Special Topic – The Changing Brain, Adult Neurogeneis

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Neurogenesis is the generation of neurons, and after 18 months it rapidly slows down to the point where it all but stops in the adult mammalian brain. However, other vertebrates such as fish and amphibians show extensive addi­tion of neurons, as well as the capacity for neuronal regeneration in adulthood (Arellano et al, 2011).

In adult mammals, only two brain regions have been determined definitively to produce neurons: the dentate gyrus of the hippocampus and the sub­ventricular region of the lateral cerebral ven­tricle (Arellano et al, 2011).

Sanai *et al.* show that the migratory cell streams departing from the sub­ventricular zone (SVZ) are present in human newborns, but disappear by the eight­eenth month after birth (Arellano et al, 2011).

There have been many indications of cell growth in infants. For example, the newborn brain contains cells at the ventricular surface that have the morphology and molecular char­acteristics of neuronal progenitors, together with a significant number of cells expressing Ki67 — a marker of proliferating cells. Additionally, researchers found a number of cells in the SVZ expressing DCX and PSA-NCAM which are indications of immature migrating neurons. Furthermore, they found EGFR, which is a marker of intermediate progenitors of neu­rons. However, Sanai and colleagues did not detect these features in infants older than 18 months, or in children and adults. It seems, therefore, that most, if not all, neurogenic activity in these areas of the human brain has disappeared by time infants are weaned (Arellano et al, 2011).

It can be seen from these experiments that neurogenesis primarily occurs in infants. Some mammals have neurogenesis even to adulthood, however humans lack this feature except for a select few regions. There are current studies being done on apes and chimpanzees, our closest relatives, in order to see whether there is reduced neurogenesis in the adult SVZ or if this is just a trait that is specific to humans (Arellano et al, 2011).

During development, neural cell proliferation and neurogenesis occurs when cells move from the ventricular zone (up a bipolar cell’s process) towards the marginal zone. Then it returns to the ventricular zone and divides either vertically or horizontally. Vertical cleavage allows for this process to be repeated, but horizontal cleavage leads to the cell migrating up to the marginal zone and forming the cell layers in an inside out arrangement. The reason for this cleavage dependent differentiation is because of the distribution of Notch-1 and Numb proteins. Notch-1 is distributed in the upper part of the cell, and Numb is in the lower part of the cell. Unequal distribution leads to differentiation, but equal distribution of Notch-1 and Numb leads to undifferentiation and continuation to divide. This can be seen in Figure 1. This is done in the developing brain, but the question arises as to whether or not the adult brain performs neurogenesis (Bear et al, 2007).

Since the 1960’s, there has been considerable research into the possibility of adult neurogenensis. Altman used 3H-thymidine labeling and light microscopy to show that there were select regions in the brain, namely the hippocampus, neocortex, and olfactory bulb, that were synthesizing DNA for the processes of growth and division of cells. Cells that do not need to divide do not continuously synthesize DNA and as such, only those cells that divide will pick up this radioactive thymidine. However, some such as Rakic, were skeptical of these findings due to the inability to repeat this in monkeys. In the 1980’s Nottenbohm used a similar method with BrdU-labeling and immunocytochemistry to confirm Altman’s results. Further studies using BrdU in sick elderly cancer patients (which labeled proliferating tumor cells) also confirmed neurogenesis in adult brains when the BrdU was found in the hippocampus and other regions that were not part of the tumor. Some studies have gone as far as localizing the origin of the new neural cells of the olfactory bulb to the subventricular zone (Gould, 2007).

As these studies progressed, cells that had adult neurogenesis were labeled neurogenic, and those that did not have neurogenesis were labeled as non neurogenic. Pre 1990, no areas were considered neurogenic. As of the early 1990’s the areas considered neurogenic were the dentate gyrus and the olfactory bulb/subventricular zone. All other areas were considered non-neurogenic. By the late 1990’s, the areas considered neurogenic have been expanded significantly, as can be seen in Figure 2 (Gould, 2007).

Neurogenesis in the neocortex is debatable, and there are both cases for and against this. When Altman used his 3H-thymidine labeling technique, he initially found what appeared to be false positives (and therefore initially not considered neurons that performed neurogenesis) of labeled cells in the neocortex, due to an overlap of cells. However, upon further review, some cells appeared to be the product of neurogenesis. Kaplan later used electron microscopy on adult rat brains, and determined that the labeled neurons from Altman’s tests were not false positives. He was even able to determine the projections off of the neurons and classify them as axons, dendrites, and synapses (Gould, 2007).

Subsequent research involved using BrdU and antibodies that are able to detect neuronal markers, (specifically NeuN – neuronal nucleus, which is a protein found in the nucleus and cytoplasm of mature neuronal cells). The researchers found several cells that had both BrdU and NeuN labeling in the adult neocortex of different types of mammals (such as hamsters and rats), but the neocortex had a lower level of these double labeled cells than the dentate gyrus. False positives were ruled out by examining the cells labeled with both BrdU and NeuN with a confocal microscope (Gould, 2007).

However, even with this labeling technique, questions have arisen about whether or not this label definitively shows neurogenesis. It detects DNA synthesis, but synthesis is possible for reasons other than growth and mitosis, namely DNA repair. However, this has not been observed in adult brains. Furthermore, after only a short period of time following the BrdU injection, the marker is found to be absent. This implies that BrdU is found during mitosis of progenitor cells which then differentiate into mature neurons. Similarly, some of the markers used are known to label some proteins found in damaged glia as well. However there are labels like NeuN that do not label glia (and using these in conjunction support the claim that the cells are neurons only, and not glia). These claims support neurogenesis as a theory (Gould, 2007).

However, although these studies seem to support the case for neurogenesis in the neocortex, the studies (both the 3H-thymidine, and BrdU/NeuN studies) have been difficult to replicate in adult mice, rats, macaque monkeys, and humans. In order to explain these different results, we must understand the techniques used to perform the experiments. In order to have BrdU bind to its antibodies, the DNA must be single stranded. In order to gain access to single strand DN, it must be denatured. This denaturation can cause damage to the cells. Differences in the denaturation and other parts of the technique for isolating single stranded DNA could compromise some tests more than others. There is evidence showing that this limits the amount of viable tissue and its quality and also lowers the signal to noise ratio that is necessary for detecting the stains. This is one possibility for the discrepancy in the different studies (Gould, 2007).

Yet, there have been other studies used to claim that there is no neurogenesis in the neocortex. These studies involve using brain areas that have been shown to have high levels of neurogenesis such as the dentate gyrus or the hippocampus as control groups for comparison with the neocortex. The hippocampus study showed that when labeling with BrdU and NeuN, the neocortex does not show the typical staining pattern because neocortical neurogeneis possibly may only occur at a level that is lower than the limit for detection, and as such the results are therefore unreliable as a positive control (see Figure 3) (Gould, 2007).

Additionally, the actual microscopic analysis of the cells that have been stained leaves room for error in detection. This analysis is very precise and difficult to perform, and the double labeled cells are hard to detect (because many are small and the stain is often not found in the cytoplasm). Therefore, we cannot rule out neurogenesis from those studies that couldn't detect the labels (Gould, 2007).

Other studies also mention size and shape of cells and use those criteria to consider the cells neuronal or non-neuronal cells. The study explains that small cells had the double label, but many large cells did not, and therefore only non-neuronal tissue was labeled. However, this is a biased assumption, because many neocortical neuronal cells are small, yet according to that study, they are not neurons. Therefore we can rule that study as inapplicable because of the different neuron classification system (Gould, 2007).

In addition to the labeling studies, which clearly have many issues and controversies, we can look at other types of studies as well. There have been other studies that focus on endogenous markers of immature neurons. Molecules such as PSA-NCAM or CRMP4 are markers for immature neurons, and have been found in adult neurons. However, it must be noted that we don't know if these neurons that are detected are new cells from neurogenesis or even if they will be functional as part of neural circuits. Either way, the fact that they are found, is still indicative of new neuron formation (Gould, 2007).

Two final techniques used to detect neurogenesis in adults involved uses retroviral labeling and 14C labeling. Neither of the techniques were able to definitively prove that there is neurogenesis in the neocortex, however, this is not conclusive and it may only be due to the limitations of the experiment itself that they have been unsuccessful as of yet. First we will discuss the retrovirus technique. This involves using retroviruses to cause proteins in new neurons to fluoresce. This protein is expressed all throughout the cells and allows researchers to see axons and dendrites. This allows them to differentiate them from glia, which have different morphological structures and processes. Therefore, if a cell is involved in neurogenesis, researchers can be sure that they are neurons and not other types. Also retroviral experimentation can be used in living slices. However, since retrovirus activity is unpredictable, it is not a dependable technique to observe neurogenesis in adult brains, because there would need to be a high level of neurogenesis in order to be detected properly. There are several other reasons that make this technique difficult to prove neurogenesis occurred (Gould, 2007).

Lastly 14C labeling involves using a compound that is already in the human brain, namely carbon, rather than adding an exogenous chemical in order to test. Testing for 14C involves using mass spectroscopy to determine the amount of this isotope of carbon in the NeuN and non-NeuN cells. The levels of BrdU and NeuN found in the dentate gyrus are 30 times larger than those in the neocortex. Therefore, it is possible that if no detection occurred in the neocortex, this may only be to the low amount of BrdU and NeuN neurons in the neocortex, and not because there is no neurogenesis. Yet again, this leads to inconclusive results (Gould, 2007).

As of present time, new experiments have been performed and new information has been discovered. The basic timeline of adult neurogenesis research as of now gives a good background on the entire scientific study:

The first tests regarding adult neurogenesis were performed on rats in the 1960’s. Subsequently testing branched out to birds and other species similar to rats. However mammal adult neurogenesis still seemed to be a false hope type of situation. The earliest study done on mammals was in 1974. It was performed on monkeys and the results were inconclusive. The real breakthrough with adult neurogenesis in mammals came in the 1990’s. Since that time, especially in the 21st century (which yielded a significant increase in technology) there has been a major boom in research of adult neurogenesis across all species of animal testing, including mammals (Wheeler, 2010).

The first major advancement occurred in 1963 by showing that cells in the dentate gyrus (DG) and a few other regions have adult neurogenesis. In 1967, researchers found signs of neurogenesis in the guinea pig hippocampus. The first study on mammals was done in the 1970’s, it was a study on monkeys that showed that new neurons do not grow in adult mammalian brains. Others found neurogenesis in a baby rat in the DG and the olfactory bulb (OB) (Wheeler, 2010).

In the 1980’s, researchers observed neurogenesis in the visual cortex of adult rats and new glia in adult rats (glia are not the focus of our discussion, but nonetheless important in showing that different brain cells have growth). More research showed that the hippocampus is also involved in neurogenesis. This study was done in both young and old mice (Wheeler, 2010).

The late 1990’s was a very successful time for neurogenesis research. Multiple experiments were performed and showed that the DG had neurogenesis even in adulthood. This included the cell proliferation decrease in adult primate DGs, yet it created a connection between the adult rat DG and performance on hippocampus dependent learning tasks. The largest breakthrough came in 1999 when researchers received confirmation of neurogenesis in adult primates. Although this neurogenesis was only recorded at low levels, it was a major breakthrough for neurogenesis research (Wheeler, 2010).

In the 21st century, it was determined that anti depressants effect hippocampal neurogenesis as well as astrocytes. Researchers noticed that when they blocked neurogenesis in the adult rat DG, it impaired hippocampus-dependent learning, but not hippocampus independent learning. There was also a trend of proliferation in primate amygdalas. In 2003, experimenters demonstrated cell proliferation at the site of damage in post mortem Huntington’s disease patients (Wheeler, 2010).

Experiments with humans significantly increased in 2004, and researchers saw neurogenesis in post-mortem Alzheimer’s patients. There was also a study that showed that astrocytes can differentiate into neurons. Additionally, it was shown that increased neurogenesis in the brain might mediate effects of anti depressants. In 2004, researchers found evidence of neurogenesis in the adult human OB (Wheeler, 2010).

In 2006, there was a study done which showed that hippocampal neurogenesis is not necessary for the behavioral effects of an enriched environment. There was also a study done on a stroke victim which suggested that vascular niches were involved in neurogenesis (Wheeler, 2010).

In 2007, researchers were first able to characterize the human rostral migratory stream (Wheeler, 2010).

Figure 4 shows a summary of the timeline (Wheeler, 2010).

Research in neurogenesis has advanced over the years. It was first thought that neurogenesis only occurred in infants, yet as experimentation progressed, it was determined that several regions in the brain such as the dentate gyrus of the hippocampus, and the olfactory bulb show neurogenesis in the adult brain. Adult neurogenesis in the neocortex is still a matter of debate. Neurogenesis even effects different regions of the brain differently and can be involved in different disorders. Neurogenesis in the brain can be found in several different types of animals as well. Although there is currently strong debate about neurogenesis in adults, and no definitive conclusions have yet to be reached, we can be certain that this topic will be of interest to many researchers for years to come.

References

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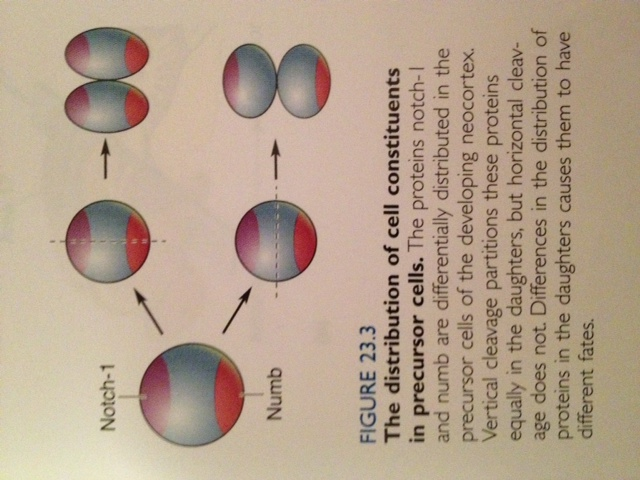
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Figure 1.

*Notch-1 and Numb distribution*

Figure 2.

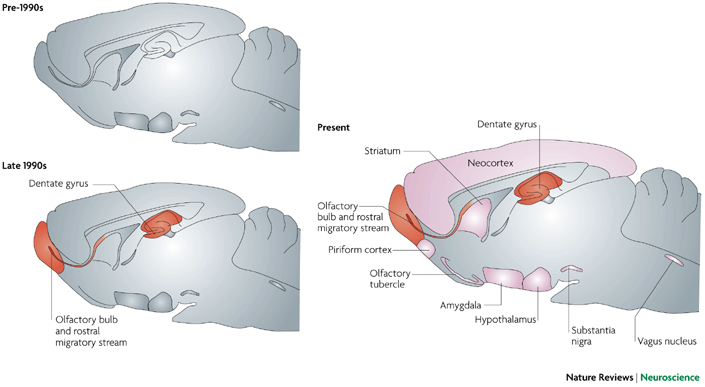
*Differences in the regions thought to be neurogenic in adults. Top shows prior to 1990’s (no neurogenesis), bottom left shows late 1990’s where the olfactory bulb and dentate gyrus are neurogenic, bottom right shows present day which includes previous regions as well as the neocortex.*

Figure 3.

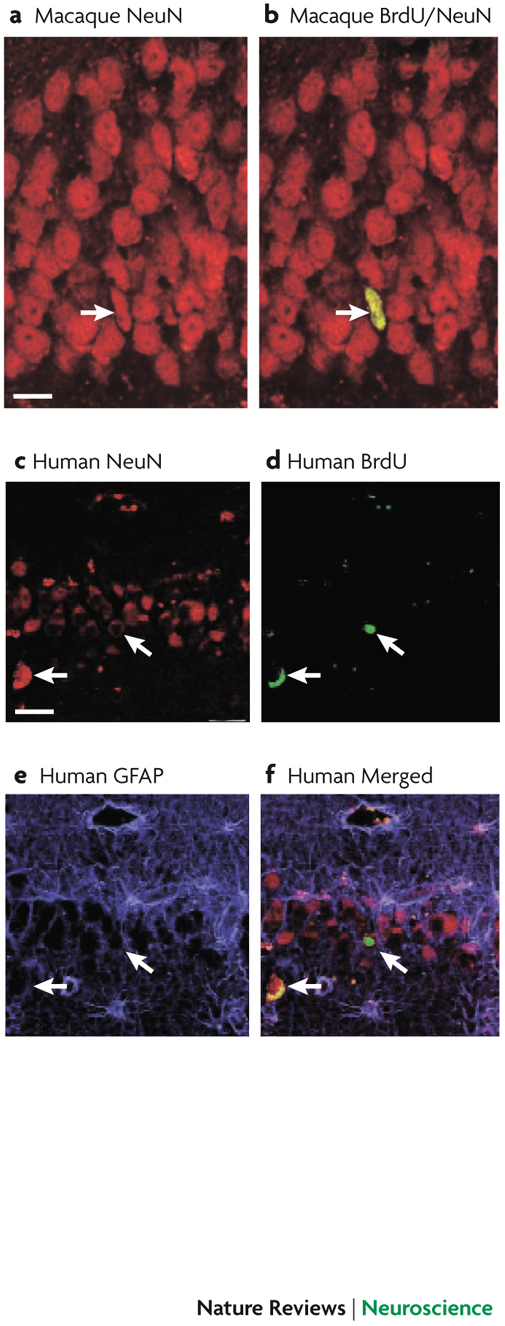
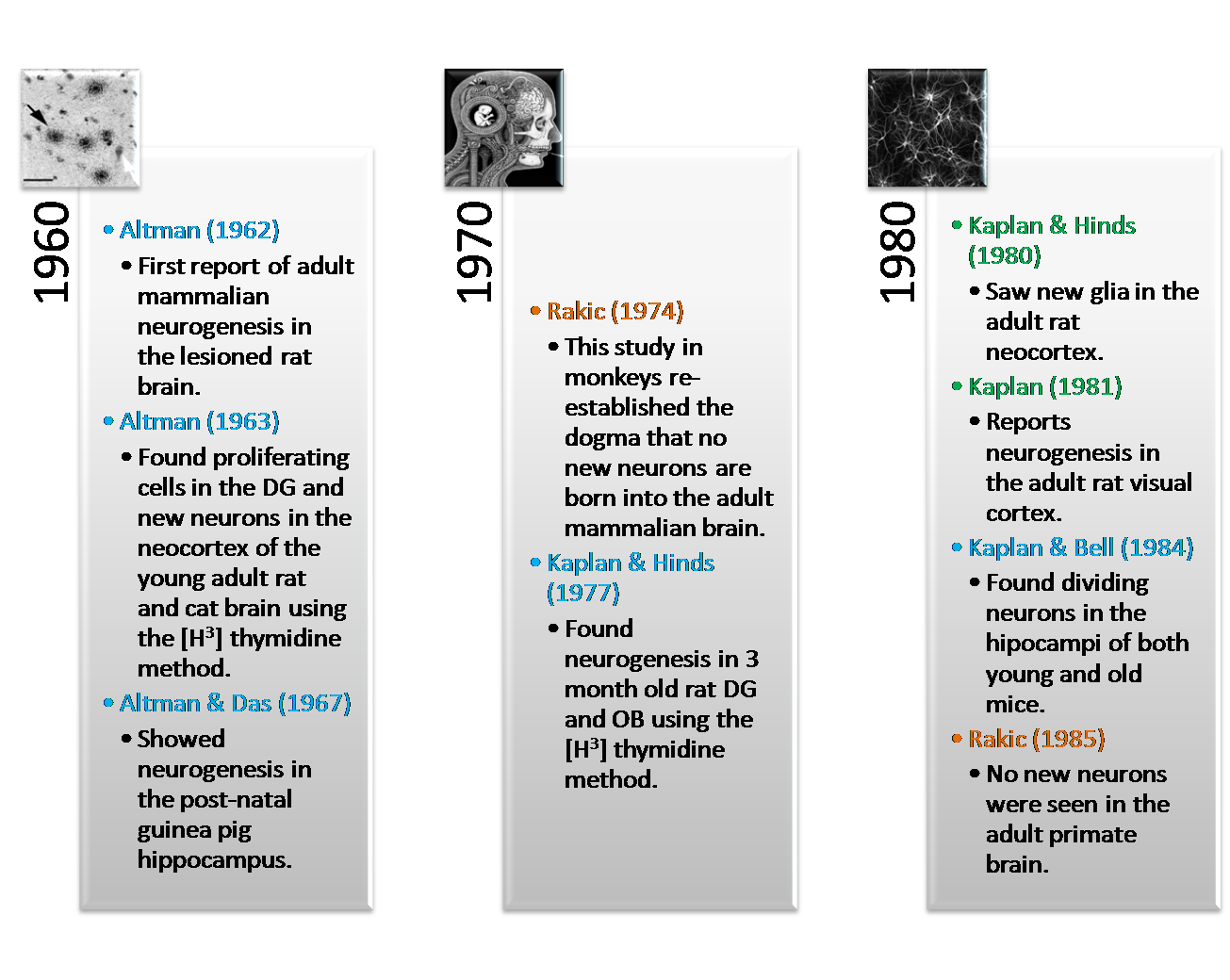
*Neurons stained with BrdU, NeuN and BrdU/NeuN*

Figure 4.

*Timeline of Neurogenesis Research*

