of neurons in the cerebral neocortex of adult Macaque monkeys.* Journal of Neuroscience, 2003. **23**(3): p. 937-942.
48. Bhardwaj, R.D., et al., *Neocortical neurogenesis in humans is restricted to development.* Proceedings of the National Academy of Sciences of the United States of America, 2006. **103**(33): p. 12564-12568.
49. Altman, J., *The Discovery of Adult Mammalian Neurogenesis*, in *Neurogenesis in the Adult Brain I: Neurobiology*. 2011. p. 3-46.

Student #7

Great answer!

We are born with all the neurons we're going to have. For many years, this statement was considered a well-known fact in the field of neuroscience. However, within the last 30 years the field has changed drastically with the finding that neurogenesis can occur in specific regions of the brain throughout adulthood. Here I will consider current ideas and controversies in the field of adult neurogenesis. In this review, I will highlight the methods used for identifying new neurons in the brain, evidence for a decreased prevalence of neurogenesis when comparing birds versus rodents and primates, the temporal and spatial constraints of postnatal neurogenesis, and methodological issues that may contribute to controversies in the field.

Adult neurogenesis: Methodology

Two common techniques used to identify newly generated neurons in the brain are ^{3}H-thymidine autoradiography and 5-bromo-2-deoxyuridine (BrdU) labeling. ^{3}H-thymidine autoradiography is an older technique. With this method, isotopically labeled thymidine (^{3}H-thymidine) is injected into an animal and ^{3}H-thymidine is incorporated into the DNA of cells going through the S-phase (synthesis phase) of cell cycle [1]. Thus, labeled cells will be those that were in the S-phase of mitosis when ^{3}H-thymidine was injected. Labeled cells are then visualized using autoradiography. Morphological characteristics of the cells must be examined to determine if the newly formed cells are neurons [2]. A newer technique for labeling proliferating cells in the brain is through the use of BrdU labeling. BrdU is a thymidine analog that is also incorporated into the DNA of cells going through the S-phase of the cell cycle [3]. Similar to ^{3}H-thymidine autoradiography, BrdU is injected into animals, animals are sacrificed

after a period of time (known as the survival time) and BrdU labeling can be detected through the use of antibodies [2]. The benefit of using BrdU is that cells can be co-labeled with neuronal markers to determine the identity of BrdU labeled cells [3]. Both of these techniques have been used in determining whether adult neurogenesis occurs in various parts of the brain.

Adult neurogenesis: Gradual decrease from birds to rodents to primates

Adult neurogenesis occurs in many animals with the distribution and amount of neurogenesis varying by taxa. Comparatively, birds are believed to have a higher prevalence of adult neurogenesis than rodents and primates. Below I will highlight the discovery of adult neurogenesis in songbirds and provide evidence as to why rodents and primates are believed to have a reduced levels of adult neurogenesis compared to birds. Note that further details on the discovery of adult neurogenesis in rodents and primates will be discussed in "Mammalian postnatal neurogenesis: Spatial constraints".

Adult neurogenesis in birds, specifically songbirds, was first noted by Nottebohm and colleagues in the mid-1980s. They discovered in adult canaries that newly divided cells in the brain were generated in ventricular zone and then migrated to areas of the telencephalon, notably the higher vocal center (HVC)[4, 5]; these cells then differentiated into neurons and some of the new neurons produced action potentials indicating that they were potentially functional within the neuronal circuitry [5, 6]. This finding was intriguing, since the HVC is a brain structure important in song acquisition and production in songbirds; specifically, the HVC projection to the RA is essential in song production [7]. Thus, adult neurogenesis in the HVC may be essential for the production of a new songs in canaries, since these birds produce a new song repertoire each year [8, 9]. In fact, neurogenesis in the HVC is highest during the times of year

in which the bird's song is modified [10] and many of the newly generated neurons project to the RA [11] further illustrating that neurogenesis in the HVC may be important in the production of new songs each year. Additionally, these new neurons are believed to replace existing neurons in the HVC, since neuronal death proceeds neuronal production in the HVC and the total number of neurons in the HVC does not change across the year [10, 12]. Notably, this high level of neurogenesis and neuronal turnover in songbirds is hypothesized to be important for establishing new neuronal circuits, so new learning can occur each year [7]. One basis for this hypothesis is that canaries have a relatively long lifespan compared to similarly sized mammals (about 5x longer than that of a mouse) and due to space limitations in the brain, higher neurogenesis may have evolved to allow new memories to be formed each year [13, 14]. Although I highlighted the importance of neurogenesis in the HVC of the canary, adult neurogenesis in the hippocampus has also been implicated in the formation of new memories each year in chickadees [15]. Thus, in birds, higher levels of adult neurogenesis may serve as a form of plasticity within the brain to enable new learning to occur each year.

Compared to songbirds and other non-mammalian vertebrates, the prevalence of adult neurogenesis in the rodent is believed to be somewhat reduced. While adult neurogenesis in birds can occur across the telencephalon, in rodents, adult neurogenesis is for the most part restricted to the hippocampal dentate gyrus and the subventricular zone (SVZ); new cells are produced in the SVZ and migrate to the olfactory bulb where they differentiate into neurons [16-18]. A reason why neurogenesis may have been retained and restricted to these brain sites over the course of evolution, is that it may have been beneficial to conserve plasticity mechanisms in areas of the brain important for detecting and encoding complex environments and novel stimuli [17]. In fact, neurogenesis is enhanced in the hippocampus of rodents maintained in an enriched

environment [19] and novel stimuli elicit greater cellular responsiveness, as measured by c-Fos, in new adult born olfactory neurons compared to preexisting olfactory neurons [20]. Thus, both of these studies suggest that neurogenesis in the hippocampus and olfactory bulb in rodents may aid in novelty learning and detection. In comparison to birds, adult neurogenesis in rodents may still serve as mechanism of plasticity, but to lesser extent.

Compared to rodents and birds, adult neurogenesis in primates is considered to be significantly decreased. In non-human primates, adult neurogenesis occurs both in the hippocampal dentate gyrus and the SVZ [21-23], however, the overall levels of neurogenesis that occur in the dentate gyrus, for example, in non-human primates are dramatically reduced compared to rodents; it is estimated that new neurons contribute to .004% of the granule cell layer of the hippocampus per day in macaques, compared to 0.1% per day in rodents [3, 21]. Although these numbers are not completely reliable, since methodological issues can contribute discrepancies in the total number of new neurons counted (see "Methodological considerations for adult neurogenesis"), it does suggest that neurogenesis in primates is decreased compared to rodents. Adult neurogenesis has also been hippocampus of humans, but the overall levels compared to rodents are believed to be reduced [24]. Thus, these studies suggest that reduced levels of adult neurogenesis may have been selected for in mammalian evolution, particularly in primates [17]. In fact Rakic has postulated that in primates, such as humans, neurogenesis may not be as advantageous, since adding or replacing neurons in a stable neuronal circuitry may disrupt already stored experiences and memories important for long-lived species, such as humans [25, 26]. Thus, although we do not currently know the functional implications of adult neurogenesis in humans, its presence suggests that adult neurogenesis has been conserved across evolution and perhaps may sub-serve some role in plasticity.

Mammalian postnatal neurogenesis: Temporal constraints

As noted above, adult neurogenesis occurs across the lifetime of many animals. However, the concept of *postnatal* neurogenesis is applied to neurogenesis that occurs after birth and in some cases across the lifespan of an animal. Thus, there are two defined periods of postnatal neurogenesis; "protracted" neurogenesis occurs for a limited time after birth and is considered as an extension of embryonic neurogenesis, while "persistent" neurogenesis occurs across the lifespan of the animal [18]. Both protracted and persistent neurogenesis occur in discrete brain areas. For example, protracted neurogenesis occurs in the cerebellum of most mammals, while persistent neurogenesis mainly occurs in the hippocampal dentate gyrus and SVZ [18]. Below I will highlight evidence for temporal constraints of both protracted and persistent postnatal neurogenesis.

Protracted postnatal neurogenesis occurs in the cerebellum of most mammals. Cerebellar postnatal neurogenesis occurs via cell proliferation in the external granular layer (EGL), a part of the cerebellum present for a short time after birth; the dividing cells that eventually differentiate into neurons reside in the EGL [18]. Cell proliferation in the cerebellum can occur from weeks to 1 year postnatally, depending on the species [27]. In mice, cell proliferation in the cerebellum, as measured by ^{3}H-thymidine labeling, is high up to postnatal day 10 with no cell proliferation observed after postnatal day 20 [28]. The last cells to develop and differentiate from these proliferating cells are small granule neurons in the cerebellum [29]. In humans, postnatal neurogenesis in the cerebellum can extend for a year after birth [27]. Thus, it is possible that the delayed production of granule neurons after birth may play an important role in motor learning during the early stages of a mammal's life, as adding new neurons and creating new neuronal

connections during this period may aid in the development of fundamental motor movements, such as walking [18].

Contrary to protracted neurogenesis, persistent neurogenesis occurs across the lifetime of an animal, but the amount of neurogenesis may change in an age-dependent fashion. In rats, cell proliferation is reduced in the hippocampal dentate gyrus in old (21 month) versus young (6 month) rats [30], indicating that although cell proliferation and neurogenesis may persist in old age, the overall levels are reduced. Levels of cell proliferation and neurogenesis are also reduced with age in the hippocampal dentate gyrus and SVZ in non-human primates [31, 32]. Notably, neurogenesis in the hippocampal dentate gyrus is significantly decreased after 3 months of age and after 1 year, the levels of neurogenesis are relatively low [31]. Thus, these studies suggest that although neurogenesis may occur across an animal's lifetime in specific brain areas, temporal constraints may be limiting the overall levels of neurogenesis during different stages of life.

Mammalian postnatal neurogenesis: Spatial constraints

The first reports of postnatal neurogenesis in rodents were noted by Altman and colleagues in the mid-1960's [29, 33], but at that time little attention was given to such findings. Other reports then came from Kaplan and colleagues in late-1970's and mid-1980's; these studies used ^{3}H-thymidine autoradiography and electron microscopy to indicated postnatal neurogenesis in hippocampal dentate gyrus of rodents [34, 35]. Again, these findings were not initially recognized by scientific community. It wasn't until Nottebohm's discovery of adult neurogenesis in songbirds [4-6, 13] that considerable attention was given to the notion of postnatal neurogenesis in rodents as well. In fact, many studies have now confirmed that

postnatal neurogenesis exists in two brain sites of rodents under normal physiological conditions, the hippocampal dentate gyrus and SVZ; newly divided cells in the SVZ migrate to the olfactory bulb via the rostral migratory stream (RMS) where the differentiate into neurons [2, 16, 36]. Both the hippocampal dentate gyrus and SVZ are known as "neurogenic" zones, since neurogenesis is recognized to occur there in adult rodents; all other regions of the brain are known as "non-neurogenic", since adult neurogenesis is not widely recognized to occur in these other brain regions [2].

Although postnatal neurogenesis in rodents became recognized in the field after the mid-1980's the idea of neurogenesis in adult primates remained a controversial issue. In 1985, Rakic used ^{3}H-thymindine autoradiographic labeling in adult rhesus monkeys to determine that adult neurogenesis does not occur in primates [25]. He further speculated that in long-lived primates, neurogenesis during adulthood may be disadvantageous, since new neurons would need to form synaptic connections and integrate into neuronal networks established by prior experiences [25]. Contrary to this notion, Eriksson et al. used BrdU and neuronal makers to establish that newly generated neurons were produced in the SVZ and hippocampal dentate gyrus of deceased adult human cancer patients; cells expressing BrdU in the dentate gyrus were co-labeled with neuronal markers indicating that these cells did in fact differentiate into neurons [24]. This study brought new insight to the field suggesting that humans do have the capability of generating new neurons in adulthood. Reevaluation of neurogenesis in adult non-human primates was performed by both Kornack and Rakic and Gould et al. in 1999; both labs found levels of adult neurogenesis in the hippocampal dentate gryus [21, 23]. Furthermore, existence a RMS contributing to new neurons in the olfactory bulb in adult non-human primates [22] and potentially in humans [37], further

illustrates that neurogenesis in primates, similar to rodents, occurs in the aforementioned neurogenic sites of the brain.

Although it is well-established that adult neurogenesis occurs in neurogenic zones, neurogenesis in non-neurogenic zones is a topic of much debate. Areas of the brain that have controversial evidence for and against neurogenesis are the neocortex, striatum, hypothalamus, amygdala, and substantia nigra [2, 18, 38]. Here, I will limit my discussion to conflicting evidence of adult neurogenesis in the neocortex.

Adult neurogenesis in the neocortex of rodents is still a controversial topic. Kaplan was one of the first to report adult neurogenesis in the neocortex using ^{3}H-thymidine autoradiography and electron microscopy [39]. However, following studies failed to find the existence of adult neurogenesis in the neocortex under normal physiological conditions; these studies found cell proliferation in the neocortex, but noted that such cells were either glial or undifferentiated cells, not neurons [40, 41]. Contrary to these results, a more recent study found adult neurogenesis in many areas of the necortex [42]. The investigators noted that many of the newly formed neurons were very small in size and since neurogenesis in the neocortex may occur at low levels, it may have made it difficult for prior investigators to find these neurons [42, 43]. In fact, these new neurons expressed GAD-67, a GABA synthesizing enzyme, indicating that new neurons generated in the neocortex may be GABA-ergic interneurons [42]. Thus, further studies in rodents may be warranted to confirm if low levels of neurogenesis in the neocortex may be contributing to the interneuron population in adulthood.

Similar to evidence in rats, adult neurogenesis in the cortex is very controversial in primates. Gould et al. reported new neurons in the prefrontal, parietal, and temporal cortex of adult macaques; some of the new proliferating cells were suggested to have divided in the

neocortex, while others were believed to have originated from newly divided cells in SVZ that migrated to the neocortex where they differentiated into neurons [44, 45]. While other studies have found proliferating cells in the neocortex of non-human primates, it was noted that many of cells failed to also express neuronal markers; those cells that appeared to be newly divided neurons were in fact two separate cells (one a newly divided cell and the other a neuron) in close approximation to one another [46, 47].

Further evidence against adult neurogenesis in the cortex came from a study using human brain tissue. Bhardwaj et al. used carbon-14 (^{14}C) dating to identify newly generated cells in human brain tissue [48]. The basis for this technique is that during the atomic bomb testing in the Cold War, atmospheric ^{14}C levels were dramatically increased; ^{14}C would have been incorporated into the human body, specifically DNA of newly dividing cells at that time. Individuals born before or after the atomic bomb testing were used to determine via ^{14}C dating that no new neurons were detected in human cortex [48]. Further, labeling for BrdU and neuronal markers in the human cortex indicated no adult neurogenesis [48]. Overall, these studies have lead some scientists to believe that adult neurogenesis does not occur in the cortex [26]. While this conclusion may be true, differences in methodological procedures may have contributed to some of the discrepancies between different studies. These methodological considerations will now be reviewed.

Methodological considerations for adult neurogenesis

Both ^{3}H-thymidine autoradiography and BrdU labeling have been widely used in studies of adult neurogenesis. ^{3}H-thymidine autoradiography is an older technique that labels cells in the S-phase of mitosis and requires that morphological features of the cells be characterized in

order to determine if the newly generated cells are in fact neurons. This technique is very time consuming and it can be difficult to perform reliably [49]. However, many of the hallmark studies establishing the existence of adult neurogenesis have used ^{3}H-thymidine autoradiography and such conclusions have been confirmed with other labeling techniques, such as double labeling with BrdU and neuronal markers [2, 19, 29, 33, 34]. Thus, I do not think that controversies in the field are based primarily on whether an "old" versus a "new" technique was used. This is not to say that one technique, such as BrdU double labeled with neuronal markers, might provide more compelling evidence of neurogenesis than another, but rather other methodological issues may be contributing to discrepancies between studies. I think there may be many issues contributing to whether a "false negative" (saying there is no effect when there is one) versus a "false positive" (saying there is an effect when there isn't one) is occurring. Below I will give examples of methodological issues that could be contributing to conflicting conclusions about adult neurogenesis.

Methodological issues could contribute to some researchers concluding prematurely that adult neurogenesis does not occur in specific brain areas. One factor that could contribute to a false negative is the amount of survival time given after the ^{3}H-thymidine or BrdU injection. Survival time issues can especially come into play when looking at a brain area that might already have low levels of neurogenesis; if the survival time is too long, the labeled neurons may have already died, while if the survival times are too short, the newly divided cells may not have had enough time to differentiate into neurons leading to the false conclusion that neurogenesis does not occur in that brain area [7]. The length of survival time may have come into play when adult neurogenesis was examined in non-human primates, since the first study claimed that adult neurogenesis does not occur (survival times ranged from 3 months to 6 years) [25], while

following studies did find adult neurogenesis in non-human primates (survival times ranged from 2hrs to 97 days) [21-23]. An additional, methodological consideration is the age of the animals used for the study. Some studies looking at neurogenesis in non-neurogenic sites use animals of varying ages [23, 25]. As stated before, levels of neurogenesis reduce with age [31, 32], so using an old subject base to determine that neurogenesis does not occur in a specific brain site may bias the results.

Another methodological issue that could contribute to a false negative result is the quality of staining and quantitative measures used for determining what is considered a new labeled neuron. If the quality of staining is poor, brain areas that may be producing new neurons, but at lower levels, may not be considered as neurogenic brain sites. Gould has indicated the importance of using a positive control, such as verifying that cells are co-labeled with BrdU and neuronal markers in the dentate gyrus, whenever looking at brain sites that are considered non-neurogenic [2]. This consideration is important in ensuring that the tissue is adequately stained to label new neurons in areas where they may be produced at low levels. Another important consideration is using a consistent standard for counting new neurons in the different brain sites. For example, many of the cells considered to be new neurons in the cortex are believed to be GABA-ergic interneurons, which have a smaller soma than surrounding cortical pyramidal neurons [42, 43]. Thus, it may not only be harder to find such newly generated neurons, but if new neurons labeled with BrdU and neuronal markers are not counted consistently across different brain sites (for example, the cortex versus the hippocampal dentate gyrus), then it may be prematurely concluded that adult neurogenesis does not exist in such areas. Thus, it is important that standards of counting double labeled cells be kept across all brain sites that are analyzed.

In addition to false negatives, false positives may occur depending on how the tissue is analyzed. It is possible that some cells stained with BrdU appear to be co-labeled with neuronal markers, but are in fact separate cells in close proximity to labeled neurons. By using confocal z-series analysis, which basically takes 3D images of stained tissue, Koketsu et al. determined that many BrdU cells that appeared to be co-labeled with neuronal markers were in fact separately labeled cells [47]. Thus, studies that do not use this technique may be inaccurately counting newly divided cells as neurons when they may not be.

Overall, a systematic method of determining whether adult neurogenesis occurs in particular areas of the brain, especially in non-neurogenic areas, should be used. The fact that different standards are used for identifying newly generated neurons in non-neurogenic zones most likely contributes conflicting results across studies. To get around this issues, multiple measures should be used to validate claims, such as double labeling cells with neuronal markers, using z-series analysis to confirm that cells are double labeled, using positive controls to validate quality of tissue staining, and using consistent standards in evaluating neurogenesis across brain areas.

1. Sanes, D.H., T.A. Reh, and W.A. Harris, *Development of the nervous system*. 3 ed. 2012: Elsevier Inc.
2. Gould, E., *Opinion - How widespread is adult neurogenesis in mammals?* Nature Reviews Neuroscience, 2007. **8**(6): p. 481-488.
3. Taupin, P., *BrdU immunohistochemistry for studying adult neurogenesis: Paradigms, pitfalls, limitations, and validation.* Brain Research Reviews, 2007. **53**(1): p. 198-214.
4. Goldman, S.A. and F. Nottebohm, *Neuronal production, migration, and differentiation in a vocal control nucleus of the adult female canary brain.* Proceedings of the National Academy of Sciences of the United States of America-Biological Sciences, 1983. **80**(8): p. 2390-2394.
5. Alvarezbuylla, A. and F. Nottebohm, *Migration of young neurons in adult avian brain.* Nature, 1988. **335**(6188): p. 353-354.

6. Paton, J.A. and F. Nottebohm, *Neurons generated in adult brain are recruited into functional circuits.* Science, 1984. **225**(4666): p. 1046-1048.
7. Nottebohm, F., *Why are some neurons replaced in adult brain?* Journal of Neuroscience, 2002. **22**(3): p. 624-628.
8. Nottebohm, F., *From bird song to neurogenesis.* Scientific American, 1989. **260**(2): p. 74-79.
9. Nottebohm, F., *Neuronal replacement in adult brain.* Brain Research Bulletin, 2002. **57**(6): p. 737-749.
10. Kirn, J., et al., *Cell death and neuronal recruitment in the high vocal center of adult male canaries are temporally related to changes in song.* Proceedings of the National Academy of Sciences of the United States of America, 1994. **91**(17): p. 7844-7848.
11. Alvarezbuylla, A., J.R. Kirn, and F. Nottebohm, *Birth of projection neurons in adult avian brain may be related to perceptual or motor learning.* Science, 1990. **249**(4975): p. 1444-1446.
12. Alvarezbuylla, A., C.Y. Ling, and F. Nottebohm, *High vocal center growth and its relation to neurogenesis, neuronal replacement and song acquisition in juvenile canaries.* Journal of Neurobiology, 1992. **23**(4): p. 396-406.
13. Nottebohm, F., *Neuronal replacement in adulthood.* Annals of the New York Academy of Sciences, 1985. **457**: p. 143-161.
14. Kirn, J.R. and F. Nottebohm, *Direct evidence for loss and replacement of projection neurons in adult canary brain.* Journal of Neuroscience, 1993. **13**(4): p. 1654-1663.
15. Barnea, A. and F. Nottebohm, *Seasonal recruitment of hippocampal neurons in adult free ranging black capped chickadees.* Proceedings of the National Academy of Sciences of the United States of America, 1994. **91**(23): p. 11217-11221.
16. Lois, C. and A. Alvarezbuylla, *Long-distance neuronal migration in the adult mammalian brain.* Science, 1994. **264**(5162): p. 1145-1148.
17. Kaslin, J., J. Ganz, and M. Brand, *Proliferation, neurogenesis and regeneration in the non-mammalian vertebrate brain.* Philosophical Transactions of the Royal Society B-Biological Sciences, 2008. **363**(1489): p. 101-122.
18. Bonfanti, L. and P. Peretto, *Adult neurogenesis in mammals - a theme with many variations.* European Journal of Neuroscience, 2011. **34**(6): p. 930-950.
19. Kempermann, G., H.G. Kuhn, and F.H. Gage, *More hippocampal neurons in adult mice living in an enriched environment.* Nature, 1997. **386**(6624): p. 493-495.
20. Magavi, S.S.P., et al., *Adult-born and preexisting olfactory granule neurons undergo distinct experience-dependent modifications of their olfactory responses in vivo.* Journal of Neuroscience, 2005. **25**(46): p. 10729-10739.
21. Kornack, D.R. and P. Rakic, *Continuation of neurogenesis in the hippocampus of the adult macaque monkey.* Proceedings of the National Academy of Sciences of the United States of America, 1999. **96**(10): p. 5768-5773.
22. Kornack, D.R. and P. Rakic, *The generation, migration, and differentiation of olfactory neurons in the adult primate brain.* Proceedings of the National Academy of Sciences of the United States of America, 2001. **98**(8): p. 4752-4757.
23. Gould, E., et al., *Hippocampal neurogenesis in adult Old World primates.* Proceedings of the National Academy of Sciences of the United States of America, 1999. **96**(9): p. 5263-5267.

24. Eriksson, P.S., et al., *Neurogenesis in the adult human hippocampus.* Nature Medicine, 1998. **4**(11): p. 1313-1317.
25. Rakic, P., *Limits of neurogenesis in primates.* Science, 1985. **227**(4690): p. 1054-1056.
26. Rakic, P., *No more cortical neurons for you.* Science, 2006. **313**(5789): p. 928-929.
27. Walton, R.M., *Postnatal Neurogenesis: Of Mice, Men, and Macaques.* Veterinary Pathology, 2012. **49**(1): p. 155-165.
28. Fujita, S., M. Shimada, and T. Nakamura, *H3-Thymindine autoradiographic studies on cell proliferation and differentiation in external and internal granular layers of mouse cerebellum.* Journal of Comparative Neurology, 1966. **128**(2): p. 191-&.
29. Altman, J., *Autoradiographic and histological studies of postnatal neurogenesis. 2. A longitudinal investigation of kinetics migration and transfromation of cells incorporating tritiated thymidine in infant rats with special reference to postnatal neurogenesis in some brain regions.* Journal of Comparative Neurology, 1966. **128**(4): p. 431-&.
30. Kuhn, H.G., H. DickinsonAnson, and F.H. Gage, *Neurogenesis in the dentate gyrus of the adult rat: Age-related decrease of neuronal progenitor proliferation.* Journal of Neuroscience, 1996. **16**(6): p. 2027-2033.
31. Jabes, A., et al., *Quantitative analysis of postnatal neurogenesis and neuron number in the macaque monkey dentate gyrus.* European Journal of Neuroscience, 2010. **31**(2): p. 273-285.
32. Leuner, B., et al., *Diminished adult neurogenesis in the marmoset brain precedes old age.* Proceedings of the National Academy of Sciences of the United States of America, 2007. **104**(43): p. 17169-17173.
33. Altman, J. and G.D. Das, *Autoradiographic and histological evidence of postnatal hippocampal neurogenesis in rats.* Journal of Comparative Neurology, 1965. **124**(3): p. 319-&.
34. Kaplan, M.S. and D.H. Bell, *Mitotic neuroblasts in the 9 day and 11 month old rodent hippocampus.* Journal of Neuroscience, 1984. **4**(6): p. 1429-1441.
35. Kaplan, M.S. and J.W. Hinds, *Neurogenesis in the adult ratL electron microscopic analysis of light autoradiographs.* Science, 1977. **197**(4308): p. 1092-1094.
36. Luskin, M.B., *Restricted proliferation and migration of postnatally generated neurons derived from the forebrain subventricular zone.* Neuron, 1993. **11**(1): p. 173-189.
37. Bedard, A. and A. Parent, *Evidence of newly generated neurons in the human olfactory bulb.* Developmental Brain Research, 2004. **151**(1-2): p. 159-168.
38. Migaud, M., et al., *Emerging new sites for adult neurogenesis in the mammalian brain: a comparative study between the hypothalamus and the classical neurogenic zones.* European Journal of Neuroscience, 2010. **32**(12): p. 2042-2052.
39. Kaplan, M.S., *Neurogenesis in the 3 month old rat visual cortex.* Journal of Comparative Neurology, 1981. **195**(2): p. 323-338.
40. Ehninger, D. and G. Kempermann, *Regional effects of wheel running and environmental enrichment on cell genesis and microglia proliferation in the adult murine neocortex.* Cerebral Cortex, 2003. **13**(8): p. 845-851.
41. Magavi, S.S., B.R. Leavitt, and J.D. Macklis, *Induction of neurogenesis in the neocortex of adult mice.* Nature, 2000. **405**(6789): p. 951-955.
42. Dayer, A.G., et al., *New GABAergic interneurons in the adult neocortex and striatum are generated from different precursors.* Journal of Cell Biology, 2005. **168**(3): p. 415-427.

43. Cameron, H.A. and A.G. Dayer, *New Interneurons in the adult neocortex: Small, sparse, but significant?* Biological Psychiatry, 2008. **63**(7): p. 650-655.
44. Gould, E., et al., *Neurogenesis in the neocortex of adult primates.* Science, 1999. **286**(5439): p. 548-552.
45. Gould, E., et al., *Adult-generated hippocampal and neocortical neurons in macaques have a transient existence.* Proceedings of the National Academy of Sciences of the United States of America, 2001. **98**(19): p. 10910-10917.
46. Kornack, D.R. and P. Rakic, *Cell proliferation without neurogenesis in adult primate neocortex.* Science, 2001. **294**(5549): p. 2127-2130.
47. Koketsu, D., et al., *Nonrenewal of neurons in the cerebral neocortex of adult Macaque monkeys.* Journal of Neuroscience, 2003. **23**(3): p. 937-942.
48. Bhardwaj, R.D., et al., *Neocortical neurogenesis in humans is restricted to development.* Proceedings of the National Academy of Sciences of the United States of America, 2006. **103**(33): p. 12564-12568.
49. Altman, J., *The Discovery of Adult Mammalian Neurogenesis*, in *Neurogenesis in the Adult Brain I: Neurobiology*. 2011. p. 3-46.

Student #7

HIGH PASS. Great response.

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Adult neurogenesis: Methodology

Two common techniques used to identify newly generated neurons in the brain are ^{3}H-thymidine autoradiography and 5-bromo-2-deoxyuridine (BrdU) labeling. ^{3}H-thymidine autoradiography is an older technique. With this method, isotopically labeled thymidine (^{3}H-thymidine) is injected into an animal and ^{3}H-thymidine is incorporated into the DNA of cells going through the S-phase (synthesis phase) of cell cycle [1]. Thus, labeled cells will be those that were in the S-phase of mitosis when ^{3}H-thymidine was injected. Labeled cells are then visualized using autoradiography. Morphological characteristics of the cells must be examined to determine if the newly formed cells are neurons [2]. A newer technique for labeling proliferating cells in the brain is through the use of BrdU labeling. BrdU is a thymidine analog that is also incorporated into the DNA of cells going through the S-phase of the cell cycle [3].

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Adult neurogenesis occurs in many animals with the distribution and amount of neurogenesis varying by taxa. Comparatively, birds are believed to have a higher prevalence of adult neurogenesis than rodents and primates. Below I will highlight the discovery of adult neurogenesis in songbirds and provide evidence as to why rodents and primates are believed to have a reduced levels of adult neurogenesis compared to birds. Note that further details on the discovery of adult neurogenesis in rodents and primates will be discussed in "Mammalian postnatal neurogenesis: Spatial constraints".

Adult neurogenesis in birds, specifically songbirds, was first noted by Nottebohm and colleagues in the mid-1980s. They discovered in adult canaries that newly divided cells in the brain were generated in ventricular zone and then migrated to areas of the telencephalon, notably the higher vocal center (HVC)[4, 5]; these cells then differentiated into neurons and some of the new neurons produced action potentials indicating that they were potentially functional within the neuronal circuitry [5, 6]. This finding was intriguing, since the HVC is a brain structure important in song acquisition and production in songbirds; specifically, the HVC projection to the RA is essential in song production [7]. Thus, adult neurogenesis in the HVC may be essential for the production of a new songs in canaries, since these birds produce a new song

repertoire each year [8, 9]. In fact, neurogenesis in the HVC is highest during the times of year in which the bird's song is modified [10] and many of the newly generated neurons project to the RA [11] further illustrating that neurogenesis in the HVC may be important in the production of new songs each year. Additionally, these new neurons are believed to replace existing neurons in the HVC, since neuronal death proceeds neuronal production in the HVC and the total number of neurons in the HVC does not change across the year [10, 12]. Notably, this high level of neurogenesis and neuronal turnover in songbirds is hypothesized to be important for establishing new neuronal circuits, so new learning can occur each year [7]. One basis for this hypothesis is that canaries have a relatively long lifespan compared to similarly sized mammals (about 5x longer than that of a mouse) and due to space limitations in the brain, higher neurogenesis may have evolved to allow new memories to be formed each year [13, 14]. Although I highlighted the importance of neurogenesis in the HVC of the canary, adult neurogenesis in the hippocampus has also been implicated in the formation of new memories each year in chickadees [15]. Thus, in birds, higher levels of adult neurogenesis may serve as a form of plasticity within the brain to enable new learning to occur each year.

Compared to songbirds and other non-mammalian vertebrates, the prevalence of adult neurogenesis in the rodent is believed to be somewhat reduced. While adult neurogenesis in birds can occur across the telencephalon, in rodents, adult neurogenesis is for the most part restricted to the hippocampal dentate gyrus and the subventricular zone (SVZ); new cells are produced in the SVZ and migrate to the olfactory bulb where the differentiate into neurons [16-18]. A reason why neurogenesis may have been retained and restricted to these brain sites over the course of evolution, is that it may have been beneficial to conserve plasticity mechanisms in areas of the brain important for detecting and encoding complex environments and novel stimuli [17]. In

fact, neurogenesis is enhanced in the hippocampus of rodents maintained in an enriched environment [19] and novel stimuli elicit greater cellular responsiveness, as measured by c-Fos, in new adult born olfactory neurons compared to preexisting olfactory neurons [20]. Thus, both of these studies suggest that neurogenesis in the hippocampus and olfactory bulb in rodents may aid in novelty learning and detection. In comparison to birds, adult neurogenesis in rodents may still serve as mechanism of plasticity, but to lesser extent.

Compared to rodents and birds, adult neurogenesis in primates is considered to be significantly decreased. In non-human primates, adult neurogenesis occurs both in the hippocampal dentate gyrus and the SVZ [21-23], however, the overall levels of neurogenesis that occur in the dentate gyrus, for example, in non-human primates are dramatically reduced compared to rodents; it is estimated that new neurons contribute to .004% of the granule cell layer of the hippocampus per day in macaques, compared to 0.1% per day in rodents [3, 21]. Although these numbers are not completely reliable, since methodological issues can contribute discrepancies in the total number of new neurons counted (see "Methodological considerations for adult neurogenesis"), it does suggest that neurogenesis in primates is decreased compared to rodents. Adult neurogenesis has also been hippocampus of humans, but the overall levels compared to rodents are believed to be reduced [24]. Thus, these studies suggest that reduced levels of adult neurogenesis may have been selected for in mammalian evolution, particularly in primates [17]. In fact Rakic has postulated that in primates, such as humans, neurogenesis may not be as advantageous, since adding or replacing neurons in a stable neuronal circuitry may disrupt already stored experiences and memories important for long-lived species, such as humans [25, 26]. Thus, although we do not currently know the functional implications of adult

neurogenesis in humans, its presence suggests that adult neurogenesis has been conserved across evolution and perhaps may sub-serve some role in plasticity.

Mammalian postnatal neurogenesis: Temporal constraints

As noted above, adult neurogenesis occurs across the lifetime of many animals. However, the concept of *postnatal* neurogenesis is applied to neurogenesis that occurs after birth and in some cases across the lifespan of an animal. Thus, there are two defined periods of postnatal neurogenesis; "protracted" neurogenesis occurs for a limited time after birth and is considered as an extension of embryonic neurogenesis, while "persistent" neurogenesis occurs across the lifespan of the animal [18]. Both protracted and persistent neurogenesis occur in discrete brain areas. For example, protracted neurogenesis occurs in the cerebellum of most mammals, while persistent neurogenesis mainly occurs in the hippocampal dentate gyrus and SVZ [18]. Below I will highlight evidence for temporal constraints of both protracted and persistent postnatal neurogenesis.

Comment [SMB1]: Good that you make this distinction.

Protracted postnatal neurogenesis occurs in the cerebellum of most mammals. Cerebellar postnatal neurogenesis occurs via cell proliferation in the external granular layer (EGL), a part of the cerebellum present for a short time after birth; the dividing cells that eventually differentiate into neurons reside in the EGL [18]. Cell proliferation in the cerebellum can occur from weeks to 1 year postnatally, depending on the species [27]. In mice, cell proliferation in the cerebellum, as measured by ^{3}H-thymidine labeling, is high up to postnatal day 10 with no cell proliferation observed after postnatal day 20 [28]. The last cells to develop and differentiate from these proliferating cells are small granule neurons in the cerebellum [29]. In humans, postnatal neurogenesis in the cerebellum can extend for a year after birth [27]. Thus, it is possible that the

delayed production of granule neurons after birth may play an important role in motor learning during the early stages of a mammal's life, as adding new neurons and creating new neuronal connections during this period may aid in the development of fundamental motor movements, such as walking [18].

Contrary to protracted neurogenesis, persistent neurogenesis occurs across the lifetime of an animal, but the amount of neurogenesis may change in an age-dependent fashion. In rats, cell proliferation is reduced in the hippocampal dentate gyrus in old (21 month) versus young (6 month) rats [30], indicating that although cell proliferation and neurogenesis may persist in old age, the overall levels are reduced. Levels of cell proliferation and neurogenesis are also reduced with age in the hippocampal dentate gyrus and SVZ in non-human primates [31, 32]. Notably, neurogenesis in the hippocampal dentate gyrus is significantly decreased after 3 months of age and after 1 year, the levels of neurogenesis are relatively low [31]. Thus, these studies suggest that although neurogenesis may occur across an animal's lifetime in specific brain areas, temporal constraints may be limiting the overall levels of neurogenesis during different stages of life.

Mammalian postnatal neurogenesis: Spatial constraints

The first reports of postnatal neurogenesis in rodents were noted by Altman and colleagues in the mid-1960's [29, 33], but at that time little attention was given to such findings. Other reports then came from Kaplan and colleagues in late-1970's and mid-1980's; these studies used ^{3}H-thymidine autoradiography and electron microscopy to indicated postnatal neurogenesis in hippocampal dentate gyrus of rodents [34, 35]. Again, these findings were not initially recognized by scientific community. It wasn't until Nottebohm's discovery of adult

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neurogenesis in songbirds [4-6, 13] that considerable attention was given to the notion of postnatal neurogenesis in rodents as well. In fact, many studies have now confirmed that postnatal neurogenesis exists in two brain sites of rodents under normal physiological conditions, the hippocampal dentate gyrus and SVZ; newly divided cells in the SVZ migrate to the olfactory bulb via the rostral migratory stream (RMS) where the differentiate into neurons [2, 16, 36]. Both the hippocampal dentate gyrus and SVZ are known as "neurogenic" zones, since neurogenesis is recognized to occur there in adult rodents; all other regions of the brain are known as "non-neurogenic", since adult neurogenesis is not widely recognized to occur in these other brain regions [2].

Although postnatal neurogenesis in rodents became recognized in the field after the mid-1980's the idea of neurogenesis in adult primates remained a controversial issue. In 1985, Rakic used ^{3}H-thymindine autoradiographic labeling in adult rhesus monkeys to determine that adult neurogenesis does not occur in primates [25]. He further speculated that in long-lived primates, neurogenesis during adulthood may be disadvantageous, since new neurons would need to form synaptic connections and integrate into neuronal networks established by prior experiences [25]. Contrary to this notion, Eriksson et al. used BrdU and neuronal makers to establish that newly generated neurons were produced in the SVZ and hippocampal dentate gyrus of deceased adult human cancer patients; cells expressing BrdU in the dentate gyrus were co-labeled with neuronal markers indicating that these cells did in fact differentiate into neurons [24]. This study brought new insight to the field suggesting that humans do have the capability of generating new neurons in adulthood. Reevaluation of neurogenesis in adult non-human primates was performed by both Kornack and Rakic, and Gould et al. in 1999; both labs found levels of adult neurogenesis in the hippocampal dentate gryus [21, 23]. Furthermore, existence a RMS contributing to new neurons

Comment [SMB2]: In which Rakic contradicts his 1985 report.

in the olfactory bulb in adult non-human primates [22] and potentially in humans [37], further illustrates that neurogenesis in primates, similar to rodents, occurs in the aforementioned neurogenic sites of the brain.

Although it is well-established that adult neurogenesis occurs in neurogenic zones, neurogenesis in non-neurogenic zones is a topic of much debate. Areas of the brain that have controversial evidence for and against neurogenesis are the neocortex, striatum, hypothalamus, amygdala, and substantia nigra [2, 18, 38]. Here, I will limit my discussion to conflicting evidence of adult neurogenesis in the neocortex.

Adult neurogenesis in the neocortex of rodents is still a controversial topic. Kaplan was one of the first to report adult neurogenesis in the neocortex using ^{3}H-thymidine autoradiography and electron microscopy [39]. However, following studies failed to find the existence of adult neurogenesis in the neocortex under normal physiological conditions; these studies found cell proliferation in the neocortex, but noted that such cells were either glial or undifferentiated cells, not neurons [40, 41]. Contrary to these results, a more recent study found adult neurogenesis in many areas of the necortex [42]. The investigators noted that many of the newly formed neurons were very small in size and since neurogenesis in the neocortex may occur at low levels, it may have made it difficult for prior investigators to find these neurons [42, 43]. In fact, these new neurons expressed GAD-67, a GABA synthesizing enzyme, indicating that new neurons generated in the neocortex may be GABA-ergic interneurons [42]. Thus, further studies in rodents may be warranted to confirm if low levels of neurogenesis in the neocortex may be contributing to the interneuron population in adulthood.

Similar to evidence in rats, adult neurogenesis in the cortex is very controversial in primates. Gould et al. reported new neurons in the prefrontal, parietal, and temporal cortex of

adult macaques; some of the new proliferating cells were suggested to have divided in the neocortex, while others were believed to have originated from newly divided cells in SVZ that migrated to the neocortex where they differentiated into neurons [44, 45]. While other studies have found proliferating cells in the neocortex of non-human primates, it was noted that many of cells failed to also express neuronal markers; those cells that appeared to be newly divided neurons were in fact two separate cells (one a newly divided cell and the other a neuron) in close approximation to one another [46, 47].

Further evidence against adult neurogenesis in the cortex came from a study using human brain tissue. Bhardwaj et al. used carbon-14 (^{14}C) dating to identify newly generated cells in human brain tissue [48]. The basis for this technique is that during the atomic bomb testing in the Cold War, atmospheric ^{14}C levels were dramatically increased; ^{14}C would have been incorporated into the human body, specifically DNA of newly dividing cells at that time. Individuals born before or after the atomic bomb testing were used to determine via ^{14}C dating that no new neurons were detected in human cortex [48]. Further, labeling for BrdU and neuronal markers in the human cortex indicated no adult neurogenesis [48]. Overall, these studies have lead some scientists to believe that adult neurogenesis does not occur in the cortex [26]. While this conclusion may be true, differences in methodological procedures may have contributed to some of the discrepancies between different studies. These methodological considerations will now be reviewed.

Methodological considerations for adult neurogenesis

Both ^{3}H-thymidine autoradiography and BrdU labeling have been widely used in studies of adult neurogenesis. ^{3}H-thymidine autoradiography is an older technique that labels cells in

the S-phase of mitosis and requires that morphological features of the cells be characterized in order to determine if the newly generated cells are in fact neurons. This technique is very time consuming and it can be difficult to perform reliably [49]. However, many of the hallmark studies establishing the existence of adult neurogenesis have used ^{3}H-thymidine autoradiography and such conclusions have been confirmed with other labeling techniques, such as double labeling with BrdU and neuronal markers [2, 19, 29, 33, 34]. Thus, I do not think that controversies in the field are based primarily on whether an "old" versus a "new" technique was used. This is not to say that one technique, such as BrdU double labeled with neuronal markers, might provide more compelling evidence of neurogenesis than another, but rather other methodological issues may be contributing to discrepancies between studies. I think there may be many issues contributing to whether a "false negative" (saying there is no effect when there is one) versus a "false positive" (saying there is an effect when there isn't one) is occurring. Below I will give examples of methodological issues that could be contributing to conflicting conclusions about adult neurogenesis.

Methodological issues could contribute to some researchers concluding prematurely that adult neurogenesis does not occur in specific brain areas. One factor that could contribute to a false negative is the amount of survival time given after the ^{3}H-thymidine or BrdU injection. Survival time issues can especially come into play when looking at a brain area that might already have low levels of neurogenesis; if the survival time is too long, the labeled neurons may have already died, while if the survival times are too short, the newly divided cells may not have had enough time to differentiate into neurons leading to the false conclusion that neurogenesis does not occur in that brain area [7]. The length of survival time may have come into play when adult neurogenesis was examined in non-human primates, since the first study claimed that adult

neurogenesis does not occur (survival times ranged from 3 months to 6 years) [25], while following studies did find adult neurogenesis in non-human primates (survival times ranged from 2hrs to 97 days) [21-23]. An additional, methodological consideration is the age of the animals used for the study. Some studies looking at neurogenesis in non-neurogenic sites use animals of varying ages [23, 25]. As stated before, levels of neurogenesis reduce with age [31, 32], so using an old subject base to determine that neurogenesis does not occur in a specific brain site may bias the results.

Another methodological issue that could contribute to a false negative result is the quality of staining and quantitative measures used for determining what is considered a new labeled neuron. If the quality of staining is poor, brain areas that may be producing new neurons, but at lower levels, may not be considered as neurogenic brain sites. Gould has indicated the importance of using a positive control, such as verifying that cells are co-labeled with BrdU and neuronal markers in the dentate gyrus, whenever looking at brain sites that are considered non-neurogenic [2]. This consideration is important in ensuring that the tissue is adequately stained to label new neurons in areas where they may be produced at low levels. Another important consideration is using a consistent standard for counting new neurons in the different brain sites. For example, many of the cells considered to be new neurons in the cortex are believed to be GABA-ergic interneurons, which have a smaller soma than surrounding cortical pyramidal neurons [42, 43]. Thus, it may not only be harder to find such newly generated neurons, but if new neurons labeled with BrdU and neuronal markers are not counted consistently across different brain sites (for example, the cortex versus the hippocampal dentate gyrus), then it may be prematurely concluded that adult neurogenesis does not exist in such areas. Thus, it is

important that standards of counting double labeled cells be kept across all brain sites that are analyzed.

In addition to false negatives, false positives may occur depending on how the tissue is analyzed. It is possible that some cells stained with BrdU appear to be co-labeled with neuronal markers, but are in fact separate cells in close proximity to labeled neurons. By using confocal z-series analysis, which basically takes 3D images of stained tissue, Koketsu et al. determined that many BrdU cells that appeared to be co-labeled with neuronal markers were in fact separately labeled cells [47]. Thus, studies that do not use this technique may be inaccurately counting newly divided cells as neurons when they may not be.

Overall, a systematic method of determining whether adult neurogenesis occurs in particular areas of the brain, especially in non-neurogenic areas, should be used. The fact that different standards are used for identifying newly generated neurons in non-neurogenic zones most likely contributes conflicting results across studies. To get around this issues, multiple measures should be used to validate claims, such as double labeling cells with neuronal markers, using z-series analysis to confirm that cells are double labeled, using positive controls to validate quality of tissue staining, and using consistent standards in evaluating neurogenesis across brain areas.

1. Sanes, D.H., T.A. Reh, and W.A. Harris, *Development of the nervous system*. 3 ed. 2012: Elsevier Inc.
2. Gould, E., *Opinion - How widespread is adult neurogenesis in mammals?* Nature Reviews Neuroscience, 2007. **8**(6): p. 481-488.
3. Taupin, P., *BrdU immunohistochemistry for studying adult neurogenesis: Paradigms, pitfalls, limitations, and validation.* Brain Research Reviews, 2007. **53**(1): p. 198-214.
4. Goldman, S.A. and F. Nottebohm, *Neuronal production, migration, and differentiation in a vocal control nucleus of the adult female canary brain.* Proceedings of the National

Academy of Sciences of the United States of America-Biological Sciences, 1983. **80**(8): p. 2390-2394.
5. Alvarezbuylla, A. and F. Nottebohm, *Migration of young neurons in adult avian brain.* Nature, 1988. **335**(6188): p. 353-354.
6. Paton, J.A. and F. Nottebohm, *Neurons generated in adult brain are recruited into functional circuits.* Science, 1984. **225**(4666): p. 1046-1048.
7. Nottebohm, F., *Why are some neurons replaced in adult brain?* Journal of Neuroscience, 2002. **22**(3): p. 624-628.
8. Nottebohm, F., *From bird song to neurogenesis.* Scientific American, 1989. **260**(2): p. 74-79.
9. Nottebohm, F., *Neuronal replacement in adult brain.* Brain Research Bulletin, 2002. **57**(6): p. 737-749.
10. Kirn, J., et al., *Cell death and neuronal recruitment in the high vocal center of adult male canaries are temporally related to changes in song.* Proceedings of the National Academy of Sciences of the United States of America, 1994. **91**(17): p. 7844-7848.
11. Alvarezbuylla, A., J.R. Kirn, and F. Nottebohm, *Birth of projection neurons in adult avian brain may be related to perceptual or motor learning.* Science, 1990. **249**(4975): p. 1444-1446.
12. Alvarezbuylla, A., C.Y. Ling, and F. Nottebohm, *High vocal center growth and its relation to neurogenesis, neuronal replacement and song acquisition in juvenile canaries.* Journal of Neurobiology, 1992. **23**(4): p. 396-406.
13. Nottebohm, F., *Neuronal replacement in adulthood.* Annals of the New York Academy of Sciences, 1985. **457**: p. 143-161.
14. Kirn, J.R. and F. Nottebohm, *Direct evidence for loss and replacement of projection neurons in adult canary brain.* Journal of Neuroscience, 1993. **13**(4): p. 1654-1663.
15. Barnea, A. and F. Nottebohm, *Seasonal recruitment of hippocampal neurons in adult free ranging black capped chickadees.* Proceedings of the National Academy of Sciences of the United States of America, 1994. **91**(23): p. 11217-11221.
16. Lois, C. and A. Alvarezbuylla, *Long-distance neuronal migration in the adult mammalian brain.* Science, 1994. **264**(5162): p. 1145-1148.
17. Kaslin, J., J. Ganz, and M. Brand, *Proliferation, neurogenesis and regeneration in the non-mammalian vertebrate brain.* Philosophical Transactions of the Royal Society B-Biological Sciences, 2008. **363**(1489): p. 101-122.
18. Bonfanti, L. and P. Peretto, *Adult neurogenesis in mammals - a theme with many variations.* European Journal of Neuroscience, 2011. **34**(6): p. 930-950.
19. Kempermann, G., H.G. Kuhn, and F.H. Gage, *More hippocampal neurons in adult mice living in an enriched environment.* Nature, 1997. **386**(6624): p. 493-495.
20. Magavi, S.S.P., et al., *Adult-born and preexisting olfactory granule neurons undergo distinct experience-dependent modifications of their olfactory responses in vivo.* Journal of Neuroscience, 2005. **25**(46): p. 10729-10739.
21. Kornack, D.R. and P. Rakic, *Continuation of neurogenesis in the hippocampus of the adult macaque monkey.* Proceedings of the National Academy of Sciences of the United States of America, 1999. **96**(10): p. 5768-5773.
22. Kornack, D.R. and P. Rakic, *The generation, migration, and differentiation of olfactory neurons in the adult primate brain.* Proceedings of the National Academy of Sciences of the United States of America, 2001. **98**(8): p. 4752-4757.

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23. Gould, E., et al., *Hippocampal neurogenesis in adult Old World primates.* Proceedings of the National Academy of Sciences of the United States of America, 1999. **96**(9): p. 5263-5267.
24. Eriksson, P.S., et al., *Neurogenesis in the adult human hippocampus.* Nature Medicine, 1998. **4**(11): p. 1313-1317.
25. Rakic, P., *Limits of neurogenesis in primates.* Science, 1985. **227**(4690): p. 1054-1056.
26. Rakic, P., *No more cortical neurons for you.* Science, 2006. **313**(5789): p. 928-929.
27. Walton, R.M., *Postnatal Neurogenesis: Of Mice, Men, and Macaques.* Veterinary Pathology, 2012. **49**(1): p. 155-165.
28. Fujita, S., M. Shimada, and T. Nakamura, *H3-Thymindine autoradiographic studies on cell proliferation and differentiation in external and internal granular layers of mouse cerebellum.* Journal of Comparative Neurology, 1966. **128**(2): p. 191-&.
29. Altman, J., *Autoradiographic and histological studies of postnatal neurogenesis. 2. A longitudinal investigation of kinetics migration and transfromation of cells incorporating tritiated thymidine in infant rats with special reference to postnatal neurogenesis in some brain regions.* Journal of Comparative Neurology, 1966. **128**(4): p. 431-&.
30. Kuhn, H.G., H. DickinsonAnson, and F.H. Gage, *Neurogenesis in the dentate gyrus of the adult rat: Age-related decrease of neuronal progenitor proliferation.* Journal of Neuroscience, 1996. **16**(6): p. 2027-2033.
31. Jabes, A., et al., *Quantitative analysis of postnatal neurogenesis and neuron number in the macaque monkey dentate gyrus.* European Journal of Neuroscience, 2010. **31**(2): p. 273-285.
32. Leuner, B., et al., *Diminished adult neurogenesis in the marmoset brain precedes old age.* Proceedings of the National Academy of Sciences of the United States of America, 2007. **104**(43): p. 17169-17173.
33. Altman, J. and G.D. Das, *Autoradiographic and histological evidence of postnatal hippocampal neurogenesis in rats.* Journal of Comparative Neurology, 1965. **124**(3): p. 319-&.
34. Kaplan, M.S. and D.H. Bell, *Mitotic neuroblasts in the 9 day and 11 month old rodent hippocampus.* Journal of Neuroscience, 1984. **4**(6): p. 1429-1441.
35. Kaplan, M.S. and J.W. Hinds, *Neurogenesis in the adult ratL electron microscopic analysis of light autoradiographs.* Science, 1977. **197**(4308): p. 1092-1094.
36. Luskin, M.B., *Restricted proliferation and migration of postnatally generated neurons derived from the forebrain subventricular zone.* Neuron, 1993. **11**(1): p. 173-189.
37. Bedard, A. and A. Parent, *Evidence of newly generated neurons in the human olfactory bulb.* Developmental Brain Research, 2004. **151**(1-2): p. 159-168.
38. Migaud, M., et al., *Emerging new sites for adult neurogenesis in the mammalian brain: a comparative study between the hypothalamus and the classical neurogenic zones.* European Journal of Neuroscience, 2010. **32**(12): p. 2042-2052.
39. Kaplan, M.S., *Neurogenesis in the 3 month old rat visual cortex.* Journal of Comparative Neurology, 1981. **195**(2): p. 323-338.
40. Ehninger, D. and G. Kempermann, *Regional effects of wheel running and environmental enrichment on cell genesis and microglia proliferation in the adult murine neocortex.* Cerebral Cortex, 2003. **13**(8): p. 845-851.
41. Magavi, S.S., B.R. Leavitt, and J.D. Macklis, *Induction of neurogenesis in the neocortex of adult mice.* Nature, 2000. **405**(6789): p. 951-955.

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42. Dayer, A.G., et al., *New GABAergic interneurons in the adult neocortex and striatum are generated from different precursors.* Journal of Cell Biology, 2005. **168**(3): p. 415-427.
43. Cameron, H.A. and A.G. Dayer, *New Interneurons in the adult neocortex: Small, sparse, but significant?* Biological Psychiatry, 2008. **63**(7): p. 650-655.
44. Gould, E., et al., *Neurogenesis in the neocortex of adult primates.* Science, 1999. **286**(5439): p. 548-552.
45. Gould, E., et al., *Adult-generated hippocampal and neocortical neurons in macaques have a transient existence.* Proceedings of the National Academy of Sciences of the United States of America, 2001. **98**(19): p. 10910-10917.
46. Kornack, D.R. and P. Rakic, *Cell proliferation without neurogenesis in adult primate neocortex.* Science, 2001. **294**(5549): p. 2127-2130.
47. Koketsu, D., et al., *Nonrenewal of neurons in the cerebral neocortex of adult Macaque monkeys.* Journal of Neuroscience, 2003. **23**(3): p. 937-942.
48. Bhardwaj, R.D., et al., *Neocortical neurogenesis in humans is restricted to development.* Proceedings of the National Academy of Sciences of the United States of America, 2006. **103**(33): p. 12564-12568.
49. Altman, J., *The Discovery of Adult Mammalian Neurogenesis*, in *Neurogenesis in the Adult Brain I: Neurobiology*. 2011. p. 3-46.