Running head: NEUROGENESIS IN AN ANIMAL MODEL OF AUTISM

The Impact of Environmental Enrichment on Neurogenesis in an Animal Model of Autism

By

Janine Reynard

A Thesis submitted to the Faculty of Graduate Studies of

The University of Manitoba

In partial fulfillment of the requirements of the degree of

MASTER OF ARTS

Department of Psychology

University of Manitoba

Winnipeg

Table of Contents

Acknowledgements

I would like to thank my supervisor Dr. Ivanco and my committee members, Dr. Larson and Dr. Frost for their expert feedback and suggestions on turning this thesis into a polished manuscript. In addition, I would like to thank the University of Manitoba and NSERC for funding this project.

I would also like to thank my family and friends. Without them, there is no way that I would have the courage, perseverance, desire, and confidence in myself to pursue my dreams. The support they have given me throughout this process has been nothing short of amazing. Whenever I doubted myself, they were always there with encouraging words and an ear to listen. Without them, this thesis would not have been possible.

List of Tables

List of Figures

List of Copyrighted Material for which Permission was Obtained

All figures can be found in Appendix II.

Figure 1. Rat brain atlas – Bregma 2.52 mm (Figure 13) – Anterior Motor Cortex

Figure 2. Rat brain atlas – Bregma 0.36 mm (Figure 30) – Middle Motor Cortex

Figure 3. Rat brain atlas – Bregma -1.32 mm (Figure 44) – Posterior Motor Cortex

Figure 4. Rat brain atlas – Bregma -2.04 mm (Figure 50) – Anterior Dentate Gyrus

Figure 5. Rat brain atlas – Bregma -2.76 mm (Figure 56) – Middle Dentate Gyrus

Figure 6. Rat brain atlas – Bregma -4.80 mm (Figure 73) – Posterior Dentate Gyrus

Abstract

Autism, a neurodevelopmental disorder, is assumed to result from early neural tube damage. Individuals with Autism exhibit macroencephaly during childhood. To examine increased neurogenesis as a factor in macroencephaly, the valproic acid (VPA) model of Autism was used to examine how exposure to enrichment affects neurogenesis in the dentate gyrus. To induce the model, pregnant rats received two 100mg/kg VPA injections on days 11, 12, and 13 of gestation. Half the pups in each group were exposed to enrichment from post-natal days 30-60. Neurogenesis was examined by fluorescence microscopy for the proliferation marker bromodeoxyuridine (BrdU) and neuronal specific nuclear marker (NeuN). Counts of double-labeled cells were done from the dentate gyrus, an area known for adult neurogenesis. Results indicate that neurogenesis is not abnormal in the VPA model and enrichment increases the neurogenesis similarly in both VPA and control animals. This research provides a better understanding of brain plasticity in the VPA model of Autism.

Chapter 1: Brain Plasticity

Plasticity is the term used to define the ability of the brain to change as a result of experience. At one time it was thought that the adult brain was incapable of change and the concept of plasticity was limited to developmental processes. Today, it is known that brain plasticity is a continual process that is integral to the normal state of the nervous system. Brain development and maturation, learning and memory, and reorganization and recovery after injury are all examples of brain plasticity that occur throughout a lifetime. Despite many laboratories investigating plasticity with a variety of techniques, there is still much to be discovered regarding the mechanisms that underlie plasticity and how normal and compromised brains are able to change and adapt as a result of experience.

In mammals, plastic changes occur most often within the cerebral cortex (Kolb, 1995; Striedter, 2005). Over the course of evolution, the cortex has adapted considerably more than other areas of the brain (Striedter, 2005). The cortex is the outermost portion of the brain that has a folded appearance with many grooves called sulci and bumps called gyri. Microscopically, the cortex is composed of six layers of interconnected neurons arranged in cortical columns. Neurons, the electrically excitable cellular components of the brain, are highly adaptable. A neuron has a cell body with many highly branched projections called dendrites that function to receive signals via connections with other neurons. Plasticity may occur through changes in strength of connection, addition of new connections, removal of old connections, or addition of new neurons, and can be examined at both the microscopic and gross anatomical levels.

Environmental Enrichment and Brain Plasticity

Throughout development, stimulation acquired through interaction with the environment plays a key role in the organization of the neuronal circuitry required for normal brain function. Hebb (1947) was among the first to propose the 'enriched environment' as an experimental paradigm designed to examine the impact that environment has on behaviour. Hebb observed rats' behaviour after exposure to environmental enrichment. Instead of using enriched laboratory conditions, Hebb allowed his rats to roam free throughout his house and treated them as pets. These free-roaming rats were found to have improved memory and better problem solving abilities than rats that remained at the laboratory in standard cages (Hebb, 1947). Hebb did not examine the rats' brains directly, but was able to demonstrate that enhanced experience changes behaviour, and it was assumed that this behavioural plasticity was a result of a plastic change in the brain. To examine whether enhanced experience does cause changes in the brain, Krech, Rosenzweig and Bennett (1962) compared the brains of rats living in an enriched environment to rats that were housed individually in normal cages. They found that rats exposed to enrichment had increased brain weights and increased cholinesterase enzyme activity in their brains, indicating that exposure to enrichment is able to induce cerebral changes. These studies were among the first to indicate how important experience is on subsequent behaviour and development.

Presently, environmental enrichment in the laboratory is defined as an environment with increased levels of sensory, motor, social, and cognitive stimulation compared to standard housing conditions. Enrichment options for rodents may include the addition of environmental stimuli such as balls, running wheels, toys, tunnels, and

ladders to the housing conditions that are often rearranged to enhance novelty in the environment (as reviewed by van Praag, Kempermann, & Gage, 2000). Enrichment also includes enhanced social stimulation, where the rats are housed in larger groups rather than individually or in pairs. Enriched environments are created by a combination of different types of stimulation, and so examination of the effect of the environment can be difficult since a single contributing factor, such as visual, motor, or sensory stimulation, cannot be easily isolated. Several studies have examined the importance of single variables and combinations of variables on the effect of environmental enrichment. For example, Rosenzweig, Bennett, Herbert, and Moriomoto (1978) found that social grouping alone was not able to account for the increased cortical weight found in animals exposed to enrichment. In addition, allowing rats to simply observe, hear, and smell other rats in an enriched environment without being allowed participation does not facilitate an increase in brain weight (Ferchmin, Bennett, & Rosenzweig, 1975). These observer rats were shown to have brain weights similar to those rats housed in standard conditions, indicating that interacting with the environment is necessary to elicit the effects of the enriched environment.

The effects of enrichment can be noticed almost immediately. In adult rats, after a period of only 40 minutes in an enriched environment, subtle effects in RNA content can be measured and after four days significant increases in cortical thickness can be measured (Ferchmin & Eterovic, 1986). These findings indicate that even short exposure may facilitate measureable changes in brain structure that may generate changes in behaviours, as well.

The plastic changes facilitated by exposure to enrichment are not permanent. When an animal is moved from an enriched condition to a standard condition the changes in the brain slowly dissipate, but statistically significant differences, such as cortical thickness have been shown to persist in rats for three weeks after removal from the enrichment (Bennett, Rosenzweig, Diamond, Morimoto, & Herbert, 1974). The greater the amount of time spent in an enriched environment, the longer the changes have been shown to persist (Bennett et al., 1974; Camel, Withers, & Greenough, 1986). Although we do not know how long the benefits of enrichment last, it is assumed that consistent exposure to enrichment would be most beneficial and would continue to facilitate plastic changes in the brain. This suggests that changes in the brain as a result of experience are related to the level of experience the animal receives.

Anatomical Changes as a Result of Exposure to Enrichment

Exposure to an enriched environment has been shown to impact the brain at the gross anatomical level. Increased wet brain weight (Bennett, Rosenzweig, & Diamond, 1969; Krech et al., 1962) and increased cortical thickness (Bennett, Diamond, Krech, & Rosenzweig, 1964) have been reported in animals exposed to enriched conditions compared to those in standard conditions. The impact of environmental enrichment can also be seen when brain tissue is examined microscopically. Several studies have found increased dendritic branching and dendritic length in the rat cortex following exposure to enrichment (Camel et al., 1986; Holloway, 1966; Kolb, Gibb, & Gorny, 2003; Restivo et al., 2005). Not only does exposure to enrichment increase the complexity of the neuron by increasing the length and volume of the dendrites, environmental enrichment has also been shown to increase the density and maturity of dendritic spines (Restivo et al., 2005;

Turner & Greenough, 1985; Turner, Lewis, & King, 2003), which enhances the signal transmission potential of the neuron. These findings are important since extending the dendritic tree and increasing the density and maturity of dendritic spines increases the cell's ability to make connections with and receive input from other neurons.

Enrichment has also been shown to change non-neuronal elements of the brain. Altman and Das (1964) housed their enriched rats in a condominium style cage with water and food locations that would change every two to three days, combined with a running wheel as their enrichment paradigm. They found increased numbers of glial cells in the cortex and an overall increased size of the cortex in animals exposed to enrichment compared to control animals. Sirevaag, Black, Shafron, and Greenough (1988) housed rats in triplets with toys in their cage that were changed daily, plus an additional 30 minutes per day in an activity box. These enriched rats were found to have greater vascular capacity compared to rats that were housed in pairs or by themselves in standard cages. These findings indicate that environmental enrichment can have a global impact on brain architecture, by affecting both neurons and non-neuronal elements in the brain.

Enrichment and Synaptogenesis

Synaptogenesis, the creation of new synapses or connections between neurons in the brain, is also affected by environmental enrichment. Turner and Greenough (1985) compared rats housed for 30 days in an enriched condition, a social condition with a cage mate but no toys, and an isolated condition. Synaptic density estimates derived from electron micrographs were used, and an increase of approximately 11% more synapses per neuron per unit area in the brains of animals reared in enriched environments were found compared to those reared in the social environment or the isolated environment

(Turner & Greenough, 1985). Increases in synaptic density have been shown to persist for an additional 30 days after removal from the enriched environment (Briones, Klintsova, & Greenough, 2004), indicating that experience is able to cause long term changes in the brain, even after the experience has ended. Synaptic alterations can be examined by measuring the amount of the synaptic protein synaptophysin. Synaptophysin is a synaptic vesicle glycoprotein that is present in neurons that participate in synaptic transmission, and is used as a target in immunostaining for estimation of number of synapses via stereology (Calhoun, Jucker, Martin, Thinakaran, Price, & Mouton, 1996). Thus, an increase in synaptophysin may indicate an increase in synaptic transmission. Synaptophysin levels have been shown to naturally decrease with age (Masliah, Mallory, Hansen, DeTeresa, & Terry, 1993). To examine whether enrichment can reduce age-related decline in synaptophysin, Frick and Fernandez (2003) exposed 28 month old female mice to enrichment for three hours a day for 14 days. An enzyme-linked immunosorbent protein assay (ELISA) was performed and hippocampal and cortical synaptophysin levels were found to be increased in the mice exposed to enrichment in comparison to younger mice and other aged mice controls. These data suggest that exposure to enrichment can restore the declining synaptophysin level in aged mice (Frick & Fernandez, 2003), which can be correlated to an increase in synapse formation indicating that the enriched environment facilitates changes in brain plasticity.

Enrichment and Brain Injury

Although plasticity is often examined in non-injured brains, investigating plasticity in injured brains can yield new insights into brain change. Beneficial effects of exposure to enrichment have been noted in animals following brain injury. Passineau,

Green, and Dietrich (2001) used a fluid percussion brain injury protocol to cause traumatic brain injury to the hippocampus, cortex, and white matter tracts in the targeted hemisphere in adult male rats. Animals recovered from surgery either in standard or enriched environments. Eleven days after surgery the rats performed the Morris water maze test. Although there was no difference in performance in the water maze, the injured rats exposed to enrichment were found to have significantly smaller cortical lesions than those rats that recovered in standard conditions (Passineau et al., 2001). This indicates that even though enrichment did not change performance in the water maze, it can facilitate rehabilitation of brain injury. In another study, Johansson and Ohlsson (1996) found that rats that were given increased social stimulation after ischemic stroke performed better than rats that were housed individually but with a running wheel. Rats that were housed in an enriched environment with social interaction and free physical activity performed the best on behavioural tasks (Johansson & Ohlsson, 1996). This evidence suggests that repeated exposure to enrichment can be a valuable tool for aiding recovery after brain injury.

Behavioural Change after Exposure to Enrichment

Environmental enrichment has been shown to facilitate behavioural change due to neuroanatomical changes. Kolb and Gibb (1991) found that enriched rats were able to recover behaviours such as claw trimming, beam walking, and running following frontal lobe lesions faster than control rats. In another example, prolonged periods of maternal separation have been shown to impact hormonal and behavioural responses to stress and fear (Kolb & Gibb, 1991). Exposure to environmental enrichment was shown to decrease the plasma corticosterone levels in mice that were separated from their mothers during

the first two weeks of life, and also those mice that were subject to behavioural and restraint stress training (Francis, Diorio, Plotsky, & Meaney, 2002). These results indicate that exposure to enrichment can also facilitate observable changes in behaviour.

Enrichment Effects in other Species

Although enrichment studies are often performed using rodents, many other species show brain and behavioural changes in response to enrichment. Changes in brain structure have been noted in adult marmosets after living for one month in an enriched environment. These animals showed increased length and complexity of dendrites, increased dendritic spine density, and increased synaptic protein levels in the hippocampus and prefrontal cortex when compared to adult marmosets living in standard cages (Kozorovitskiy et al., 2005). Changes in behaviour have been demonstrated in adult orange-winged Amazon parrots after exposure to enrichment (Meehan, Garner, & Mench, 2004). These parrots show behavioural stereotypies when living in captive environments, but when exposed to physical enrichments (e.g. alternate perch sites, moveable objects) and foraging enrichments (e.g. manipulate objects through holes, chew through barriers to food sources) the stereotyped behaviours decreased when compared to birds from standard cages (Meehan et al., 2004). Other species such as fruit flies, fish, birds, gerbils, squirrels, cats, bears, and humans (as reviewed by Mohammed et al., 2002) have also shown similar effects to those that have been found in rodents, such as increases in dendritic growth and branching (Kolb & Gibb, 1991) indicating that enriched environments have similar effects across different species. With many species demonstrating similar results, comparison between species is possible, allowing for generalization of effects to humans and other species.

Summary

Plasticity is an intrinsic property of the nervous system that allows for organization of neural networks, learning, and adaptation to the changing environment. One of the main goals in brain plasticity research is to understand the mechanisms that underlie plasticity. One of the experimental paradigms used in the laboratory to research the phenomenon of brain plasticity is environmental enrichment. Laboratory enrichment paradigms provide the comparison of increased levels of sensory, motor, social, and cognitive stimulation to standard laboratory conditions, which include no extra stimulation. This paradigm is useful in investigating the effect of a changing environment on the brain compared to a static environment to observe how the brain adapts to change.

Chapter 2: Neurogenesis

One example of brain plasticity is neurogenesis, the creation of new neurons in the brain. Initially, neurogenesis was thought to be restricted to prenatal and early postnatal development; the adult brain was assumed to not have the capacity to facilitate this process. In the 1960s, the concept of neurogenesis in adults was examined in rats, but interest in neurogenesis peaked in the late 1990s when it was demonstrated that new neurons were produced in the adult human hippocampus. A study by Eriksson and colleagues (1998), examined the brains of individuals with cancer who had been injected with BrdU to label proliferating tumor cells. Upon post-mortem examination of the brains, neurogenesis was found to occur in the adult hippocampus. Since the patients were sick and relatively old, the authors concluded that these findings were likely an underestimation of the neurogenic potential of the healthy adult brain. Other studies (Cameron & McKay, 2001; Kornack & Rakic, 1999; Markakis & Gage, 1999; Ramirez-Amaya, Marrone, Gage, Worley, & Barnes, 2006; van Praag, Shubert, Zhao, & Gage, 2005; Zhao, Teng, Summers, Ming, & Gage, 2006) confirmed the finding that neurogenesis does occur in the adult hippocampus. These studies are important to the field of brain plasticity because they indicate that the adult brain is capable of generating new neurons. The addition of new neurons to the adult brain provides a mechanism for plasticity in the adult and a new opportunity for research.

Development of the Human Central Nervous System

It is now well known that cell genesis is necessary for an organism's development after conception. After fertilization, a series of mitotic cell divisions converts the fertilized egg into a blastula, a 128 cell sphere. At this stage of development (around day

14 of gestation), a process called gastrulation transforms this ball of undifferentiated pluripotent cells into a flattened, disc-shaped embryo with defined dorsal-ventral, anterior-posterior, and right-left axes. This process also forms three distinct cell layers, ectoderm, mesoderm, and endoderm, which give rise to all the cells of the body. These layers are called germ layers and form what is known as the trilaminar disc. The ectoderm forms the epidermis and the nervous system, the endoderm forms the digestive and respiratory systems, and the mesoderm forms the bones, connective tissues, blood, and blood vessels.

The onset of gastrulation is signalled by the formation of the primitive streak on the dorsal surface of the embryo. The primitive streak is a thickening at the posterior end of the embryo that extends anteriorly. Within the primitive streak, the cells change conformation and form a depression called the primitive groove. At the anterior end, the collection of cells thickens and forms the primitive node. At the center of the node a primitive pit forms. The primitive pit provides a location for ingression of cells to form the mesoderm layer. Near the end of the cell ingression phase, the primitive streak begins to regress toward the posterior end of the embryo. At this point, chordamesoderm cells in the mesoderm layer condense and form a solid cylinder called the notochord. In the human embryo, gastrulation and notochord formation is complete around day 18 and at that point, the neural tube begins to form.

The process that gives rise to the neural tube is known as neurulation. Neurulation is a very sensitive mitotic process that is susceptible to being disrupted (Juriloff & Harris, 2000). This process begins at embryonic day 18 and is complete by day 28. Neurulation is divided into primary and secondary phases (Lemire, 1969; van

Allen et al., 1993). Primary neurulation forms the brain and most of the spinal cord, whereas secondary neurulation forms the caudal, sacral, and coccygeal regions of the spinal cord. The first step in primary neurulation involves the formation of the neural plate, which is a thick, flat layer of ectodermal cells that forms above the notochord. The cells in the neural plate then lengthen and ridges form along the anterior-posterior midline of the trilaminar disc, forming the neural folds, which line the neural groove (Shum & Copp, 1996). Over the course of several days, induction from the notochord enables these neural folds to bend, fold over, and fuse to form a hollow tube, known as the neural tube (Smith & Schoenwolf, 1991). The first fusion of the neural folds occurs at the lower medulla at approximately 22 days of gestation. Closure proceeds rostrally and caudally from this point until the entire tube is fused (Sausedo, Smith, & Schoenwolf, 1997; Schoenwolf, 1988). The anterior portion closes at approximately 24 days, and the posterior portion closes at approximately 26 days.

Secondary neurulation, which begins after primary neurulation is complete, forms the most caudal segment of the spinal column (Schoenwolf & DeLongo, 1980). The process of secondary neurulation is different than primary neurulation. The cells first form a solid cylinder then hollow out to form a canal. Secondary neurulation lasts until approximately the seventh week of gestation and ends when both parts of the neural tube have fused into one structure. Gastrulation and neurulation are two very important and complex processes that determine the fate of the embryo. If either process is not completed properly, serious neural tube defects may occur (van Allen et al., 1993) that cause irreversible damage to the development of the embryo.

Around the time of neural tube closure the human brain consists of a layer of actively proliferating cells called the neuroepithelium that surrounds a large ventricle. At around 4–5.2 weeks gestation, the human brain grows dorsally due to increased cell proliferation (Bayer, Altman, Russo, & Zhang, 1993). Between the second month and the time of birth, the telencephalic region, which forms the cortex, expands rapidly due to the migration of the newly formed cells.

Although neurogenesis occurs throughout the lifetime, the main phase of prenatal neural cell proliferation occurs between two to five months gestation (Clancy, Darlington, & Finlay, 2001). Neuroblast cells divide, resulting in proliferative neuronal-glial progenitor cells in the ventricular and subventricular zones which create columns that migrate together and form columns in the cortex (Angevine & Sidman, 1961). Cells from the ventricular zone are the earlier-formed neurons, followed by the cells from the subventricular zone, which are the later-formed neurons, and glial cells. As a result of neural migration, the six-layered cerebral cortex forms. Neurons move from their place of origin in the ventricular and subventricular zones to their locations in the cortex. The earliest generated cells form the deepest cortical layers, whereas the later generated neurons form the more superficial layers of the cortex (Angevine & Sidman, 1961). Peak neuronal migration occurs between the third and fifth months of gestation (Sidman & Rakic, 1973).

The next stage involves neuronal differentiation and organization into functioning circuits. These circuits form the functional system of the brain, which processes and integrates information. This stage occurs around six months of gestation and lasts for three years after birth. Axons and dendrites grow and form synapses with other cells,

glial cells proliferate, and regressive events such as apoptosis and pruning, all occur at this stage in development.

Myelination, the last stage of brain development, begins in the second trimester and continues throughout adult life. These complex events of brain development do not occur as distinct and separate processes, but overlap and the outcome of one step may affect subsequent steps. This indicates that damage or abnormalities early in gestational development may have a profound effect on later stages of brain development. The human brain, when compared to other species, has a long period of embryonic development (Bayer et al., 1993) and is thus one of the most vulnerable organs to teratogenic insult.

Areas of the brain that are known for neurogenesis are known as neurogenic regions. The adult brain has two known, well-researched neurogenic regions, the subventricular zone of the lateral ventricles and the subgranular zone of the dentate gyrus of the hippocampus. Cells produced by the subventricular zone of the lateral ventricles migrate via the rostral migratory stream to the olfactory bulb where they differentiate into interneurons (Lois & Alvarez-Buylla, 1993; Luskin, 1993). Cells produced by the subgranular zone migrate locally and differentiate into neurons of the dentate granule cell layer (Cameron, Wooley, McEwan, & Gould, 1993). In the past decade there has been research indicating that other regions such as the cortex (Dayer, Cleaver, Abouantoun, & Cameron, 2005; Gould, Reeves, Graziano, & Gross, 1991), striatum (Dayer et al., 2005), amygdala, and hypothalamus (Fowler, Liu, Ouimet, & Wang, 2002) are also potential neurogenic regions for study.

Adult-born cells follow the same maturation process as cells created during embryonic development, and are therefore vulnerable in the same way neurons formed during embryonic development. In the adult brain, granule cells migrate a short distance from the subgranule zone into the granule cell layer of the hippocampus where they make connections with other neurons (Markakis, & Gage, 1999; Stanfield & Trice, 1988; Zhao et al., 2006). Within five months, these neurons are morphologically indistinguishable from the older neurons (van Praag, Schneider, Christie, Toni, Palmer & Gage, 2002). New cells are more responsive to stimulation than older cells (Ramirez-Amaya et al., 2006), indicating that new cells may also be involved in and integral to neuronal plasticity.

Human Development versus Rat Development

The development of humans and rats is not comparable using basic chronology since gestation in the rat is 22 days compared to nine months in humans. Developmental events can be compared using anatomical features and histological landmarks (Bayer et al., 1993). In a study that linked development of the rat central nervous system to the development human central nervous system, Bayer and colleagues (1993) showed that the period of 3.5–7 weeks gestation in humans is the comparable developmental time period to embryonic days 11–15 in rats. More specifically, approximately 4–5.2 weeks for human development can be matched with a late embryonic day 12. Neural tube formation is complete at approximately gestational day 11.5–12 in rats and gestational day 27–29 in humans. The generation of cells that will form the cortex begins on embryonic day 12 and continues until about day 21 in the rat. Developmental neurogenesis is complete at day 22, which is the time of birth of the rat (Bayer et al.,

1993). Understanding the comparisons in development between humans and rats makes it possible to compare developmental injury.

Neurogenesis and BrdU

Research has shown that although the adult brain has lost most of its ability for neurogenesis, cells are still able to proliferate and migrate (Eriksson et al., 1998). Proliferating cells can be labeled with bromodeoxyuridine (BrdU), halogenated thymidine. Thymidine is the only nucleotide that uniquely occurs in DNA. BrdU is made by adding a halogen molecule to thymidine. During the synthesis phase of mitosis BrdU is incorporated into the newly formed DNA, thus labeling the DNA in the nucleus and indicating that the cell is a newly formed cell (Kuhn & Cooper-Kuhn, 2007). Other nucleotides, adenine, guanine, and cytosine, would not specifically target DNA since they can also be found in RNA.

It is important to note that BrdU does not label cell proliferation, but acts as a marker of DNA synthesis. It is estimated that BrdU is available for labeling in the brain for approximately two hours after it is introduced (usually by systemic injection), so the cells must be actively synthesizing DNA near the time of introduction in order to be labeled. The labeled DNA is detectable over long periods of time, providing that the cell survives and that it does not rapidly proliferate, which would dilute the label below the detection level (Kuhn & Cooper-Kuhn, 2007). BrdU-labeled cells can be visualized using immunochemistry and can be combined with other cell markers to confirm the identity of the new cells, which allows for quantification and examination of neurogenesis within the brain.

Function of Neurogenesis

Although there are many hypotheses about neurogenesis, the exact role of neurogenesis in the brain remains unknown. Theories and research suggest it is likely that neurogenesis functions in learning and memory (Altman, 1967; reviewed by Cayre, Canoll, & Goldman, 2009). Altman was one of the first to suggest that neurogenesis had a role in learning and memory. Altman suggested that since neurogenesis was found in structures that are known to function for sensory processing and the encoding of memories, this form of plasticity may function in learning and memory processing that is executed by these structures (Altman, 1967).

Studies using birds were among the first to show the relationship between learning and neurogenesis. For example, in chickadees, a seasonal fluctuation of neurogenesis occurs in response to the need for increased spatial memory for seed storage and retrieval (Barnea & Nottebohm, 1994). In rats, it was demonstrated that the number of adult generated neurons doubles in the hippocampus in response to hippocampal-dependent learning tasks, whereas tasks that do not require the hippocampus do not result in new neurons. These results indicate that new neurons in the hippocampus are affected by, and possibly involved in, memory (Gould, Beylin, Tanapat, Reeves, & Shors, 1999). Other studies have examined the number of new neurons in relation to task performance and found that generally, those animals with the fewest new neurons perform the poorest, and those with the most new neurons perform the best (Kempermann & Gage, 2002). These results indicate that the hippocampus is able to respond to cognitive demands, such as learning and memory, by producing more neurons.

In order to examine the role that hippocampal neurogenesis plays in spatial learning and memory, Jessberger et al. (2009) used a lentivirus-based strategy to specifically block neurogenesis by inhibiting WNT gene signaling in the dentate gyrus of adult rats. WNT signaling has been shown to be necessary for generation of new neurons. By blocking the WNT pathway, the formation of new neurons can be stopped. Animals were injected with the lentivirus vector and began behavioural testing eight weeks later. After sacrifice, the lentivirus exposed animals were categorized into two groups based on the degree of neurogenesis knockout: high knockout and low knockout. The performance on the behavioural tasks was correlated to the animal and group. The water maze was used to test for spatial learning. All groups followed normal learning progression for the task, but when tested for retention, the high knockout rats performed the worst in comparison to the low knockout and control groups (Jessberger et al., 2009). In an object recognition task, this same pattern of performance was found. In tests of retention, the high knockout group performed the worst, followed by the low knockout group. These findings suggest that a function for neurogenesis may be memory and long-term retention tasks in both spatial learning and object recognition.

Spatial learning and object recognition are important abilities in humans as well, especially for taxi drivers (Maguire et al., 2000). An MRI study in humans showed that the posterior hippocampi of London taxi drivers were significantly larger than that of control subjects who did not drive a taxi. The posterior hippocampus has been theorized to store a spatial representation of the environment. In addition, this study found that hippocampi volume correlated with the amount of time spent as a taxi driver, indicating that the adult human hippocampus is responsive to environmental demands and functions

in spatial memory. This study did not examine neurogenesis as the mechanism for the increased volume, however, based on the data from rats in a water maze (Jessberger et al., 2009; van Praag, Kempermann, & Gage, 1999), it appears that increased neurogenesis is responsible for the volume change and ultimately the enhanced spatial memory in these individuals.

Although there are theories about the function of neurogenesis, the functional relevance remains uncertain. There is mounting evidence to show that neurogenesis is involved in learning and memory (Gould et al., 1999; Jessberger et al., 2009). Increased neurogenesis and enhanced performance on hippocampal dependent tasks suggests a correlation between neurogenesis and performance. More evidence for this correlation is found in experiments when neurogenesis is blocked and spatial memory capacity declines, providing more verification for link between neurogenesis and memory.

Factors Known to Affect Neurogenesis

The process of neurogenesis is sensitive to intrinsic and extrinsic factors. Conditions of stress and depression, aging, prenatal stress, and prenatal alcohol exposure can result in decreased neural proliferation. In contrast, exercise and exposure to antidepressant medication have been shown to positively affect neurogenesis by increasing proliferation and survival of new neurons.

Stress and Depression

Stress is known to alter normal physiological homeostasis and cause changes in the brain. Both chronic and acute stress has been shown to negatively affect hippocampal neurogenesis by suppressing the formation of granule cells (Gould, Tanapat, McEwen, Galea, & Fuchs, 1997; Veena, Srikumar, Raju, & Shankaranarayana Rao, 2009; Veena,

Srikumar, Mahati Raju, Bhagya, & Shankaranarayana Rao, 2009). Veena, Srikumar, Mahati and colleagues (2009) found that after 21 days of restraint stress, the number of BrdU positive cells in the dentate gyrus of adult rats was significantly reduced by approximately 70% compared to the number of BrdU positive cells in control animals. Another study found that chronic restraint stress significantly decreased the amount of neurons generated and decreased the number of BrdU positive cells that survived in the dentate gyrus (Veena, Srikumar, Raju, et al., 2009). In addition, when examining acute stress the same pattern emerged: sacrificing animals 24 hours after the last BrdU injection and labeling for BrdU and a neuronal marker (NeuN), they found less new cell proliferation in the dentate gyrus (Heine, Maslam, Zareno, Joels, & Lucassen, 2004). Taken together, these data indicate that stress is a very important factor that can have very deleterious effects on neurogenesis.

Chronic stress has been a proposed risk factor for development of depression (Katz, Roth, & Carroll, 1981). Individuals with depression have been shown to have reduced hippocampal volumes (Campbell, Marriot, Nahmias, & MacQueen, 2004). This loss of hippocampal neurons has been implicated as a factor in the reduced size of hippocampus in depressed individuals. This size reduction may be a contributing factor to the symptomology of depression (as reviewed by Duman, Malberg, & Thome, 1999). Drug treatment for depression is prescription of anti-depressant medication. Malberg, Eisch, Nestler, and Duman (2000) examined what effects chronic antidepressant treatment had on new neurons in the dentate gyrus in rats. The antidepressants tested included a monoamine oxidase inhibitor (tranylcypromine), a selective serotonin reuptake inhibitor (fluoxetine), and a norepinepherine reuptake inhibitor (reboxetine). They also

tested the effect of treatment with an antipsychotic (haloperidol) to determine whether the increase in BrdU labeled cells is specific to antidepressants. Chronic administration of antidepressant treatment was found to significantly increase the number of BrdU labeled cells in the dentate gyrus of rats. The BrdU labeled cells were found to be characteristic of immature cells undergoing cell division indicating an increase in cell proliferation. Administration of the antipsychotic was not found to increase BrdU labeled cells. This indicates that the increase in BrdU labeled cells may be specific to antidepressant treatment. By comparing several classes of antidepressants, results indicate that increasing neurogenesis in the dentate gyrus may be a common action of antidepressant medication (Malberg et al., 2000). Additional evidence has shown that when antidepressant-induced neurogenesis is disrupted through irradiation by low dose x-ray to the hippocampus in rats, the beneficial behavioural response to the antidepressant medication disappears. This disruption in neurogenesis persisted for eight weeks after delivery of the final x-ray dose (Santarelli et al., 2003). These results indicate that neurogenesis may have a functional and adaptive role in the brain to compensate for the damaging effects of stress and depression.

Developmental Stress

Prenatal stress has been hypothesized to predispose individuals to illness and disease later in life. Prenatal stress has been shown to reduce neurogenesis in the dentate gyrus of rats (Lemaire, Koehl, LeMoal, & Abrous, 2000). Animals exposed to stress *in utero* showed impairment in hippocampal-related spatial tasks compared to control animals that did not receive any prenatal stress (Lemaire et al., 2000). Coe et al. (2003) examined prenatal stress in both early and late pregnancy in rhesus monkeys. Pregnant

rhesus monkeys were acutely stressed through an acoustic startle paradigm on days 50–92 post-conception (early pregnancy), or days 105–147 post-conception (late pregnancy). The offspring were evaluated at 2–3 years of age and the researchers found that prenatal stress in both early and late pregnancy resulted in elevated cortisol levels, reduced hippocampal volumes, and inhibited neurogenesis in the dentate gyrus (Coe et al., 2003). These data indicate that factors that affect neurogenesis which occur prenatally can have severe effects on subsequent brain structure and development, and learning and memory that persist throughout the lifetime.

Aging

The rate of granule cell proliferation has been shown to naturally decrease as we age. Kuhn, Dickinson-Anson, and Gage (1996) examined how aging affects hippocampal neurogenesis by injecting animals with BrdU six times over three consecutive days. The animals were sacrificed four weeks after the last BrdU injection. It was found that the number of BrdU-positive cells in the dentate gyrus of rats decreased in old rats (12 to 27 months old) in comparison to young rats (6 months old), indicating a reduction in neurogenesis in aging rats (Kuhn et al., 1996). In another study, animals were injected with BrdU once a day for 12 consecutive days and were sacrificed four weeks after the last BrdU injection. It was found that old mice (18 months old) were shown to have less BrdU-positive cells than young mice (6 months old) (Kempermann, Kuhn, & Gage, 1998). These findings indicate that although there is a natural decrease in neurogenesis with age, the ability to create new neurons remains suggesting that this type of plasticity may have functional significance in an older population.

Prenatal Exposure to Alcohol

Fetal alcohol syndrome results from maternal alcohol use during gestation and is among the most commonly diagnosed developmental disorders (Denny, Tsai, Floyd, & Green, 2009). In humans, it affects physical and cognitive functioning across the lifespan. It is also shown that fetal alcohol syndrome has co-morbidity with Autism (Nanson, 1992). Individuals with fetal alcohol syndrome have characteristic dysmorphic facial features such as small head, short but upturned nose, widely spaced eyes, smooth and wide philtrum with a thin upper lip. In both human (Mattson, Riley, Gramling, Delis & Jones, 1998) and animal studies (Becker, Randall, Salo, Saulnier, & Weathersby, 1994), associations between fetal alcohol syndrome and poor learning and performance on memory tasks have been shown. In an MRI analysis of total brain volume and hippocampal volume, three groups of participants were examined. Individuals whose mothers admitted to consuming alcohol during pregnancy, individuals whose mothers admitted to consuming alcohol during pregnancy and who show the characteristic dysmorphic facial features, and age matched control individuals were compared. It was found that dysmorphic individuals performed poorest of the three groups on verbal and nonverbal learning and memory tasks. In addition, all alcohol exposed individuals showed lower total brain volumes and lower hippocampal volumes than individuals in the control group who were not exposed prenatally to alcohol (Coles et al., 2011). These results indicate that the related cognitive impairment in fetal alcohol syndrome seems to be related to the severity of the underlying brain damage that occurred prenatally.

Neurogenesis rates have been shown to decrease and be correlated to long-term deficits when brain damage occurs prenatally. For example, in an animal model of

human third trimester binge-like alcohol exposure, rats were exposed to a daily dose of 5.25g/kg alcohol on postnatal days 4–9. The animals were then injected with BrdU every second day from day 30–50. Exposed animals were found to have significantly decreased number of mature neurons in their adult hippocampus after 80 days (Klintsova, Helfer, Calizo, Dong, Goodlett, & Greenough, 2007). When animals are exposed prenatally to high-ethanol diets during gestational days 5–21 the number of BrdU-positive cells in the dentate gyrus generated daily is significantly reduced compared to low-ethanol diet fed rats (Miller, 1995). These findings indicate that the prenatal brain damage caused by alcohol exposure persists and causes long-term deficits in hippocampal neurogenesis. These cellular deficits may be the underlying cause of the hippocampal-dependent cognitive behavioural problems that are noted in this model and in humans with fetal alcohol spectrum disorder.

Exercise

Physical exercise has been shown to not only improve the physical health of individuals, but to also improve cognition and brain function. Van Praag et al. (1999) compared water-maze learning, voluntary wheel running, enrichment, and standard housed groups of mice. They found that running doubled the amount of new cells in all other groups, except for the enrichment group (van Praag et al., 1999). Olson, Eadie, Ernst, and Christie (2006) examined the combination of environmental enrichment and voluntary exercise, which has been found to increase adult hippocampal neurogenesis. They hypothesize that while enrichment increases the survival of the newly formed cells, exercise increases the level of proliferation of the new cells. This suggests that participating in voluntary exercise increases the amount of neurogenesis in the dentate

gyrus, but adding enrichment to the exercise paradigm increases neurogenesis even more. These findings illustrate a potential mechanism to induce proliferation and survival of new cells, which may contribute to better understanding of brain damage.

Neurogenesis and Environmental Enrichment

One of the main factors known to impact neurogenesis is exposure to an enriched environment, which presumably provides a larger number of opportunities for learning than standard laboratory housing. Mice exposed to environmental enrichment have been shown to have significantly more neurons in the dentate gyrus than littermates who were reared in standard laboratory cages (Kempermann, Kuhn, & Gage, 1997). Half of the mice were raised in an enriched environment and half in a control environment (standard laboratory housing) for 40 days. During the last 12 days the mice received a daily injection of BrdU. Twenty-four hours after the last injection, a subset of mice was sacrificed to evaluate the effect enrichment has on neuron proliferation. The remaining mice survived in their respective environments for an additional 28 days, to allow for maturation and differentiation of the BrdU-labeled cells. During this time period, mice were tested in the Morris water maze, which is a spatial learning task. Mice in enriched environments were found to have a shorter swim path, indicating that they were able to learn faster than the control animals. Upon examination of proliferation of neurons in the brains of the first group of animals sacrificed, they found no significant difference between control and animals in enrichment, indicating that enrichment does not alter cell proliferation. When examining the brains of the rest of the animals, they found a significant difference in the amount of new neurons. The animals exposed to enrichment showed 57% more labeled cells per dentate gyrus than the control animals. When the

hippocampus was examined, approximately 15% increase in hippocampal depth was found, which was attributed to enhanced arborization of dendrites, varying sizes of neuronal nuclei, or more glial cells in those animals exposed to enrichment (Kempermann et al., 1997). Although proliferation rates were similar, exposure to enrichment facilitated enhanced survival for the newly developed cells in comparison to those animals that did not receive enrichment.

A recent study (Iso, Simoda, & Matsuyama, 2007), examined how neurogenesis is affected in mice when the environment changes from a standard environment to one with enrichment and vice versa. This experiment used four different groups of mice. One group was housed in an enriched environment for the duration of the experiment; another was housed in standard conditions. The other two groups switched environments, one group of animals spent the first six weeks in the enriched environment, then the last two weeks in the standard environment and the other spent the first six weeks in a standard environment, then enrichment for the last two weeks. Brain analysis showed that the enriched group and the impoverished-enriched group had heavier brains than the impoverished group and the enriched-impoverished group. The enriched group and the impoverished-enriched groups also had more neurons in the hippocampus than the other two groups (Iso et al., 2007). This pattern is important as the more recent condition determines the effect seen in the brain, indicating that the brain is capable of plasticity within a few weeks.

Conditions that increase hippocampal neurogenesis are associated with improved memory performance and, conversely, conditions that decrease neurogenesis are associated with cognitive deficiencies. Not only does exposure to enrichment increase

the amount of neurogenesis in the dentate gyrus by approximately 70%, but also improves long-term recognition memory in rats (Bruel-Jungerman, Laroche, & Rampon, 2005). To test this, rats were injected with methylazoxymethanol acetate (MAM), an anti-mitotic agent that prevents cells from dividing for a short period, is used to reduce enrichment-induced neurogenesis. The rats were tested using an object recognition task that compares the time exploring new objects to the time exploring old objects. The MAM exposed rats were found to have substantial decreases in neurogenesis in the dentate gyrus when raised in standard laboratory conditions, and profound loss of neurogenesis was found in rats exposed to enriched conditions, so that the residual neurogenesis level resembled that of the rats raised in standard conditions. MAM was also found to decrease the enrichment-induced memory improvement (Bruel-Jungerman et al., 2005). These results indicate that enrichment enhances neurogenesis in the dentate gyrus of adult rats and that neurogenesis in the dentate gyrus likely functions in memory.

Exposure to environmental enrichment is also able to overcome the negative effects of stress on neurogenesis. Enrichment was found to reduce the depressive symptoms in chronically stressed rats (Veena, Srikumar, Mahati, et al., 2009). In addition to a reduction of stress, exposure to enrichment restored hippocampal cell proliferation and improved the rat's ability to learn a radial arm maze (Veena, Srikumar, Mahati, et al., 2009). These results indicate that exposure to environmental enrichment is able to mediate negative effects of some factors such as stress and restore normal neurogenesis in the rat hippocampus.

Summary

Once thought to be solely a developmental process, neurogenesis has been shown to occur in the subventricular zone of the lateral ventricles and the subgranule zone of the dentate gyrus in the adult brain. Exposure to environmental enrichment has been shown to increase the neurogenic potential of the adult rat brain by promoting survival of new neurons, which is important in forming new neural connections in response to experience.

Insight into how the damaged brain responds to experience is integral to understanding the mechanism of plasticity. The importance of understanding brain plasticity and neurogenesis is to develop possible mechanisms to repair brain damage. A model disorder to study this response to experience is Autism. Autism is a neurodevelopmental disorder assumed to result from prenatal neurological damage. This disorder provides researchers with a model for both examination of the effects of early brain injury on neurogenic potential and the effects of environmental enrichment as a tool to enhance brain plasticity.

Chapter 3: Autism

Autism is a complex neurodevelopmental disorder characterized by a triad of symptoms, including abnormal social interactions, impairments in communication (both verbal and non-verbal), and by repetitive stereotyped patterns of behaviour. Individuals with Autism show extreme symptom heterogeneity that ranges from debilitating impairments to mild personality traits and for that reason, is diagnosed on a spectrum. Approximately half of children with Autism demonstrate some type of intellectual disability and have severely impaired speech. In addition, epilepsy is often co-morbid with Autism. One third of individuals with Autism have had at least two epileptic seizures (Rapin, 2002). Autism has several known risk factors, but the underlying cause remains unknown. More research involved in understanding how the brain in individuals with Autism is able to change and adapt to the environment is important in understanding the disorder.

It is estimated that approximately one in 110 individuals in the United States are diagnosed with an Autism Spectrum Disorder (ASD) (Rice, 2009). Prevalence numbers vary, but epidemiological data indicates that the rates of Autism diagnosis have risen by 300% in the last three decades (Fombonne, 2003). This increase is largely due to the greater understanding of the symptoms of Autism, and to the broadening of diagnostic criteria used for diagnosis to reflect the symptom presentation that occurs along the Autism spectrum, but the true prevalence remains unknown.

History and Symptomology

The word Autism comes from the Greek word "*autos*" meaning "self." In 1911, Bleuler used the term Autism to describe a group of symptoms of schizophrenia. In

1943, the word Autism was used by Kanner (1943) to describe children with a disorder that caused disturbance of social development, where the child was unable to relate to others and to the environment in an ordinary way and had an obsessive desire for sameness. Kanner suggested that Autism is brought about by a deficiency in the biological systems responsible for the development of affective associations with others (Kanner, 1971). Despite Kanner's description of Autism, the disorder was not accepted into the Diagnostic and Statistical Manual of Mental Disorders (DSM) until 1980. Being listed in the DSM meant that Autism had a set of diagnostic criteria that could be used by professionals, which allowed for more consistency in diagnosis across professionals.

The diagnostic criteria currently used for diagnosis of Autism have changed slightly from Kanner's original description. Autism is now described as a behaviourally defined pervasive developmental disorder of brain function, and is diagnosed based on behavioural criteria alone since no specific biological markers are known. Currently, in the DSM-IV-TR, Autism is the most severe of five disorders on the Autism Spectrum that fall under the umbrella category of pervasive developmental disorders (PDD). Although there are strict criteria for diagnosis, Autism is a heterogeneous disorder. No two individuals present with the same constellation of symptoms, and thus, the variable intensity and variety of symptoms within the core domains is why Autism is diagnosed along a spectrum of illness.

Autism is present at birth, but is usually not diagnosed until between two and three years of age (Kemper & Bauman, 1993). Parents become concerned when their child begins to miss higher-order cognitive developmental milestones and decide to seek answers (see Appendix I for review of the Measles-Mumps-Rubella vaccine

controversy). Brain structures responsible for higher-order social, emotional, language, attention, and cognitive functions do not begin to develop until around two to three years of age. In Autism, these structures form abnormally during a critical period when neural systems are beginning to form their circuitry (as reviewed by Courchesne, Redcay, Morgan, & Kennedy, 2005). These social deficits are not obvious in infancy, but become gradually more evident and concerning as the child ages.

Neuroanatomical Abnormalities

Due to the great phenotypic variability between individuals with Autism, the symptoms of Autism do not directly suggest a specific brain region or brain system that when damaged would result in the development of the diagnostic set of behaviours (Rodier, Ingram, Tisdale, Nelson, & Romano, 1996). In addition, no unifying pathology related to all cases of Autism has been reported (Bailey et al., 1998). It is likely that the associated neurological abnormalities occur early during embryonic development (Kemper & Bauman, 1993). To determine the neurobiology of Autism, both non-invasive imaging techniques such as MRI and fMRI, and post-mortem examination have been used to help determine the brain regions that may be involved in Autism.

Brain Volume

One of the most consistent findings in Autism research is that of macroencephaly. Macroencephaly is defined as having a head circumference greater than the 98th percentile (Mosconi, Zwaigenbaum, & Piven, 2006). Brain volume is highly correlated to head circumference, and therefore an easy way to estimate brain volume (Mosconi et al., 2006). The earliest record of brain enlargement was in Kanner's original paper where he noted that the children with Autism that he was studying had abnormally large heads

(Kanner, 1943). Now with the advent of MRI technology, researchers are able to make more accurate evaluations of brain size than simply measuring head circumference.

At birth, brain size in children with Autism appears to be normal (Hazlett et al., 2005; Lainhart et al., 1997; Stevenson, Schroer, Skinner, Fender, & Simensen, 1997), but brain size increases rapidly in children with Autism. Children between the ages of two and four have been frequently reported to have brain enlargement in comparison with age matched control groups (Courchesne et al., 2001; Courchesne, Carper, & Akshmoomoff, 2003; Hazlett et al., 2005; Stevenson et al., 1997). Lainhart and colleagues (1997) reported that by two to four years of age, approximately 90% of those with Autism had larger than average brain volumes, and 37% met the criteria for macroencephaly (Lainhart et al., 1997). When comparing individuals with Autism 12 years old and older with age matched controls, this disparity in brain size is gone. There is no difference in brain size between adolescents with Autism and controls (Aylward, Minshew, Field, Sparks, & Singh, 2002). These results indicate that brain development in Autism is abnormal. At birth in individuals with Autism, there is no apparent difference in brain size. In early development, there is abnormally accelerated brain growth, which slows around adolescence with brain size normalizing.

Many MRI studies show overall brain enlargement in children with Autism. One MRI study compared a large sample of three to four year old children with ASD to age matched control groups who were either typically developing or developmentally delayed children (Sparks et al., 2002). Researchers found that children who were diagnosed with Autism had significantly increased cerebral volume when compared to both control groups. Brain development in Autism is abnormal with respect to both normally

developing children and children who are developmentally delayed indicating that the Autism brain development is different even though these children also often have delayed development.

The consequence of an enlarged brain is not well understood. It is possible that this overgrowth is at the root of the impairments in higher cognitive functioning noted in Autism (Courchesne et al. 2001). During development, the normal brain undergoes periods of synaptogenesis, neurogenesis, rapid dendritic arborization, and pruning of existing synaptic connections. These processes shape neural connectivity and are influenced by learning and experience. It is proposed that in Autism, this growth and elaboration of connectivity occurs too early without being guided by learning and experience (Courchesne et al., 2001) and thus, abnormalities in cortical connectivity are likely to occur.

Cerebellum

Abnormalities of the cerebellum in individuals with Autism are well documented. Approximately 90% of autopsies on individuals with Autism show some sort of cerebellar abnormality (Allen & Courchesne, 2003). Both increases in cerebellar volume in Autism (Piven, Saliba, Bailey, & Arndt, 1997) and decreases in volume (Hazlett et al., 2005) have been noted, but volume studies of the cerebellum can be difficult to interpret and results remain inconclusive.

One consistent finding in the cerebellum is a decrease in Purkinje cell density (Bailey et al., 1998; Bauman & Kemper, 1985; Fatemi, Halt, Realmuto, Earle, Kist, Thuras et al., 2002; Ingram, Peckham, Tisdale & Rodier, 2000). Purkinje cells are GABAergic neurons located in the cerebellum. They regulate neuronal excitability, are

responsible for muscle tone, coordinate motor output with the frontal and motor cortices, and are associated with shifting and orienting attention. Damage to this area has been associated with difficulty with posture and gait (Thach, Goodkin, & Keating, 1992). A reduction in the number of these cells has been shown to increase the amount of repetitive behaviour in a mouse model of Purkinje cell loss (Martin, Goldowitz, & Mittleman, 2010). These results in humans and mice indicate that the decrease in Purkinje cells may be an underlying cause for both motor output issues and restricted and repetitive behaviours noted in Autism.

Atrophy of the cerebellar cortex has also been noted at autopsy in both infants and adults with Autism. This type of atrophy in the cerebellum usually corresponds to a loss of olivary neurons (Fatemi et al., 2002), which normally function for motor control via connections, in a transitory zone, to the Purkinje cells in the cerebellum. Autopsy studies on individuals with Autism show that these olivary neurons are present in adequate numbers (Bauman & Kemper, 1985). After 30 weeks gestation, the transitory zone disappears in the human fetus (Rakic & Sidman, 1970) therefore, it is likely that the cerebellar lesion occurred before this time point in human gestation, indicating that Autism has prenatal beginnings (Bauman & Kemper, 1985; Bauman, Filipek, & Kemper, 1997). These data suggest that the damage is prenatal, rather than damage from an acquired lesion, which may be responsible for the underlying cerebellar damage noted in Autism.

Brain Stem

Abnormalities in the brainstem have been indicated as a possible cause for Autism. Stromland, Nordin, Miller, Akerstrom, and Gillberg (1994) examined a group of

individuals with Autism whose mothers had ingested thalidomide during pregnancy. They noted a presence of ear abnormalities but an absence in limb abnormalities in these individuals. Upon comparison of the disrupted gestational processes to a normal gestational developmental timeline, these developmental abnormalities point to postnatal injury between 20–24 days after conception (Stromland et al., 1994). This is also the time period when the neural tube is closing forming the connection between the spinal cord and the brain, suggesting that the brainstem is also involved.

Further evidence for brainstem involvement in Autism has been found in autopsy studies. Rodier and colleagues (1996) performed an autopsy on a 21 year old female who met the DSM-IV criteria for Autism and found that her brain stem lacked the majority of the facial nucleus. This structure is responsible for facial expressions, movements of the face and that of the tongue. In addition, the superior olive, which functions to relay auditory information, was found to be abnormal (Rodier et al., 1996). In a different post mortem study of six autistic brains, developmental abnormalities of the brainstem resulting from developmental damage to the neural tube were found (Bailey et al., 1998). Additionally, Hashimoto et al. (1995) noted that both the cerebellum and the brainstem volume increased with development from birth in both children with Autism and those without, suggesting that the size difference in the children with Autism is not due to a degenerative process after birth. Autopsy analysis has also shown abnormalities in the brain stem in individuals with idiopathic Autism (Hashimoto et al., 1995). The brain stem is an extremely important structure in the nervous system that functions as an information conduit, houses the nuclei of the cranial nerves, and is responsible for

integration of functions. Damage to the brain stem may also contribute to impairments noted in Autism.

Hippocampus and Amygdala

In a large MRI study, 3–4 year old children with Autism were compared to age matched controls who were developing normally or who were diagnosed as developmentally delayed but without Autism. The children with Autism were shown to have larger hippocampi and amygdala in comparison to both control groups (Sparks et al., 2002). Another MRI study found a reduction in volume of the hippocampi and amygdala in adolescents with Autism when compared to control subjects (Aylward et al., 1999). These differences in size may indicate that these structures follow the same abnormal brain growth pattern seen in cerebral volumes of children with Autism.

Abnormalities in the hippocampus and amygdala in Autism are likely due to curtailed development and not a result of an acquired brain lesion. Damage to the amygdala affects the evaluation of emotional stimuli intensity, specifically for fear. Individuals with Autism have been shown to have reduced social cognition and are often inept at emotional understanding (Travis & Sigman, 1998). Damage to the hippocampus is implicated in the dysfunction of memory systems in Autism, such as reduced use of cognitive organizing strategies that support memory (Minshew & Goldstein, 1993). Due to the importance of these two structures in normal social and cognitive functioning, improper connections between the hippocampus and amygdala with the rest of the cerebral cortex is expected, which may cause the some of the deficits in higher-order cognitive functions seen in Autism (Minshew & Goldstein, 1993).

Motor Cortex

In addition to the characteristic symptoms of Autism, motor dysfunctions, such as delays in achieving motor milestones, clumsiness, and difficulty carrying out organized movements and actions in parallel, have been noted in a number of cases of Autism (Teitelbaum, Teitelbaum, Nye, Fryman, & Maurer, 1998). These motor symptoms have been classified as associative symptoms (Ming, Brimacombe, & Wagner, 2007). In an fMRI study comparing individuals with Autism to age matched controls, it was found that individuals with Autism had decreased activation patterns during a visually paced finger movement task (Muller, Pierce, Ambrose, Allen, & Courchesne, 2001). This indicates that there may be a neurological reason for the characteristic motor deficits noted in Autism.

One study compared clumsiness in individuals with Autism, Asperger syndrome (mildest syndrome on the Autism spectrum), and pervasive development disorder not otherwise specified (PDD-NOS) (Ghaziuddin & Butler, 1998). All three groups showed coordination deficits. Those with Asperger syndrome had less severe deficits than those with Autism or PDD-NOS, suggesting that as the severity of the disorder increases so does the amount of motor impairments. In analysis of fine and gross motor functions, balance, coordination, and oral motor functions, high functioning children with Autism were shown to have more motor problems than control children (Noterdaeme, Mildenberger, Minow, & Amorosa, 2002). Motor disturbances are one of the earliest behavioural symptoms of the disorder and recent research has suggested that a valuable approach to proper diagnosis of where on the Autism spectrum the individual falls may be through the study of motor coordination (Teitelbaum et al., 1998; Mostofsky, Burgess,

& Gidley Larson, 2007). These dysfunctions may be due to abnormalities noted in the cerebellum and in the frontal lobe where the motor cortex is located.

Abnormalities at the Cellular Level

At the cellular level, there are also marked differences between normal brain tissue and brain tissue from patients with Autism. Cells in the hippocampus, amygdala, medial septal nucleus, mammillary nuclei, and the cortex of the anterior cingulate gyrus tend to be smaller and more densely packed in autistic brain tissue compared to normal brain tissue in these areas (Kemper & Bauman, 1998). In addition to being smaller and more densely packed, neurons in the hippocampus of individuals with Autism have shown reduced complexity of their dendritic trees (Raymond, Bauman, & Kemper, 1996). The size and complexity of the neuron is important for its ability to form connections with other neurons and may reflect its maturity. Smaller and less complex neurons are likely to not be able to make extensive connections with other neurons, which impacts brain function. Pyramidal neurons have been shown to have small cell bodies and reduced dendritic arborization (Raymond et al., 1996). Cell minicolumns, although greater in number in individuals with Autism, are reduced in width and are significantly smaller and less compact. This is thought to create excess "noise" in the cortex that would overload the system leading to inability of the brain to meaningful connections between neurons (Casanova, Buxhoeveden, Switala, & Roy, 2002). These results indicate that there are also microscopic differences in the brains of individuals with Autism.

Normal brain development consists of both progressive and regressive processes. Neurogenesis, axon growth, and formation of synapses are progressive processes.

Apoptosis, axonal pruning, and synapse elimination are regressive processes. Both progressive and regressive process abnormalities have been theorized in Autism. Increased progressive processes are implicated in the increased brain growth in early development in children with Autism (Courchesne et al., 2001). Decreased degenerative processes, such as decreased levels of dendritic pruning, have been found in a rat model of Autism (Snow, Hartle, & Ivanco, 2008). Other issues that contribute to abnormalities in the brains of individuals with Autism are reduced programmed cell death, altered cell migration, abnormal cell differentiation, reduced neuronal size, and altered synaptogenesis.

Caveats to Anatomical Studies

Anatomical studies are useful to help determine what brain regions are involved in Autism, but these studies do not always find similar results. Errors in measurement, inconsistencies in design, small sample sizes, and insufficient control groups have all been noted as potential sources for discrepancy (Akshoomoff, Pierce, & Courchesne, 2002). In addition, each individual with Autism presents a unique constellation of symptoms and a unique brain composition that may not facilitate comparison. There may also be issues with comparing studies across time since the definition and diagnostic criteria of Autism have changed over the years. These factors, taken together, illustrate that care must be taken in defining and designing the experiment as well as the diagnostic criteria used so that comparisons may be made.

Importance of Research on Autism

Research on Autism is not only important for the individual affected and the families involved, but Autism also has devastating effects on society. The cost to care for

an individual with Autism over his or her life can be upwards of $3.2 million dollars (Ganz, 2007). In the United States, the health care cost of Autism is estimated at $35 billion dollars annually (Ganz, 2007). As of yet, there is no cure for Autism, there are however treatment options. Therapies generally focus on educational and behavioural interventions given the limited information available on the neurobiology of Autism. The first few years of life are crucial in human brain development and are a time of particular vulnerability to abnormal events and conditions. The main objective of neurobiological examination of Autism is to determine the brain regions that are most severely affected. Once these regions are identified they can be examined in relation to symptom presentation, then strategies can be developed for possible intervention and early diagnosis.

Risk Factors

Although the etiology of Autism remains unknown, there are several known environmental and genetic risk factors associated with Autism. Autism is known to have a strong genetic basis, which is complex due to multiple gene and environmental interactions. Autism has also been linked to exposure to several environmental agents during gestation that disrupt normal development.

Genetics

It is known that Autism has a strong genetic component. Monozygotic twins (identical twins) have a concordance rate of approximately 65–95%, whereas dizygotic twins (fraternal twins) have a concordance rate of only 3–8% (as reviewed by Dawson, 2008). Autism is known to be among the most heritable of all neurodevelopmental disorders (Kumar & Christian, 2009). There is no one gene that has been labeled, but

there is strong evidence for a multiple gene interaction and also an environmental contribution that increases susceptibility to Autism (Dawson, 2008).

One of the most noticeable genetic characteristics of Autism is the sex ratio of those diagnosed. Recent male to female ratio estimates range from 5.5:1.4 to 16.8:4.0 of individuals diagnosed with ASD (Rice, 2009). This sex discrepancy has prompted most researchers to focus on boys. When girls are included, the numbers are often too small to include as a statistical factor. Hartley and Sikora (2009) examined the difference between girls and boys with Autism in developmental profiles, Autism symptoms, and coexisting behavioural problems. It was found that although there were similar patterns of strengths and weaknesses, there are subtle differences between the sexes. Girls seem to be more deficient in communication skills, have more sleep problems, and have more affective issues, whereas boys tend to have more stereotyped behaviours (Hartley & Sikora, 2009). This indicates that although overall symptom presentation may be similar between boys and girls, a sex specific set of diagnostic criteria may be useful for diagnosis and treatment prescription.

Some known genetic disorders show an autistic phenotype. Fragile X syndrome, tuberous sclerosis, and Rett syndrome all have a known genetic cause and have a high co-morbidity for Autism. These disorders are referred to as syndromic causes of Autism (Folstein & Rosen-Sheidley, 2001). Fragile X is a genetic disorder caused by a chromosomal deficit on the X chromosome in males that silences transcription of the FMR1 gene causing limited or no production of the Fragile X Mental Retardation Protein (FMRP) (Pieretti et al., 1991). The FMR1 gene encodes for fragile X mental retardation protein, which is responsible for mRNA at the synapse. FMR1 becomes silenced in

fragile X syndrome resulting in inherited mental retardation (Pieretti et al., 1991). Approximately 50% of individuals with Fragile X meet the diagnostic criteria for Autism (Demark, Feldman, & Holden, 2003). Using Fragile X knockout mice, Irwin et al. (2001) examined the dendritic morphology of layer V pyramidal neurons in the visual cortex and found greater immature spine density on these neurons. These results parallel those found in human cases of Fragile X syndrome and may be indicative of a deficit in pruning or spine maturation (Irwin et al., 2001), which may account for cognitive, motor, and sensory problems.

Tuberous sclerosis is an autosomal dominant disorder caused by mutations in either of the genes TSC1 or TSC2. These genes encode for tumour suppressing proteins. Mutations in these genes cause benign tumours to grow in the brain and other vital organs in the body. Approximately 50% of individuals with tuberous sclerosis show learning deficiencies (Ridler et al., 2007), and approximately 60% of individuals meet the criteria for Autism (Harrison & Bolton, 1997).

Rett syndrome, caused by a mutation in the MECP2 gene, is a severe neurodevelopmental disorder (Guy, Gan, Selfridge, Cobb, & Bird, 2007). Rett syndrome is one of the most common causes of mental retardation in females. The MECP2 gene is located on the X-chromosome and, therefore, occurs predominantly in females as it is lethal in hemizygous males (Hagberg, Aicardi, Dias, & Ramos, 1983). The MECP2 gene encodes for methyl-CpG-binding protein 2 (MeCP2) that is essential for normal postnatal brain development (Amir, van Den Veyver, Wan, Tran, Francke, & Zoghbi, 1999). Girls with Rett syndrome develop normally until about the age of 6–18 months, then development begins to regress with loss of speech and purposeful hand use, and the

development of stereotypic hand movements (Hagberg et al., 1983). During regression, symptoms similar to Autism develop and sometimes children are misdiagnosed with Autism. Rett syndrome, like Autism, falls under the pervasive developmental disorder category in the DSM-IV. Although similar to Autism, Rett syndrome is not diagnosed on the Autism spectrum.

Teratogens

A teratogen is an agent that is capable of interfering with the normal development of a fetus, causing birth defects (Koren, Pastuszak, & Ito, 1998). Studies have linked two well known teratogens to the development of Autism. Exposure to teratogenic agents such as Thalidomide (Stromland et al., 1994) and Valproic Acid (Williams, King, Cunningham, Stephan, Kerr, & Hersh, 2001) has been proposed risk factors for the development of Autism.

In the late 1950s Thalidomide was prescribed to pregnant women to alleviate morning sickness. As a result, approximately 5,000 children were born with Thalidomide defects in West Germany alone (Stromland et al., 1994). Depending on when in the pregnancy the mother used Thalidomide, deformities varied from upper and lower limb defects to defects in organ systems. In addition to these deformities, the rate of Autism was found to be 50 fold higher in individuals whose mother ingested Thalidomide approximately 20–24 days after conception in comparison to the general population (Stromland et al., 1994). When mothers ingested Thalidomide during pregnancy over any other time period than 20–24 days after conception the rate of Autism in exposed children was 0% (Rodier et al., 1996). These results indicate that there are critical time

periods for development during gestation, and that one of the most critical time periods for the development of Autism appears to be during the first trimester.

Valproic acid (VPA) exposure has also been found to be a risk factor for Autism when exposed *in utero*. Valproic acid is an anticonvulsant medication that has been used to effectively control various types of seizure disorders. Doses of valproic acid for seizure control range from 300–2000 mg, aiming to achieve therapeutic plasma levels of 50–100 μg/ml (Ornoy, 2009). However, during pregnancy, the clearance of valproic acid is increased and may result in umbilical cord valproic acid levels that are up to five times higher than maternal serum levels at term (Froescher, Gulger, Niesen, & Hoffmann, 1984). Thus, the teratogenic potential of valproic acid is well known and women taking VPA during pregnancy are at an increased risk of having a child with a congenital abnormality.

Due to ingestion of antiepileptic drugs during pregnancy, a group of disorders known as fetal anticonvulsant syndromes have been described in which children exposed to antiepileptic drugs during the first trimester show malformations, developmental disorders, and have various medical issues occur in association with a characteristic facial appearance (Moore, Turnpenny, Glover, Lloyd, Montgomery, & Dean, 2000). Valproic acid is commonly implicated in fetal anticonvulsant syndrome and is thus considered the most teratogenic anticonvulsant (Froescher et al., 1984). Characteristic facial features such as epicanthal folds, broad nose with a flat bridge and anterverted nostrils, thin upper lip and thick lower lip, and a shallow philtrum are noted in children with fetal anticonvulsant syndrome (Moore et al., 2000). Exposure to VPA *in utero* can also cause developmental delays such as speech delay, gross motor delay, learning difficulties,

congenital heart defects, finger abnormalities, and development of autistic spectrum and hyperactivity behaviours (Moore et al., 2000). Due to the severity of the outcomes to the child, taking anti-epileptic medications during pregnancy can be very harmful.

A relationship has been shown between fetal anticonvulsant syndrome and Autism. In a population based study, the long term effects of prenatal exposure to antiepileptic medication was examined in 260 children in Scotland (Rasalam et al., 2005). Of these 260 children exposed to antiepileptic medication in utero, 26 children were reported by their parents to have behavioural and/or social difficulties. Of these 26, 11 children met DSM-IV criteria for Autism. Prenatal exposure to valproic acid was found to be the drug most commonly associated with development of Autism, 8.9% of the children in the study were exposed to valproic acid monotherapy (anti-epileptic medication is solely valproic acid in comparison to polytherapy where other types of anti-epileptics are used in combination) and were diagnosed with Autism. Prenatal exposure to antiepileptic medication, especially to valproic acid, has been strongly implicated as a risk factor for the development of Autism.

Animal Model of Autism

An animal model of Autism has been created in rats using prenatal exposure to valproic acid. Foundation for this model comes from examining the studies of the effects of both Thalidomide and valproic acid on humans. Prenatal exposure to both these drugs has been shown to increase the incidence of Autism in humans (Stromland et al., 1994; Rasalam et al., 2005). Thalidomide does not have the same teratogenic effects on rodents as it does in humans. For that reason, prenatal exposure to valproic acid is used to experimentally induce Autism in rats.

Although an animal model cannot replicate a human disease exactly, primary symptoms can be approximated for evaluating theories about the causes of the human disease. An animal model needs to reflect features of the disease such as genetics, pathology, behaviour, etiology, or molecular changes (van Kooten, Hof, van Engeland, Steinbusch, Patterson, & Schmitz, 2005) in order for it to be an appropriate model for human disease. Animal models are useful for determining the roles of pathogens, genes, environment, and testing potential therapies.

There is an increasing amount of evidence to show that the valproic acid animal model of experimentally induced Autism is an accepted model (Dufour-Rainfray et al., 2010; Kim et al., 2011; Rodier et al., 1996; Schneider & Przewłocki, 2005; Schneider, Turczak, & Przewłocki, 2006; Wagner, Reuhl, Cheh, McRae, & Halladay, 2006). VPA exposed rats show brain and behavioural abnormalities consistent with those generally found at autopsy and in brain imaging studies of human Autism patients. Therefore, the VPA model of Autism seems to parallel both anatomical and functional pathology that is reported in human cases of Autism and as such, is an invaluable tool for the study of neurodevelopment in Autism.

VPA and Neural Tube Closure

VPA is found to be an inhibitor of histone deacetylases, which enables it to interrupt the cell cycle and induce apoptosis and growth arrest. This cell cycle interruption has an inhibitory effect on cell proliferation (Phiel, Zhang, Huang, Guenther, Lazar, & Klein, 2001), which would cause abnormalities in developing structures at the time of ingestion. Experimental evidence has suggested that prenatal VPA exposure inhibits proliferation of neural stem cells and migration in the cerebral cortex (Kuwagata,

Ogawa, Shioda, & Nagata, 2009), indicating that VPA exposure *in utero* can lead to abnormal brain and neural tube development and be a possible risk factor in Autism.

During early pregnancy, one of the most important events is the development of the neural tube. It has been shown that different exposure times during early pregnancy result in different neural tube defects (Vorhees, 1987b). For example, in rodents, exposure on day nine can cause neural tube defects consistent with spina bifida (Ehlers, Sturje, Merker, & Nau, 1992) and exposure to VPA on day 12, the day the neural tube closes, can cause the deficits seen in Autism (Rodier et al., 1996). Therefore, the date of exposure to VPA is very important for formation of injury.

As the neural tube is closing, the production of the first neurons that form the motor nuclei of the cranial nerves is occurring (Rodier, Ingram, Tisdale, & Croog, 1997). In addition, the first developing progenitor neurons for other brain regions are also likely to be affected during this time period. Thus, developmental abnormalities resulting from damage in these brain regions could become apparent as symptoms in an individual with Autism.

Brain Abnormalities

Rats exposed to VPA prenatally show brain abnormalities resembling those generally found in humans with Autism at autopsy and in brain imaging studies (Rodier et al., 1996). A decrease in Purkinje cells in the cerebellum is one of the most robust findings in both the VPA animal model and in individuals with Autism (Bailey et al., 1998; Hashimoto et al., 1995; Ingram et al., 2000; Martin et al., 2010; Rodier et al, 1996). It has also been shown that VPA-exposed rats have abnormal brain stems with diminished motor neuron numbers in the nuclei of the cranial nerves, similar to the

abnormalities noted in humans with Autism (Rodier et al., 1996). In addition, dendritic arborization has been found to be greater in apical dendrites of layer II pyramidal cells in the primary motor cortex (Snow et al., 2008). This is the first report of altered development of cortical dendrites in the VPA model. These findings are consistent with suggested abnormal cortical development and proposed lack of pruning in individuals with Autism.

Behavioural Abnormalities

VPA rats also show many behavioural characteristics similar to those of human Autism. Many studies have found that prenatal exposure of rats to VPA on day 12.5 leads to decreased amount of social behaviour, locomotor and repetitive or stereotypic hyperactivity, lower exploratory activity, lower sensitivity to painful stimuli, and increased anxiety in a novel environment (Markram, Rinaldi, LaMendola, Sandi, & Markram, 2008; Pierce & Courchesne, 2001; Schneider & Przewłocki, 2005; Schneider, Labuz, & Przewłocki, 2001), all of which are symptoms noted in human Autism cases. In addition, when conditioned for the eye-blink reflex VPA exposed rats show more rapid acquisition and stronger, faster blinks consistent with findings of humans with Autism. These results indicate that there are behavioural parallels between human Autism and VPA induced Autism, with respect to, injury in circuitry between the brainstem and the cerebellum, but also behaviour parallels that result from the same injury (Stanton, Peloso, Brown, & Rodier, 2007). These parallels indicate that the VPA model is a sufficient analog to Autism in humans.

Delays in Maturation

In addition to the brain and behavioural similarities, VPA rats have been shown to exhibit delays in development and maturation. These delays include slower motor development, nest seeking response, surface and mid-air righting, motor activity in water maze, and lower body weight (Schneider & Przewłocki, 2005; Wagner et al., 2006). Similar delays have been noted in a VPA model of Autism using mice. Roullet, Wollaston, DeCatanzard, and Foster (2010) found delays in physical development such as lower weight, delayed eye opening, and delayed nest seeking. In another study that used a mouse VPA model, the exposed mice were found to have delayed motor maturation (Wagner et al., 2006). Corresponding delays in maturation in human cases of Autism have also been noted (Dewey, Cantell, & Crawford, 2007).

VPA-exposure has also been shown to affect the progression of pregnancy in rats. Birth rate of VPA-exposed animals has been shown to be up to 25% lower than the saline injected dams, and thus, weight during pregnancy is also reduced for the VPA-exposed dam (Markram et al., 2008). Unfortunately, comparable data in humans is not available since we are not able to diagnose Autism *in utero*. Yet the aforementioned delays are important to identify because they can be used as diagnostic aids.

Motor Cortex

VPA-exposed rats have been shown to have abnormalities within the motor cortex. Pyramidal neurons in all layers of the motor cortex have been shown to have increased excitability due to abnormalities in the glutamatergic system (Markram, Rinaldi, & Markram, 2007; Rinaldi, Silberberg, & Markram, 2008). These pyramidal neurons also seem to create over 50% more synapses to other neurons, but have smaller

minicolumn width when compared to control animals (Casanova et al., 2002). These abnormalities within the motor cortex and the abnormalities noted within the cerebellum may explain the motor disturbances noted in individuals with Autism. Therefore, the study of the motor cortex and motor learning in Autism is necessary to aid with diagnosis before the age of three, and to increase our understanding of underlying brain abnormalities. The many anatomical and behavioural similarities between the VPA model of Autism and human Autism cases provides evidence and support for the use of VPA as an appropriate animal model to study the neuropathology of Autism.

Environmental Enrichment and the Animal Model of Autism

Environmental enrichment not only affects brain plasticity in intact brains, but also can mitigate the effects of brain damage. Following frontal lobe lesions, exposure to enriched conditions enhances and speeds recovery (Williams, Gharbawie, Kolb, & Kleim, 2006). Exposure to enrichment is even able to reduce the effects of genetic and environmental risk factors on brain and behavioural development (as reviewed by Dawson, 2008). Therefore, research into how the environment is able to ameliorate the symptoms and pathology of Autism is of high value.

Exposure to an enriched environment has been found to reverse many of the behaviour aberrations found in the VPA model (Schneider et al., 2006). VPA exposed rats reared in enriched conditions were found to have an increased nociceptive threshold and increased diminished prepulse inhibition. Enrichment also alleviated the increased locomotor and repetitive activity, increased exploratory activity, social play, and social explorations, and decreased anxiety in an elevated maze when compared to the VPA exposed rats reared in standard conditions (Schneider et al., 2006). These findings

indicate that although Autism seems to cause brain damage prenatally, environmental influences are able to ameliorate the symptoms of the disorder given that the autistic rats exposed to the enriched environment functioned similar to control animals.

Environmental enrichment has also been shown to delay the onset and/or symptoms of some genetic disorders. The onset of Huntington's disease in mice can be delayed using enriched environments. Huntington's disease is a genetic disorder in which there is a progressive neurodegeneration. Transgenic mice have been developed to model the human disease. When compared to control mice reared in standard cages, exposure to environmental enrichment delayed the degenerative loss of cerebral volume (van Dellen, Blakemore, Deacon, York, & Hannan, 2000). Environmental enrichment has also been shown to improve the behaviours of Fragile X knockout mice. Fragile X knockout mice have been noted to have altered patterns of open field activity and do not show habituation to stimuli (Restivo et al., 2005). Upon brain tissue examination, these mice show reduction of basilar dendrite length and complexity and increased numbers of immature dendritic spines along the apical dendrites. After exposure to environmental enrichment, the knockout mice show restored behavioural patterns of exploration and habituation. Examination of the brain tissue shows increased basilar dendritic branching, length, and increased apical spine density (Restivo et al., 2005). These findings indicate that interaction with a stimulating environment can override some detrimental symptoms caused by genetic mutations. This finding is important as it indicates that experience is able to override genetics to some degree, which is extremely important in Autism since it has a large genetic component.

Summary

Autism is a complex disorder that is extremely variable in symptom presentation, making diagnosis and research on an underlying cause(s) difficult. MRI and autopsy studies have identified many brain regions that are abnormal in Autism, but these findings are inconsistent. Most studies show an enlargement of the brain early in life, small densely packed cells in the limbic system structures, damage to the neural tube, and Purkinje cell number decreases in the cerebellum. The development of the brain in Autism has been shown to be abnormal, but more research is needed to better understand these abnormalities.

Research on Autism is ongoing, but the cause and underlying brain pathology remains unknown. Valproic Acid is used as a teratogen to experimentally induce Autism. This model is gaining acceptance across the scientific community because of its brain and behaviour parallels to human patients with Autism. When exposed to environmental enrichment, the behavioural aberrations that the VPA rats have were shown to decrease and become more normal. The current literature does not investigate neurogenesis in the VPA model of Autism, and there has been no research on the effect of enriched environments on neurogenesis in this model. The current study investigates this gap in the literature and will contribute to the understanding of brain pathology and plasticity in Autism.

Chapter 4: Materials and Methods

Justification and Hypothesis

Precisely how neurogenesis is affected by experience in the VPA model of Autism is unknown. VPA exposure may increase neurogenesis in the dentate gyrus compared to control animals housed in standard laboratory conditions. This increase in new neurons may be a model to explore whether neurogenesis is responsible for the brain enlargement noted in Autism. Exposure to an enriched environment has been shown to ameliorate the behavioural consequences noted in the animal model of Autism in rats, and has also shown to be effective in aiding recovery following injury. How the brain adapts to early neurodevelopmental injury, as seen in Autism, is currently unknown. Analysis of neurogenesis in this model may help to examine this issue. It is hypothesized that VPA exposed rats will have abnormal amounts of neurogenesis in the dentate gyrus and that with exposure to enrichment the enrichment, the animals will have comparable amounts of neurogenesis as the control rats.

Timed Breeding

Adult, female Long Evans (*Rattus norvegicus*) rats were paired with adult, male Long-Evans rats in suspended cages. To confirm mating, the presence of a vaginal plug was confirmed and that day was designated as day one. Seven days after conception females were separated from the males and placed in standard opaque plastic laboratory cages. Colony lighting was fluorescent, and a light/dark cycle of 12:12 hour was maintained. Temperature of the colony was kept constant at 22° C, and the humidity level was maintained at 41%. Food and water were provided *ad libitum*. All procedures conformed to the standards set forth by the Canadian Council on Animal Care, and were

approved by the Protocol Management and Review Committee of the University of Manitoba.

Valproic Acid Injection

The pregnant females were randomly assigned to either the treatment group or the control group. The experimental group received valproic acid injections to induce the model. Valproic acid (Sodium valproate, Sigma Chemical) was dissolved in 0.9% saline at 100 mg/ml at a pH of 7.3 (as per Snow et al., 2008). Two pregnant rats received intraperitoneal (IP) injections of 100 mg/kg sodium valproate twice per day on day(s) 11, 12, and 13 of gestation (at 9:00 a.m. and 3:00 p.m.). A smaller, but repeated, dose was chosen to minimize the potentially toxic effects that have occurred in the model when a single total 600 mg/kg dose is used (Vorhees, 1987a). The control dams were injected with a similar volume of saline at the same gestational age as the treatment group. Dams were housed individually and the mothers were allowed to raise their own litters. No pups were culled. Litters were evaluated to ensure they were grossly normal upon visual inspection. The offspring were weaned from their mothers on postnatal day 21, and were housed with same sex littermates in standard cages.

Environmental Enrichment

On postnatal day 30, all animals were further separated and put in smaller groups of two or three same-sex littermates. Eight animals in the VPA-exposed group and three animals from the control group were exposed to environmental enrichment from postnatal day 30 until day 60, whereas the control pups were housed in standard laboratory cages without any enrichment. Enrichment consisted of a plastic tube and a nyla-bone within the cage at all times, plus 10 minutes per day in an enrichment box

where the animals experienced social interaction with all of their littermates, stimulation of exploratory behaviour with objects such as colourful plastic toys (i.e., plastic bowling pin set, plastic food set, and a plastic medical set). In addition to the standard food and water, enriched rats received occasional treats. Five control animals and four VPA-exposed animals remained in their standard laboratory housing for the duration of the experiment. These animals were handled for a short period of time daily to control for handling effects.

Tissue Preparation

Bromodeoxyuridine (BrdU) was prepared using 60 mg BrdU (#B5002, Sigma) in 1 ml of 0.007 M sodium hydroxide (NaOH). Beginning on the seventh day of enrichment all of the rats in both the enriched condition and the control condition received weekly BrdU injections of 60 mg/kg for four weeks to label newly formed cells. On day 61, all animals were perfused with saline through the heart with 0.1 M buffered saline (0.9% physiological saline). One hemisphere was removed and placed in 4% paraformaldehyde for processing via immunohistochemistry to quantify for neurogenesis, and the other hemisphere was placed in golgi-cox staining solution for processing and analysis by a lab member at a later date (as per Gibb & Kolb, 1998).

Immunohistochemistry

Immunofluorescent double labeling was performed on the tissue. BrdU was used to label the cell as a newly formed cell and a marker for neuronal nuclei (NeuN) was used to label neurons. One hemisphere from each animal was cut into coronal sections using a freezing stage microtome into 30 µm sections. The sections were placed sequentially

into a 24 well plate filled with 0.1M phosphate buffer (PB) with 0.1% sodium azide and refrigerated until ready to stain.

The following procedure was carried out at room temperature and all incubations were performed on a rotator. Sections containing the dentate gyrus and sections containing the motor cortex were stained. Sections were washed PB containing 0.1% Tween-20 five times for five minutes each. The sections were then transferred into a blocking solution containing 10% normal goat serum in PB for 20 minutes. The sections were then incubated with a mouse anti-BrdU monoclonal antibody (MAB3510, Chemicon) (1:200) for one hour. Sections were transferred into wells containing PB buffer with Tween for five washes for five minutes each. The tissue was then transferred into an antibody solution containing the BrdU secondary, Goat anti-mouse conjugated to Rhodamine-TRITC (AP124R, Chemicon) (1:500), and Mouse anti-NeuN AlexaFluor® 488 conjugated monoclonal antibody (MAB377X, Chemicon; Molecular Probes Inc.; Millipore) (1:200) for one hour. Sections were then washed in PB five times for five minutes each, and then mounted on gelatin-coated slides. Slides were allowed to dry then were cover-slipped using Dako Fluorescent Mounting Media. Slides were stored in the dark at 4°C.

Golgi-Cox Staining

One hemisphere from each brain was immersed in Golgi-cox solution for analysis of neuron morphology. Golgi-cox stain is desirable in this situation because it randomly stains approximately 1–4% of the neurons, which is useful since there are so many neurons in the brain. The tissue remained in Golgi-cox solution for 21 days in the dark. On the 21st day, the tissue was transferred into 30% sucrose for seven days. After two

days in the 30%, sucrose solution the solution was changed and left for five days. The tissue was then sectioned at 200 µm using a vibrotome and mounted on gelatin coated slides. The slides were incubated in the refrigerator for two days in a humid slide box. The slides were then processed by immersing them in ammonium hydroxide for 30 minutes and Kodak rapid fix solution in the dark for 30 minutes. This causes a precipitate to form within the cells, allowing for visualization using a light microscope. The slides were then dehydrated in an increasing series of alcohol washes then immersed in Histoclear and cover slipped using Permount. The slides then sat at room temperature in the dark in a desiccation chamber until ready to be viewed under the microscope. This tissue was prepared to be analyzed at a later date. It will not be part of the thesis, but it will complement the thesis.

Data Collection

Sections of the motor cortex and dentate gyrus were observed using immunofluorescence techniques on an Olympus BX51 light microscope paired with an Olympus high-pressure mercury burner. The brain regions of interest were defined using Paxinos and Watson's Rat Brain Atlas (2005) (see Appendix II for specific brain atlas images that were used). Cells in the granule cell layer and those that were two cell body diameters below the granule cell layer were classified as cells in the dentate gyrus. Through immunofluorescence, when viewed through the green (570 nm) filter, cells labeled by NeuN appeared green and indicated mature neurons. When viewed through the blue (505 nm) filter, cells labeled by BrdU appeared red and indicated cells with newly formed DNA that were formed during the experiment (days 30-60). Erythrocytes, debris, and pigment deposits can un-specifically bind antibodies and create a broad signal

that fluoresces under all filters. Autofluorescence was controlled for by using the ultraviolet (400 nm) filter and any cell or particle that was detectable under this filter was omitted from analysis.

All BrdU labeled cells and BrdU-NeuN double labeled cells were counted within the motor cortex and dentate gyrus. Six sections for the dentate gyrus and three sections for the motor cortex for each animal were counted. All cell counts were conducted in a blinded fashion with the animals and slides coded. The data from the motor cortex contained too few counted cells to be analyzed statistically and was, thus, excluded from analysis.

Statistical Analysis

All statistical analyses were completed using Statistica (StatSoft, Tulsa, OK). The number of labeled cells in the dentate gyrus was assessed with 2 x 2 ANOVA. The independent variables were environment (2) and group (2) and the dependent measure was number of cells counted. The repeated measure was sections. In total, three 2 x 2 ANOVAs were performed to assess number of BrdU-labeled cells, number of BrdU-NeuN-labeled cells, and percent of BrdU-NeuN-labeled cells. The level of significance used was $p = 0.05$.

Chapter 5: Results

The single control dam litter size per dam was 13 pups (nine were used for this experiment). The two VPA exposed dam litter sizes were nine (four were used, five were culled by the mother) and eight (eight were used). One dam re-absorbed her pups, which is normal in a random number of animals. There were 20 animals in total for this experiment. The birth rate of the animals used in this experiment was not ideal. In March 2009 there was a fire in the building where the animals were housed. Entry into the building was restricted and the breeding protocol did not begin until September 2009. None of the animals were lost in the experiment and these were the ones used to breed for this experiment. Eleven male-female animal pairings were attempted from September until November; only three pairings (27%) produced pups for the experiment. See Table 1 for group numbers. Raw data can be found in Appendix III.

Neurogenesis in the dentate gyrus was examined using incorporation of BrdU into the DNA of newly formed cells and by NeuN immunostaining. Visualization of NeuN stained cells (Figure 1A) was not counted specifically. All BrdU labeled cells were counted (Figure 1B) indicating newly formed cells but did not differentiate type of cell (i.e., neuron or glia). Co-labeling of BrdU and NeuN cells were considered new neurons (Figure 1C).

Effect of Environment

Exposure to environmental enrichment significantly enhanced the formation of new cells and formation of new neurons in the dentate gyrus of the rat. Analysis of the effect of environmental enrichment revealed significant main effects for both BrdU-

labeled (F (1, 16) = 9.147, p = 0.008) cells (see Figure 2) and double-labeled (F (1, 16) = 8.862, p = 0.009) cells (see Figure 3).

Effect of VPA Exposure

Prenatal exposure to VPA did not significantly facilitate the generation of new cells in the dentate gyrus, F (1, 16) = 1.341, p = 0.264 (see Figure 4). However, VPA was shown to significnatly decrease the number of newly formed neurons in the dentate gyrus, F (1, 16) = 5.259, p = 0.036 (see Figure 5).

Effect of VPA Exposure and Environment

No significant effect was found for the interactions between group and environment for total number of BrdU-labeled cells, F (1, 16) = 0.628, p = 0.440 (see Figure 6) or for total number of double labeled cells, F (1, 16) = 0.487, p = 0.495 (see Figure 7). Other studies that report on amount of neurogenesis convert the number of double labeled cells into a percentage. Here, percentage was calculated by dividing the number of BrdU-NeuN labeled cells by the total number of BrdU labeled cells. No significant effect was found for the interaction between group and environment for percentage double labeled cells, F (1, 16) = 0.001, p = 0.981 (see Figure 8).

Examination of the raw data does not show any abnormal patterns of counted cells. There are no obvious outliers for either count of BrdU labeled cells or BrdU-NeuN double labeled cells. The maximum number of BrdU labeled cells counted was 17 cells, from a VPA exposed animal in enriched conditions. The minimum number of BrdU labeled cells counted was nine, from a VPA exposed animal in standard conditions. The maximum number of double labeled cells counted was eight, from a control animal in enriched conditions. The minimum number of double labeled cells counted was three,

from a VPA exposed animal in enriched conditions. The largest range of BrdU counted cells in one animal was seven, the smallest range was three cells, and the most common range was four cells. The largest range of double labeled cells counted in one animal was four, the smallest range was one cell, and the most common range was three cells. Raw data for each animal is included in Appendix III.

BrdU and BrdU-NeuN Labeled Cells in the Motor Cortex

Cell counts in this region were too few to perform any type of statistical calculations on and, thus, are not included.

Analysis of a Sex Effect

Analysis of a possible sex effect in this experiment was planned. Given the number of animals in the experiment, there were too few animals in each group to run a statistical analsysis on sex as a factor.

Chapter 6: Discussion

The goal of this research was to understand neurogenesis in the brains of rats after VPA exposure. Specifically, we wanted to determine whether exposure to an enriched environment was beneficial to VPA exposed rats by regulating dentate gyral neurogenesis. The results show that neurogenesis in VPA-exposed rats was not different than in control rats. The results demonstrate that environmental enrichment does have a beneficial effect on neurogenesis in the VPA-exposed rats by increasing neurogenesis to amounts similar to control animals. As expected, and consistent with the literature, all rats exposed to enrichment had significantly more BrdU-labeled cells and double-labeled (BrdU-NeuN) labeled cells than rats in standard conditions. The interactions between group and environment remained non-significant when analyzing both BrdU-labeled cells and double labeled cells indicating that group effects and environmental effects are independent in this experiment.

Enrichment Affects Neurogenesis in the Dentate Gyrus

The dentate gyrus was studied because of its documented potential for neurogenesis in adult animals. The current study found that neurogenesis persists in the dentate gyrus of animals exposed to VPA. Although neurogenesis was found to be significantly different between control and VPA exposed animals, due to the small and uneven experimental group sizes these results may not represent the true influence of VPA on neurogenesis, suggesting that neurogenesis in the dentate gyrus may not be affected in this model. In addition, although statistically significant, the differences in neurogenesis noted between groups are small. The combination of small group sizes and

small disparity between groups means that the results may not be as meaningful as the statistics indicate.

The environmental enrichment paradigm was used to study the effect of extra environmental stimulation on neurogenesis in the brains of VPA exposed rats. As expected, both groups of animals that received enrichment had significantly more BrdU-labeled cells and double-labeled cells in their dentate gyrus, regardless of condition. Animals in the control group that were exposed to enrichment showed significantly more new neurons compared to VPA-exposed animals. Enrichment, as a factor that increases new cells, is a robust finding and is consistently reported across different enrichment paradigms, experimental manipulations, and different animal models (as reviewed by Nithianantharajah & Hannan, 2006). Brown et al. (2003) examined how exposure to enrichment would affect neurogenesis in the lateral ventricular wall and olfactory bulb and the dentate gyrus in mice. They found that exposure to enrichment significantly enhanced neurogenesis in the dentate gyrus, but not in the olfactory bulb. This finding is not unexpected. Neurogenesis in the lateral ventricular wall and olfactory bulb is solely for the purposes of olfactory stimulation. Nilsson, Perfilieva, Johansson, Orwar, and Eriksson (1999) also found increased neurogenesis in the rat dentate gyrus after exposure to long term enrichment.

In another experiment, Sandeman and Sandeman (2000) examined how the crayfish brain adapts to an enriched environment. Enrichment in this experiment was a larger living space and a cage mate; the control environment was a small living space with singly housed crayfish. They found more neurons in the brains of crayfish in the enriched environment compared to their siblings in the non-enriched environment

(Sandeman & Sandeman, 2000). These results show that increased neurogenesis after exposure to enrichment is a robust finding.

The results of this research indicate that environmental enrichment enhances the regulation of neurogenesis in both the VPA model and in control animals. As a result, survival of newly formed cells may have been promoted. The brains were harvested two days after the BrdU injection period (four injections over 28 days) to examine for the presence of BrdU-labeled cells. We chose not to process the brains of the animals immediately after BrdU injection because this would yield data on proliferation of new cells and it has been shown that enrichment has no effect of proliferation rates, but enhances the survival of the newly generated cells (Kempermann et al., 1998) and we were interested in examining an overall assessment of the new neurons created. In the present study, BrdU injections were spaced out so that the first injection was 30 days before sacrifice, therefore it is assumed that the exposure to enrichment may have enhanced the survival of the newly formed cells that were labeled by the first or second BrdU injection. In the present study, significantly more cells double labeled with NeuN were found in animals exposed to enrichment, indicating that survival of new neurons may have been promoted by the enrichment.

In the current study, both the VPA-exposed and control groups responded similarly to enrichment, thus it is possible that the enrichment intervention employed was not strong enough to elicit noteworthy effects in the VPA model. The animals were housed in pairs, in a small cage with a tube and toy, and received only 10 minutes per day in an activity box with other rats. To address this issue in future experiments, a more intensive enrichment paradigm would be required. For example, Schneider and

colleagues (2006) used a pre and post-weaning enrichment method. During pre-weaning the rats were subjected daily to 25 minutes of multisensory stimulation through placement on textured or altered temperature surfaces, training for behavioural tasks, and were intensely handled. During post-weaning enrichment the rats were housed in groups of 12 in a large aquarium full of toys, ladders, wheels, coloured blocks, and mazes that were changed every two days.

Enrichment has been shown to act as a behavioural intervention strategy for the VPA model of Autism (Schneider & Przewłocki, 2005). VPA exposed rats are documented to have lower sensitivity to pain, higher sensitivity to non-painful stimuli, diminished acoustic prepulse inhibition, repetitive and stereotyped behaviours, lower exploratory activity, decreased social behaviours, and increased latency to engage in social behaviours (Schneider et al., 2001; Schneider & Przewłocki, 2005). VPA-exposed rats were housed in enriched conditions for two weeks from postnatal day 22–35. Upon analysis of these behaviours after enrichment, the VPA-exposed rats were found to have normalized reactions to pain, prepulse inhibition, behaviours, exploratory activity, anxiety, and number and latency for social behaviour (Schneider et al., 2006). Enrichment works as an intervention strategy by restoring the behavioural aberrations in the model animals to behaviour comparable to control animals.

Neurogenesis in the VPA Model of Autism

In humans with Autism, one of the most consistent findings is increased cell packing density in structures like the hippocampus and amygdala (Kemper & Bauman, 1993). In the present study, there was no noticeable difference in cell packing density between VPA exposed animals and control animals. The results indicate that

neurogenesis in the dentate gyrus was decreased in the VPA exposed animals since more new neurons were counted in control animals. This may be that increased cell density is a finding in humans specifically, which is not replicated in the animal model.

To examine whether the production and migration of new neurons to the cortex is a mechanism for the brain overgrowth that is noted in Autism, the present study examined the motor cortex for new neurons. The abnormal pattern of brain growth in children with Autism is well documented. Aylward and colleagues (2002) found that there is an acceleration of brain growth in early childhood resulting in above average brain volumes, followed by a normalization of brain size around adolescence (Aylward et al., 2002). Although the motor cortex is not a site of active neurogenesis, Magavi, Leavitt, and Macklis (2000) found that new neurons produced in the dentate gyrus are able to migrate out to areas in the cortex. The present study found too few cells in the motor cortex to perform statistical analysis, indicating that excess migration of new cells to the cortex may not be the mechanism for the brain overgrowth noted in children with Autism.

In typically developing children, there is a brain growth spurt from birth to age two to three (Dobbing & Sands, 1973), however, this growth spurt remains under control and brain volumes remain within a normal range. In the rat, a corresponding growth spurt occurs around postnatal day 35 (Dobbing & Sands, 1973). The animals in the present experiment began the BrdU injections at postnatal day 37, indicating that the time period of accelerated growth is captured in the experimental design.

Although the results of the present study suggest that there is no difference in neurogenesis between the animal model of Autism and control animals, current human

research suggests otherwise. Aylward and colleagues (2002) suggested that the increase in brain volume is a result of increased neurogenesis and reduced pruning in the brains of individuals with Autism (Aylward et al., 2002). The pattern of densely packed but smaller cells in limbic structures is typically noted in earlier stages of brain development, and may reflect features of an immature brain (Raymond et al., 1996). This lack of proper brain development and streamlining of neural connections may be at the root of the symptoms of Autism. Although the brain has more neurons, proper connections are not made and thus, the beneficial effects of increased neurogenesis are not seen in these individuals.

The current study suggests that increased neurogenesis may not be the sole mechanism for brain over-growth noted in Autism. The overgrowth could also be caused by increased gliogenesis, increased synaptogenesis, disturbed migration of neurons, decreased apotosis, decreased pruning, or a combination of these processes. A study by Snow and colleagues (2008) found that there was increased arborization of dendrites in the VPA model indicating that there may be deficient pruning in the brain in Autism. Children normally have more neurons in their brains than adults do. Proper brain development requires removal of large numbers of neurons through dendritic pruning. It is assumed that pruning eliminates faulty connections and those connections that are not used or redundant, which optimizes brain function (Frith, 2003). The apparent lack of dendritic pruning in the brains of those with Autism may be the cause for increased brain size and abnormal functioning of the autistic brain. It is unclear whether the hypothesized excess of synaptic connections is due to initial over generation, or to a failure of pruning mechanisms, which normally refines brain circuitry (as reviewed by

Eigsti & Shapiro, 2003). Due to the severity of the brain overgrowth in Autism, it is likely that both processes are involved.

During the first year of life, the normal brain is developing at a rapid rate. Knickmeyer et al. (2008) examined human brain development from birth to two years of age. They found that the total brain volume increases by 101% in the first year, followed by 15% in the second year of life. In the first year, grey matter increased by 149%, white matter increased by 11%, and cerebellum volume increased by 240%. Despite younger brains being more plastic, there is a critical time window during which, disruption of the processes of migration, neurogenesis, synaptogenesis, and pruning would result in long lasting and permanent detrimental effects. It is possible that in Autism, these important processes are not completed as required and the resulting characteristic symptoms present in the individual when these underlying brain regions fail to develop normally.

It is also possible that maturation the dentate gyrus in Autism is delayed, which may be the reason why the current study found less neurogenesis in this area. The dentate gyrus is one of the last regions to complete neurogenesis (Bayer et al., 1993), this region is found to be immature at 15 months of age (Seress, 1992). The dentate gyrus has been shown to be smaller in children with Autism compared to control children, and it is hypothesized that the delay in maturation of the dentate gyrus due to slower neurogenesis may disrupt connections forming in this region and cause memory and cognitive problems (Saitoh, Karns, & Courchesne, 2001).

Efficacy of the VPA Model of Autism

For an animal model to qualify as a satisfactory replicate of the human condition it must show construct validity, face validity, and predictive validity (Roullet et al.,

2010). Construct validity is the experimental demonstration that a test or experiment is measuring the construct or attribute it claims to be measuring. This type of validity is supported in this model based on the classic presentation of Autism symptoms and underlying brain abnormalities. Diagnosis of Autism is based on abnormal social interaction, impairment in communication, and stereotyped behaviour with restricted interests. Such diagnostic criteria can be observed in VPA exposed rats. VPA rats demonstrate similar deficits in decreased social responsiveness and stereotyped hyperactivity (Schneider & Przewłocki, 2005). Neuroanatomical abnormalities in VPA treated rats can be compared to those in autopsy observations of individuals with Autism, for example a decrease in Purkinje cells in the cerebellum is found in both humans and rats with Autism (Rodier et al., 1996). Face validity examines whether the test is valid and if it appears reasonable. Face validity is supported in the VPA model because human children exposed to VPA *in utero* suffer from fetal valproate syndrome. Approximately 10% of individuals with fetal valproate syndrome have been diagnosed with Autism (Rasalam et al., 2005). Predictive validity is the ability of the test to predict results on some measure. Support for predictive validity comes from Schneider and colleagues (2006) who reversed some of the behavioural deficits observed in VPA exposed rats through exposure to environmental enrichment.

The method of injection used in the current study was designed to mimic normal drug taking behaviours in humans. It is possible that this method of injections produced a milder Autism model than desired. The current study injected 100mg/kg VPA twice per day on days 11, 12, and 13. In a study by Wagner and colleagues (2006), the injection protocol indicated that the mice would be injected with 200 mg/kg on days 12–17 (a total

of 1200mg/kg). In these animals, only minor deficits were found when tested for negative geotaxis on postnatal days 6–14 compared to control animals. Otherwise, they found no other significant difference between the VPA pups given low doses over several days compared to control animals. When animals were given a single 600 mg/kg injection on day 13, they found that VPA exposed mice showed longer latencies of righting, shorter latency to fall on a hanging wire grip task, and slower learning in a water maze task. Although animals that received multiple injections had a total exposure oftwice as much VPA, the lack of severity of their behavioural symptoms does not suggest a very strong model for Autism compared to the single injection animals. Most researchers who use the VPA model utilize a single VPA injection of 500–600 mg/kg (Schneider & Przewłocki, 2005; Markram, et al, 2008; Rinaldi, Kulangara, Antoniello, & Markram, 2007). This dose has been shown to result in maximum levels (900µl/ml) in maternal plasma (Binkerd, Rowland, Nau, & Hendrickx, 1988). It is likely that the multiple small injection doses do not result in a high enough maternal plasma level to cause damage to the fetus. It would be interesting to note the differences in this experiment if the single injection method was used.

One question that remains is what constitutes ‘enrichment’ for humans. Are models of animal enrichment comparative to intensive early intervention strategies for children with neurodevelopmental disorders? The underlying framework of both paradigms is the same, and includes active engagement in complex sensory and motor tasks that will produce meaningful changes in behaviour, social interaction, and play abilities (Reynolds, Lane, & Richards, 2010). Many children with neurodevelopmental disorders are exposed to enriched environments at home and school, but they lack

meaningful engagement with the environment. Animal studies have shown that simple exposure to the environment is not enough, but physical and social engagement with the environment is necessary to bring about neurological changes (Reynolds et al., 2010).

Conclusion

Autism is a challenging disorder to study experimentally in humans, but the use of the VPA animal model has proven to be useful for the study of the brain in Autism. Similarities in behavioural and neuroanatomical pathology in VPA rats and Autism provide evidence for the utility of this model. The use of this model allows for evaluation of effects of environmental enrichment paradigms on brain plasticity and behavioural outcomes. Although a significant difference between VPA-exposed and control rats was not found, this experiment shows that brain plasticity persists in the VPA animal model of Autism. Enrichment was found to significantly enhance the amount of newly formed neurons in the dentate gyrus. In future experiments, having larger animal group sizes may enhance the relevance of the statistical results. In addition, using a larger dosage of VPA to induce the model of Autism and a more intense enrichment protocol would likely enhance the findings of this experiment and may reveal a significant difference between VPA exposed and control animals.

References

Akshoomoff, N., Pierce, K., & Courchesne, E. (2002). The neurobiological basis of Autism from a developmental perspective. *Development and Psychopathology, 14*, 613–634.

Allen, G. & Courchesne, E. (2003). Differential effects of developmental cerebellar abnormality on cognitive and motor functions in the cerebellum: An fMRI study of Autism. *American Journal of Psychiatry, 160*(2), 262–273.

Altman, J. & Das, G.D. (1964). Autoradiographic examination of the effects of enriched environment on the rate of glial multiplication in the adult rat brain. *Nature, 204,*1161-1163.

Altman, J. (1967). Postnatal growth and differentiation of the mammalian brain, with implications for a morphological theory of memory. In G.C. Quarton, T. Melneckuk, & F.O. Schmitt (Eds.), *The Neurosciences a study program* (pp. 723-743). New York, NY: The Rockefeller University Press.

Amir, R.E. , van Den Veyver, I.B., Wan, M., Tran, C.Q., Francke, U., & Zoghbi, H.Y. (1999). Rett syndrome is caused by mutations in X-linked MECP2, encoding methyl-CpG-binding protein 2. *Nature, 23*, 185–188.

Angevine, J.B. & Sidman, R.L. (1961). Autoradiographic study of cell migration during histogenesis of cerebral cortex in the mouse. *Nature, 192,* 766–768.

Aylward, E.H., Minshew, N.J., Field, B.A., Sparks, B.S., & Singh, B.S. (2002). Effects of age on brain volume and head circumference in Autism. *Neurology, 59,* 175–183.

Aylward, E.H., Minshew, N.J., Goldstein, G., Honeycutt, N.A., Augustine, A.M., Yates, K. et al. (1999). MRI volumes of amygdala and hippocampus in non-mentally retarded autistic adolescents and adults. *Neurology, 53*(9), 2145–2150.

Bailey, A., Luthert, P., Dean, A., Harding, B., Janota, I., Montgomery, M. et al. (1998). A clinicopathological study of Autism. *Brain, 121,* 889–905.

Barnea, A. & Nottebohm, F. (1994). Seasonal recruitment of hippocampal neurons in adult free-ranging black-capped chickadees. *Proceedings of the National Academy of Sciences, 91,* 11217–11221.

Bauman, M. & Kemper, T.L. (1985). Histoanatomic observations of the brain in early infantile Autism. *Neurology, 35*(6), 866–874.

Bauman, M., Filipek, P.A. & Kemper, T.L. (1997). Early infantile Autism. *International Review of Neurobiology, 41,* 367–386.

Bayer, S.A., Altman, J., Russo, R. J., Zhang, X. (1993). Timetables of neurogenesis in the human brain based on experimentally determined patterns in the rat. *NeuroToxicology, 14*(1), 83–144.

Becker, H.C., Randall, C.L., Salo, A.L., Saulnier, J.L., & Weathersby, R.T. (1994). Animal research: Charting the course for FAS. *Alcohol Health and Research World, 18,* 10-16.

Bennett, E.L., Diamond, M.C., Krech, D. & Rosenzweig, M.R. (1964). Chemical and anatomical plasticity of brain. *Science, 146,* 610-619.

Bennett, E.L., Rosenzweig, M.R., & Diamond, M.C. (1969). Rat brain: effects of environmental enrichment on wet and dry weights. *Science, 163,* 825-826.

Bennett, E.L., Rosenzweig, M.R., Diamond, M.C., Morimoto, H., & Herbert, M. (1974). Effects of successive environments on brain measures. *Physiology and Behaviour, 12,* 621-631.

Binkerd, P.E., Rowland, J.M., Nau, H., & Hendrickx, A.G. (1988). Evaluation of valproic acid developmental toxicity and pharmokinetics in Sprague-Dawley rats. *Fundamental and Applied Toxicology, 11*(3), 485-493.

Briones, T.L., Klintsova, A.Y., & Greenough, W.T. (2004). Stability of synaptic plasticity in the adult rat visual cortex induced by complex environment exposure. *Brain Research,1018,* 130-135.

Brown, J., Cooper-Kuhn, C.M., Kempermann, G., van Praag, H., Winkler, J., Gage, F.H., et al. (2003). Enriched environment and physical activity stimulate hippocampal but not olfactory bulb neurogenesis. *European Journal of Neuroscience, 17,* 2042-2046.

Brown, K., Kroll, J.S., Hudson, M.J., Ramsay, M., Green, J., Long, S. et al. (2010). Factors underlying parental decisions about combination childhood vaccinations including MMR: A systematic review. *Vaccine, 28,* 4235-4248.

Bruel-Jungerman, E., Laroche, S., & Rampon, C. (2005). New neurons in the dentate gyrus are involved in the expression of enhanced long-term memory following environmental enrichment. *European Journal of Neuroscience, 21,* 513-521.

Calhoun, M.E., Jucker, M., Martin, L.J., Thinakaran, G., Price, D.L., & Mouton, P.R. (1996). Comparative evaluation of synaptophysin-based methods for quantification of synapses. *Journal of Neurocytology,25*(12), 821-828.

Camel, J.E., Withers, G.S., & Greenough, W.T. (1986). Persistence of visual cortex dendritic alterations induced by postweaning exposure to a "superenriched" environment in rats. *Behavioural Neuroscience, 100(*6), 810-813.

Cameron, H.A., & McKay, R.D. (2001). Adult neurogenesis produces a large pool of new granule cells in the dentate gyrus. *The Journal of Comparative Neurology, 435,* 406-417.

Cameron, H.A., Wooley, C.S., McEwen, B.S., & Gould, E. (1993). Differentiation of newly born neurons and glia in the dentate gyrus of the adult rat. *Neuroscience, 56*(2), 337-344.

Campbell, S., Marriot, M., Nahmias, C., & MacQueen, G.,M. (2004). Lower hippocampal volume in patients suffering from depression: A meta-analysis. *American Journal of Psychiatry,161,* 598-607.

Casanova, M.F., Buxhoeveden, D.P., Switala, A.E., & Roy, E. (2002). Minicolumnar pathology in Autism. *Neurology, 58,* 428-432.

Cayre, M., Canoll, P., & Goldman, J.E. (2009). Cell migration in the normal and pathological postnatal mammalian brain. *Progress in Neurobiology, 88,* 41-63.

Clancy, B., Darlington, R.B., & Finlay, B.L. (2001). Translating developmental time across mammalian species. *Neuroscience, 105*(1), 7-17.

Coe, C., Kramer, M., Czeh, B., Gould, E., Reeves, A., Kirschbaum, C. et al. (2003). Prenatal stress diminishes neurogenesis in the dentate gyrus of juvenile rhesus monkeys. *Biological Psychiatry, 54,* 1025-1034.

Coles, C.D., Goldstein, F.C., Lynch, M.E., Chen, X., Kable, J.A., Johnson, K.C. et al. (2011). Memory and brain volume in adults prenatally exposed to alcohol. *Brain and Cognition, 75,* 67-77.

Courchesne, E., Carper, R., & Akshoomoff, N. (2003). Evidence of brain growth in the first year of life in Autism. *Journal of the American Medical Association, 290*(3), 337-344.

Courchesne, E., Karns, C.M., Davis, H.R., Ziccardi, R., Carper, R.A., Tigue, Z.D. et al. (2001). Unusual brain growth patterns in early life in patients with autistic disorder: An MRI study. *Neurology, 57*(2), 245-254.

Courchesne, E., Redcay, E., Morgan, J. T., & Kennedy, D.P. (2005). Autism at the beginning: Microstructural and growth abnormalities underlying the cognitive and behavioural phenotype of Autism. *Development and Psychopathology, 17,* 577-597.

Dawson, G. (2008). Early behavioural intervention, brain plasticity, and the prevention of Autism spectrum disorder. *Development and Psychopathology, 20*(3), 775-803.

Dayer, A.G., Cleaver, K.M., Abouantoun, T. & Cameron, H.A. (2005). New GABAergic interneurons in the adult neocortex and striatum are generated from different precursors. *Journal of Cell Biology, 168*(3), 415-427.

Demark, J.L., Feldman, M.A., & Holden, J.J. (2003). Behavioural relationship between Autism and fragile X syndrome. *American Journal of Mental Retardation, 108,* 314-326.

Denny, C.H., Tsai, J., Floyd, R.L., & Green, P.P. (2009). Alcohol use among pregnant and non-pregnant women of childbearing age. *Morbidity and Mortality Weekly Update, 58*(19), 529-532.

Dewey, D., Cantell, M., & Crawford, S.G. (2007). Motor and gestural performance in children with Autism spectrum disorders, developmental coordination disorder, and/or attention deficit hyperactivity disorder. *Journal of the International Neuropsychological Society, 13,* 246-256.

Dobbing, J., & Sands, J. (1973). Quantitative growth and development of human brain. *Archives of Disease in Childhood, 48,* 757-767.

Dufour-Rainfray, D., Vourc'h, P., LeGuisquet, A., Garreau, L., Ternant, D., Bodard, S., et al. (2010). Behaviour and serotonergic disorders in rats exposed prenatally to valproate: A model for Autism. *Neuroscience Letters, 470,* 55-59.

Duman, R.S., Malberg, J., & Thome, J. (1999). Neural plasticity to stress and antidepressant treatment. *Biological Psychology, 46,* 1181-1191.

Ehlers, K., Sturje, H., Merker, H., & Nau, H. (1992). Valproic acid induced spina bifida: A mouse model. *Teratology, 45,* 145-154.

Eigsti, I., & Shapiro, T. (2003). A systems neuroscience approach to Autism: Biological, cognitive, and clinical perspectives. *Mental Retardation and Developmental Disabilities Research Reviews, 9,* 206-216.

Eriksson, P.S., Perfilieva, E., Bjork-Eriksson, T., Alborn, A., Nordborg, C., Peterson, D., et al. (1998). Neurogenesis in the adult human hippocampus. *Nature, 4*(11), 1313-1317.

Fatemi, S.H., Halt, A.R., Realmuto, G., Earle, J., Kist, D.A., Thuras, P. et al. (2002). Purkinje cell size is reduced in cerebellum of patients with Autism. *Journal of Cellular and Molecular Neurobiology, 22*(2), 171-176.

Ferchmin, P.A., Bennett, E. L., & Rosenzweig, M.R. (1975). Direct contact with enriched environment is required to alter cerebral weights in rats. *The Journal of Comparative and Physiological Psychology, 88*(1), 360-367.

Ferchmin, P.A. & Eterovic, V.A. (1986). Forty minutes of experience increase the weight and RNA content of cerebral cortex in periadolescent rats. *Developmental Psychobiology, 19*(6), 511-519.

Folstein, S.E. & Rosen-Sheidley, B. (2001). Genetics of Autism: complex aetiology for a heterogeneous disorder. *Nature Reviews, 2*, 943-955.

Fombonne, E. (2003). Epidemiological surveys of Autism and other pervasive developmental disorders: an update. *Journal of Autism and Developmental Disorders, 33*(4), 365-382.

Fowler, C.D., Liu, Y., Ouimet, C., & Wang, Z. (2002). The effects of social environment on adult neurogenesis in the female prairie vole. *Journal of Neurobiology, 51*(2), 115-128.

Francis, D.D., Diorio, J., Plotsky, P.M., & Meaney, M.J. (2002). Environmental enrichment reverses the effects of maternal separation on stress reactivity. *The Journal of Neuroscience, 22*(18), 7840-7843.

Frick, K., & Fernandez, S. (2003). Enrichment enhances spatial memory and increases synaptophysin levels in aged female mice. *Neurobiology of Aging, 24,* 615-626.

Frith, C. (2003). What do imaging studies tell us about the neural basis of Autism? *Novartis Foundation Symposium, 251,* 149-176.

Froescher, W., Gugler, R., Niesen, M., & Hoffmann, F. (1984). Protein binding of valproic acid in maternal umbilical cord serum. *Epilepsia, 25*(2), 244-249.

Ganz, M.L. (2007). The lifetime distribution of the incremental societal costs of Autism. *Archives of Pediatrics and Adolescent Medicine, 161*(4), 343-349.

Gibb, R., & Kolb, B. (1998). A method for vibratome sectioning of golgi-cox stained whole rat brain. *Journal of Neuroscience Methods, 79*(1), 1-4.

Ghaziuddin, M. & Butler, E. (1998). Clumsiness in Autism and Asperger syndrome: a further report. *Journal of Intellectual Disability Research, 42*(1), 43-48.

Gould, E., Beylin, A., Tanapat, P., Reeves, A., & Shors, T. (1999). Learning enhances adult neurogenesis in the hippocampal formation. *Nature, 2*(3), 260-265.

Gould, E., Reeves, A.J., Graziano, M.S.A., & Gross, C.G. (1991). Neurogenesis in the neocortex of adult primates. *Science, 286*(5439), 548-552.

Gould, E., Tanapat, P., McEwen, B.S., Galea, L., & Fuchs, E. (1997). Neurogenesis in the dentate gyrus of the adult tree shrew is regulated by psychosocial stress and NMDA receptor activation. *Journal of Neuroscience, 17,* 2492-2498.

Guy, J., Gan, J., Selfridge, J., Cobb, S., & Bird, A. (2007). Reversal of neurological defects in a mouse model of Rett syndrome. *Science, 315,* 1143-1147.

Hagberg, B., Aicardi, J., Dias, K., & Ramos, O. (1983). A progressive syndrome of Autism, dementia, ataxia, and loss of purposeful hand use in girls: Rett's syndrome: Report of 35 cases. *Annals of Neurology, 14,* 471-479.

Harrison, J.E. & Bolton, P.F. (1997). Annotation: Tuberous sclerosis. *Journal of Child Psychology and Psychiatry, 38*(6), 603-614.

Hartley, S.L., & Sikora, D.M. (2009). Sex differences in Autism Spectrum Disorder: An examination of developmental functioning, autistic symptoms, and coexisting behaviour problems in toddlers. *Journal of Autism and Developmental Disorders, 39,* 1715-1722.

Hashimoto, T., Tayama, M., Murakawa, K., Yoshimoto, T., Miyazaki, M., Harada, M., et al. (1995). Development of the brainstem and cerebellum in autistic patients. *Journal of Autism and Developmental Disorders, 25*(1), 1-18.

Hazlett, H.C., Poe, M., Gerig, G., Smith, R.G., Provenzale, J., Ross, A. et al. (2005). Magnetic resonance imaging and head circumference of brain size in Autism. *Archives of General Psychiatry, 62,* 1366-1376.

Hebb, D. O. (1947). The effects of early experience on problem solving at maturity. *American Psychologist, 2,* 737-745.

Heine, V.M., Maslam, S., Zareno, J., Joels, M., & Lucassen, P.J. (2004). Suppressed proliferation and apoptotic changes in the rat dentate gyrus after acute and chronic stress are reversible. *European Journal of Neuroscience, 19,* 131-144.

Holloway, R.L. (1966). Dendritic branching: some preliminary results of training and complexity in rat visual cortex. *Brain Research, 2,* 393-396.

Ingram, J.L., Peckham, S.M., Tisdale, B., & Rodier, P.M. (2000). Prenatal exposure of rats to valproic acid reproduces the cerebellar anomalies associated with Autism. *Neurotoxicology and Teratology, 22,* 319-324.

Irwin, S., Patel, B., Idupulapati, M., Harris, J., Crisostomo, R., Larsen, B. et al. (2001). Abnormal dendritic spine characteristics in the temporal and visual cortices of patients with Fragile-X syndrome: A quantitative examination. *American Journal of Medical Genetics, 98,* 161-167.

Iso, H., Simoda, S., & Matsuyama, T. (2007). Environmental change during postnatal development alters behaviour, cognitions and neurogenesis of mice. *Behavioural Brain Research, 179,* 90-98.

Jessberger, S., Clark, R.E., Broadbent, N.J., Clemenson, G.D., Jr., Consiglio, A., Chichung Lie, D. et al. (2009). Dentate gyrus-specific knockdown of adult neurogenesis impairs spatial and object recognition memory in adult rats. *Learning & Memory, 16,* 147-154.

Johansson, B.B. & Ohlsson, A.L. (1996). Environment, social interaction, and physical activity as determinants of functional outcome after cerebral infarction in the rat. *Experimental Neurology, 139,* 322-327.

Juriloff, D.M., & Harris, M.J. (2000). Mouse models for neural tube closure defects. *Human Molecular Genetics, 9*(6), 993-1000.

Kanner, L. (1943). Autistic disturbances of affective contact. *The Nervous Child,* 217-250.

Kanner, L. (1971). Follow-up study of eleven autistic children originally reported in 1943. *Journal of Autism and Childhood Schizophrenia, 1*(2), 119-145.

Katz, R.J., Roth, K.A., & Carroll, B.J. (1981). Acute and chronic stress effects on open field activity in the rat: Implications for a model of depression. *Neurocience & Behaviour, 5*(2), 247-251.

Kemper, T.L., & Bauman, M. (1993). The contribution of neuropathologic studies to the understanding of Autism. *Neurologic Clinics, 11,* 175-187.

Kemper, T.L. & Bauman, M. (1998). Neuropathology of infantile Autism. *Journal of Neuropathology and Experimental Neurology, 57*(7), 645-652.

Kempermann, G. & Gage, F.H. (2002). Genetic influence on phenotypic differentiation in adult hippocampal neurogenesis. *Developmental Brain Research, 134*, 1-12.

Kempermann, G., Kuhn, H. G., & Gage, F. H. (1997). More hippocampal neurons in adult mice living in an enriched environment. *Nature, 386*(6624), 493-495.

Kempermann, G., Kuhn, H. G., & Gage, F. H. (1998). Experience-induced neurogenesis in the senescent dentate gyrus. *The Journal of Neuroscience, 18*(2), 3206-3212.

Kim, K.C., Kim, P., Go, H.S., Choi, C.S., Yang, S., Cheong, J.H. et al. (2011). The critical period of valproate exposure to induce autistic symptoms in Sprague-Dawley rats. *Toxicology Letters, 201*(2), 137-142.

Klintsova, A.Y., Helfer, J.L., Calizo, L.H., Dong, W.K., Goodlett, C.R., & Greenough, W.T. (2007). Persistent impairment of hippocampal neurogenesis in young adult rats following early postnatal alcohol exposure. *Alcoholism: Clinical and experimental research, 31*(12), 2073-2082.

Knickmeyer, R.C., Gouttard, S., Kang, C., Evans, D., Wilber, K., Smith, J.K. et al. (2008). A structural MRI study of human brain development from birth to two years. *The Journal of Neuroscience, 28*(47), 12176-12182.

Kolb, B. (1995). *Brain Plasticity and Behaviour.* New Jersey: Lawrence Erlbaum Associates.

Kolb, B. & Gibb, R. (1991). Environmental enrichment and cortical injury: Behavioural and anatomical consequences of frontal cortex lesions. *Cerebral Cortex, 1*, 189-198.

Kolb, B., Gibb, R., & Gorny, G. (2003). Experience-dependent changes in dendritic arbour and spine density in neocortex vary qualitatively with age and sex. *Neurobiology of Learning and Memory, 79*(1), 1-10.

Koren, G., Pastuszak, A., & Ito, S. (1998). Drugs in pregnancy. *The New England Journal of Medicine, 338*(16), 1128-1137.

Kornack, D.R. & Rakic, P. (1999). Continuation of neurogenesis in the hippocampus of the adult macaque monkey. *Proceedings of the National Academy of Science, 96,* 5768-5773.

Kozorovitskiy, Y., Gross, C.G., Kopil, C., Battaglia, L., McBreen, M., Stranahan, A. M., et al. (2005). Experience induces structural and biochemical changes in the adult primate brain. *Proceedings of the National Academy of Sciences, 102*(48), 17478-17482.

Krech, D., Rosenzweig, M.R., & Bennett, E.L. (1962). Relations between brain chemistry and problem solving among rats raised in enriched and impoverished environments. *Journal of Comparative and Physiological Psychology, 55*(5), 801-807.

Kuhn, H. G., & Cooper-Kuhn, C. M. (2007). Bromodeoxyuridine and the detection of neurogenesis. *Current Pharmaceutical Biotechnology, 8*(3), 127-131.

Kuhn, H.G., Dickenson-Anson, H., & Gage, F.H. (1996). Neurogenesis in the dentate gyrus of the adult rat: Age related decrease of neuronal progenitor proliferation. *The Journal of Neuroscience, 16*(6), 2027-2033.

Kumar, R.A. & Christian, S.L. (2009). Genetics of Autism spectrum disorders. *Current Neurology and Neuroscience Reports, 9,* 188-197.

Kuwagata, M., Ogawa, T., Shioda, S., & Nagata, T. (2009). Observation of fetal brain in a rat valproate-induced Autism model: a developmental neurotoxicity study. *International Journal of Developmental Neuroscience, 27*, 399-405.

Lainhart, J.E., Piven, J., Wzorek, M., Landa, R., Santangelo, S.L., Coon, H. et al. (1997). Macroencephaly in children and adults with Autism. *Journal of the American Academy of Child and Adolescent Psychiatry, 36*(2), 282-290.

Lemaire, V., Koehl, M., Le Moal, M., & Abrous, D.N. (2000). Prenatal stress produces learning deficits associated with an inhibition of neurogenesis in the hippocampus. *Proceedings of the National Academy of Sciences, 97*(20), 11032-11037.

Lemire, R.J. (1969). Variations in development of the caudal neural tube in human embryos (Horizons XIV-XXI). *Teratology, 2,* 361-370.

Lois, C. & Alvarez-Buylla, A. (1993). Proliferating subventricular zone cells in the adult mammalian forebrain can differentiate into neurons and glia. *Proceedings of the National Academy of Sciences, 90,* 2074-2077.

Luskin, M.B. (1993). Restricted proliferation and migration of postnatally generated neurons derived from the forebrain subventricular zone. *Neuron, 11,* 173-189.

Madsen, K.M., Hviid, A., Vestergaard, M., Schendel, D., Wohlfahrt, J., Thorsen, P et al. (2002). A population based study of measles, mumps, and rubella vaccination and Autism. *The New England Journal of Medicine, 347*(19), 1477-1482.

Magavi, S.S., Leavitt, B.R., & Macklis, J.D. (2000). Induction of neurogenesis in the neocortex of adult mice. *Nature, 405,* 951-955.

Maguire, E.A., Gadian, D.G., Johnsrude, I.S., Good, C.D., Ashburner, J., Frackowiak, R.S.J. et al. (2000). Navigation-related structural change in the hippocampi of taxi drivers. *Proceedings of the National Academy of Sciences, 97*(8), 4398-4403.

Malberg, J.E., Eisch, A.J., Nestler, E.J., & Duman, R.S. (2000). Chronic antidepressant treatment increases neurogenesis in adult rat hippocampus. *The Journal of Neuroscience, 20*(24), 9104-9110.

Markakis, E.A & Gage, F.H. (1999). Adult-generated neurons in the dentate gyrus send axonal projections to field CA3 and are surrounded by synaptic vesicles. *The Journal of Comparative Neurology, 406,* 449-460.

Markram, H., Rinaldi, T., Markram, K. (2007). The intense world syndrome—an alternative hypothesis for Autism. *Frontiers in Neuroscience, 1*(1), 77-96.

Markram, K., Rinaldi, T., LaMendola, D., Sandi, C., & Markram, H. (2008). Abnormal fear conditioning and amygdala processing in an animal model of Autism. *Neuropsychopharmacology, 33,* 901-912.

Martin, L.A., Goldowitz, D., & Mittleman, G. (2010). Repetitive behaviour and increased activity in mice with Purkinje cell loss: a model for understanding the role of cerebellar pathology in Autism. *European Journal of Neuroscience, 31*, 544-555.

Masliah, E., Mallory, M., Hansen, L., DeTeresa, R., & Terry, R. (1993). Quantitative synaptic alterations in the human neocortex during normal aging. *Neurology, 43,* 192-197.

Mattson, S.N., Riley, E.P., Graming, L., Delis, D.C., & Jones, K.L. (1998). Neuropsychological comparison of alcohol-exposed children with or without physical features of fetal alcohol syndrome. *Neuropsychology, 12*(1), 146-153.

Meehan, C.L., Garner, J.P., & Mench, J.A. (2004). Environmental enrichment and development of cage stereotypy in Orange-winged Amazon parrots. *Developmental Psychobiology, 44,* 209-218.

Miller, M.W. (1995). Generation of neurons in the rat dentate gyrus and hippocampus: Effects of prenatal and postnatal treatment with ethanol. *Alcoholism: Clinical and Experimental Research, 19*(6), 1500-1509.

Ming, X., Brimacombe, M., & Wagner, G.C. (2007). Prevalence of motor impairment in Autism spectrum disorders. *Brain & Development, 29*, 565-570.

Minshew, N., & Goldstein, G. (1993). Is Autism an amnesic disorder? Evidence from the California Verbal Learning Test. *Neuropsychology, 7*, 209-216.

Mohammed, A. H., Zhu, S. W., Darmopil, S., Hjerling-Leffler, J., Ernfors, P., Winblad, B. et al. (2002). Environmental enrichment and the brain. *Progress in Brain Research, 138,* 109-133.

Moore, S.J., Turnpenny, A.Q., Glover, S. Lloyd, D.J., Montgomery, T., & Dean, J.C. (2000). A clinical study of 57 children with fetal anticonvulsant syndromes. *Journal of Medical Genetics, 37*, 489-497.

Mosconi, M., Zwaigenbaum, L., & Piven, J. (2006). Structural MRI in Autism: findings and future directions. *Clinical Neuroscience Research, 6*, 135-144.

Mostofsky, S.H., Burgess, M.P., & Gidley Larson, J.C. (2007). Increased motor cortex white matter volume predicts motor impairment in Autism. *Brain, 130*(8), 2117-2122.

Muller, R.A., Pierce, K., Ambrose, J.B., Allen, G., & Courchesne, E. (2001). Atypical patterns of cerebral motor activation in Autism: A functional magnetic resonance study. *Biological Psychiatry, 49,* 665-676.

Nanson, J.L. (1992). Autism in fetal alcohol syndrome: A report of six cases. *Alcoholism: Clinical and Experimental Research, 16*(3), 558-565.

Nilsson, M., Perfilieva, E., Johansson, U., Orwar, O., & Eriksson, P. (1999). Enriched environment increases neurogenesis in the adult rat dentate gyrus and improves spatial memory. *Journal of Neurobiology, 39*(4), 569-578.

Nithianantharajah, J. & Hannan, A.J. (2006). Enriched environments, experience dependent plasticity and disorders of the nervous system. *Nature, 7,* 697-709.

Noterdaeme, M., Mildenberger, K., Minow, F., & Amorosa, H. (2002). Evaluation of neuromotor deficits in children with Autism and children with a specific speech and language disorder. *European Child & Adolescent Psychiatry, 11,* 219-225.

Olson, A., Eadie, B., Ernst, C., & Christie, B. (2006). Environmental enrichment and voluntary exercise massively increases neurogenesis in the adult hippocampus via dissociable pathways. *Hippocampus, 16,* 250-260.

Ornoy, A. (2009). Valproic acid in pregnancy: How much are we endangering the embryo and fetus? *Reproductive Toxicology, 28,* 1-10.

Ortqvist, A., Blennow, M., Carlsson, R.M., Hanson, L.A., Lindberg, A., Lindqvist, L., et al. (2010). Vaccination of children – a systematic review. *Acta Pediatrica, 99,* 1-192.

Passineau, M.J., Green, E.J., & Dietrich, W.D. (2001). Therapeutic effects of environmental enrichment on cognitive function and tissue integrity following severe traumatic brain injury in rats. *Experimental Neurology, 168,* 373-384.

Paxinos, G. & Watson, C. (2005). *The Rat Brain in Stereotaxic Coordinates.* Boston: Elsevier Academic Press.

Phiel, C.J., Zhang, F., Huang, E. Guenther, M.G., Lazar, M., & Klein, P. (2001). Histone deacetylase is a direct target of valproic acid, a potent anticonvulsant, mood stabilizer, and teratogen. *Journal of Biological Chemistry, 276*(39), 36734-36741.

Pieretti, M., Zhang, F., Fu, Y., Warren, S.T., Oostra, B.A., Caskey, C.T. (1991). Absence of expression of the FMR-1 gene in fragile-X syndrome. *Cell, 66,* 817-822.

Pierce, K. & Courchesne, E. (2001). Evidence for a cerebellar role in reduced exploration and stereotyped behaviour in Autism. *Biological Psychiatry,49*(8), 655-664.

Piven, J., Saliba, K., Bailey, J., & Arndt, S. (1997). An MRI study of Autism: The cerebellum revisited. *Neurology, 49,* 546-551.

Public Health Agency of Canada. (2006). *Immunization Schedules.* Retrieved May 30, 2010 from http://www.phac-aspc.gc.ca/im/is-cv/index-eng.php

Rakic, P. & Sidman, R.L. (1970). Histogenesis of cortical layers in human cerebellum, particularly the lamina dissecans. *Journal of Comparative Neurology, 139,* 473-500.

Ramirez-Amaya, V., Marrone, D.F., Gage, F.H., Worley, P.F., & Barnes, C.A. (2006). Integration of new neurons into functional neural networks. *The Journal of Neuroscience, 26*(47), 12237-12241.

Rapin, I. (2002). Perspectives: The Autism-spectrum disorders. *New England Journal of Medicine, 347,* 302-303.

Rasalam, A.D., Hailey, H., Williams, J.H.G., Moore, S.J., Turnpenny, P.D., Lloyd, D.J., et al. (2005). Characteristics of fetal anticonvulsant syndrome associated autistic disorder. *Developmental Medicine & Child Neurology, 47*(8), 551-555.

Raymond, G.V., Bauman, M.L., & Kemper, T.L. (1996). Hippocampus in Autism: A golgi analysis. *Acta Neuropathologica, 91*(1), 117-119.

Restivo, L., Ferrari, F., Passino, E., Sgobio, C., Bock, J., Oostra, B.A. et al. (2005). Enriched environment promotes behavioural and morphological recovery in a mouse model for the fragile X syndrome. *Proceedings of the National Academy of Sciences, 102*(32), 11557-11562.

Reynolds, S., Lane, S.J., & Richards, L. (2010). Using animal models of enriched environments to inform research on sensory integration intervention for the rehabilitation of neurodevelopmental disorders. *Journal of Neurodevelopmental Disorders,2*(3), 120-132.

Rice, C. (2009). Prevalence of Autism spectrum disorders. *Morbidity and Mortality Weekly Report, 58*(SS-10), 1-20.

Ridler, K., Suckling, J., Higgins, N.J., deVries, P.J., Stephenson, C.M.E., Bolton, P.F., et al. (2007). Neuroanatomical correlates of memory deficits in tuberous sclerosis complex. *Cerebral Cortex, 17,* 261-271.

Rinaldi, T., Kulangara, K., Antoniello, K., & Markram, H. (2007). Elevated NMDA receptor levels and enhanced postsynaptic long-term potentiation induced by prenatal exposure to valproic acid. *Proceedings of the National Academy of Sciences, 104*(33), 13501-13506.

Rinaldi, T., Silberberg, G., & Markram, H. (2008). Hyperconnectivity of local neocortical microcircuitry induced by prenatal exposure to valproic acid. *Cerebral Cortex, 18,* 763-770.

Rodier, P.M., Ingram, J.L., Tisdale, B., & Croog, V.J. (1997). Linking etiologies in humans and animal models: Studies of Autism. *Reproductive Toxicology, 11*(2/3), 417-422.

Rodier, P.M., Ingram, J.L., Tisdale, B., Nelson, S., & Romano, J. (1996). Embryological origin for Autism: developmental anomalies of the cranial nerve motor nuclei. *The Journal of Comparative Neurology, 370,* 247-261.

Rosenzweig, M. R., Bennett, E. L., Herbert, M., & Morimoto, H. (1978). Social grouping cannot account for cerebral effects of enriched environments. *Brain Research, 153,* 563-576.

Roullet, F.I., Wollaston, L., DeCantanzaro, D., & Foster, J.A. (2010). Behavioural and molecular changes in the mouse in response to prenatal exposure to the anti-epileptic drug valproic acid. *Neuroscience, 170,* 514-522.

Saitoh, O., Karns, K., & Courchesne, E. (2001). Development of hippocampal formation from 2 to 42 years: MRI evidence of smaller area dentate in Autism. *Brain, 124,* 1317-1324.

Sandeman, R. & Sandeman, D. (2000). Impoverished and enriched living conditions influence the proliferation and survival of neurons in crayfish brain. *Journal of Neurobiology, 45*(4), 215-226.

Santarelli, L., Saxe, M., Gross, C., Surget, A., Battaglia, G., Dulawa, S. et al. (2003). Requirement of hippocampal neurogenesis for the behavioural effects of antidepressants. *Science, 301,* 805-809.

Sausedo, R.A., Smith, J.L., & Schoenwolf, G.C. (1997). Role of non-randomly oriented cell division in shaping and bending of the neural plate. *The Journal of Comparative Neurology, 381,* 473-488.

Schoenwolf, G.C. (1988). Microsurgical analysis of avian neurulation: Separation of medial and lateral tissues. *The Journal of Comparative Neurology, 276,* 498-507.

Schoenwolf, G.C. & DeLongo, J. (1980). Ultrastructure of secondary neurulation in the chick embryo. *American Journal of Anatomy, 158*(1), 43-63.

Schneider, T., Labuz, D., & Przewłocki, R. (2001). Nociceptive changes in rats after prenatal exposure to valproic acid. *Polish Journal of Pharmacology, 53,* 531-534.

Schneider, T., & Przewłocki, R. (2005). Behavioural alterations in rats prenatally to valproic acid : Animal model of Autism. *Neuropsychopharmacology, 30*(1), 80-89.

Schneider, T., Turczak, J., & Przewłocki, R. (2006). Environmental enrichment reverses behavioural alterations in rats prenatally exposed to valproic acid: Issues for a therapeutic approach in Autism. *Neuropsychopharmacology, 31*(1), 36-46.

Seress, L. (1992). Morphological variability and developmental aspects of primate granule cells: Differences between the rodent and primate dentate gyrus. In C.E. Riback, C.M. Gall, & I. Mody (Eds.), *The Dentate Gyrus and its Role in Seizures,* (pp. 3-28). Amsterdam: Elsevier Science Publishers.

Shum, A.S. & Copp, A.J. (1996). Regional differences in morphogenesis of the neuroepithelium suggest multiple mechanisms of spinal neurulation in the mouse. *Anatomy of Embryology, 194,* 65-73.

Sidman, R.L. & Rakic, P. (1973). Neuronal migration, with special reference to developing human brain: A review. *Brain Research, 62,* 1-35.

Sirevaag, A.M., Black, J.E., Shafron, D., & Greenough, W.T. (1988). Direct evidence that complex experience increases capillary branching and surface area in visual cortex of young rats. *Brain Research, 471*(2), 299-304.

Smith, J.L. & Schoenwolf, G.C. (1991). Further evidence of extrinsic forces in bending of the neural plate. *The Journal of Comparative Neurology, 307,* 225-236.

Snow, W.M., Hartle, K., & Ivanco, T.L. (2008). Altered morphology of motor cortex neurons in the VPA rat model of Autism. *Developmental Psychobiology, 50*(7), 633-639.

Sparks, B.F., Friedman, S.D., Shaw, D.W., Aylward, E.H., Echelard, D., Artru, A.A., et al. (2002). Brain structural abnormalities in young children with Autism spectrum disorder. *Neurology, 59,* 184-192.

Stanfield, B.B. & Trice, J.E. (1988). Evidence that granule cells generated in the dentate gyrus of adult rats extend axonal projections. *Experimental Brain Research, 72,* 399-406.

Stanton, M. E., Peloso, E., Brown, K. L., & Rodier, P. (2007). Discrimination learning and reversal of the conditioned eye-blink reflex in a rodent model of Autism. *Behavioural Brain Research, 176*(1), 133-140.

Stevenson, R.E., Schroer, R.J., Skinner, C., Fender, D., & Simensen, R.J. (1997). Autism and macroencephaly. *The Lancet, 349,* 1744-1745.

Striedter, G. F. (2005). *Principles of Brain Evolution.* Massachusetts: Sinauer Associates, Inc Publishers.

Stromland, K., Nordin, V., Miller, M., Akerstrom, B., & Gillberg, C. (1994). Autism in thalidomide embryopathy: A population study. *Developmental Medicine and Child Neurology, 36*(4), 351-356.

Taylor, B., Miller, E., Farrington, C.P., Petropoulos, M., Favot-Mayaud, I., Li, J. et al. (1999). Autism and measles, mumps, and rubella vaccine: no epidemiological evidence for a causal association. *The Lancet, 353,* 2026-2029.

Teitelbaum, P., Teitelbaum, O., Nye, J., Fryman, J., & Maurer, R. (1998). Movement analysis in infancy may be useful for early diagnosis of Autism. *Proceedings of the National Academy of Science, 95,* 13982-13987.

Thach, W.T., Goodkin, H.P., & Keating, J.G. (1992). The cerebellum and the adaptive coordination of movement. *Annual Reviews in Neuroscience, 15,* 403-442.

Travis, L.L., & Sigman, M. (1998). Social deficits and interpersonal relationships in Autism. *Mental Retardation and Developmental Disabilities, 4,* 65-72.

Turner, A. M., & Greenough, W. T. (1985). Differential rearing effects on rat visual cortex synapses. I. synaptic and neuronal density and synapses per neuron. *Brain Research, 329*(1-2), 195-203.

Turner, C.A., Lewis, M.H., & King, M.A. (2003). Environmental enrichment: Effects on stereotyped behaviour and dendritic morphology. *Developmental Psychobiology, 43,* 20-27.

van Allen, M.I., Kalousek, D.K., Chernoff, G.F., Juriloff, D., Harris, M., McGillivray, B.C. et al. (1993). Evidence for multi-site closure of the neural tube in humans. *American Journal of Medical Genetics, 47,* 723-743.

van Dellen, A., Blakemore, C., Deacon, R., York, D., & Hannan, A.J. (2000). Delaying the onset of Huntington's in mice. *Nature, 404,* 721-722.

van Kooten, I.A.J., Hof, P.R., van Engeland, H., Steinbusch, H.W.M., Patterson, P.H., & Schmitz, C. (2005). Autism: Neuropathology, alterations of the GABAergic system, and animal models. *International Review of Neurobiology, 71,* 1-26.

van Praag, H., Kempermann, G., & Gage, F.H. (1999). Running increases cell proliferation and neurogenesis in the adult mouse dentate gyrus. *Nature Neuroscience, 2*(3), 266-270.

van Praag, H., Kempermann, G., & Gage, F.H. (2000). Neural consequences of environmental enrichment. *Nature Reviews, 1,* 191-198.

van Praag, H., Scheinder, A.F., Christie, B.R., Toni, N., Palmer, T.D., & Gage, F.H. (2002). Functional neurogenesis in the adult hippocampus. *Nature, 415,* 1030-1034.

van Praag, H., Shubert, T., Zhao, C., & Gage, F.H. (2005). Exercise enhances learning and hippocampal neurogenesis in aged mice. *The Journal of Neuroscience, 25*(38), 8680-8685.

Veena, J., Srikumar, B.N., Mahati, K., Bhagya, V., Raju, T.R., & Shankaranarayana Rao, B.S. (2009). Enriched environment restores hippocampal cell proliferation and ameliorates cognitive deficits in chronically stressed rats. *Journal of Neuroscience Research, 87,* 831-843.

Veena, J., Srikumar, B.N., Raju, T.R. & Shankaranarayana Rao, B.S. (2009). Exposure to enriched environment restores the survival and differentiation of newborn cells in the hippocampus and ameliorates depressive symptoms in chronically stressed rats. *Neuroscience Letters, 455,* 178-182.

Vorhees, C.V. (1987a). Teratogenicity and developmental toxicity of valproic acid in rats. *Teratology, 35,* 195-202.

Vorhees, C.V. (1987b). Behavioural teratogenicity of valproic acid: selective effects on behaviour after prenatal exposure to rats. *Psychopharmacology, 92,* 173-179.

Wakefield, A.J., Murch, S.H., Anthony, A., Linnell, J., Casson, D.M., Malik, M. et al. (1998). Ileal-lymphoid nodular hyperplasia, non-specific colitis, and pervasive developmental disorder in children. Retracted. *The Lancet, 351,* 637-641.

Wagner, G.C., Reuhl, K.R., Cheh, M., McRae, P., & Halladay, A.K. (2006). A new neurobehavioural model of Autism in mice: Pre- and postnatal exposure to sodium valproate. *Journal of Autism and Developmental Disorders, 36*(6), 779-793.

Williams, G., King, J., Cunningham, M., Stephan, M., Kerr, B., Hersh, J.H. (2001). Fetal valproate syndrome and Autism: additional evidence of an association. *Developmental Medicine and Child Neurology, 43*(3), 202-206.

Williams, P.T.J., Gharbawie, O.A., Kolb, B., & Kleim, J.A. (2006). Experience-dependent amelioration of motor impairments in adulthood following neonatal medial frontal cortex injury in rats is accompanied by motor map expansion. *Neuroscience, 141,* 1315-1326.

Zhao, C., Teng, M., Summers, R.G., Jr., Ming, G., & Gage, F.H. (2006). Distinct morphological stages of dentate granule neuron maturation in the adult mouse hippocampus. *The Journal of Neuroscience, 26*(1), 3-11.

Tables

Table 1

Number of Animals per Group

	Standard Environment		Enriched Environment	
Group	Male	Female	Male	Female
Control	2	3	2	2
VPA-exposed	3	0	6	2

Figures

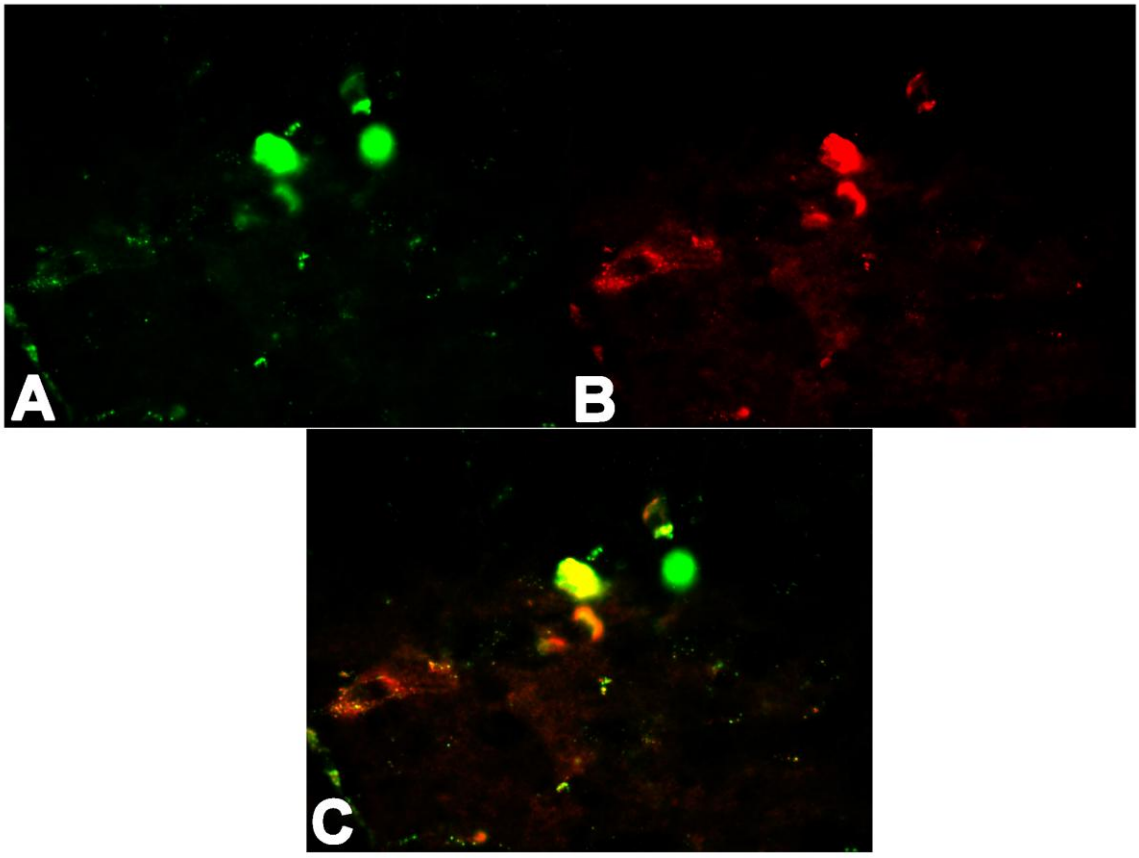


Figure 1. Fluorescent microscopy analysis of BrdU and NeuN immunochemistry. BrdU stained section from the dentate gyrus of a VPA-exposed animal from the enriched environment condition **(A),** when viewed through the green (570 nm) filter cells labeled by NeuN fluoressed green indicating that the cell is a neuron. When viewed through the blue (505 nm) filter cells labeled by BrdU fluoressed red indicating a newly formed cell **(B)**. A composite image **(C)** created by ImagePro software superimposes **A** and **B** to show a cell that is labeled by both BrdU and NeuN (yellow-orange), indicating a new neuron. The fluorescent green circle noted in both figures **A** and **C** is not a cell but an autofluorescent artifact of staning.

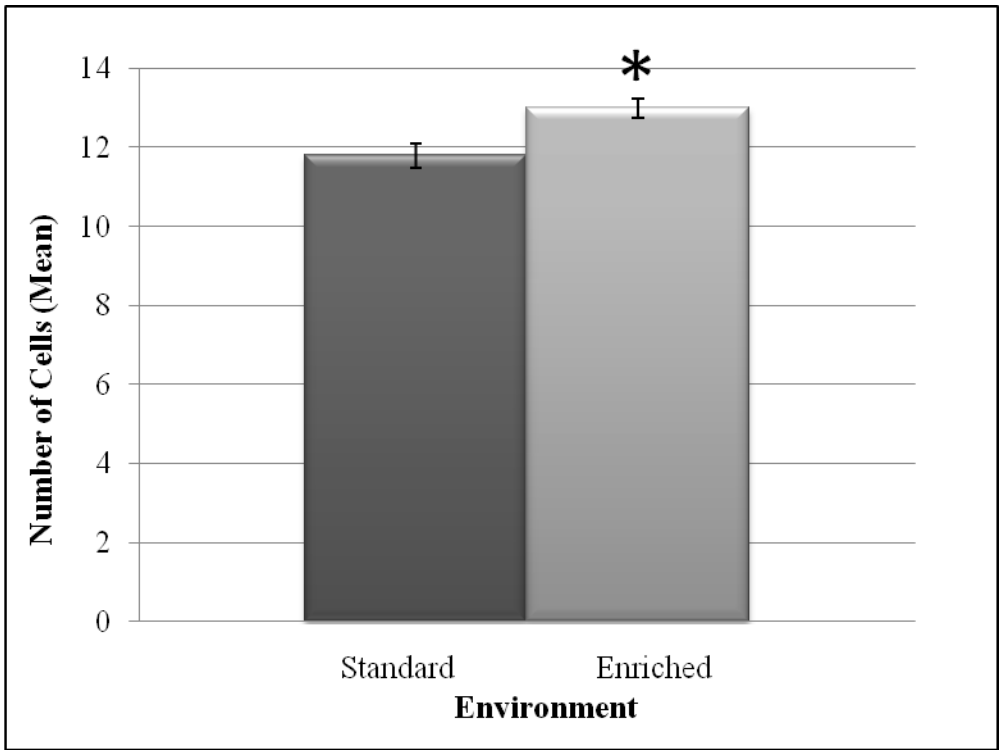


Figure 2. Effect of enrichment on number of BrdU labeled cells. Mean number (+/- SEM) of BrdU labeled cells in the dentate gyrus for all animals in both the standard (n = 8) and enriched environments (n = 12). Mean number of BrdU labeled cells is located on the Y-axis and Environment on the X-axis. The animals in the enriched environment were found to have significantly more BrdU labeled cells than control animals ($p > .05$). An * indicates a significant finding.

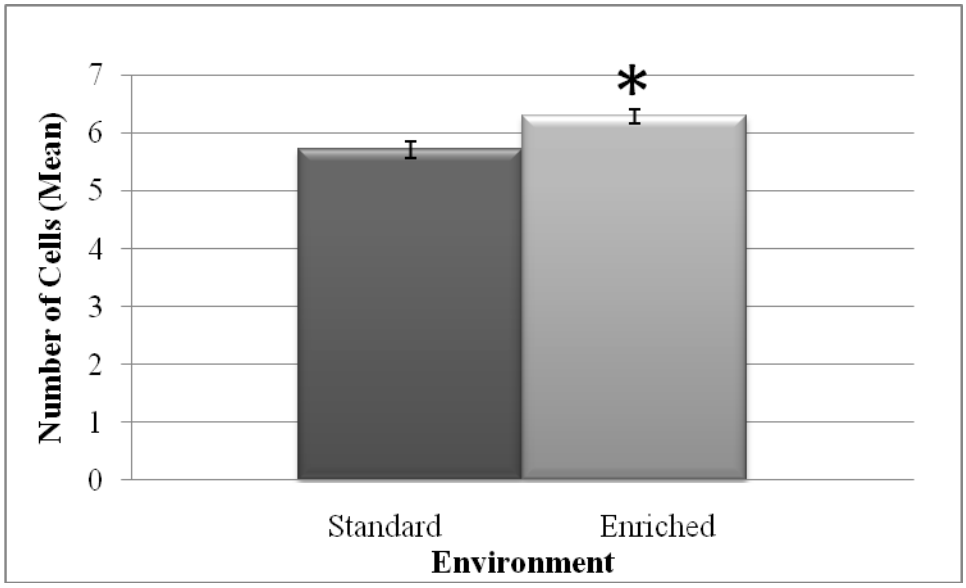


Figure 3. Effect of enrichments on number of double labeled cells. Mean number (+/- SEM) of double labeled cells in the dentate gyrus for animals in both the standard (n = 8) and enriched environments (n = 12). Number of double labeled cells is located on the Y-axis and Environment on the X-axis. The animals in the enriched environment were found to have significantly more double labeled cells than control animals ($p > .05$). An * indicates a significant finding.

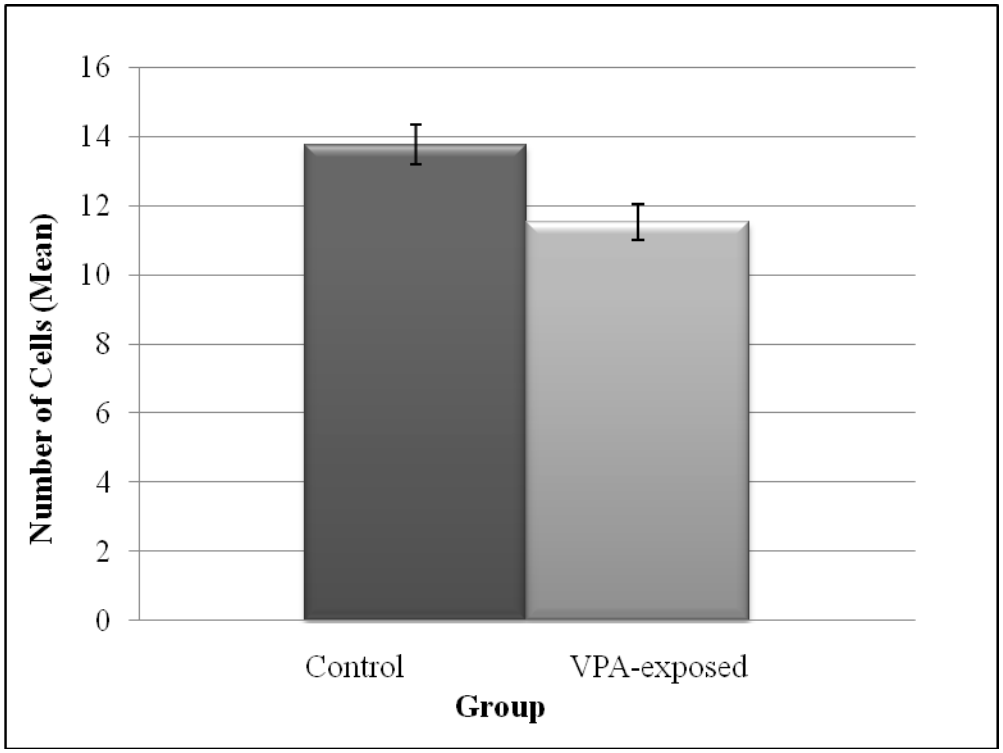


Figure 4. Effect of VPA on number of BrdU labeled cells. Mean number (+/- SEM) of BrdU labeled cells in the dentate gyrus for animals in both the standard (n = 8) and enriched environments (n = 12). Mean number of double labeled cells is located on the Y-axis and Group on the X-axis. No significant difference was found ($p > .05$).

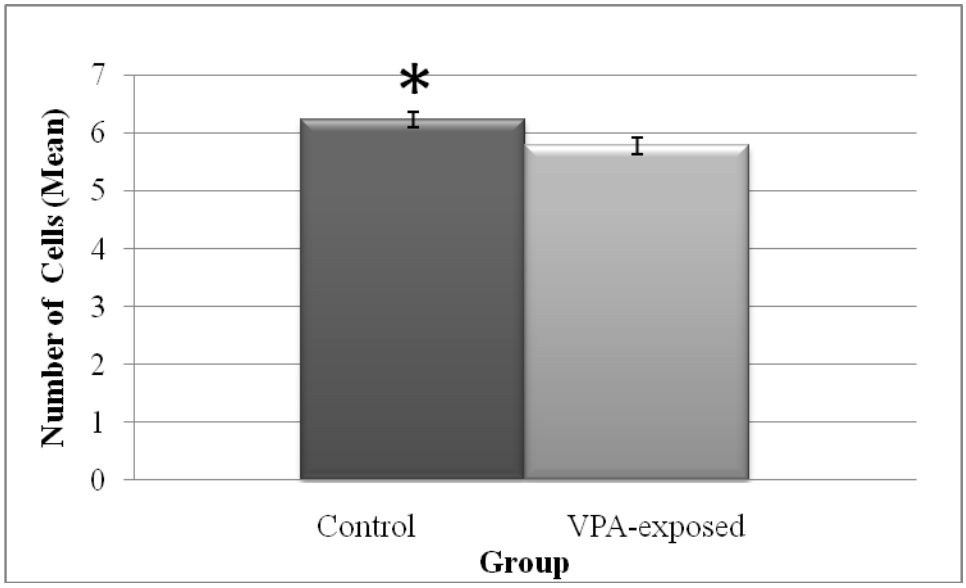


Figure 5. Effect of VPA on number of double labeled cells. Mean number (+/- SEM) of double labeled cells in the dentate gyrus for animals in both the standard (n = 8) and enriched environments (n = 12). Mean number of double labeled cells is located on the Y-axis and Group on the X-axis. The control animals were found to have significantly more double labeled cells than VPA exposed animals ($p > .05$). An * indicates a significant finding.

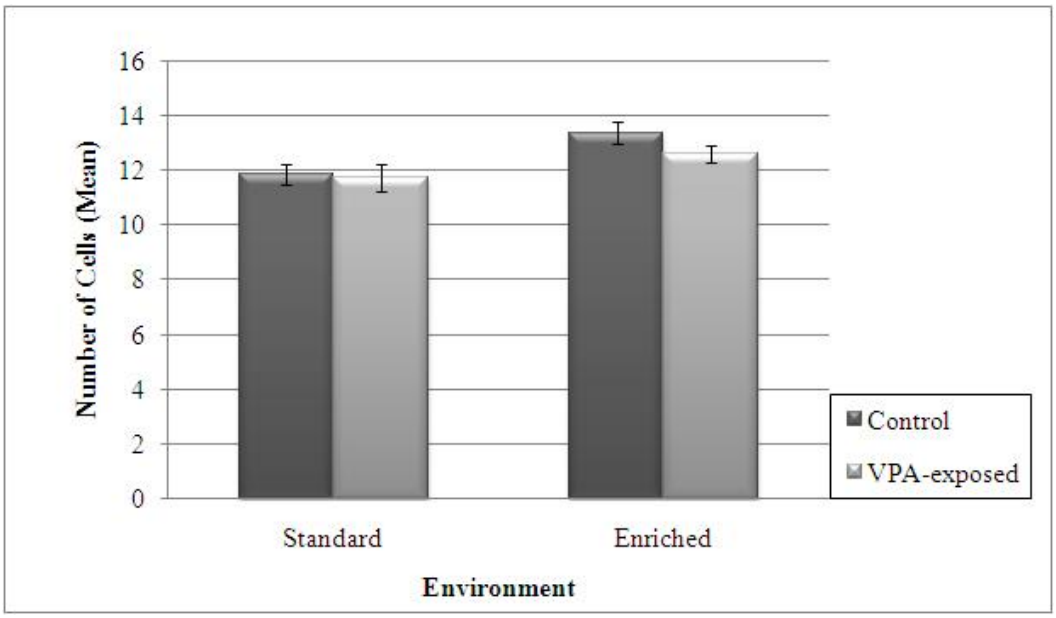


Figure 6. Interaction of group and environment on number of BrdU labeled cells. Mean number (+/- SEM) of BrdU labeled cells in standard and enriched environments for both the VPA exposed (n =11) and control (n = 9) animal groups. Mean number of BrdU labeled cells is located on the Y-axis and Environment on the X-axis. No significant difference was found for the interaction of group and environment ($p > .05$).

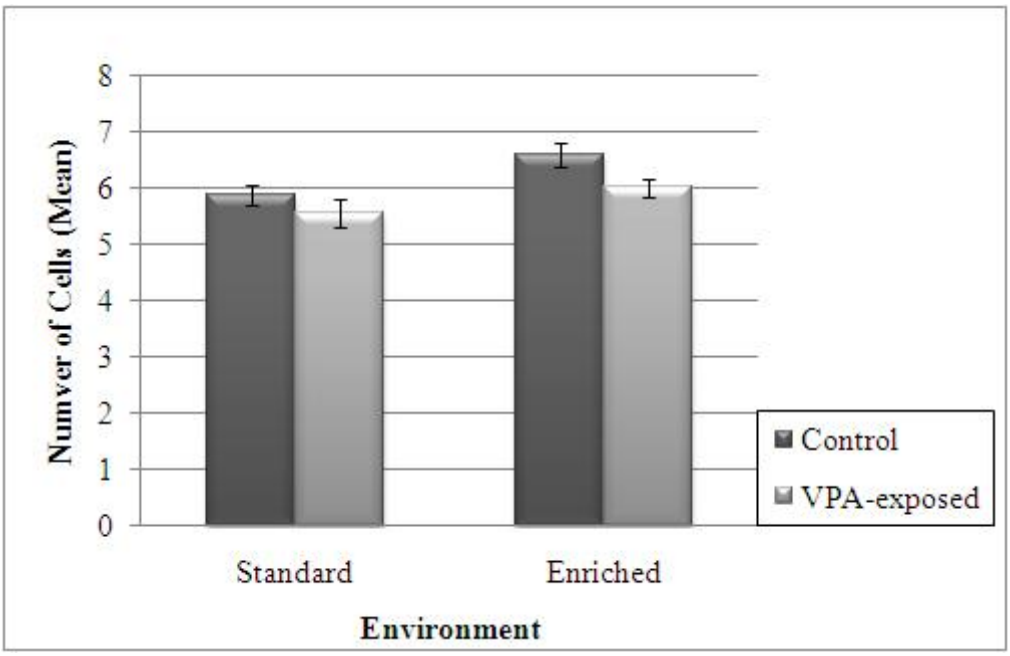


Figure 7. Interaction of group and environment on number of double labeled cells. Mean number (+/- SEM) of double labeled cells in standard and enriched environments for both the VPA exposed (n =11) and control (n = 9) animal groups. Mean number of double labeled cells is located on the Y-axis and Environment on the X-axis. No significant difference was found for the interaction of group and environment ($p > .05$).

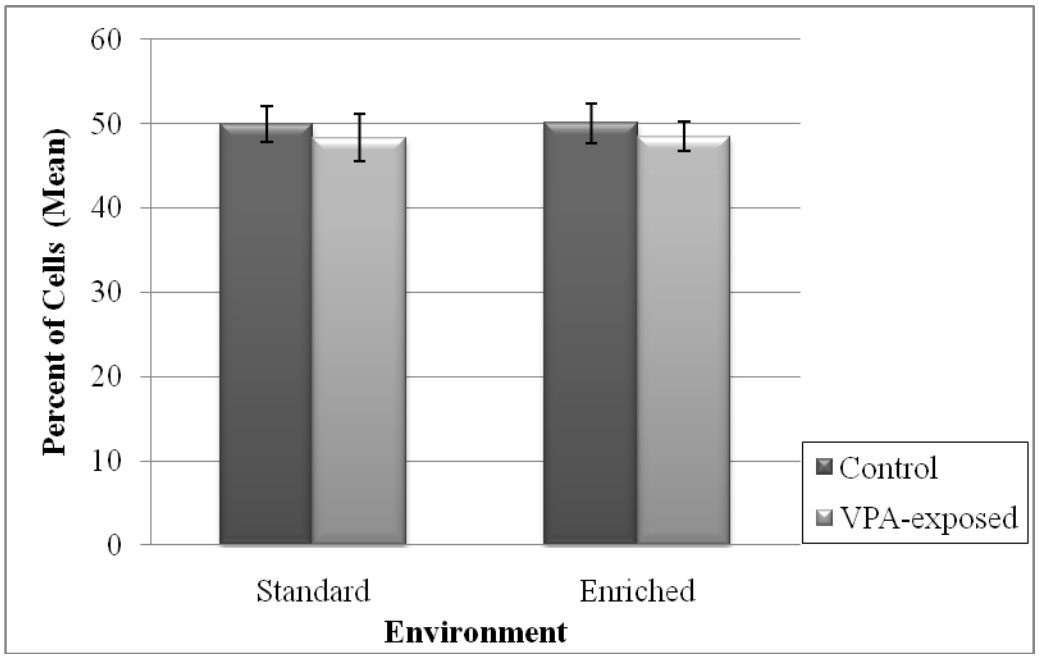


Figure 8. Interaction of group and environment on percentage of double labeled cells. Mean number (+/- SEM) of percent NeuN labeled cells in standard and enriched environments for both the VPA exposed (n =11) and control (n = 9) animal groups. Percent NeuN was calculated by dividing the number of NeuN labeled cells by the total number of BrdU labeled cells, then multiplying by 100. Percent NeuN labeled cells is located on the Y-axis and Environment is on the X-axis. No significant difference was found for the interaction of group and environment ($p > .05$).

Appendix I

Measles-Mumps-Rubella Vaccine Controversy

One of the more recent controversies associated with Autism is its reported link to the measles-mumps-rubella (MMR) vaccine. Most countries administer the first dose of this vaccine at 12-18 months of age, and the age of the second dose varies from country to country (Ortqvist et al., 2010). This vaccination is scheduled by the Public Health Agency of Canada to be given at 12 months of age and again at 18 months or between 4-6 years of age (Public Health Agency of Canada, 2006). Coincidentally, injections occur around the time of first symptom presentation and diagnosis of Autism. Claims of the connection between the vaccine and Autism were published in the Lancet in 1998 (Wakefield et al., 1998). Vaccination rates dropped dramatically because of this claim. This sparked a fury of research, which found no link between Autism and the vaccine (e.g. Taylor et al., 1999; Madsen et al., 2002). Despite compelling scientific research that there is no connection between the vaccine and Autism, parents are still refusing to have their children vaccinated (as reviewed by Brown et al., 2010), which puts the child at risk for potentially life threatening illnesses.

Appendix II

Rat Brain Atlas Figures

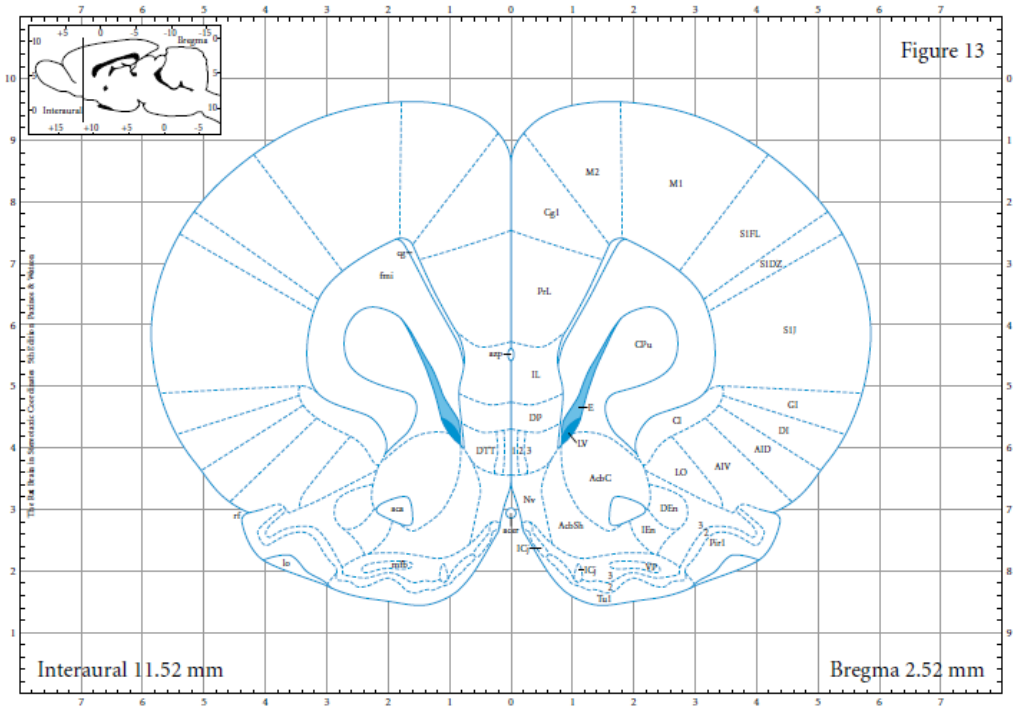


Figure 1. Rat Brain Atlas Bregma 2.52mm – Anterior Motor Cortex. Image based on 290g male Wistar rat. Cronal sections cut at 40mm thickness. Interval between atlas images is 0.12mm. Image was used to define region of interest (primary motor cortex – M1) by comparison of landmarks. Used with permission.

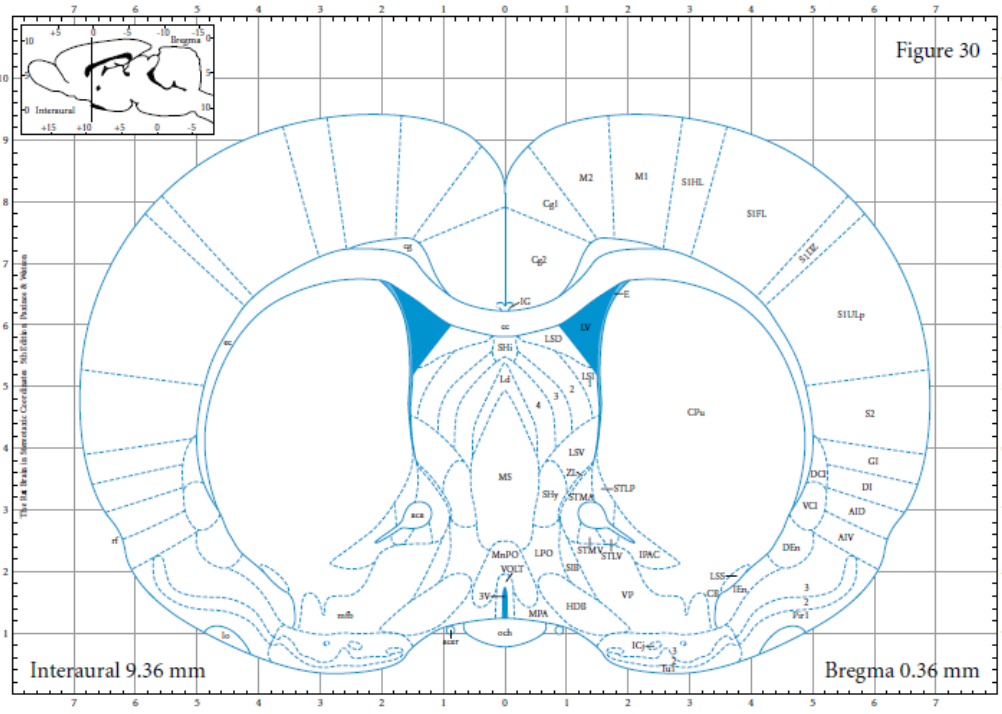


Figure 2. Rat Brain Atlas Bregma 0.36mm – Middle Motor Cortex. Image based on 290g male Wistar rat. Cronal sections cut at 40mm thickness. Interval between atlas images is 0.12mm. Image was used to define region of interest (primary motor cortex – M1) by comparison of landmarks. Used with permission.

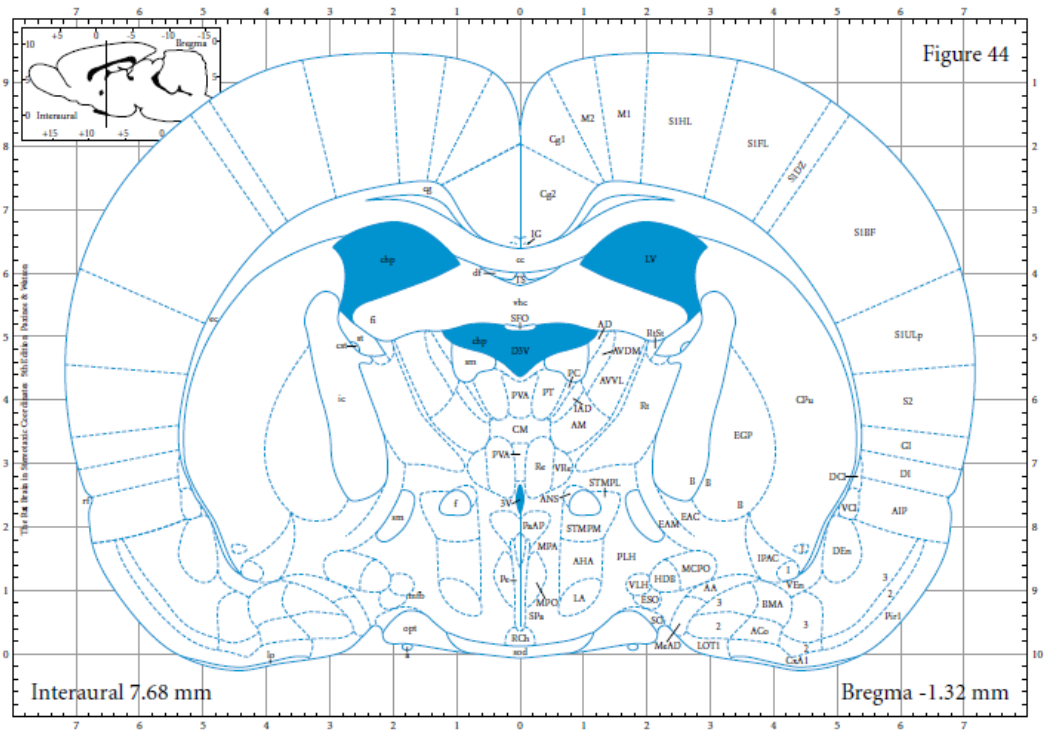


Figure 3. Rat Brain Atlas Bregma -1.32mm – Posterior Motor Cortex. Image based on 290g male Wistar rat. Cronal sections cut at 40mm thickness. Interval between atlas images is 0.12mm. Image was used to define region of interest (primary motor cortex – M1) by comparison of landmarks. Used with permission.

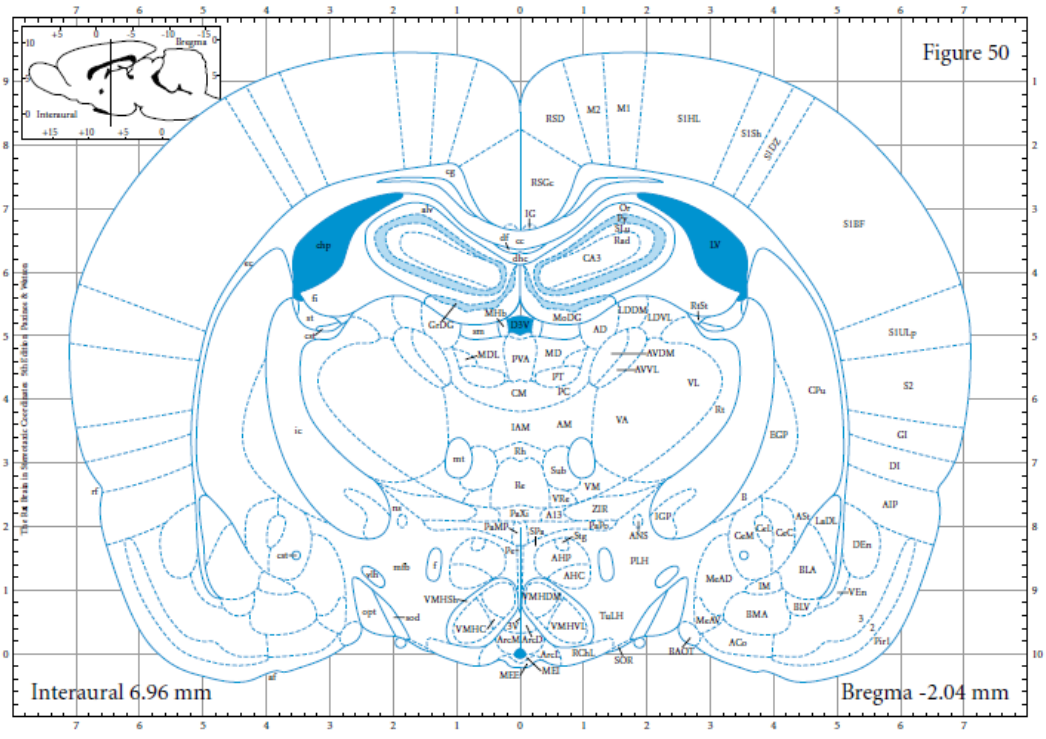


Figure 4. Rat Brain Atlas Bregma -2.04mm – Anterior Hippocampus (Dentate Gyrus). Image based on 290g male Wistar rat. Cronal sections cut at 40mm thickness. Interval between atlas images is 0.12mm. Image was used to define region of interest (dentate gyrus of the hippocampus - GrDG) by comparison of landmarks. Used with permission.

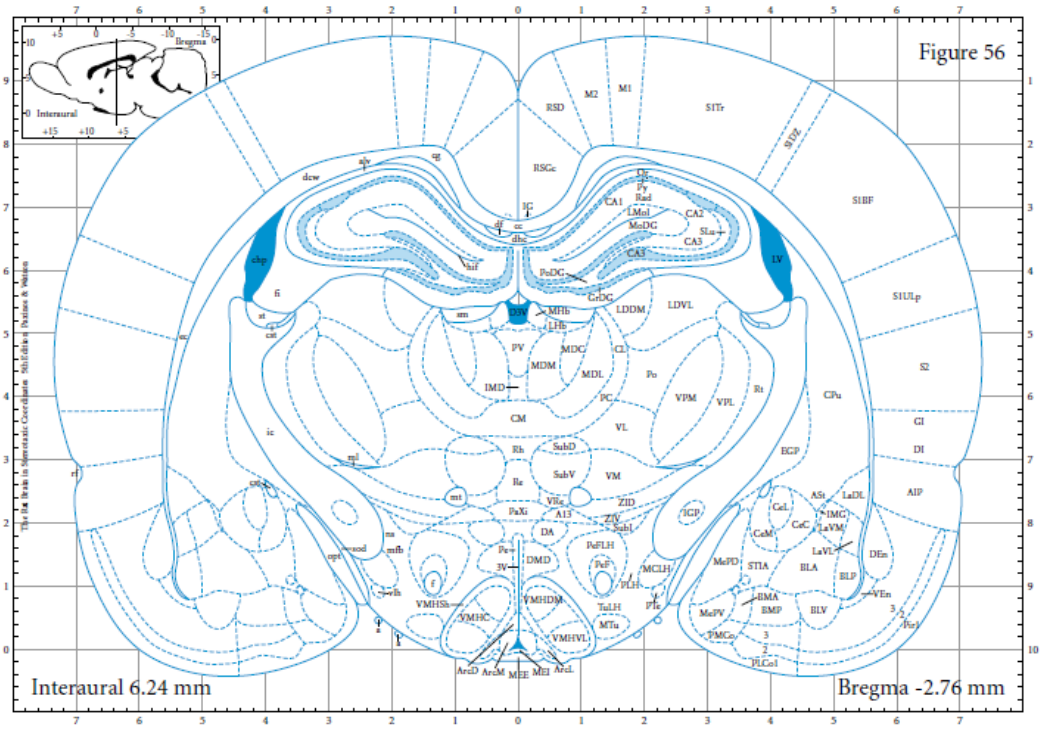


Figure 5. Rat Brain Atlas Bregma -2.76mm – Middle Hippocampus (Dentate Gyrus). Image based on 290g male Wistar rat. Cronal sections cut at 40mm thickness. Interval between atlas images is 0.12mm. Image was used to define region of interest (dentate gyrus of the hippocampus - GrDG) by comparison of landmarks. Used with permission.

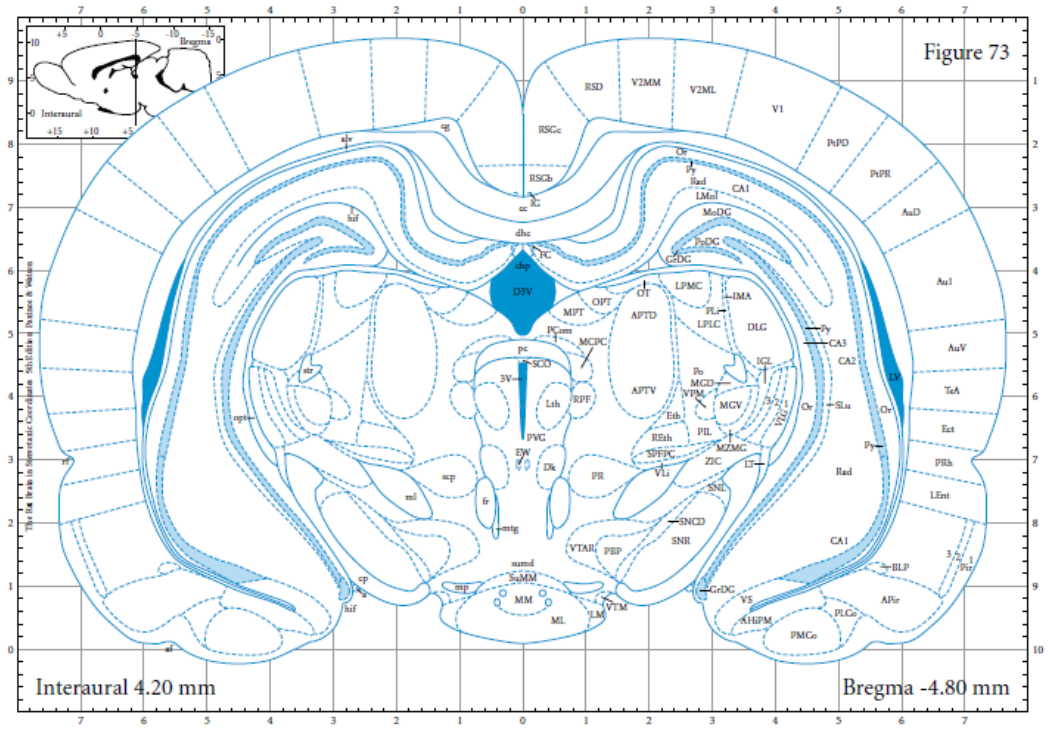
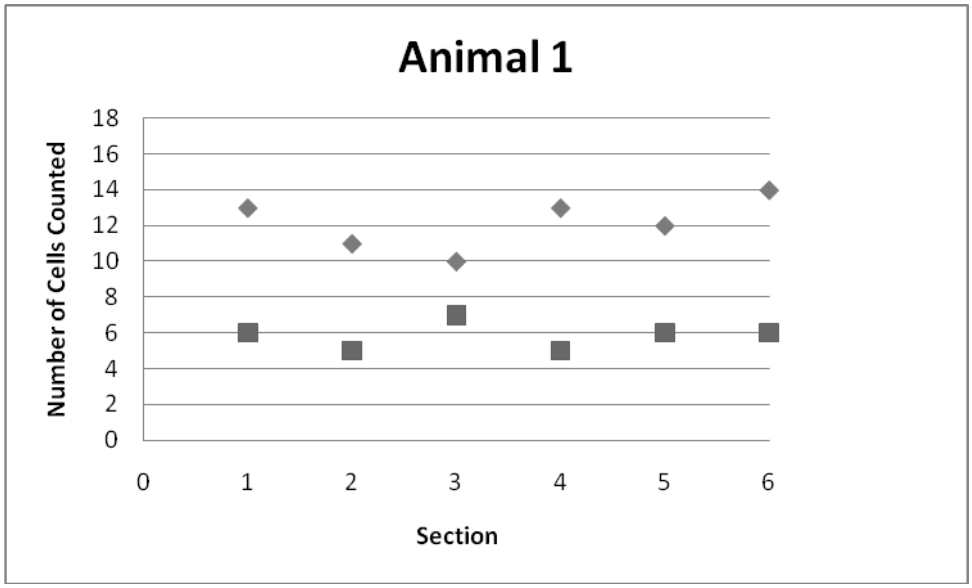


Figure 6. Rat Brain Atlas Bregma -4.80mm – Posterior Hippocampus (Dentate Gyrus). Image based on 290g male Wistar rat. Cronal sections cut at 40mm thickness. Interval between atlas images is 0.12mm. Image was used to define region of interest (dentate gyrus of the hippocampus - GrDG) by comparison of landmarks. Used with permission.

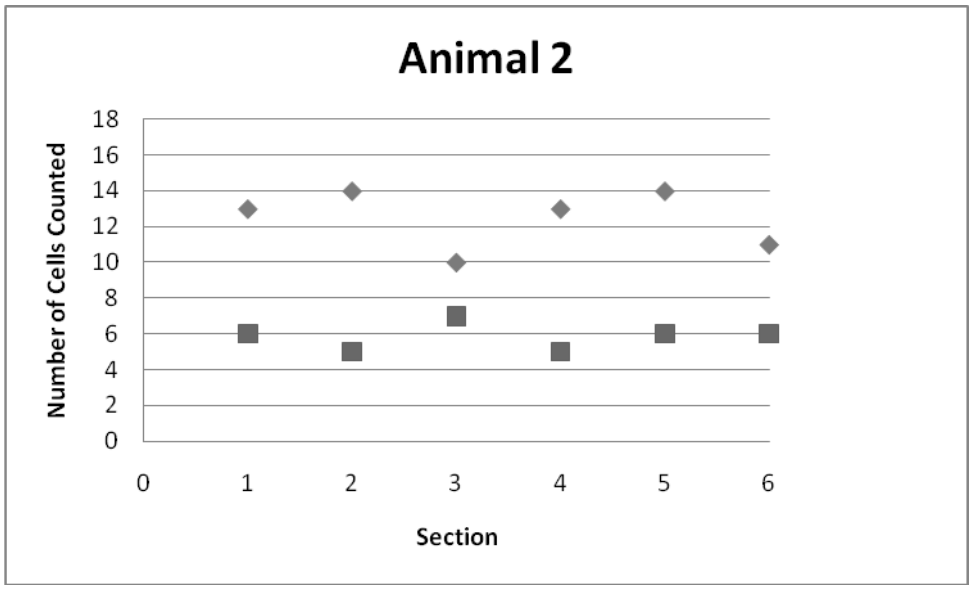
Appendix III

Raw Data

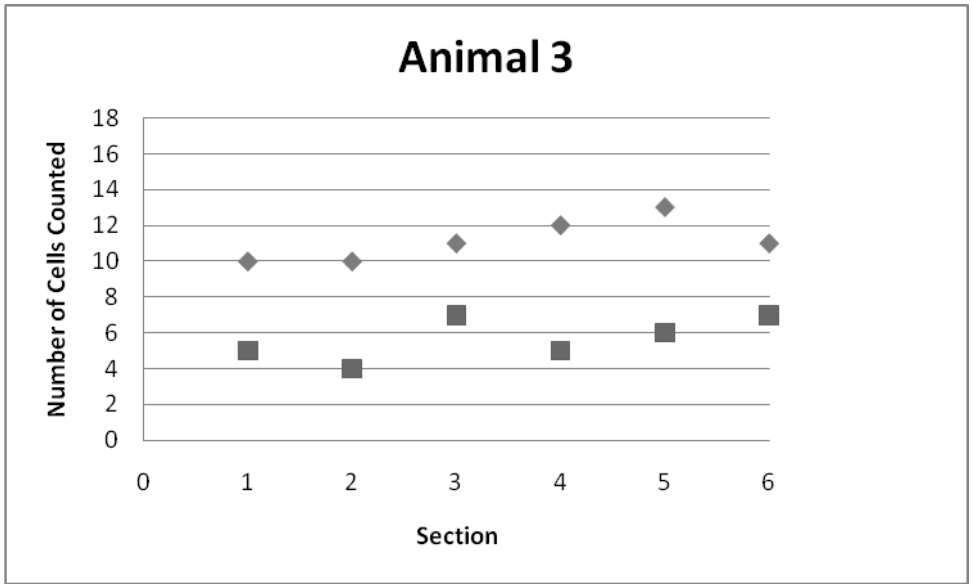
Diamonds indicate how many BrdU labeled cells were counted. Squares indicate how many double-labeled cells (BrdU-NeuN) were counted. Below each graph, the condition, environment, and sex for that animal is noted.



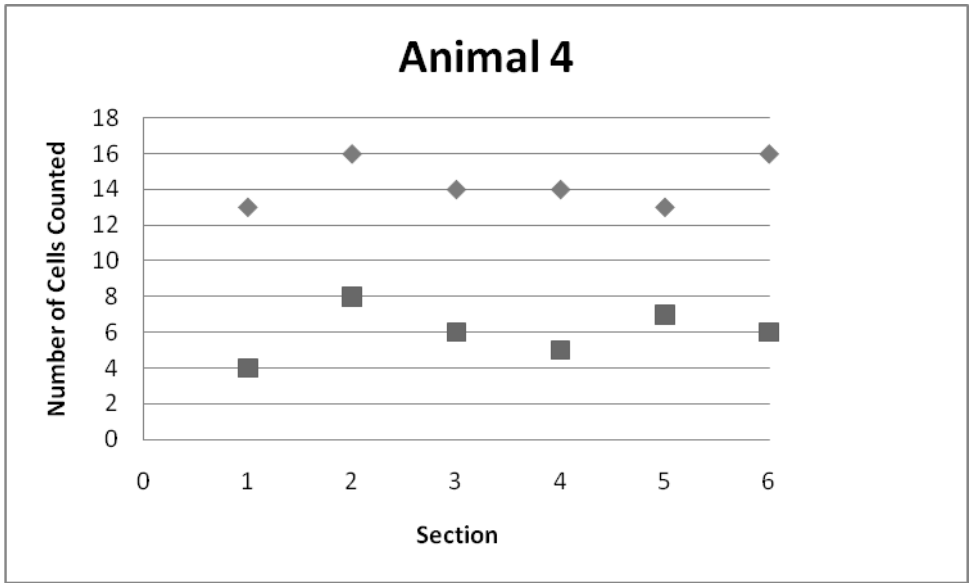
Control condition, Standard environment, Male



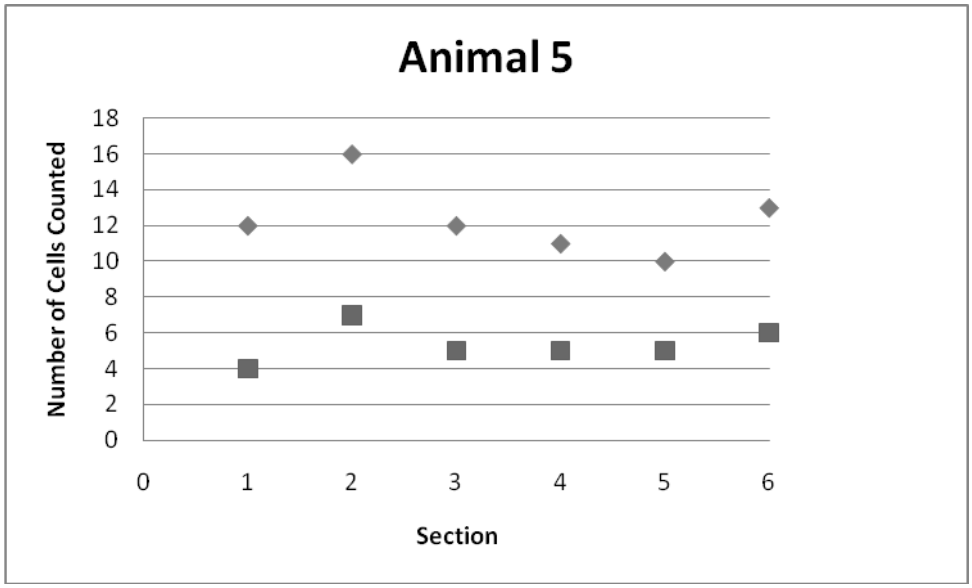
Control condition, Enriched environment, Female



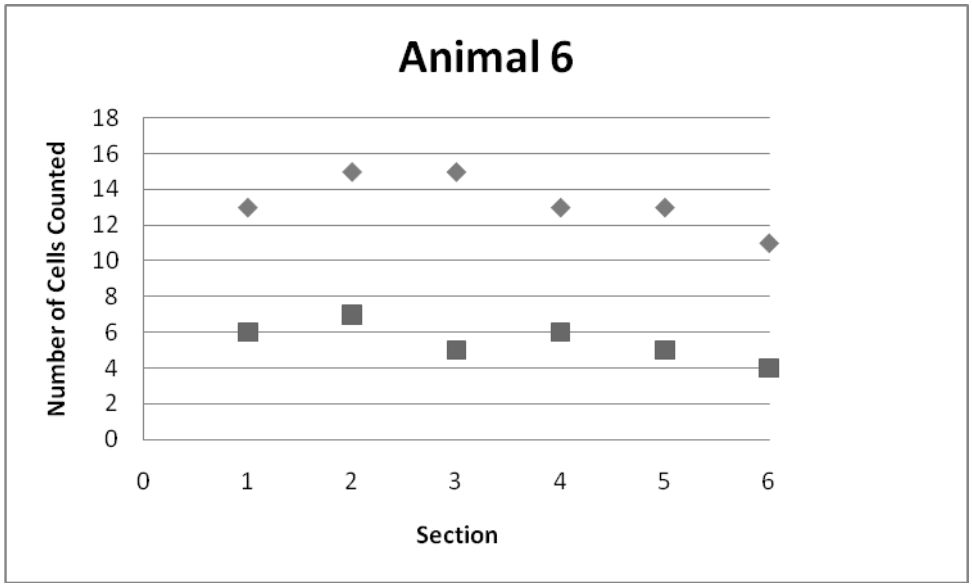
Control condition, Standard environment, Male



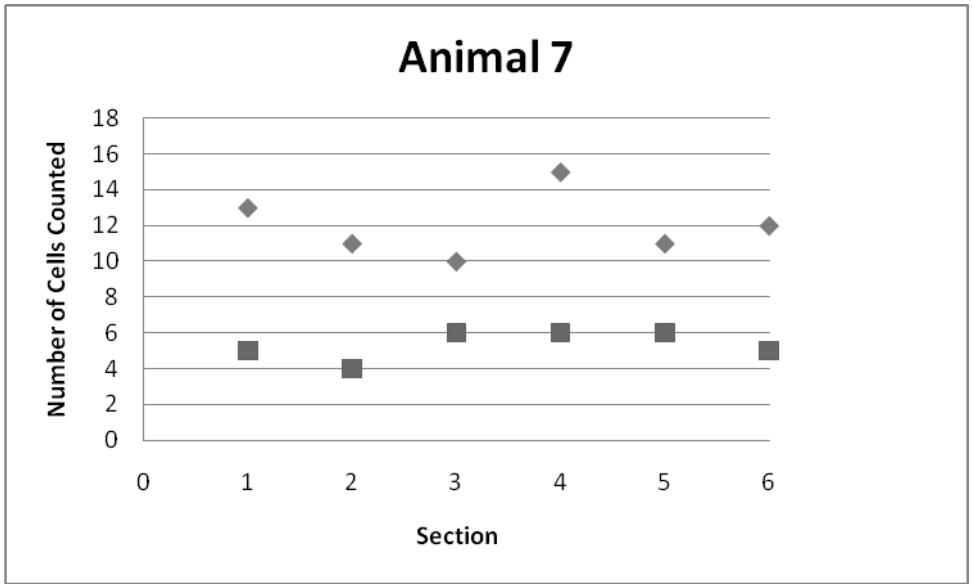
Control condition, Enriched environment, Male



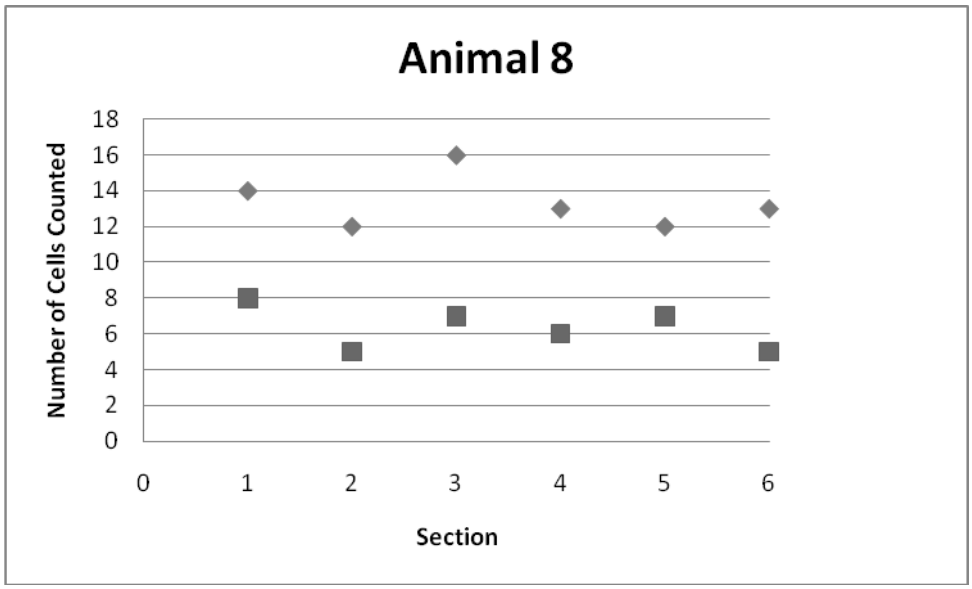
Control condition, Enriched environment, Female



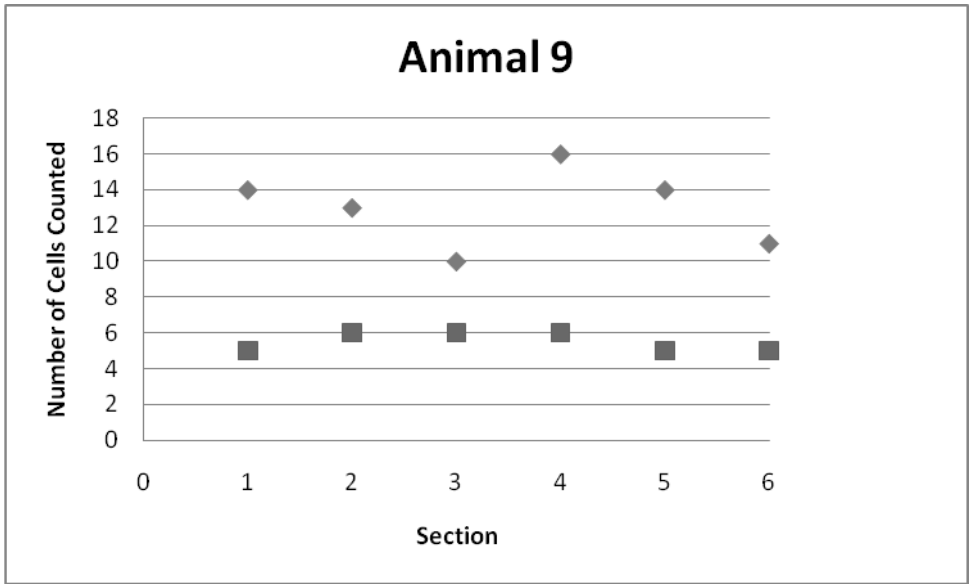
Control condition, Enriched environment, Female



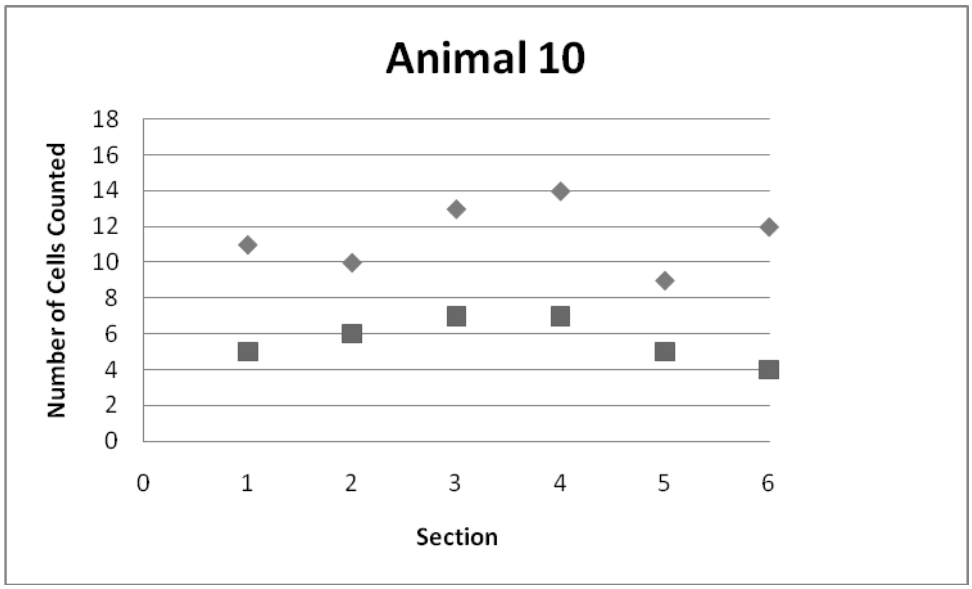
Control condition, Standard environment, Female



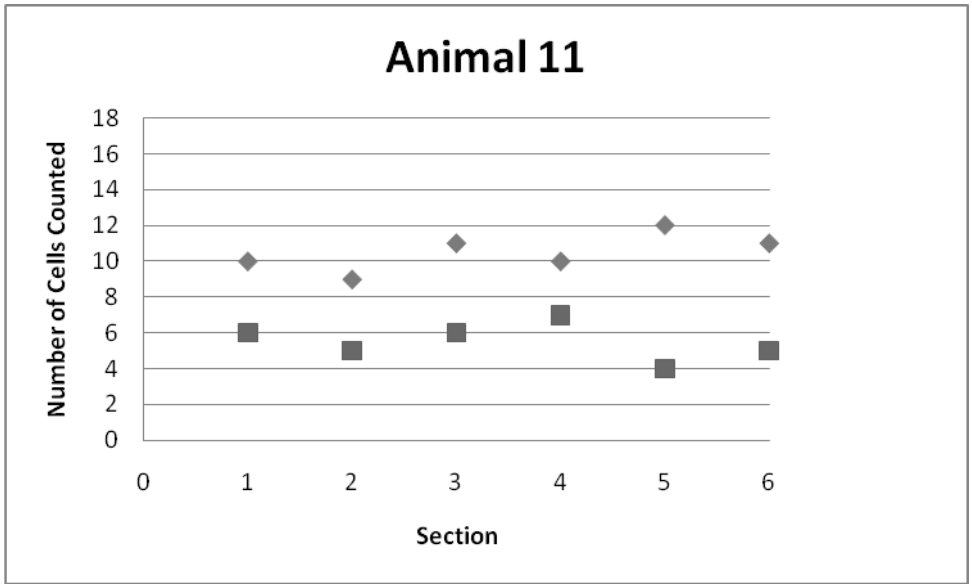
Control condition, Enriched environment, Male



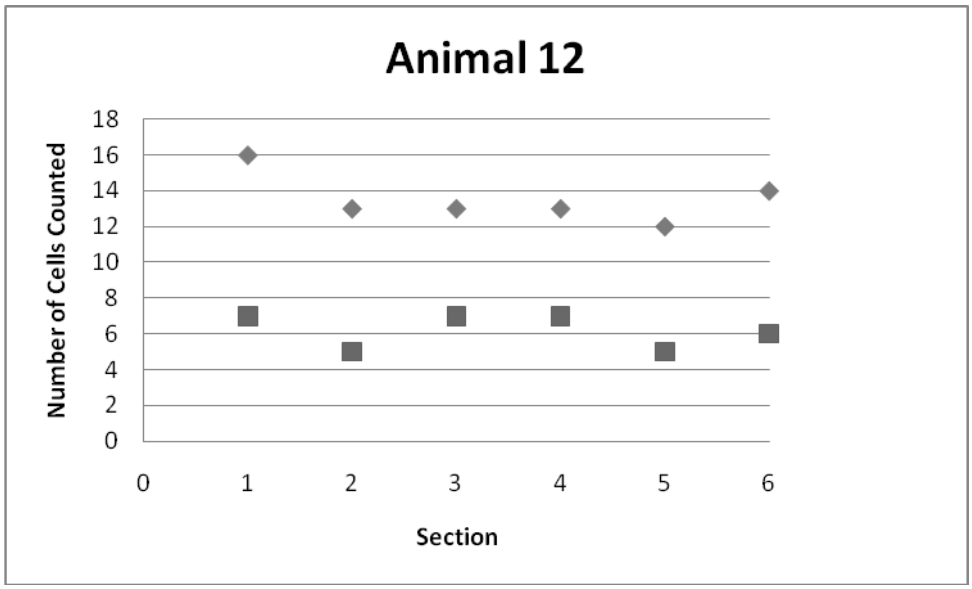
VPA condition, Enriched environment, Female



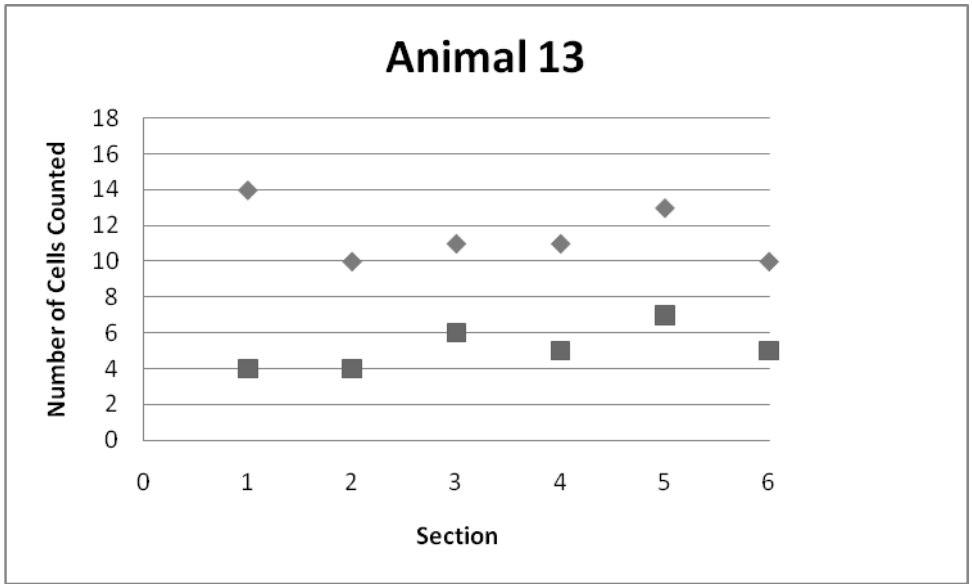
Control condition, Standard environment, Female



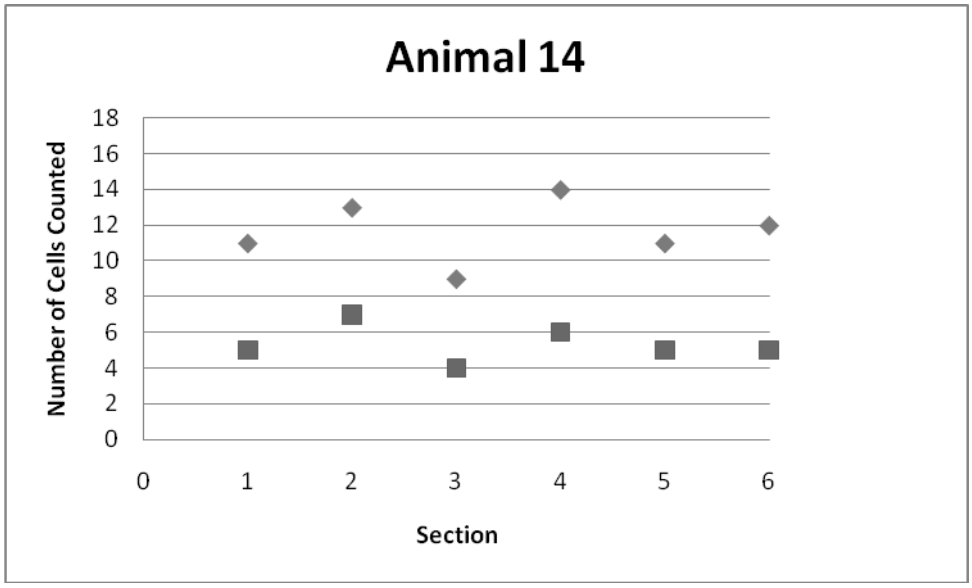
VPA condition, Enriched environment, Male



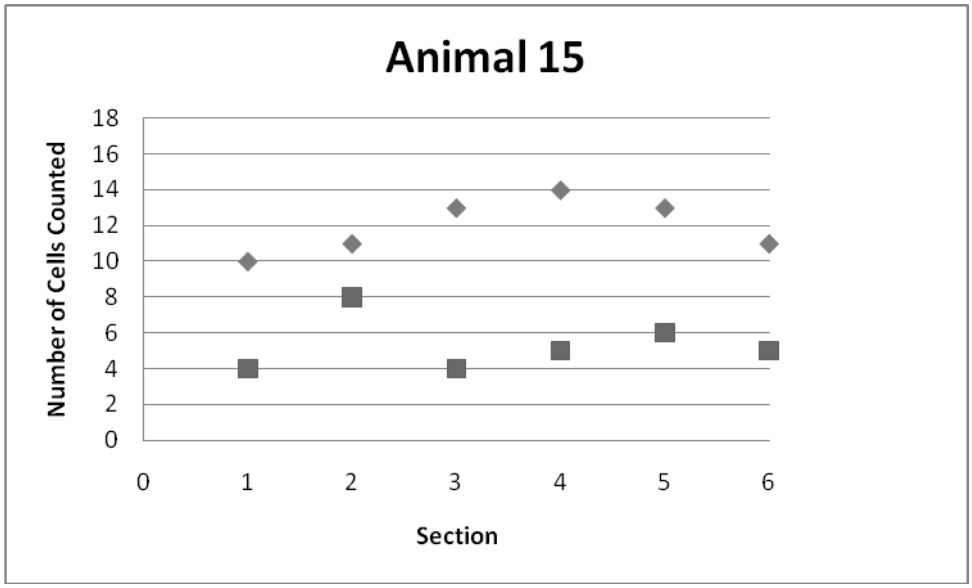
VPA condition, Enriched environment, Male



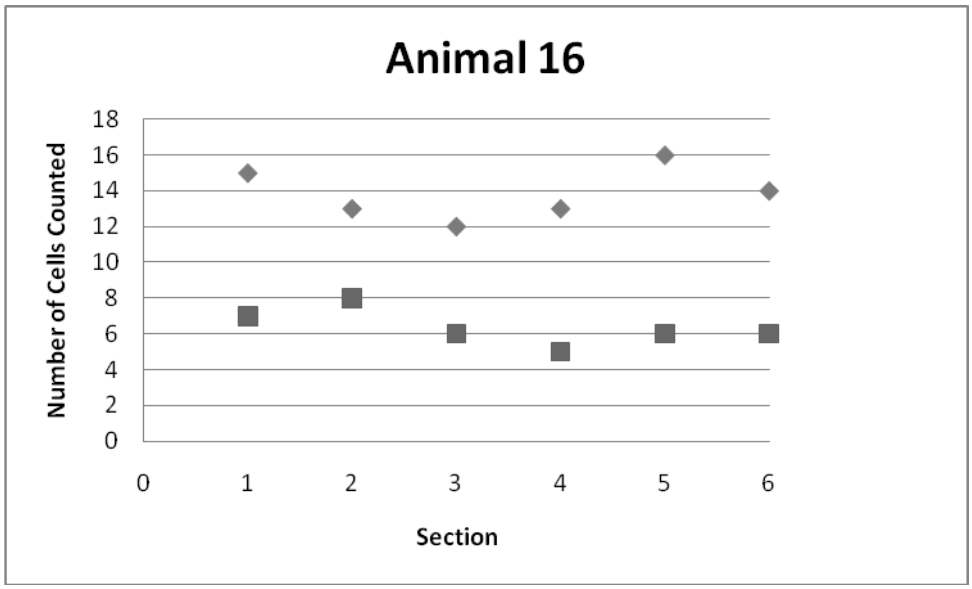
VPA condition, Standard environment, Male



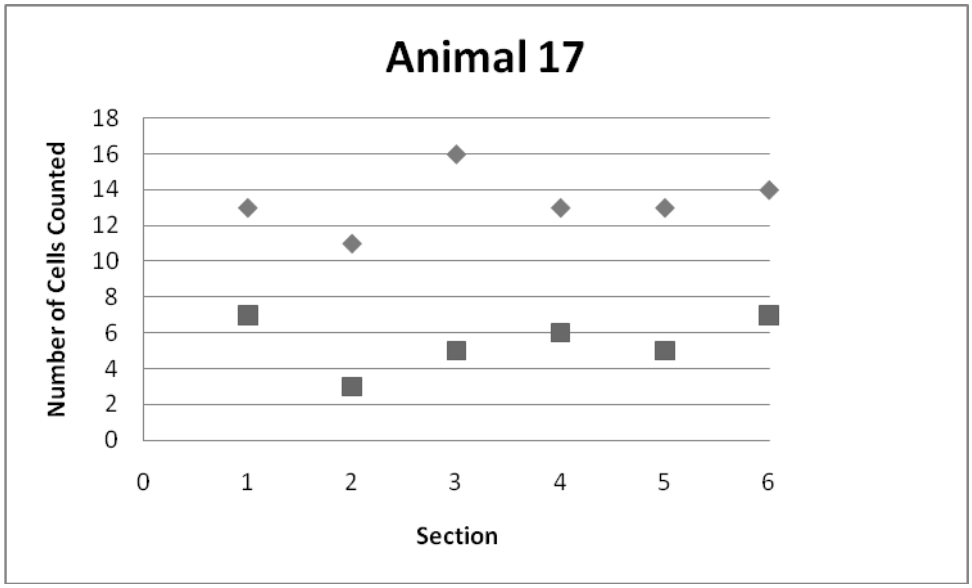
VPA condition, Standard environment, Male



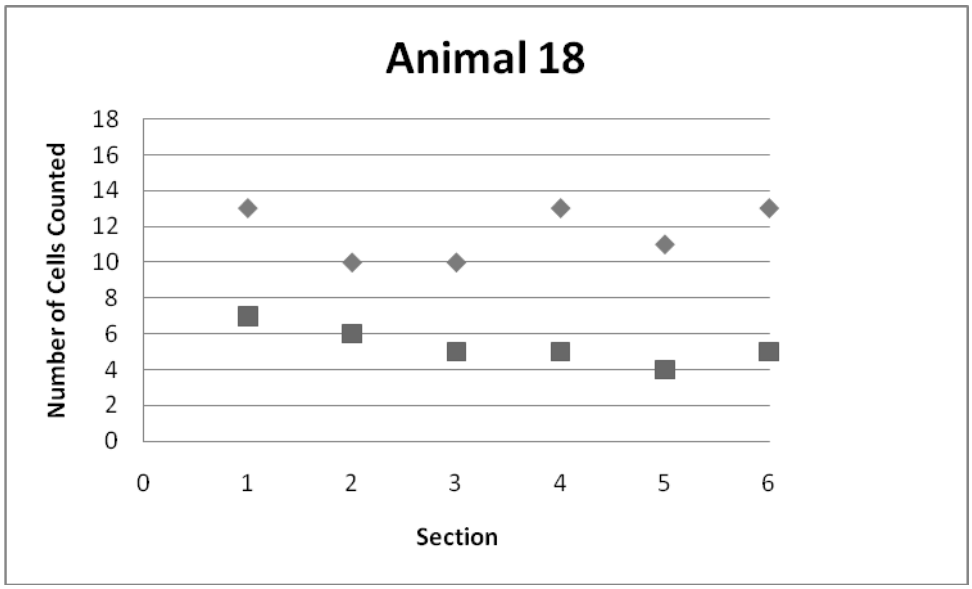
VPA condition, Standard environment, Male



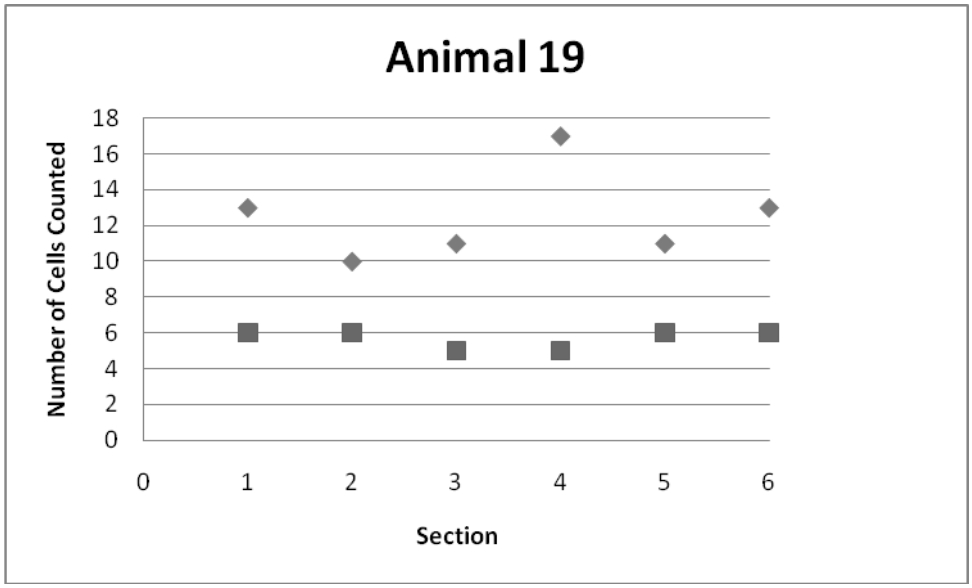
VPA condition, Enriched environment, Male



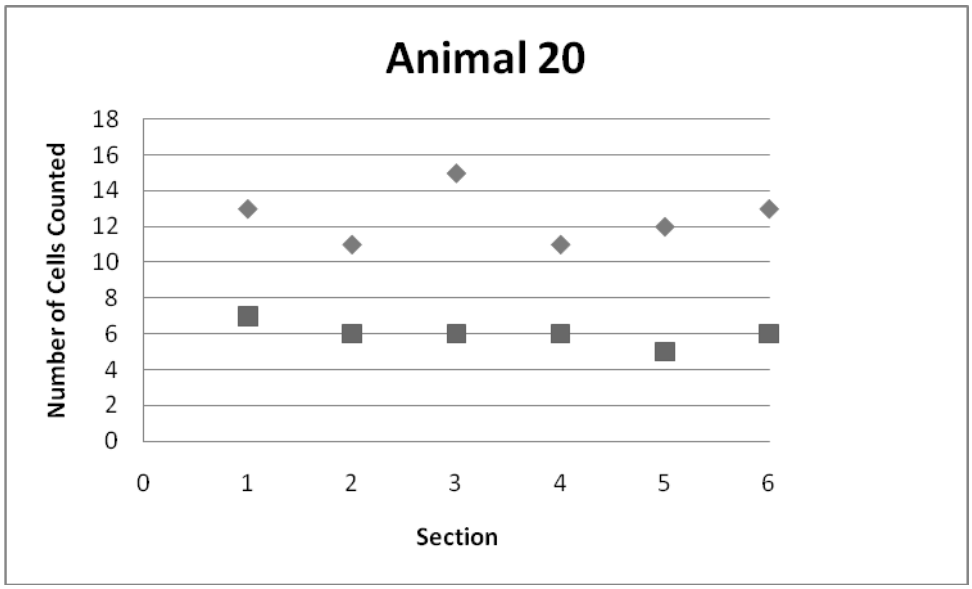
VPA condition, Enriched environment, Male



VPA condition, Enriched environment, Male



VPA condition, Enriched environment, Female



VPA condition, Enriched environment, Female

Appendix IV

List of Abbreviations

ASD	Autism Spectrum Disorders
BrdU	Bromodeoxyuridine
DSM	Diagnostic and Statistical Manual of Mental Disorders
FMR1	Gene that encodes for Fragile X Mental Retardation Protein – a mutation in which causes Fragile X Mental Retardation Syndrome
FMRP	Fragile X Mental Retardation Protein
IBI	Intensive Behavioural Intervention
MECP2	X-linked gene that codes for the production of MeCP2 protein
MeCP2	Methyl-CpG-binding protein 2
NeuN	Neuronal specific nuclear protein
PBS	Phosphate buffered saline
PDD	Pervasive Developmental Disorder
PDD-NOS	Pervasive Developmental Disorder Not Otherwise Specified
VPA	Valproic acid