

Seminar: Cognitive and Behavioral Neuroscience Seminar (603)
Meeting Date: April 25, 2002

Chairs: Herbert S. Terrace, Peter Balsam, Jon Horvitz, and Yaakov Stern

Speaker: Elizabeth Gould

Topic: Neurogenesis in the Adult Mammalian Brain

Seminar Participants:

Herbert S. Terrace, Columbia University, Psychology
Rae Silver, Columbia University, Psychology
Jacqui Rick, Columbia University, Psychology
Rohan Sobby, New York State Psychiatric Institute
Joseph Le Souter, Bermerol College
Michael Antle, Columbia University
Sinae Pitts, Columbia University, Psychology
Lori Asarian, Columbia University, Psychology
Ilia Karatsoreos, Columbia University, Psychology
Lance Kriegsfeld, Columbia University, Psychology
Christopher Weidenmeyer, Columbia University
Colin Beer, Rutgers University
Tammy Moscrip, Columbia University, Psychology
James Lee, Columbia University
Weisstaub Noeliz, Columbia University
Robert Thompson, Hunter College, CUNY
Luca Santarelli, Columbia University
SH Lisanby, Columbia University
Yaakov Stern, Columbia University, Sergievsky Center
A Kreigstein, Columbia University
B. Clinton, Columbia University
Amy Hale, Columbia University, Psychology
Mike Drew, Columbia University, Psychology
John H. Hilton, Columbia University, Sergievsky Center
Jennifer Mangels, Columbia University, Psychology
Jon Horvitz, Columbia University, Psychology
Emily Stern, Columbia University, Psychology
Johannes Schwaninger, Columbia University, Psychology
Brady Butterfield, Columbia University, Psychology
Peter Balsam, Barnard College, Psychology
Yaakov Stern, Columbia University, Sergievsky Center
Janet Metcalfe, Columbia University, Psychology
Won Yung Choi, Columbia University, Psychology
Mona Khalil, Columbia University, Psychology
(others attended, but did not sign in on our attendance sheet)

Rapporteur: Kate Lynch

Dr. Gould lectured on neurogenesis in the adult mammalian brain. Dr. Gould first provided some historical context in which to place this discussion and then showed evidence that new cells are produced in certain parts of the brain. In the 1960's Altman reported new cells that had the morphology of three brain regions, the hippocampus, the olfactory bulb and the neocortex of adult rats and cats, using ³H-thymidine methods. In the 1970s and 80s others extended these findings, again using the thymidine method, and found that there were new cells in the same three brain regions reported by Altman, the hippocampus, the olfactory bulb and the neocortex. These cells were reported to have the overall structural characteristics of neurons, including synapses on the cell bodies and dendrites. However at the time, these studies had limited impact. It wasn't until the 1990s that people really started to investigate these issues using newer techniques and that significance and interest in this phenomenon came to light. Dr. Gould emphasized that despite the renewed interest in this area of research, there is still controversy and a lot that is unknown. However, at least some of the findings from long ago are now considered well established.

Dr. Gould contrasted two methods used to investigate neurogenesis: Thymidine radiographic methods used in older studies and a newer method, bromodeoxyuridine (BrdU). Both methods are based on the application of exogenous nucleotide like molecules, which are picked up by cells that are synthesizing DNA in preparation for division. Both methods are used to mark new cells and their progeny. BrdU gives a better estimate of the number of cells actually produced in a certain region. BrdU is a newer technique that allows more than just a few cells to be seen. This method has allowed for the discovery that many thousands of new cells are produced per day in the adult rat. A second advantage of the BrdU method is that it can be easily combined with different staining methods to determine whether the new cells generated in adulthood have neuronal characteristics.

Studies done decades ago combined with newer studies have provided a good idea of how new cells are produced in hippocampus. In adulthood, the progenitor cells are located in undefined region of the hippocampus called the subgranular zone. These progenitor cells divide asymmetrically and produce two daughter cells, one of which retains the ability to divide, and the other which begins to differentiate into an immature neuron. These cells migrate a short distance to granule cell layer where they extend dendrites and axons into the CA3 region, a region of the hippocampus. This migration occurs relatively quickly.

Dr. Gould reported that there is good evidence that the new cells in the dentate gyrus (DG) of adult rats are actually neurons. These new cells have the morphology and the ultrastructure of neurons, with axons extending into the CA3 region. A recent study has shown that these new cells generate action potentials.

Dr. Gould discussed a technical point that is relevant to understanding the functional studies and some of discrepancies found in the literature. All of the original studies on adult neurogenesis that were done using the BrdU method based their experimental design on developmental studies. The developmental studies assumed correctly that BrdU enters the brain readily. It was assumed that low levels of BrdU would label all cells in the S phase, and further that high doses would be toxic to the animals.

In these initial studies low doses were used. However, the assumptions about the low dosage turned out to be incorrect. Lower doses only label a fraction of the cells and even extremely high doses do not produce toxic effects. Thus the use of lower dosages has led to an underestimation of the number of new cells produced. Studies using higher doses of BrdU combined with radioactive thymidine methods found that over 9000 progenitor cells were produced in the adult rat DG, over a 24 period. The majority of these cells contain the characteristics of at least immature neurons. Dr. Gould proposed the question of how the brain is able to produce so many new neurons without outgrowing the skull. It turns out that there is a lot of cell death in all regions in which neuron addition has been found. Death of cells depends on the type of experiences the animal has.

Dr. Gould made some points about neurogenesis in various regions before switching the focus of her talk to the hippocampus. Dr. Gould reported that new cells with neuronal characteristics have been discovered in the DG of virtually all mammals that have been studied, including non-human primates and humans. The new cells that are added to the olfactory bulb and differentiate into granule neurons in rodent and nonhuman primates, actually originate from progenitors in the subventricular region. On the other hand, DG cells originate from progenitors within the DG. Cells with neuronal staining characteristics have been found in the neocortex of adult rats and macaques.

It is easy to distinguish false positives (newly generated satellite cells next to a neuron) from true cells, because the true cells are double labeled with BrdU, used to count the number of new cells, and a stain, which indicates that the cell has neuronal characteristics. New glial cells have also been found in the adult neocortex, DG, and olfactory bulb.

Dr. Gould reported that the many more BrdU labeled cells have been found in the DG, compared to prefrontal and inferior temporal areas and neocortical regions in general. At least in rodents the olfactory bulb has many more than BrdU labeled cells than the DG.

Part II

Dr. Gould shifted her focus to hippocampus and reported finding regarding the different types of experiences that modulate neurogenesis in adulthood. Several factors have been known to effect the numbers of new cells in the DG in rodents, and in some cases primates. Stress affects adult neurogenesis, at least in the hippocampus. Stress affects not only the number of cells produced in adulthood, but also the number of cells produced developmentally as well. Early prenatal and postnatal stress in rodents and macaques has been found to affect the number of new cells in the DG during adulthood. On the positive side of things, the degree of environmental complexity has been found to increase the

number of new cells in the DG. Both environmental complexity and physical activity have been shown to increase the production and survival of BrdU labeled cells in the DG.

Dr. Gould described the first study, done in collaboration with McEwen and Flugge, looking at the effects of stress on the number of new cells in the DG in tree shrews. Tree shrews form dominance hierarchies immediately once they are together in the same environment. The dominant animals are very aggressive toward the subordinate animals. The subordinate animals show physiological and behavioral signs of extreme stress, such as elevated cortisol levels and heart rate, hyper vigilance and hyperactivity when exposed to dominant tree shrews.

The subordinate animals also showed significantly decreased numbers of new cells in the DG after acute exposure to a dominant animal. Dr. Gould reported an unusual finding, that these animals do not adapt to the stress of interaction with a dominant animal. The stress response persists over the course of hours and days. In this study the lag from the stressful event until the decrease in new cells was found was about three hours. Dr. Gould reported result on the long term effects of developmental stress in which it was found that prenatal stress to macaques (mother was exposed to acoustic startle) produces a persistent reduction of new cells into adulthood.

Dr. Gould next discussed the mechanisms of stress-induced suppression of neurogenesis. The studies done to investigate these mechanisms were done mostly in rodents with naturalistic stressors. Gould worked to characterize some more naturalistic stressors that were not yet well characterized including the effects of odors of natural predators (fox) on the number of new cells in adult rats. Exposure to a predator's odor leads to elevated glucocorticoid levels and inhibits cell proliferation in the adult rat. Behavioral effects such as digging were also found.

Gould raised the question of whether glucocorticoids are responsible for the stress-induced decrease in the production of new cells. Glucocorticoids themselves seem to have a suppressive effect on neurogenesis in the DG. In order to look at the combined effects of glucocorticoids and stressful situations, the glucocorticoid levels of adult rats were normalized. The main glucocorticoids were replaced in the drinking water. This helped to maintain the diurnal rhythm in glucocorticoid levels, but prevented the stress-induced arousal arising from glucocorticoids. This procedure eliminated the fox odor induced suppression of new cell proliferation. In the sham operation control condition, this reduction did not occur. Thus, it is clear that glucocorticoids play a role in mediating the stress-induced reduction in cell proliferation, at least in this paradigm.

Next Dr. Gould discussed studies looking at later time points after exposure to the stressor in order to better characterize the cells which were produced by the neurogenesis and to determine the functional impact of these cells. The results of this investigation were surprising and disturbing. Dr. Gould looked at several different time points after fox odor exposure and BrdU labeling. After one week, the difference between the control group and fox-odor exposure group was maintained. Staining was used to determine which cells had an immature neuronal phenotype. It was found that the decrease in the

number of new cells was due to a decrease in the number of immature neurons. Three weeks after the exposure, the effect was lost.

Dr. Gould concluded that the drop off in the number of cells between one and three weeks, was probably due to the death of newly generated cells. The effect of the stressor disappeared. Dr. Gould commented that if the newly generated cells end up dying and have no lasting impact, the neurogenesis is not nearly as compelling.

After looking through the literature on environmental complexity, Dr. Gould considered that the loss of newly generated cells in the control animals may have occurred because these animals are housed in such limited environments. Studies have shown that living in enriched environments increases the survival rate of newly generated cells in the hippocampus of black hat chickadees, adult mice and rats. In the first study black hat chickadees living in wild were caught, set free, and then recaptured (Barnes and Nottenaum, 1994). The number of cells that survived in the animals which were out in the wild was much greater than the number of cells which survived in birds that were held in captivity for the duration of the experiment. The same effect was found in the DG when using an enriched vs. controlled laboratory setting.

Next, Dr. Gould used the Visible Burrow System (VBS) paradigm created at the University of Hawaii to apply the findings concerning the effects of more complex environments to the studies of effects of stress. The VBS consisted of interconnected tunnels and tubes, and an open field area for food and water. Albino rats were put in the VBS with conspecifics. Within 3 nights a dominant emerges in each cohort and a dominance hierarchy was formed. The dominant animals exhibited offensive, aggressive behavior and spent more time in the open field than subordinates. The subordinate animals had lower levels of BrdU labeled cells in the DG, likely because of the stressful nature of the subordinate position.

In another study animals were categorized as dominant or subordinate, new cells were labeled with BrdU, and then animals were placed either in their home cage or the more complex VBS environment. After two weeks, animals in the VBS environment had more BrdU labeled cells in the DG than those animals housed in their home cage. Animals in this more complex environment retained the difference between dominant and subordinate in terms of the numbers of BrdU labeled cells. Those animals housed in the home cage did not retain the effect. Dr. Gould concluded that some aspects of a more complex environment are necessary for the survival of the newly produced cells.

Dr. Gould became interested in determining the important variables in the maintenance of new cells in the DG, and maintenance of the difference in the number new of cells in subordinate vs. dominant animals. Dr. Gould was also interested in applying these results to primate studies in naturalistic settings. Primates in the laboratory setting have limited space and in most cases have very limited social and cognitive experiences. Dr. Gould wanted to study adult neurogenesis in general, and the effects of social stress on adult neurogenesis in particular, in a situation in which the animals live in more naturalistic conditions. Dr. Gould chose to use adult marmosets to investigate the effects of social

stress on structural plasticity in the brain in animal housed in natural conditions. Adult marmosets weigh less than adult rats. The marmosets were housed in a space which is 64 X the space of average an macaque. The marmosets lived in enriched environments with other marmosets, trees to climb and ample foraging opportunities. The data for this study is still being collected.

Dr. Gould has recently begun to study another stressor, sleep deprivation. The research was motivated by an interest in the relationship between sleep deprivation and learning. In the first study, rats were placed on a small platform surrounded by water for 24 hours. If the animal fell asleep, it would fall into the water, wake up and climb back onto the platform. Twenty-four hours of sleep deprivation lead to a decrease in the number of BrdU labeled cells in the DG. Sleep deprivation is a stressful experience and thus elevated glucocorticoid levels were predicted. A study currently being run to see whether eliminating the changes in glucocorticoids would eliminate the effect of sleep deprivation on the number of BrdU labeled cells.

Dr. Gould raised the question, what are these new cells doing in the system? Thousands of new cells are produced in the DG everyday in adult rats. Dr. Gould pointed out that this is a large energy expenditure and so it is likely that these cells are there for some purpose. Furthermore it is likely that these cells perform some function which cannot be preformed by the cells produced during development. Dr. Gould suggested that immaturity of the new neurons may be important for some kind of unique function. For example, it is possible that the new neurons form connections rapidly than mature neurons and have different electrophysiological properties than mature neurons. The cells in the inner granule layers tend to be immature neurons, and these neurons appear to exhibit LTP. Finally, adult generated cells have less divergent axons than those generated during development. A post doc, Hastings, in Dr. Gould's lab found that only granule cells generated during development have axons that diverge widely in the horizontal plane. No adult generated cells have been found with this type of axon. Thus there seem to be structural difference between cells produced during development and those produced during adulthood. The question of the functional correlate of these observations has not yet been determined.

In summary, Dr. Gould has shown that new cells with neuronal characteristics are produced in the hippocampus, olfactory bulb, and neocortex. Stress inhibits cell proliferation in the hippocampus. Complex environments are necessary for the survival of new cells. Lastly, new granule cells have unusual properties and may participate in hippocampal function.

Questions:

Balsam asked if the progenitor cells express any of these effects.

Gould replied that when looking at short survival times we are looking at progenitor cells. She explained that fewer of those cells go from G1 into S when the animal is stressed.

Balsam then asked whether the number of progenitor cells is dampened in the long term.

Gould replied that the answer to that is not yet known. Even when looking at effects in adulthood or prenatal stress, there are fewer BrdU cells. However, it is unclear whether there are more progenitor cells that just are not entering into S.

Terrace asked Gould to make further comments on a previous slide which mentioned learning effects.

Gould reported that a study was performed in her lab that corroborated data of other groups looking at the production and survival of these new cells. In both cases it was shown that with certain learning tasks you can enhance the survival of newly generated cells in the DG. Another group has also shown a cell proliferation effect, an increase in the production of these cells as well as enhanced survival.

Terrace asked specifically about spatial learning tasks in which the hippocampus is known to be more involved.

Gould responded that there does not seem to be anything special about spatial learning.

Stern asked whether new cells actually help the animals perform better.

Gould responded that there is no specific evidence that the new neurons help the animals perform better. However, Gould reported that there is some evidence the new cells may be necessary for the animals to perform a very difficult task. In you decrease the production of these new cells, you can get an impairment on certain hippocampally dependent tasks. However, these tasks are very difficult and require hundreds of trials for the animals to acquire them. Thus, the link between new cells and learning is unclear. On certain easier tasks (e.g. spatial navigation tasks) impairment is not seen.

Balsam asked whether short delay conditioning would produce an increase in the newly generated cells.

Gould responded that short delay conditioning does not increase the number of new cells. Gould emphasized that this may be because the task is easier. It is difficult to match task difficulty because the hippocampally dependent version of a task is usually much more difficult than the hippocampally independent version.

Le Sauter asked whether the total number of cells changes over the life of the animal.

Gould said that the answer to this question depends on the strain of rats. In certain strains there is an increase in the number of cells over the animal's lifetime. However in the strains of rats used in Gould's laboratory, the number of cells over the animal's lifetime is tightly regulated. Gould posed the question of whether a more complex environment would lead to an increase in the number of cells over the animal's lifetime, or whether a

different population of cells would die when new cells are added. The answers to this are unknown. Gould pointed out that a lot of the effects seen in these studies may be deprivation effects. Gould suggested that better characterization of paradigms is necessary to understand what is going on under normal conditions.

Silver asked whether functions have been established for new neurons in the olfactory bulb, where they have been studied longer.

Gould clarified that the new neurons in the olfactory bulb have not been studied longer than those in the hippocampus. However, Gould reported that a recent study showed PCA n-camp knocked out mice. And these new cells that are produced in the subventricular zone express the something from of n-camp while they are migrating and you get a lot less neuronal migration and integration with the olfactory bulb in animal that don't PCS n-camp. And those animals it seems have deficits in olfactory discrimination. Those animals without PSA n-camp, throughout their entire lives, and through development, so not really clean study either. People are working on this now.

Khalil asked if anyone has investigated whether the neurons that are already there are supporting the new cells or rejecting them.

Gould replied that the answer to that is not yet known. However, Gould reported that if you selectively kill some mature granule cells, a compensatory increase in the production of those cells. This suggests that the dying cells are sending a signal that causes the progenitor cells to divide at a faster rate. Gould reported that she is not aware of any studies done looking at the local cues of mature cells.

Antle asked how long the animals went without sleep in the sleep deprivation studies.

Gould responded that the animals were sleep deprived for 24 hours.

Antle asked whether it was total sleep deprivation just REM sleep deprivation.

Gould explained that animals on a larger platform were REM sleep deprived, while animals on a smaller platform were totally sleep deprived. There is more REM sleep deprivation on the smaller platform.

Terrace asked whether the small increase in the neocortex is just stress related, or whether it is observed under other circumstances.

Gould responded that they have not looked at the changes in the neocortex in response to stress because the numbers are quite low. However, some data on the effect of stressors in the subventricular zone. Gould said that she is planning to branch out into other areas of the brain.

