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PLASTICITY AND AUTISM: BEHAVIOURAL AND ANATOMICAL CORRELATES OF A RAT MODEL OF AUTISM USING VALPROIC ACID IN UTERO

BY

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A Thesis submitted to

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In Partial Fulfillment of the Requirements for the Degree

of

MASTER OF ARTS

Department of Psychology

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Abstract

Autism is a developmental disorder that is etiologically diverse. The underlying syndrome, however, is assumed to be a result of early neural damage. An animal model of autism has been proposed that uses an antiseizure medication, valproic acid (VPA), to induce neuroanatomical correlates of autism in rats. The social behaviour of these animals, however, has not been characterized. Evidence of cortical pathology is also lacking. The goal of this research was to evaluate how social behaviour is affected in this model as well as its use as a model of damage from which to study neuronal plasticity. Female rats were given VPA on gestational day 12.5, which corresponds to a time point in human gestation that has been linked to the development of autism. A control group received saline injections. Social play behaviour of offspring was analyzed to determine the frequency of play solicitations, non-play social interaction (i.e. sniffing behaviour), and responses to both types of interactions. Morphology of cells in layer II of the somatomotor cortex was also examined in both groups to elucidate any differences in dendritic length after VPA exposure. No significant differences were found between the two groups on any of the

behavioural measures or on dendritic development in primary motor cortex. Behavioural impairments have been found in other studies using a higher dosage level of VPA. In conjunction with these studies, the present study indicates that VPA dosage level is critical in producing behavioural changes associated with autism. Cortical development, as assessed by dendritic length, is not affected at this dosage. The development of an animal model of autism will assist in the study of possible treatments, behavioural, or pharmacological, and may yield new insights into the brain's response to insult during early development.

PLASTICITY AND AUTISM: BEHAVIOURAL AND ANATOMICAL CORRELATES OF A RAT MODEL OF AUTISM USING VALPROIC ACID IN UTERO

Chapter 1

Introduction

The brain has an inherent ability to change, adapt, and reorganize in response to environmental experiences such as in development and learning, as well as to damage or trauma, as in stroke or brain injury. Over the last two decades, a great deal of knowledge has been gained about brain plasticity. The most important implication of the accumulated research has been a shift in the perception of the very nature of the adult brain from being a very static and rigid organ to one that shows a large capacity for adaptation. It was once believed that the developing brain had a monopoly on plasticity and that this plastic ability was punctuated by critical periods for neural development throughout the early years. There is growing knowledge to support the existence of plasticity in the adult brain, although quantitatively different from that seen in early development.

A vital assumption in the study of brain plasticity is that the same mechanisms within the brain account for the various types of plasticity, such as developmental plasticity, plasticity underlying learning, and plastic change after damage or trauma. The brain is assumed to have a redundant tendency, whereby the same mechanisms are initiated for structural and functional modifications in response to various environmental inputs. Thus, researchers can examine plasticity using a number of different paradigms.

Enriched Environments and Plasticity

Learning and environmental experiences have long been speculated to serve as catalysts for changes in behaviour, and thus, for the underlying changes in the brain. Among the first to research the impact of learning and experience on brain-behaviour relationships was Hebb (1947), who observed rats' behaviour after exposure to enrichment conditions. In a landmark study, one group of rats was blinded at birth by removal of the eyes, whereas a second group was blinded at maturity to assess the impact of timing on behavioural outcomes. The two groups were then tested using two protocols. The first protocol examined

"learning" by the rats by assessing the error scores on tasks that required extensive training before mastery could be achieved. Secondly, the same two groups were assessed using a "testing" approach, in which the tasks involved required very little in the way of training. Hebb found that the group blinded at maturity was significantly better during the testing trials than the group blinded at birth. No significant differences, however, were found in the learning trials. This illustrates the importance of critical periods in development and their influence on subsequent behaviour.

Hebb (1947) concurrently examined the impact of the environment on the behaviour of rats. He compared rats reared in laboratory cages to those reared in an enriched environment (his home, where they were able to run free a great deal of the time), and found that those raised in the more complex environment were better able to perform the "test" tasks than the caged group. The importance of Hebb's research lies in the fact that it was among the first to test the importance of experience on subsequent behaviour and development. Later researchers (e.g. Diamond, Lindner, Johnson, Bennett, & Rosenzweig, 1975; Greenough & Volkmar, 1973; Rosenzweig, Bennett, & Diamond, 1969) began

to examine the same environmental variables in relation to changes in the brain, which were assumed to account for the associated behavioural changes. This concept of behaviour affecting the structure of the brain, which, in turn, affects brain function and behaviour, is a key assumption in the study of plasticity today. The cerebral cortex is also assumed to be the most likely candidate for neuronal plasticity, as this is, in phylogenetic terms, the newest part of the brain (MacLean, 1990).

Bennett, Rosenzweig, and Diamond (1969) found an increase in cortical weight of rats reared in enriched settings versus those reared in isolation, with the greatest difference in the occipital region. Increases in cortical thickness (Rosenzweig & Bennett, 1978) have also been reported, providing initial support for what we now assume-that environmental enrichment does alter brain anatomy.

Another key assumption lies in what constitutes evidence of plasticity in the brain. Neurons contain the messengers of the nervous system and consist of cell bodies, axons, and dendrites. The majority of connections between neurons occur via axons that extend from the cell body and transmit information to synapses, where neurons

receive information via dendrites. Communication within the neuron down the axon is electrochemical, whereas communication between neurons at the synapse is chemical. For the purpose of investigating plasticity, one can look for structural changes at the synaptic level, which may be represented by dendritic changes. More dendritic material reflects more opportunities for synaptic transmission. Furthermore, dendrites have been shown to exhibit a high degree of responsiveness to changes in firing rates of and injury to other neurons, making them a good candidate for providing evidence of cortical alteration (Kolb & Whishaw, 1998).

Volkmar and Greenough (1972) were among the first to thoroughly examine changes in dendritic fields as a result of enriched environments. This study examined these effects in response to three housing conditions. Rats in the enriched condition (EC) were reared in groups of 12 in large cages containing various stimuli, with novel stimuli being introduced daily. The isolated group (IC) of littermates was housed in standard cages, and a third group of social littermates (SC) was reared in pairs in standard cages. The authors found a linear relationship between the amount of stimulation provided and the extent of higher-

order (orders three, four, and five) dendritic branching. Dendrites branching from the cell body were considered order one branching. Order two branching was further dendritic growth from the order one dendrites, and so on. Few differences were found in order one and two dendrites, or dendrites that branched from or near the cell body. Here, as in previous enrichment studies, the greatest amount of branching occurred within, but was not limited to, the occipital cortex.

Environmental conditions have also been shown to affect the morphology of the temporal cortex in rats. Greenough, Volkmar, and Juraska (1973) found a significant increase in branching of basal dendrites, which are the dendrites extending from the bottom of the cell body, of layers IV and V pyramidal cells of the temporal cortex in rats reared in enriched environments. Pyramidal cells are the principal cells of the cortex and are the major output cells of this structure (Kolb & Whishaw, 1998). Greenough and Chang (1989), using the same three housing conditions, also reported an increase in the number of dendrites for rats reared in the enriched condition. An increase in the number of dendrites increases the area available for the formation of synapses at the corresponding neuron, thus

strengthening the communicative ability of the neural network in question (Kolb & Whishaw, 1998).

Dendritic changes have been shown as a result of experience, not only in the adult mammalian brain, but also in the mushroom bodies of insects. In a recent study, Farris, Robinson, and Fahrbach (2001) examined the extent of dendritic branching of the Kenyon cells, which constitute the mushroom body, relative to age and experience in adult worker honeybees. Adult worker bees perform in-hive tasks for the first three weeks of development (nursing behaviour). Subsequently, foraging behaviour outside of the hive is prevalent. Age was accounted for by examining a group of same-age bees at different behavioural stages, from those with no foraging experience to highly experienced foragers. Those with the most flight and foraging experience exhibited more dendritic segments of the Kenyon cells than less experienced foragers. These differences were greater than could be accounted for by age alone. An increase in neuropil volume was also associated with increased flight experience, with a detectable and significant increase in volume noted after the first flight. These data not only suggest a substantial capacity for plasticity, but also

suggest that this plastic ability in response to environmental input is a primitive characteristic, one that has most likely been conserved through evolution due to its efficacy in promoting survival.

Motor Learning and Plasticity

The effects of environment on the brain and behaviour have been well documented. This impact is not exclusive to complex learning environments, but also to more specific tasks where motor learning of a particular behaviour or behaviours may occur. Structural changes have been found to occur in neurons of the hippocampus and motor cortex after self-stimulation rewarding experience involving motor learning (Shankaranarayana, Desiraju, & Raju, 1993). Electrodes were implanted in three groups of rats in the lateral hypothalamus, a structure of the limbic system important in motivated behaviours, and in the substantia nigra-ventral tegmental, which sends a large portion of its axons to the motor cortex. The self-stimulation (SS) group received training in pedal pushing to activate the electrodes and receive stimulation. The experimenter-administered electrical stimulation group (EA) was not allowed the opportunity for self-stimulation, but received

stimulation at the hands of the experimenter. The sham control (SH) group underwent implantation of the electrodes but did not receive any stimulation. A fourth group of rats, the normal control (NC) group, was not implanted with electrodes. In the SS group, dendritic branching occurred in the hippocampus, an area that plays a key role in learning and memory, as well as in layer V motor cortical pyramidal neurons. Similar structural changes were not observed in the EA group, providing support for the importance of self-stimulation, nor were such effects seen in the SH or NC groups. These structural changes were found to be persistent both 30 and 60 days post treatment (Shankaranarayana, Raju, & Meti, 1998).

The plastic ability of the brain is affected by many factors, including hormones and stress (Kolb & Whishaw, 1998). The perception of an experience as rewarding by the subject may be a factor in the magnitude of plastic change elicited. Robinson and Kolb (2001) found that rats allowed to self-administer cocaine for a one-month period showed increased dendritic branching as well as increased dendritic spine growth as compared to rats that worked for food. These structural changes were found in the nucleus

acumbens, a structure involved in reward and punishment (Robinson & Kolb, 2001).

Morphological changes within the cortex, including dendritic branching, may assist with the acquisition of a learned motor skill, possibly by assisting with reorganization of cortical representation. Kleim, Barbay, and Nudo (1998) demonstrated that skilled reach training produced a functional reorganization of the motor cortex. The skilled reaching condition (SRC) animals showed a greater cortical representation of the wrist and digit, contralateral to the trained paw, relative to animals in the unskilled reaching condition (URC). Functional reorganization has also been illustrated by research on the somatosensory area of the cortex. Fingers adjacent to an amputated digit show increased cortical representation (Gilbert, 1998). In essence, the remaining digits appear to utilize the available neural resources, which speaks to the efficient nature of the brain.

A number of other studies have elucidated plastic effects in the cerebellar cortex subsequent to motor learning. The cerebellum is known to play a role in motor activity. Kleim et al. (1998) reported an increase in the number of synapses in this area of the brain after rats

were trained in an acrobatic condition. Compared to rats that performed voluntary exercise and an inactive group, the acrobatic conditioned group exhibited a significantly greater number of parallel fibers, one of the two excitatory inputs of the cerebellar cortex, to the Purkinje cell synapses. These neurons constitute the single inhibitory output of this structure. This study differs from the aforementioned research, as in this case, evidence for plastic change involved increases in synapses rather than dendritic branching. Here, evidence of plasticity is found in modifications at the synapse, which is the major site of communication within the nervous system. Hence, an increase in the number of functional synapses within a neural network is assumed to improve the communicative ability and information processing ability of the brain.

In an attempt to determine if synaptic alterations of the cerebellar cortex endure without subsequent or continuous training, Kleim, Vij, Ballard, and Greenough (1997) trained one group of rats in an acrobatic condition (AC). Each rat in this group was pair-matched with an animal that performed a motor activity (MC). Pairs were then assigned to one of three additional training groups. One group received continuous training for 38 days; a

second group received training for ten days and was sacrificed 28 days later, with no additional training, whereas the third group was sacrificed after the initial 10-day training period. In all three of these training conditions, the AC group had significantly more synapses of Purkinje cells than the MC group, illustrating that motor activity alone is not in and of itself sufficient to produce plastic change and that motor learning is a prerequisite. No differences were observed in the number of synapses per cell as a function of the length of overall training received; animals that received the shortest training period had the same number of synapses per cell as did those that received continuous training. This suggests persistence in this rise in synapses, and thus, the corresponding motor skills, without the necessity for continued training.

Motor learning has also been shown to have a therapeutic effect on the motor abilities of adult rats after exposure to alcohol postnatally (Klintsova, Goodlett, & Greenough, 1999). On postnatal days four through nine, rats were given either alcohol (AE), a maltose/dextrin solution (gastrostomy controls; GC), or were allowed to suck as normal (SC). At six months of age, animals from all

three groups were randomly assigned to rehabilitative conditions (RC) or inactive conditions (IC). Subsequent testing of motor abilities demonstrated significant improvement for the AE group that received the rehabilitative condition. An increase in parallel fiber synapses per Purkinje neuron was also reported for the AE/RC group, lending support to the hypothesized resilience of neural plasticity. A rat's neural development is less advanced at birth than that of a human infant (Bayer, Altman, Russo, & Zhang, 1993). As such, experimental manipulations conducted on a rat during early postnatal days have effects that are similar in their potential developmental consequences to damage occurring during the third trimester in humans, making rats useful subjects for researching teratogenic effects at early developmental stages (Bayer et al., 1993).

To assess whether exercise alone induced the same rehabilitative effects, Klintsova et al. (1998) introduced a third condition of motor activity (MC). Results indicated that motor learning, not motor activity, was necessary for plasticity and therapeutic effects to occur. In a follow-up study using the aforementioned protocols, Klintsova, Scamra, Hoffman, Napper, Goodlett and Greenough

(2002) reported significant increases in the molecular layer of the paramedian lobule (PML) of the cerebellum after motor skill training in all three postnatal conditions. The AE group showed lower overall volume of the PML in comparison to the GC and SC groups. These results provide additional evidence that the adult brain is much more dynamic than previously thought.

Neural Damage and Plasticity

The study of how the human brain adapts and reorganizes in response to damage, as in stroke, traumatic brain injury, illness, or in cases of developmental pathology, has had significant implications for investigating normal brain functioning. When damage to particular areas of the brain correlates with certain behavioural deficits, it can be inferred that the areas in question contribute to normal brain functioning. This has become a key assumption in neuroscientific study, and to this end, the lesion method has been applied in laboratory settings to induce damage in vitro. A localist view of the lesion method argues that a direct relationship exists between a specific site of damage and the resulting behavioural deficits. Proponents of an aggregate viewpoint

take a more Gestalt position of the brain structures and behaviours, asserting that these structures have a much more complex and dependent interaction with one another in terms of behavioural outcomes (Moses & Stiles, 2001).

In support of the aggregate view of brain functioning, a number of studies have reported cortical modifications after injury in areas connected to the damaged area (Jones, Kleim, & Greenough, 1996), suggesting the integrated nature of brain plasticity. Regardless of the position one takes, the efficacy of studying damage as a means of understanding normal brain functioning and its dynamic nature has been well documented. An added advantage of this approach lies in the fact that morphological changes that accompany learning are often subtle, whereas damage induces similar changes but on a grander scale. Hence, examining plasticity using damage as the experiential catalyst has the advantage of elucidating effects that may not be detectable using learning paradigms.

Jones, Kleim, and Greenough (1996) found an increase in the number of synapses per neuron and increased dendritic volume of the layer V motor cortex contralateral to the lesioned forelimb area of the rodent sensorimotor cortex. These structural changes were time-dependent.

Insult in this area reduces the use of the contralateral forelimb, and increases the use of the ipsilateral forelimb (Jones & Schallert, 1992), illustrating the brain's response not only to damage, but also to the resulting compensatory behavioural outcomes and highlighting the brain-behaviour interdependence. It is important to note that the term recovery is often utilized quite broadly where cases of brain damage have resulted in behavioural deficits. Rather, true "recovery" may not be possible, but the compensatory behaviours lead to endpoints previously attained (Kolb & Whishaw, 1989).

An interesting finding in the work of Jones and Schallert (1992) is related to the onset of behavioural compensation and the time-dependent increases in cortical plasticity. The number of synapses per neuron in layer V 30 days post-lesion was increased compared to shams, however this was not the case at ten or 18 days post lesion. In contrast, dendritic volume in this area was shown to increase in lesioned animals compared to shams after 18 days only. No significant increases were detected at ten or 30 days. The onset of behavioural improvements in the use of the forelimb of rats, however, has been shown to occur one-day post lesion. This difference can possibly be

attributed to the methods used to identify these cortical modifications, which may not be sophisticated enough currently to detect subtle neural change.

The size of the lesion has a pronounced effect on behavioural outcomes in rats. Kolb and Cioe (2000) induced lesions of various sizes of the medial frontal lobe in rats on postnatal day two. Subsequently, the rats, upon reaching maturity, were trained on two tasks to measure behavioural endpoints reached after damage. The Morris water task (Morris, 1984), in which rats search for a platform disguised in clouded water, was used to assess spatial abilities. The Whishaw reaching task (Whishaw, Connor, & Dunnett, 1986) assesses use of the forepaw through a series of specific movements exhibited by normally functioning rats in their reaching behaviours. Lesion size was found to affect the extent of behavioural deficits, as smaller lesions resulted in less impairment of function. Lesion size, however, was not found to significantly affect the subsequent amount of dendritic branching or spine density of layer III pyramidal cells. Postmortem analysis of the brains of rats trained on these two tasks revealed a large reduction in both spine density and dendritic branching in both the large and small lesion

size group. In contrast, an effect of lesion size was shown for brain weights as well as cortical thickness. Larger lesions produced more pronounced decreases in both neural indices. Such early insult to the medial frontal cortex resulted in decreased sparing of function and decreased dendritic arborization. The authors noted the contrast of these results to previous work, where similar lesions induced later in development, between postnatal days 7-12, resulted in considerable behavioural improvements and extensive plastic change (Kolb & Whishaw, 1989). These data suggest that the brain's response to damage varies as a function of the timing of the damage.

The existence of critical periods in development has been demonstrated for a number of years. Kennard (1936) examined behavioural outcomes in monkeys after lesions to the motor cortex and found that sparing of function was greater when lesions were given in infancy as compared to similar lesions in adulthood. The assumption that the earlier the damage to the brain, the better the functional "recovery" came to be known as the Kennard principle. This principle assumes greater plasticity in the young brain versus the adult brain. The results of Kolb et al. (2000), however, contradict this assumption, as early frontal

cortical damage in the rodent resulted in a greater degree of abnormal morphological changes and less behavioural compensation than later-occurring damage.

The timing of not only the injury but also any rehabilitative efforts has been shown to play a critical role in functional recovery as well as morphological changes. Kozlowski, James, and Schallert (1996) lesioned rats in the forelimb representation of the sensorimotor cortex and subsequently cast either the impaired, nonimpaired, or neither limb. Data consisted of behavioural observations and analysis of the neural tissue. When movement was restricted in the nonimpaired limb, behavioural outcomes and dendritic growth in homotopic regions in the contralateral cortex were comprised, and the extent of neural damage was increased. When movements of the limb ipsilateral to the lesion were restricted, causing forces use of the damaged limb, behavioural deficits were much more extensive. Movement restriction of the impaired limb, however, did not result in any significant neural modifications, and behavioural deficits were only slightly greater than was the case in lesioned rats with unrestricted movement. Restricted movement of a limb in rats with no cortical damage did not result in any

detectable deficits in behaviour, nor was there evidence of neural change. This was among the first studies to show exaggerated cortical damage as a result of behavioural influences. It suggests that forced use of the damaged limb, which could occur as a result of rehabilitative therapies, can cause overstimulation of the living cells adjacent to the lesion and lead to a rapid deterioration of that hemisphere. Clearly, the study of the damaged brain has important implications for the development of treatment models.

The advantages of an enriched environment versus an "impoverished" one have been previously discussed in relation to their ability to induce plastic changes in the brain. Enriched environments have also been implicated in potential treatments after damage. Biernaskie and Corbett (2001) examined the effects of environmental enrichment coupled with task-specific training on behavioural improvement and compensation in rats after focal ischemic injury or strokes. The ischemic group housed in the enriched environments (IE) received daily rehabilitative training while the ischemic group housed in the standard housing (IS) did not. Rehabilitative treatment was delayed for 15 days post insult. Subsequent assessment revealed

that the IE group showed a 30% improvement in a stair-reaching task, and functional recovery was found in a beam-traversing task as compared with the IS group, which continued to show significant deficits. This treatment effect was noted at nine weeks after treatment. Since behavioural outcomes reached after damage often occur via increased reliance on the ipsilateral side, neural processes were examined in layer V pyramidal cells in the motor cortex contralateral to the damaged hemisphere. This area showed an increase in dendritic growth in the IE group over a sham-operated group as well as the IS group. Bury and Jones (2002) found similar neural changes in the contralateral motor cortex and improvements in behavioural outcomes as a result of an increased dependence on the non-impaired limb after task-specific training following unilateral lesions in the sensorimotor cortex.

Johansson and Belichenko (2002) also examined enriched environments and plasticity after ischemic injuries in rats. Again, evidence of neural change was found in the side contralateral to the site of insult, but in the somatosensory cortex. Increased spines were found in pyramidal cells in cortical layers II/III of ischemic rats housed in the enriched environments versus a group housed

in standard cages. These differences, however, did not extend to layers V/VI. Enriched environments led to an increase in spine density of pyramidal cells in all layers of the intact rodent somatosensory cortex but were found only in the more superficial layers in rats with ischemic injury.

The method chosen to damage the brain can affect the results. Watson, Dietrich, Busto, Wachtel, and Ginsberg (1988) developed an animal model of stroke, whereby rose Bengal dye is injected into the animal, and a high-wattage light, delivered through a fiber optic bundle in the brain area of interest, is turned on. This causes a reaction with the dye and produces a blood clot in the area, thus mimicking a thromboembolic stroke. The resulting lack of oxygen causes neural tissue death. The damage induced is consistently reproducible, noninvasive, and decreases the risk of death to experimental animals. These are advantages over other methods used to induce stroke, such as middle cerebral artery occlusion, which involves blocking the blood flow to the artery by tying it with a surgical thread (Ohlsson & Johansson, 1995). Such studies are key in the development of protocols for the treatment of human ischemic injuries and highlight the fact that

improving the efficacy of treatment necessitates the inclusion of environmental conditions and timing of delivery in development.

Long-term Potentiation and Plasticity

Long-term potentiation (LTP) has been used as a model of learning and memory for the past three decades since it was first discovered by Bliss and Lomo (1973). LTP is induced by artificially stimulating specific brain areas with electrodes. This repeated stimulation of neural circuits is hypothesized to mimic that which may occur naturally in cases of environmental learning and memory storage. As noted earlier, the method of communication within a neuron is electrochemical, and by monitoring electrophysiological output, the firing of cells can be detected.

Bliss and Lomo (1973) first demonstrated LTP in the rabbit hippocampus, a substrate thought to be important in learning and memory. They found that repeated tetanization of hippocampal pathways led to an increase in the efficiency of these pathways, which outlasted the duration of the testing procedure. Numerous studies have since used LTP as a paradigm for the study of plasticity, including

Engert and Bonhoeffer (1999), who found that LTP in the hippocampus resulted in increased spine growth. Weeks, Ivanco, LeBoutillier, Racine, and Petit (1998) found a positive correlation between the degree of LTP induced and the number of synapses per neuron present in rat hippocampus, providing additional evidence that LTP produces neural alterations.

To determine if LTP produces morphological changes in cortical areas, Ivanco, Racine, and Kolb (2000) induced LTP in the sensorimotor cortex of freely moving rats via tetanization of the corpus callosum, which is a bundle of neural tissue connecting the left and right hemispheres. Postmortem analysis revealed an increase in dendritic branching as well as in spine density in Layer III pyramidal cells of the sensorimotor cortex over control animals. LTP was produced in both hemispheres as a result of the location of tetanization. This study provides support for the utility of LTP as a model of learning, as comparable neural changes have been found in animals housed in enriched environments (Kolb & Whishaw, 1998).

The aforementioned studies using LTP differ in the neural pathways involved. The hippocampus is thought to be involved in short-term, rapid, temporary information

storage, while the neocortical areas contribute to long-term storage (Ivanco & Racine, 2000). Hippocampal induction has been shown to occur at a faster rate than induction in the neocortex (Ivanco, Racine, & Kolb, 2000). The existence of different induction rates of LTP in these regions provides further support for LTP as a model of not only learning, but also information storage. In addition, these data support consolidation theories of memory, whereby the hippocampus acts as the site of short-term storage, whereas the cortex is associated with long-term memory storage (Fries, Fernandez, & Jensen, 2003).

Neurogenesis and Plasticity

The adult brain has long been considered static in terms of its capacity for neural proliferation. In recent years, however, a growing number of studies have supported the concept of neurogenesis as a means of generating plastic change within the adult brain. This phenomenon has been well documented in the rodent brain (Kuhn, Palmer & Fuchs, 2001), and recent research has shown neurogenesis in other species of animals.

Environmental conditions have been shown to affect the rate of neural growth. Kempermann, Kuhn, and Gage (1997)

found that mice housed in enriched environments had more neurons as well as more glial cells, the supporting cells of the central nervous system, in the dentate gyrus of the hippocampus than did those housed in standard laboratory settings. The survival of these newly developed cells was also increased in the enriched group. Behavioural assessment also showed that the mice exposed to complex environments performed better in a Morris water maze, presumably as a result not only of the increase in neurons but also in synapses and dendrites, collectively increasing the efficiency of this neural circuitry in communication.

Neurogenesis within the dentate gyrus of the hippocampus has also been reported in Old World primates (Gould et al., 1999). This proliferation was not exclusive to the younger primates but was also evident in 23-year-old animals, although to a lesser extent. Eriksson et al. (1998) reported that humans have the same regenerative ability in the dentate gyrus. Thus, the data support the notion of neurogenesis as a mechanism of plastic change in mammals.

Although the evidence of neurogenesis demonstrates structural change within the brain, resulting functional changes in behaviour are still speculative. The existence

of newly developed neurons does not provide evidence that these neurons are functional. As noted earlier, the utility of a neuron lies in its ability to communicate with other neurons. Thus, neurogenesis does not necessarily imply an increased efficiency of neural networks, as evidence is needed to determine if the newly-generated neurons have the ability to fire. More research is needed to discover the functionality of these neurons and their implications for plasticity.

The alleged ability of the mammalian brain to generate new neurons, in addition to its capacity for synaptogenesis and dendritic changes, have added to the growing knowledge of neural plasticity. A multidisciplinary approach, examining these changes in response to enriched environments, motor learning, and trauma or injury, has increased the understanding of the brain's striking capacity for adaptation, in both the young brain and the adult mature brain. The examination of the damaged brain has enhanced our understanding of the functionally intact brain, yet our knowledge of the brain's plastic ability is insignificant relative to what remains undiscovered. Autism, a behavioural syndrome assumed to result from early neurological damage, provides researchers with a model for

examination of the effects of early insult to the brain across the lifespan and subsequent behavioural effects on the individual.

Chapter 2

Autism

Autism is a pervasive developmental disorder, and, according to the latest epidemiological study in Canada, affects one in 1000 children (Bryson, Clark, & Smith, 1988). The prevalence rates vary across different countries, but recent epidemiological data indicate that these rates have increased 300% in the last 30 years (Fombonne, 2003). The rise and reliance on chemicals, including drugs, in our environment suggest a higher exposure level to toxins that may influence the development of the fetus and may be a contributing factor in the increases seen in developmental disorders (Bertrand, Mars, Boyle, Yeargin-Allsopp, & Decoufle, 2001; Chakrabarti & Fombonne, 2001; Fombonne, 2003). Autism affects males at a rate 4 times higher than females (Bryson et al., 1988), however the symptoms of females with the disorder tend to be more severe (Huebner, 1992).

Symptoms of the disorder essential for diagnosis include impairments in three key areas: i) social interaction, with a lack of social reciprocity, impairment in use the of nonverbal communication, and decreased social play behaviour; ii) verbal communication and language

usage, ranging from a delay in language acquisition to mutism (Peeters & Gillberg, 1999); and iii) behavioural patterns, interests, and activities that are repetitive, restrictive, and stereotyped, such as hand flapping or a preoccupation with objects (Fein, Pennington, Markowitz, Braverman & Waterhouse, 1986). Symptom onset in one of the categories must occur before the age of three (Diagnostic and Statistical Manual of Mental Disorders, 4th ed., 1994). Widespread variation exists in the degree of impairment; those diagnosed may have considerable functional abilities and intellect, whereas others afflicted suffer from debilitating impairments, making independent living unattainable (Peeters & Gilberg, 1999). The majority of those afflicted exhibit moderate to severe impairment (Gray & Tonge, 2001).

Etiology & Risk Factors

Since it was first described by Kanner in 1943 as early infantile autism, much research has been done in efforts to determine the etiology(ies) and resulting neuropsychological impairments. Traditionally, behavioural symptoms associated with the disorder were assumed to be a consequence of the child-rearing abilities of cold and

distant parents (eg. Bettleheim, 1967). Some treatments in use only a decade ago were based on the assumption that the etiological basis is not insult to the central nervous system but relational problems (Zappella, 1990). The disorder has a high comorbidity rate with a number of neurological and medical conditions including mental retardation (Fragile X), blindness, deafness, and epilepsy (Bauman & Kemper, 1994), leading some to propose that the basis of autism is neurobiological and stems from some form of insult (Rodier, Ingram, Tisdale, Nelson, & Romano, 1996).

Although it is now widely agreed that brain insult is the precipitating cause of autism, the precise mechanism(s) underlying this insult is (are) not fully understood. Autism appears to be etiologically diverse, as exposure to mercury (Enayati, Redwood, Roger, & Binstock, 2001), teratogenic agents, including thalidomide (Stromland, Nordin, Miller, Akerstrom, & Gilberg, 1994) and valproic acid (Williams, Cunningham, Stephan, Kerr, & Hersh, 2001), as well as perinatal brain-affecting viral infections, such as rubella virus and cytomegalovirus (Pletnikov, Rubin, Vasudevan, Moran, & Carbone, 1999), have all been proposed as potential risk factors for the development of the

disorder. A genetic component exists for the disorder, as evidenced by the higher incidence rate among siblings. This link, however, is not a direct one that is attributable to a single gene, as the concordance rate for monozygotic twins is not 100% (Rodier, Ingram, Tisdale, & Croog, 1997), suggesting multiple genetic interactions (Akshoomoff, Pierce, & Couchesne, 2002).

The identification of several risk factors, such as sex, genetic liability, and exposure to teratogens, coupled with the diverse nature of symptoms in those diagnosed, suggests that a number of factors contribute to the phenotypic expression of the disorder. The general presentation of symptoms is similar, but the specific combination of risk factors involved likely changes the overall quality of the disorder in each case.

Neuroanatomical Abnormalities

Research into the causes of autism has been hindered by the heterogeneous expression of symptoms in autism, suggestive of multiple focal areas of insult (Acosta, 2003; Bailey et al., 1998; Rodier et al., 1996). This difficulty is compounded by the subtlety of damage associated with autism, as few gross neuroanatomical anomalies have been

reported (Acosta, 2003; Rodier et al., 1996). Additionally, no distinct pathology related to all cases of autism has been reported (Bailey et al., 1998). Overall brain volumes appear relatively normal in postmortem examination of autism, although cases of megaencephaly and microencephaly have been reported (Askshoomoff et al., 2002).

One neuroanatomical finding that is well documented is abnormalities of the cerebellum (Bailey et al., 1998; Bauman & Kemper, 1985; Courchesne, Yeung-Courchesne, Press, Hesselink, & Jernigan, 1988; Courchesne, Hesselink, Jernigan, & Young-Courchesne, 1987; Hashimoto et al., 1995; Ingram, Peckham, Tisdale, & Rodier, 2000; Pierce & Courchesne, 2001; Sparks, Friedman, & Shaw, 2002), which have been found in ninety-five percent of autopsies of autistic individuals (Allen & Courchesne, 2003). This brain structure, located posterior to the brain stem, has long been assumed to be primarily involved in motor activities such as coordination, gait, and posture (Allen & Courchesne, 2003). Cerebellar abnormality in autism includes cases of both hyperplasia, resulting in greater cell packing density, as well as hypoplasia (Ingram et al., 2000), defined as a permanent decrease in neuronal number

of an area (Bayer et al., 1993). The latter, however, is the dominant finding and is characterized by a reduction of Purkinje cells of the posterior vermis portion on the midline of the cerebellum (Ingram et al., 2000). These cells constitute a primary class of cerebellar neurons and are the exclusive source of output for the cerebellar cortex (Allen & Courchesne, 2003). Damage to the vermis is most closely associated with difficulty with gait and adjustment of posture (Thach, Goodkin, & Keating, 1992).

Other areas of the cerebellum have been found to show pathology. Evidence of atrophy of the cortex of the cerebellum (neocerebellum), with a reduction in both Purkinje and granule cells, has been described (Bauman & Kemper, 1985). This cerebellar cell loss has been reported in the absence of gliosis (Bauman, 2001), which is indicative of neuronal death. Gliosis occurs when neural space is filled with supporting cells called glia in areas previously inhabited by neurons after their death. Neuronal loss in the cerebellum, as a result of maldevelopment, would not lead to this gliosis, as the absence of cells would not be due to death but to other factors during development, such as impaired neuronal migration (Bauman, 2001). Migration of these cerebellar cells begins in the

eighth week of gestation in man and embryonic day 15 in the rodent (Bayer, Altman, Russo, & Zhang, 1993). If the loss of cells resulted from insult, and hence, cell death, gliosis would be expected. These findings suggest that cerebellar pathology related to autism may occur at an early point during development rather than as a result of an acquired lesion (Bauman, 2001).

Despite the consistency of findings regarding cerebellar morphology in autism, contradiction exists regarding the direction of abnormality, as both hypoplasia and hyperplasia have been found in cases of autism (Ingram et al., 2000). A reduction in the number of cells within a given structure can be assumed to decrease that structure's communicative ability and cause dysfunction, however, greater cell density and cell packing within a structure may also contribute to impairment (Waterhouse, Fein, & Modahl, 1996). In addition, other studies have not found similar anatomical abnormalities associated with the cerebellum. Errors in measurement, insufficient control groups, and small sample size have all been cited as potential sources of this discrepancy (Akshoomoff et al., 2002).

The growing evidence for cerebellar pathology in autism suggests that dysfunction of this structure is a critical feature of the disorder. How this neuroanatomic finding relates to the deficits in higher-order capacities such as language and socialization, and the diverse presentation of symptoms, however, has yet to be elucidated. One possible explanation relates to the functionality of the cerebellum. As stated, the cerebellum is most closely linked to motor behaviours. New technology that allows functional imaging of the brain, such as functional magnetic resonance imaging (fMRI), has expanded the postulated role of the cerebellum; evidence suggests it has a much more diffuse role in human behaviour, including mediation of cognitive processing (Akshoomoff & Courchesne, 1992) and attention (Allen & Courchesne, 2003). In fact, cognitive impairments in attention are frequently exhibited in autism (Courchesne et al., 1994).

One of the principal behavioural symptoms in autism is a restriction in the range of interests, behaviours, or movements, and exploratory behaviour is consequently often absent or limited (Pierce & Courchesne, 2001). To assess the role of the cerebellum in exploratory behaviour, Pierce and Courchesne (2001) used magnetic resonance imaging to

document the degree of cerebellar hypoplasia in the vermis. The same children were then rated in terms of time exploring a novel environment as measured by duration of exploration, number of containers explored, and motor activity. Children with autism spent substantially less time in exploratory behaviour than control children, and this temporal difference was negatively correlated with the magnitude of hypoplasia. This differentiation was independent of overall motor activity, which did not significantly differ between the two groups.

Allen and Courchesne (2003) used functional magnetic resonance imaging to observe cerebellar activation during a selective attention task and during a motor task and found that the cerebellar cortex of individuals with autism showed decreased activation during the attention task but increased activation during the motor task when compared to healthy controls. These researchers previously demonstrated that regions of the cerebellum differentially respond to aspects of attention and motor involvement (Allen, Buxton, Wong, & Courchesne, 1997).

The cerebellar damage seen in autism appears to involve aberrant neural circuitry and is likely to have a detrimental effect on the functioning of interdependent

structures and systems (Carper & Courchesne, 2000). The cerebellum receives multiple inputs from almost all regions of the central nervous system, including the frontal, prefrontal, and parietal cortical areas (Middleton & Strick, 1998). These cortical areas subserve a broad range of functions. The cerebellum, in turn, projects to all portions of the motor system, except the basal ganglia (Thach et al., 1992). Thus, the cerebellum appears to have functional relevance in many domains of human behaviour, including non-motor functions, and its pathology may have more extensive implications for functional impairments in autism than previously speculated.

Despite the expansive role of the cerebellum in cognitive functioning, cerebellar dysfunction is insufficient to account for the diversity of core symptoms or for the high degree of associated impairments in autism. Other neurological abnormalities have been implicated, including malformation of the brain stem (Bailey et al., 1998; Bayer et al., 1993; Hashimoto et al., 1995; Rodier et al., 1996;). The brain stem houses the nuclei of the cranial nerves. Analysis of post-mortem tissue of individuals with autism has revealed malformations of the inferior olives and impaired migration of neurons, or

ectopia (Bayer et al., 1993), of the olivary complex (Bailey et al., 1998). In one autopsy case of an autistic individual, the brain stem lacked the majority of the facial nucleus, a structure responsible for movements of the face and tongue used in facial expressions and involved in the motor output of crying, and the superior olive, which serves as a relay station for auditory stimuli from the ear to the brain (Rodier et al., 1996). In addition, the distance between the trapezoid body and the inferior olive was markedly shortened compared to a control (Rodier et al., 1996). The largest MRI study of the brain in autism found reduced volumes in all areas of the brain stem and the vermis of the cerebellum but not in any other neural regions (Hashimoto et al., 1995).

In addition to the neuroanatomical abnormalities of the brain stem in autism, behavioural abnormalities consistent with brain stem dysfunction have been found. Wong and Wong (1991) used evoked potentials to assess auditory pathways in individuals with autism. These potentials mark different stages of sensory processing. Those with autism had slower brain stem transmission times. Factor analysis was done to account for the effects of other variables, including age, sex, and degree of

retardation. The only factor accounting for these auditory brain stem response abnormalities was autism characteristics. Rosenhall, Nordin, Brantberg, and Gillberg (2003) reported similar findings, also suggestive of possible brain stem dysfunction.

Experimental Induction of Autism using Valproic Acid

As previously stated, no single etiology has accounted for the brain abnormalities associated with autism. One potential risk factor for developing the disorder is exposure to teratogenic agents (Rodier et al., 1996). Stromland et al. (1994) noted that individuals exposed to thalidomide, an anti-nausea drug once administered to pregnant women, during the 20-24th day of gestation had an incidence rate of 30% for developing autism, a rate much higher than the 0.1% rate seen in the general population. Based on such evidence, Rodier and colleagues (1996) devised a method to experimentally induce neuropathology in rats analogous to that seen in humans with autism. Thalidomide does not affect developing rats in the same manner as in humans. The anti-seizure medication valproic acid, however, is teratogenic in rats, has similar chemical properties to thalidomide, and results in malformations

similar to those seen after human thalidomide exposure (Rodier et al., 1996). Rodier et al. (1996) administered VPA to pregnant rats on gestational day 12.5, which corresponds to the day of neural tube closure in human development and a time point in gestation associated with the development of autism. The resulting offspring had similar neural abnormalities as seen in human cases of autism, in particular, aberrations of brain stem, including the motor and cranial nuclei (Rodier et al., 1996). Despite abnormal brain stem development, which is often fatal (Rodier et al., 1996), exposed offspring had survival rates equivalent to controls, showed no external malformations, and brain stem deficits remained into adulthood (Rodier et al., 1996). In addition to brain stem abnormalities, prenatal exposure to VPA during neural tube closure leads to anatomical abnormalities of the cerebellum similar to those seen in human cases of autism, including fewer Purkinje cells in the cerebellar vermis and a reduced volume of the cerebellum (Ingram et al., 2000). These marked similarities in brain morphology of prenatally exposed rats and humans with autism lend support for the use of VPA to experimentally induce the neuroanatomical parallels of autism (Teitelbaum, 2003).

Behavioural Effects of Prenatal VPA-exposure

Research has shown that prenatal exposure to VPA is behaviourally teratogenic as well, as may be expected based on the resulting brain abnormalities. Rats exposed prenatally during neural tube closure exhibited evidence of hypoalgesia at both juvenile and adult stages, and this decreased sensitivity to painful stimuli is present in some cases of autism (Schneider, Labuz, & Prezlocki, 2001). Offspring exposed to VPA during gestational days 7-18 also showed decreased overall activity, reduced spontaneous alteration and exploration in a T-maze, and a decreased level of startle responding to both auditory and tactile stimuli compared to controls (Vorhees, 1987). VPA exposure also negatively affected the performance of offspring in straight channeling swimming tests, as evidenced by increased swim times relative to controls. A group x sex interaction was found for swimming maze errors; exposed females made more errors than controls. The exposure, however, did not seem to impair the males' ability to maneuver through a swimming maze, as no differences on these measures were reported for males (Vorhees, 1987). This interaction effect is an additional parallel to the human condition, as differences in the severity of symptoms

exist in autism, with females exhibiting more severe symptoms than males (Huebner, 1992).

Social Play Behaviour in an Animal Model of Autism

Behavioural research into the teratogenicity of VPA has yielded many correlates to that seen in autism. Verification of behavioural deficits after VPA exposure more primary to the disorder, including social interaction impairments, in addition to the reported anatomical findings, may provide additional evidence for the utility of VPA in experimentally inducing autism as a means of creating an animal model of the disorder.

Social play behaviour is a major form of social interaction in children (Gray & Tonge, 2001), and is a crucial factor in the development of affectivity towards others (Daenan, Woterink, Gerrits, & Van Ree, 2002). Deficits in social play behaviour are frequent in children with autism. This behaviour, however, has yet to be examined in a VPA model of autism. Characterizing the social behaviour of VPA exposed offspring using play analysis is useful and appropriate for a number of reasons. This form of social interaction is comparable in its goals

and purpose among mammalian species (Pletnikov, Rubin, Vasudevan, Moran, & Carbone, 1999). Moreover, rodent play behaviour has been researched extensively and is well defined in terms of its onset and kinematics (Pellis, Field, Smith, & Pellis, 1997; Pellis & Pellis, 1996;). The frequency of play solicitation in rats peaks during the juvenile period between 30 - 40 days (Pellis & Pellis, 1996). As this behaviour occurs naturally and spontaneously, no training is required. Social play behaviour in the rodent is comparable to rough-and-tumble play in humans. There is a paucity of published research, however, on this type of play in children with autism. Examining social play behaviour in the rodent, however, still provides a useful index upon which to measure species-specific impairments in juvenile rodent sociability in a VPA model of autism.

Cortical Involvement in Autism

Autism has been linked to neural insult, particularly of the cerebellum (Ingram et al., 2000) and brain stem (Rodier et al., 1996). Damage to both brain areas has been found as a result of prenatal VPA exposure. The cerebellum is highly interconnected with many areas of the brain,

including the frontal cortex. This brain area is highly involved in aspects of higher-order processing and important in governing planning, communication, and social behaviour. Given that the cerebellum projects diffusely to the frontal cortex (Schmahmann, 1996), damage to the cerebellum undoubtedly has a negative impact on the functioning of the later-developing frontal cortex, (Muller, Pierce, Ambrose, Allen, & Courchesne, 2001), providing an additional hypothesis for the presentation of symptoms.

Evidence of frontal lobe abnormalities has been reported in magnetic resonance imaging studies of autism. Carper and Courchesne (2000) found an inverse relationship between cerebellar and frontal lobe abnormality. Frontal lobe cortical volume was increased in some individuals with autism, and this increase was correlated with the extent of cerebellar pathology. Research has found impairments in executive function and memory, abilities associated with frontal lobe functioning, in those with localized lesions of the cerebellum (Schmahmann & Sherman, 1998).

The motor cortex is a region of the frontal cortex that is involved with motor acquisition, and, in addition to the cerebellum, constitutes part of the motor system in

the brain. Impaired cerebellar development may contribute to or be associated with functional and anatomical deficits in this particular cortical area. Motor dysfunctions have been seen in a number of cases of autism, including clumsiness (Ghaziuddin & Butler, 1998), and a wide range of motor stereotypies, such as hand flapping, pacing, and spinning (Rapin, 1997). Motor disturbances are one of the earliest behavioural symptoms of the disorder (Teitelbaum, Teitelbaum, Nye, Fryman, & Maurer, 1998). One study found that children with autism showed less activation of supplementary motor area than controls during a finger movement task (Muller et al., 2001), a finding that suggests a decreased level of plastic change associated with learning in autism.

Although research implicates supplementary areas of the motor cortex in autism, there is a paucity of research on the degree of pathology, if any, in primary cortex. No published study has examined the development of dendritic processes in this area in autism. Given the motor disturbances commonly exhibited by those afflicted and the high degree of connectivity between the cerebellum and frontal cortex, morphology of neurons in the primary motor cortex is likely altered after prenatal VPA exposure. More

research is needed to determine the degree of involvement of the motor cortex in the presentation of autistic symptoms and could be accomplished using the VPA model of autism.

Additionally, this teratogen model of autism is an excellent candidate for studying neuronal plasticity as a result of maldevelopment. Much research has been directed at studying plasticity in primary cortical areas, including motor cortex, but these studies often initiate localized damage. Examining dendritic development in the primary motor area in this model will allow researchers to assess the extent of reorganization after early neurodevelopmental damage on later developing neurons and structures.

Precisely how dendritic growth or pruning is affected in this model is unknown. VPA exposure may induce neural stunting of the primary motor cortex. The cerebellum and primary motor cortex have a high degree of interconnectedness. Cerebellar information reaches the primary motor cortex via the ventrolateral nucleus of the thalamus (Molinari, Filippini, & Leggio, 2002). Damage to a cerebellar hemisphere leads to decreased activity of pyramidal neurons in the contralateral motor cortex (Di Lazzaro et al., 1994). VPA-exposure in rats produces

cerebellar abnormalities and, thus, may lead to a prolonged decrease in excitability in cortical pyramidal neurons. This likely alters their morphological state over time. Hence, neuropathology of the cerebellum that occurs early in development likely results in subsequent neuropathology of areas connected to these structures, including the motor cortex. A consequence of this may be stunted cortical dendritic development.

An alternative to the prediction that VPA exposure results in decreased dendritic length of pyramidal neurons in motor cortex is that exposure to this teratogen will induce neural growth. Decreased afferents to a cell can lead to a decline in dendritic material of that cell (Kolb & Whishaw, 1998). To compensate for impaired connectivity, surrounding neurons expand their dendritic material and field of influence after damage (Kolb & Whishaw, 1998), allowing for a cell to communicate with more neurons than prior to damage. This occurs at the site of damage as well as surrounding neural tissue (Kolb & Whishaw, 1998), suggesting that localized damage can result in structural changes more distal to the injury. Pyramidal neurons have vast horizontal connections with other cortical areas (Johannson & Belichenko, 2002). This connectivity is

considered an integral part of cortical reorganization (Hess, Aizenman, & Donoghue, 1996) and may account for the morphological changes reported not only at the site of damage but also at distal sites. It follows, then, that a similar plasticity may be evident between different neural structures that are intricately connected. The response of the brain to early neurodevelopmental damage, as seen in autism, is unknown, and examination of the morphology of cortical cells in the VPA model of cerebellar damage could begin to address this uncertainty.

Chapter 3

Behavioural and Anatomical Correlates of VPA-exposed Rats

Experiment 1

Behavioural Analysis

Rationale

An animal model of autism has been developed via exposure of rat embryos to VPA at the time of gestation when the neural tube closes. Such exposure produces neuroanatomical correlates of autism. The social behaviour of these animals, however, has not been characterized. The goal of this research was to determine how social behaviour is affected in this model.

Materials and Methods

Timed breeding

Fourteen adult female Long-Evans (Rattus norvegicus) rats were paired with one of four adult male Long-Evans rats at three months of age in suspended cages. The presence of a vaginal plug was used to confirm mating and was designated day one. Females were separated from males and placed in standard opaque plastic laboratory cages (46 x 25.5 x 20.5 cm^3) five days after conception and were

randomly assigned to either the treatment group or the control group.

Injection

Valproic acid (Sodium valproate, Sigma Chemical) was dissolved in 0.9% saline at 250-mg/mL at a pH of 7.3. Seven dams received a single intraperitoneal (IP) injection of 350-mg/kg sodium valproate on day 12.5 of gestation (as per Rodier et al., 1996). The seven control dams were injected with a similar volume of saline at day 12.5 of gestation. Dams were housed individually and allowed to raise their own litters. Litters were evaluated to ensure they were grossly normal upon visual inspection. The offspring were weaned from the mothers on postnatal day 28 and were housed in same-sex groups of four in standard cages. Animals were then moved between postnatal days 45 and 48 and housed in pairs in standard cages thereafter. Colony lighting was fluorescent, and a light/dark cycle of 12:12 hour was maintained, with lighting turned on at 06:00 CST. Temperature of the colony room was kept constant at 22°C, and humidity level was maintained at 41%. Recording of social behaviour was done during the animals' light cycle. Food and water were provided *ad libitum*.

Behavioural Recording

Subjects were Long Evans rat pups from the VPA-exposed dams and control dams that received saline. A balanced experimental design was employed by testing four pups from each dam (N=54) to control for any litter effects. To control for any sex differences in social behaviour, the number of males and females were matched across both groups. One dam, however, had a small litter, and only two pups were chosen for testing (VPA-exposed: n= 28; control: n=26). Behaviour was recorded on two separate occasions between postnatal days 30 and 33 for a total of 40 minutes of observation. Prior to testing, each rat was isolated from its littermates for approximately one-half hour. The tail of each rat was marked for identification purposes. Each juvenile rat was then randomly paired with a same-age pup with the same teratogen status. Both rats were placed in an opaque plastic cylinder, 42 cm high, and 43.5 cm in diameter. The behaviour was recorded on videotape from the top with a Canon ZR50 camcorder with a shutter-speed of 1000 frames/s. Behaviour was analyzed from the videotapes using a flat screen television and a VCR with frame-by-frame capabilities to detect rapid movements. The observer was unaware of the teratogen status of animals.

Social Play Behaviour

Evaluation of the animals' social behaviour was conducted by assessing the characteristic behaviour of play initiation in the rodent, which is gaining access to the nape of the neck of a *defender* via the snout (Pellis et al., 1996). Each occurrence of a nape attack was recorded and tallied for each pair of animals to determine the total frequency of play initiations per pair.

Responses to nape attacks that consisted of rotation along the longitudinal axis to supine were termed a complete rotation (as per Pellis et al., 1996). Responses to nape attacks were termed partial rotations if they consisted of a defender rotating along its longitudinal axis, with the hind paws remaining on the ground (as per Pellis et al., 1996). Avoidance responses to nape attacks, in which the defender simply moves away from the attacker, were also noted. Instances where the receiver failed to respond to playful contact were also recorded. The total number of responses to play solicitation was calculated, and the percentage of occurrence among each of the four response categories was computed to elucidate the most typical response. This percentage was averaged over both

animals to yield a measure of each type of response per pair of animals.

Non-play Social Interactions

Sniffing behaviour, a form of non-play social interaction, was also recorded for the same 40 minute time period and quantified by examining the frequency of occurrence. To determine if qualitative differences in social interaction exist between the teratogen and control groups, regions of the body that were the target of sniffing were noted. These regions were the head, nape, thoracic, lumbar, sacral, and tail areas (as per Pellis et al., 1996). A measure of total sniffing was calculated by summing the frequency of occurrence of sniffing to each of the six bodily regions. Instances of avoidance to sniffing were also recorded. The degree of avoidance behaviour to sniffing by a conspecific is likely to be affected by the amount of sniffing exhibited by that conspecific. To determine the level of avoidance behaviours exhibited by subjects, a ratio of avoidance behaviours exhibited to sniffing received was calculated per animal for each of the bodily regions of interest. A measure of overall avoidance to sniffing was calculated by computing the ratio of

avoidance behaviour to sniffing of all bodily regions to the total frequency of sniffing received. These ratios were averaged to yield measures of avoidance to sniffing per pair. Due to high interdependence of social behaviours, all measures were examined per pair (VPA-exposed: n=14; controls: n=13), and the pair was used as the statistical unit of analysis.

Statistical Analysis

All behavioural data were evaluated to determine normality of distribution and homogeneity of variance. Normality was assessed by computing a value of skewness and dividing this value by the standard error of skewness. The data were considered normally distributed if this calculated value was between -2 and +2. Levene's test was applied to determine if variances across samples were equal on the behavioural measures. All measures satisfied the assumption of homogeneity of variance. Only nape attacks, however, were considered normally distributed, and a repeated-measures ANOVA was used to test for differences between the groups. The nonparametric Mann-Whitney test was used to evaluate differences between the control group and VPA-group on measures of sniffing, avoidance to

sniffing, and responses to playful contacts, as these failed to meet the necessary parametric assumptions required for ANOVA[1]. All tests were two-tailed, and statistical significance was set at $p < 0.05$.

Results

Social Play Behaviour

No significant main effect of group was found for frequency of nape attacks, F (1,25) = 0.046, p = 0.831 (see Figure 1). Mann-Whitney U tests were used to test for significant differences in responses to play (see Table 1 for a summary of the results). No significant differences were found in the percentage of occurrence of any of the four response types analyzed (see Figure 2). Both the VPA-exposed animals and the controls, however, had a low level of typical responses to the nape attacks.

Non-play Social Interaction

Mann-Whitney U tests were used to test for significant differences on measures of sniffing and responses to sniffing (see Table 1 for a summary of the results). The two groups did not differ in the frequency of sniffing

[1] The behavioural measures assessed here are often analyzed in the literature using ANOVA.

behaviour to any of the bodily regions or in total sniffing behaviour towards conspecifics (see Figures 3 and 4, respectively).

No significant differences were found in avoidance behaviours to sniffing directed at any of the body regions (see Figure 5). The ratio of overall avoidance to sniffing by another rat was not significantly different between groups (see Figure 6). The social behaviour of animals exposed to VPA in utero did not differ significantly from controls. Table 2 displays means and standard errors of measurement for all forms of social behaviour examined for both VPA-exposed and control groups.

Discussion

Social Play Initiations

The goal of this research was to characterize the social behaviour of rats after prenatal VPA exposure and to determine if behavioural similarities to autism in humans exist. Rats exposed to valproic acid in utero did not differ in frequency of social play initiation relative to the control group, nor did they respond atypically to play initiations or non-play social interaction of conspecifics. Deficits in social interactions were quantified by

examining social play behaviour, as the study of social play may reveal insight into an animal's ability to recognize the goal or intention of another (Bekoff & Byers, 1998), an ability postulated to be central to the dysfunction in autism (Baron-Cohen, Tager-Flusberg, & Cohen, 2000). VPA-exposure in utero was predicted to affect social behaviour of rats in a manner similar to that seen in humans, as children with autism exhibit inappropriate social interactions and deficits in play behaviour (Gray & Tonge, 2001).

The present study assessed play solicitations in the rodent in terms of the frequency of occurrence. A proposed rat model of autism that induces lesions of the amygdala examined social behaviour by measuring the duration of play episodes rather than frequency of occurrence (Daenan, Wolterink, Gerritts, & Van Ree, 2002). These authors found significant differences between controls and experimental groups using duration of social play episodes, suggesting that duration is a valid measure of sociality in rats. For the present study, frequency of play solicitation was used, as it was reasoned that examining the frequency of initiations would give a more accurate depiction of overall level of social play behaviour. For example, in a given

dyad, one animal could initiate a high number of play episodes, but if the receiver of these attacks is non-responsive, the overall duration of play will be brief. A different animal could solicit fewer instances of play, but with a responsive partner, the duration of play here may be longer. Thus, frequency of occurrence is assumed to be an appropriate measure of social play behaviour and may offer insight into the appetitive and motivational aspects of play behaviour in a rodent model of autism.

One possible explanation for the lack of decreased frequency of play initiations as well as a failure to produce deficits in non-play social interaction, as measured by sniffing, in this model could be attributed to the overall level of VPA given to the dam. A dosage of 350 mg/kg delivered at gestational day 12.5 is sufficient to produce brain stem abnormalities similar to those seen in the human condition (Rodier et al., 1996), but may be insufficient to produce noticeable impairments in social functioning. The cerebellar abnormalities associated with this teratogen, however, were reported after administration of a single larger dose (600 mg/kg) on the same day of gestation. Behavioural research using VPA in utero with the higher dose is behaviourally teratogenic (Schneider &

Przewlocki, 2004; Vorhees, 1987). Rats exposed repeatedly to VPA during gestational days 7-18, during neural tube closure, explored their environment less, responded at a decreased level to stimuli in multiple modalities, and made more errors during measures of motor functioning (Vorhees, 1987). Schneider and Przewlocki (2004) reported a number of behavioural deficits in rats after a single exposure of 600 mg/kg to VPA on gestational day 12.5, including decreased play frequency, reduced exploration, and decreased pain sensitivity. These studies found behavioural parallels to those seen in children with autism using a stronger dose than that used in the present study. Although social behaviour may be affected at the lower dose, it may be in a manner more subtle and not detectable by the methods chosen for the present study. Thus, an important consideration in teratology studies is the dosage used. A stronger dose of any teratogen is likely to yield more dramatic and detectable outcomes. It is, however, relevant to identify the minimum dosage required to elicit detrimental affects on the fetus. Comparison of social behaviour using dosage as a factor would allow for a correlational examination of the dosage level and could assist with determining appropriate dose administration to

pregnant epileptic women in order to minimize a child's risk of developing autism or other pathology.

Responses to Social Play

There were an unexpectedly low number of responses to play solicitations in both groups in the present study. The predominant response to nape attacks in the juvenile phase is the complete rotation, occurring in response to approximately 60% of play solicitations (Pellis & Pellis, 1997). In both groups of rats in the present experiment, play solicitations frequently failed to initiate any responses from the receiver, and complete rotations accounted for less than 1% of the responses in both groups.

The type of lighting conditions under which the animals were tested may have contributed to this reduced response pattern. Testing of play behaviour in the rodent has been done under dim illumination conditions, using a red 25W light (e.g. Knutson, Burgdorf & Panksepp, 1998; Pellis et al., 1997; Pellis & Pellis, 1996), as high-illumination is an aversive stimulus to rodents, and, consequently, results in decreased social behaviour in both frequency of nape attacks and vocalizations emitted during play (Knutson et al., 1998). Behavioural testing of

animals in the current study was done under higher illumination conditions using cold white lighting. The sociability of both groups was likely affected by this stimulus and may have contributed to the lack of responsiveness in both groups. Future testing of social play behaviour would best be done under dim lighting conditions to ensure that alterations due to environmental stimuli in behaviours of interest are minimized. Dim lighting, however, minimizes the type of data one can reliably collect and makes kinematic analysis arduous. This was an important consideration, as without prior exposure to social play behaviour in rodents on the part of the observer, it can be difficult to distinguish sniffing directed at the nape with nips, or playful contact, directed at this region, under low-illumination conditions.

Increasing the time spent in isolation prior to behaviour testing, which was 30 minutes in the present study, may also increase the occurrence of complete rotations, which tend to prolong the play episode, and may result in more nape attacks overall. Pellis et al. (1997) found that increased isolation time, from one hour to 24 hours, resulted in a decrease in avoidance responses and increase in complete rotations. Isolation increases the

motivation for play (Pellis et al., 1997), and assessing whether typical play responses increase as a function of isolation in animals exposed to VPA prenatally may elucidate how motivation to socialize is affected in this model.

Future Research

The present study examined social play behaviour by evaluating the frequency of nape attacks in the rodent during the juvenile phase. Another aspect of this type of social interaction in the rodent is the emission of ultrasonic vocalizations during play (Knutson et al., 1998). Prenatal VPA-exposure may influence vocalizations in the pups and may represent a qualitative difference in social play behaviour not detected by the methods used in the present study.

The examination of vocalizations in VPA-exposed rats may offer insight into social deficits associated with play, but may also be a rich source of data to evaluate the model on a multi-symptomatic level. The vocalizations emitted during play are high-frequency (~55 kHz) (Knutson et al., 1998). Vocalizations are also emitted upon maternal separation, but these are in the mid-frequency

range (~40kHz)(Knutson et al., 1998). These vocalizations provide a measure of bonding behaviour in these animals (Kahne, Tudorica, Borella, Shapiro, Johnstone, Huang, & Whitaker-Azmitia, 2002). Children with autism have been shown to bond less with caregivers (Kahne et al., 2002). Thus, this line of research could provide an additional parallel to the disorder and could be investigated using the VPA model.

Examination of vocalizations in the rodent may serve as an additional source of evidence for the behavioural teratogenicity of VPA related to autism. One of the key areas of behavioural impairment in autism is impaired communication. This aspect of social interaction has been studied in the rat. Conspecifics communicate food preferences to each other after one of the rats has experienced adverse effects from ingestion of a noxious food (Beck & Galef, 1989). A similar experiment using rats exposed to VPA in utero may serve as a species-specific measure of impairment in communicative behaviour in a rodent model of autism and may reflect impaired communication after exposure. The evaluation of species-specific behavioural measures in an animal model to

elucidate correlates to the human condition is vital to allow for any degree of generalizability.

Caveats

An additional consideration in the generalizability of findings from the present study to the human condition is the nature of play behaviour exhibited by the rat. Social play in the rat most closely resembles rough-and-tumble play in humans. The research on rough-and-tumble play in children with autism is scarce, and much of the information on this type of play is anecdotal. A search of three databases, PsychInfo, PubMed, and EbsoHost yielded only one published study involving this type of play behaviour in the context of communication in autism. Whitaker and Reynolds (2000) taught children with autism hand signalling as means of improving communication skills. The facilitator initiated rough-and-tumble play, and the researchers found that children did exhibit and respond to instances of rough and tumble play. This study, however, did not examine this type of behaviour in a control group, so no inferences can be drawn relating the frequency of these behaviours as compared to neurologically intact children. If this type of social behaviour is not affected

to a large degree in autism, an animal model of the disorder, in which the predominant type of play exhibited by the animal is rough-and-tumble, may fail to accurately depict the social play behaviour deficits accompanied by the disorder. Other studies investigating animal models of autism in rats (Daenan et al., 2002; Pletnikov et al., 1999; Schneider & Przewlocki, 2004) and guinea pigs (Caston, Yon, Mellier, Godfrey, Delhaye-bouchaud, & Mariani, 1998), however, have used social play behaviour as an index of general sociability using methods similar to those used in the current research.

Human behaviours, including social interactions, are highly complex, and one cannot draw exact parallels with similar behaviours in any animal research. Animal models, nonetheless, are excellent tools for examining neurodevelopmental disorders by means not possible in human studies. Investigation of social interactions in a rat model of autism still allows researchers to assess whether teratogen exposure produces species-specific impairments in rodent sociability and to assist with characterizing the phenotypic expression of the disorder. This is crucial for assessment of the model's efficacy for further research into the disorder, such as investigation of pharmacological

and behavioural interventions and their subsequent effect on the brain.

In summary, the present research investigated the effects of prenatal VPA exposure on rat social behaviour and found no significant differences in frequency of play solicitation, responses to play, or non-play social investigation. Adjusting the dosage and environmental conditions of behavioural recording may result in the detection of aberrant play in a VPA model of autism.

Chapter 4

Experiment 2

Quantitative Morphology

Rationale

Exposure to VPA in utero interrupts normal neural development, resulting in pathology of the brain stem and cerebellum. To determine the level of plasticity in autism after such damage, the morphology of cells of the somatomotor cortex was also examined to elucidate any differences in dendritic length after VPA exposure. The motor cortex was selected because of its interconnectedness with the cerebellum and its known ability to reorganize after experience, including damage. The pyramidal cells of layer II were chosen, as these cells receive input from other structures, including the cerebellum, and project to other cortical regions.

Materials and Method

Tissue Preparation

Tissue from 18 Long Evans rats (VPA-exposed: n=9; control: n=9) used in the behavioural experiment was collected and used for drawing. After behavioural recordings were taken, animals were sacrificed between

postnatal day 36 and postnatal day 40 with a lethal dose of sodium pentobarbital. The animals were then perfused with saline through the heart, and brains extracted and immersed in Golgi-cox solution (as per Gibb & Kolb, 1998). Fixation time was 21 days, after which time the tissue was infiltrated with a sucrose solution for seven days. The processed tissue was sectioned at 200 µm with a vibrotome, placed onto glass slides, and allowed to sit for 48 hours. Tissue sections were then processed using ammonium hydroxide, which causes the formation of a precipitate within the cell, and in 1-4% of cells, this precipitate fills the cell in its entirety. This allows for visualization of the cell and its components using light microscopy.

Data Collection

Pyramidal cells were drawn from layer II of the motor cortex from pups from nine dams per group. Cells that met the following criteria was chosen for data collection: i) the cell was located in primary motor cortex; ii) the cell was stained sufficiently to allow for accurate visualization of processes; iii) the cell was not obscured by other material, such as glia, vasculature, and other

neurons; and iv) the cell was largely intact, with few truncated or cut processes. Five cells were drawn from each of the left and right hemispheres, yielding a total of 90 cells/group for analysis. These cells were traced at 40x magnification using the software program Neurolucida (MicroBrightField Inc.) and a special microscope equipped with a motorized stage.

The drawer was blind to the teratogen status during data collection. Tissue samples from all dams used in behavioural testing were not available, as some of the tissue was not suitable for drawing due to variations with the Golgi-cox penetration into cells and due to unexplainable poor preservation of tissue after processing.

Analysis

Comparison of differences in total dendritic length was accomplished using a variation of Sholl analysis, which involves using a series of concentric, equidistant circles. In traditional Sholl analysis, a cell is drawn manually with paper and pen, and the cell drawing is placed under an acetate sheet of rings such that the first ring is centered over the cell body. The number of dendrites that intersect each ring are counted and multiplied by the distance

between each ring, 10 μm, to provide an estimate of total dendritic length. Analysis was conducted using the NeuroExplorer (MicroBrightField, Inc.) software program, which uses a more sophisticated form of the basic technique behind Sholl. When using the computerized method of cell drawing, dendrites are drawn through different focal planes in three-dimensional space. Instead of a series of concentric circles, the software employs concentric spheres around the cell body and conducts a three-dimensional version of Sholl, thus providing a measure of total dendritic length at each ring. As the analysis conducted on these measures in NeuroExplorer takes into account the three-dimensionality of the cell drawing, a more accurate approximation of length at each intersection is obtained as compared to estimation based on the number of intersections at each ring.

Statistical Analysis

Morphological data were assessed to determine normality of distribution and homogeneity of variance using methods described in Experiment 1. The assumptions of normality and homogeneity of variance were satisfied. To determine if any morphological differences were significant

between groups, a 2 x 10 x 15 (Group x Cell x Sphere) analysis of variance (ANOVA) was used. Data were taken from the first 20 spheres analyzed using Sholl for basilar dendrites, and this was extended to include the first 25 spheres in the analysis for estimates of apicals, as apicals tend to be longer. Tests were two-tailed, and statistical significance was set at $p < 0.05$.

Results

No significant main effect of group was found for basilar dendrites, F (1,9) = 0.6, p = 0.433, or apical dendrites, F (1,9) = 1.5, p = 0.239 (see Figure 7). No significant group x sphere interaction was found for either basilar, F (1,19) = 0.6, p = 0.991 (see Figure 8), or apical dendrites, F (1,24) = 0.34, p = 0.999 (see Figure 9). Table 3 displays means and standards errors of the mean for the morphological data.

Discussion

To investigate cortical involvement in an animal model of autism, dendritic arborization was examined in rats exposed to VPA, a known teratogen. No significant differences were detected in length of either basilar or

apical dendrites in pyramidal cells of primary motor area, an area highly responsive to experience.

It was reasoned that VPA exposure would i) induce neural growth, ii) induce neural stunting, or iii) not affect cortical development. The nonsignificant findings of the present research may be evidence that VPA-exposure does not influence motor cortical development. The fact that no detectable differences were found between the two groups, however, may be attributable to the dosage used, as with the behavioural data. The dosage used in the present study results in brain stem anomalies associated with autism (Rodier et al., 1996), but no other gross neural abnormalities were found. Although decreased cell counts in the cerebellum have been found after VPA exposure (Ingram et al., 2000), the extent of maldevelopment in the cerebellum has not been examined with the dosage used in the present study. The level of toxicity embryos were exposed to may not lead to extensive damage to other neural areas, including the cerebellum.

In addition to the dosage used, another factor contributing to the non-significant findings of the current research may be the time point of data collection. The animals from which morphological data were collected were

sacrificed between postnatal day 36 and postnatal day 40, by which time rats have reached maturity and brain development has ceased (Kolb & Whishaw, 1998). During infancy, the brain has the most capacity for plasticity, and early neurodevelopmental damage may result in a compensatory rebound in neurogenesis (Rodier et al., 1996). This may have occurred in the present study, such that no differences in quantitative morphology of neurons exist in this area by the time morphological data were obtained during the juvenile period. Examining length of dendritic material at a much earlier time point, shortly after gestation, may provide a more accurate depiction of the brain's response to this teratogen, as a real difference in dendritic morphology after VPA-exposure may exist that was obscured by the time of sacrifice.

An additional consideration in morphological examination of this area after VPA exposure is the structural aspects of the cell examined. Primary motor cortex may be affected post VPA-exposure, but the resulting pathology may be altered spine density rather than decreased dendritic length. Decreased spine density of apical dendrites of pyramidal frontal cortical neurons has been reported in cases of autism (Williams, Hauser,

Purpura, DeLong, & Swisher, 1980). As spines are the major sites of chemical connectivity between axodendritic excitatory synapses (Huttenlocher, 1991; Kolb & Whishaw, 1998), decreased density of spines likely affects synaptic activity and could, in part, account for observed functional impairments (Huttenlocher, 1991). Examination of dendritic spines may elucidate pathology in primary motor cortex in a VPA model of autism.

Future Research

Further examination of dendritic development in the cortex in a model of autism should include data collection via the Golgi method across different time points in development. This neural area changes significantly in humans during the first two postnatal years of life by way of pruning and synapse reorganization during the normal course of development (Huttenlocher, 1991). In the rat, postnatal days 14-35 are characterized by rapid axonal and dendritic growth in the cerebral cortex (Eayrs, 1964). In the present study, tissue was collected immediately following this expansive period, between postnatal days 36-40. This period could have compensated for any earlier maldevelopment. Thus, assessing dendritic morphology

throughout the process of development could reveal more specific time points whereby this normal developmental process may be curtailed.

The VPA model of autism has been found to produce neuropathology of the cerebellum (Ingram et al., 2002) and brain stem in rodents (Rodier et al., 1996). Future research could utilize histopathology to confirm neuropathology of these brain centers associated with autism at different doses. The model could be used to examine neocortical areas putatively involved in autism, including supplementary motor area (Muller et al., 2001) and anterior cingulate cortex (Kemper & Bauman, 1998), as well as limbic forebrain structures, including the hippocampus and amygdala (Kemper & Bauman, 1993). These data could provide additional support for the efficacy of this model.

The development of an animal model of autism allows for researchers to investigate the disorder itself, but also aids in the investigation of brain plasticity. The present research examined the morphological effects of VPA exposure in utero on cortical development and found that the dendritic length of cells in layer II of the primary motor cortex was unaffected. Increasing the dosage used

and examining cells at different points in development may give a more complete picture of the level of cortical involvement in the autistic phenotype.

Chapter 5

General Discussion

At present, research into autism has yet to produce an effective animal model of the disorder that possesses behavioural and neuroanatomical correlates to the human condition (Teitelbaum, 2003). Prenatal exposure to VPA in rats results in behavioural deficits and neuropathology that parallel that seen in the human condition. The present research examined the behavioural effects of VPA exposure on sociability and morphology of pyramidal cells within primary motor cortex and found no significant differences between exposed animals and controls.

The behavioural findings of the present study do not support the prediction of aberrant social behaviour after VPA exposure. Given the brain alterations found after exposure to this teratogen (Ingram et al., 2000; Rodier et al., 1996), and the findings of other behavioural research using prenatal VPA exposure and rats, the most likely explanation for the findings relates to the dosage used. A study published recently (Schneider & Przewlocki, 2004) indicates that a higher VPA dosage exposure at the same gestational time produces behavioural consequences, including alterations in play behaviour. Thus, although the

dosage used in the development of the model is sufficient to induce noticeable neuropathology of a similar nature to that seen at autopsy in human cases of autism, the frequency of social play solicitation and responsiveness to play does not seem to be affected in the present study.

To determine the affect of VPA on cortical development, the second experiment examined dendritic length of neurons in primary motor cortex, an area responsive to environmental experiences, including damage. This brain area is likely related to both motor behaviour and social behaviour in the rat, as it is also classically described, behaviourally and anatomically, in the rat as a "frontal" region. Given the cerebellar damage associated with VPA exposure (Ingram et al., 2000) and the connectivity of the cerebellum and primary motor cortex, cortical development is likely affected, but the result may be changes in spine formation and density rather than dendritic length. Spines may be stunted, as has been found at autopsy in autism (Williams et al., 1980). Conversely, spines may show an increase in number and/or density after such exposure. Cortical reorganization occurs after other forms of neural insult, such as ischemia (Kolb & Whishaw, 1998). A similar type of plasticity may occur in a

VPA model of autism, where neuropathology is associated with maldevelopment.

Although the current research focused on prenatal VPA-exposure as a potential model of autism, other animal models involving lesioning of the amygdala and hippocampus during early postnatal development have been devised (Bachevalier, 1994). The subsequent social behaviour of these animals is abnormal. The lesion approach, however, negates the possible relevance of the sequence of damage and its cascading morphological impact. Evidence suggests that autism results from damage to neural areas early in development that impacts the organism throughout the lifespan. This early damage is likely to impair the subsequent development of later forming structures and circuitry, possibly in subtle ways not easily detected, as few gross abnormalities have been found in cases of autism (Rodier et al., 1996). As such, a teratogen model of autism is more appropriate for replicating damage to the brain in this nature, and hence, may more closely resemble the pattern of neural damage in the human condition.

There are other clear benefits to using a teratogen model of autism in the study of the disorder and the

development of potential treatments. Such a model is appropriate for investigation of other disorders. Morphological analysis of cells after VPA-exposure could offer insight into the mechanisms behind dendritic alterations in mental retardation. Autism has a high comorbidity with mental retardation (Baumann & Kemper, 1994). The presence of differences found in length of dendritic arborization using this model could allow for the conclusion that VPA-exposure in utero does alter neural morphology. If the resulting alteration post-exposure was an overall decrease in dendritic material, however, it may be a function of some form of mental retardation in a subset of animals rather than autism alone, as mental retardation is characterized by stunted dendritic arborization in numerous brain areas (Kaufmann & Moser, 2000). Decreased dendritic arborization, however, has been noted in autism in hippocampal subregions CA1 and CA4 (Raymond, Bauman & Kemper, 1996). As the case studies used did not suffer from seizures, the hippocampal pathology identified could not be attributed to any detrimental effects of seizure activity. To dissociate potential effects of neuropathology related to autism and to mental retardation on dendritic pattern, future research could

involve a comparative examination of neuronal morphology using the VPA-model and a model of mental retardation. An animal model of mental retardation has yet to be devised in the rodent, but a model of Fragile X syndrome, a genetic form of mental retardation, has been developed in mice (Bakker et. al., 1994). The VPA-model could be extended to mice to induce the neuroanatomical correlates of autism. Morphological data could then be collected from both neuropathological models to dissociate between the effects of each condition on dendritic development.

A VPA model of autism could also be used to gain a greater understanding of the functions of specific neural areas. Of particular interest in neuroscience research is the cerebellum (Allen & Couchesne, 2003). Traditionally, the cerebellum was deemed important for motor coordination and precision (Allen & Courchesne, 2003). Recent research that implicates this substrate in aspects of cognitive functioning has generated a heightened interest in this brain structure. An animal model of autism is an excellent candidate for examining the functional relevance of the cerebellum in different domains, due to the cerebellar pathology associated with the disorder.

The study of brain function could benefit from the VPA model, but this model is also well suited to examine how the brain reorganizes after damage and the mechanisms underlying plasticity. Studies of plasticity have used the lesion method to a great extent, and a vast amount of knowledge has been gained as a result. What is lacking, however, in the literature, is research examining the secondary effects of neural damage on other brain areas. The VPA model, whereby damage occurs when very little of the central nervous system is present, makes it a useful tool for understanding neurodevelopmental sequelae in the presence of abnormal brain development. Moreover, examining the morphological consequences of any original pathology on remote brain areas offers great potential for understanding brain connectivity and the limits of brain plasticity.

Conclusion

A VPA model is a useful model for neuroscientific inquiry on many levels. The ability to develop and test effective behavioural and pharmacological treatments of autism will be enhanced by the development of an appropriate animal model of autism. As the disorder is multi-symptomatic, an effective animal model demands

examination of deficits in multiple behavioural domains. A number of brain areas have been implicated in autism, but identifying brain areas consistently damaged in autism has been difficult, as such studies have low replication rates. Thus, an animal model of autism will be particularly useful in confirming pathology in areas linked to the disorder as well as investigation into areas not yet identified. Using a multi-dimensional approach, whereby both behavioural and neuroanatomical correlates of autism are identified, will provide converging evidence for the efficacy of this animal model. This model could be applied to studies of brain function, as this will assist in understanding how neuropathology relates to behavioural expression, not only in autism but also in other disorders where the brain is compromised. Furthermore, examination of dendritic arbour using this model of neural maldevelopment could elucidate the brain's response to damage during early brain development.

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Table 1

Mann-Whitney U Tests on Social Behaviours for Pairs of Animals

Behaviour	U	p	p*
Responses to Play			
Complete Rotations	91	1.0	1.0
Partial Rotations	90	.98	1.0
Avoidance	66.5	.24	1.0
No Response	69	.30	1.0
Sniffing			
Head	61	.16	1.0
Nape	81	.65	1.0
Thoracic	74.5	.43	1.0
Lumbar	74.5	.43	1.0
Sacral	58.0	.12	1.0
Tail	81.0	.65	1.0
Total	68.5	.28	1.0
Avoidance to Sniffing			
Head	79.5	.58	1.0
Nape	78	.55	1.0
Thoracic	76	.49	1.0
Lumbar	85.5	.79	1.0
Sacral	64	.202	1.0
Tail	89	.943	1.0
Total	83.5	.72	1.0

Note: Reported p values are corrected for ties.
N=27; (VPA: n=14, Control: n=13).
p* : p values corrected for minimum simultaneous significance using sequential Bonferroni

Table 2

Descriptive Statistics on Measures of Social Interaction

Behaviour	Control		VPA-Exposed	
	Mean	SEM	Mean	SEM
Nape Attacks	23	5.18	19.92	4.23
Responses to Play				
Complete Rotations	.38	.38	.18	.18
Partial Rotations	1.48	1.39	.52	.43
Avoidance	20.59	5.26	15.17	5.48
No Response	70.42	6.20	76.43	6.52
Sniffing				
Head	114.36	14.09	82.54	12.01
Nape	76.79	11.14	66.70	8.33
Thoracic	66	8.58	57.62	8.67
Lumbar	63.5	8.20	54.92	7.83
Sacral	106.86	13.84	85.31	14.41
Tail	102.07	14.50	99.08	17.70
Total	529.57	65.07	446.15	63.92
Avoidance to Sniffing				
Head	13.59	1.72	13.52	1.77
Nape	10.68	1.67	9.15	1.7
Thoracic	7.98	4.09	4	1.33
Lumbar	3.9	1.78	2.49	.79
Sacral	9	2.17	5.96	1.66
Tail	2.1	.73	2.19	.94
Total	7.88	1.72	6.22	.75

Note: Nape attack and sniffing are expressed as the frequency of occurrence during the 40 minutes of observation. Data for responses to play are expressed as the percentage of occurrence, and avoidance to sniffing is expressed as the percentage sniffs avoided to sniffs received. All data were reported per pair of animals.
N=27; (VPA: n=14, Control: n=13).

Table 3

Descriptive Statistics on Layer II Cells of Motor Cortex

Dendrite	VPA-Exposed		Control	
	Mean	SEM	Mean	SEM
Basilar	2105.11	95.26	1938.22	97.92
Apical	1294.76	46.25	1380.05	52.19

Note: Mean length per cell.
N=18; (VPA: n=9, Control: n=9).

Figure Captions

Figure 1. Mean frequency of occurrence (+/-SEM) of nape attacks per pair of animals for VPA exposed (n = 14), and control (n = 13) groups over 40 mins. The two groups did not differ significantly in the amount of play initiations exhibited ($p > .05$).

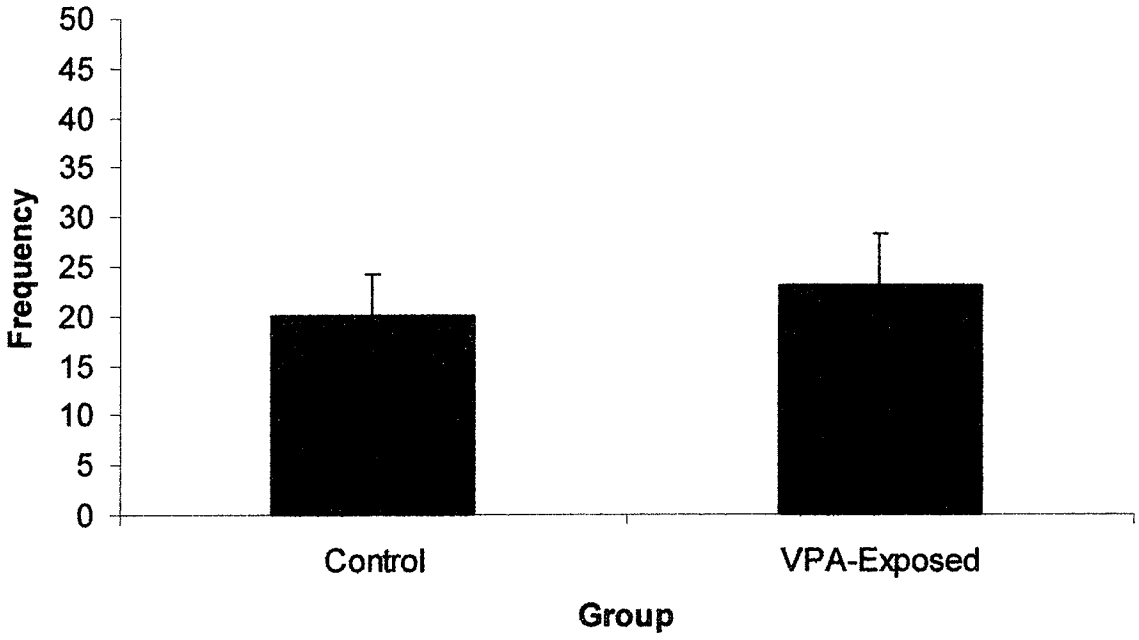

50
45
40
35
30
25
20
15
10
5
0
Frequency
Control
VPA-Exposed
Group

Figure 2. Percentage of occurrence (+/-SEM) of each type of response to a nape attack per pair of animals for VPA exposed (n = 14), and control (n = 13) groups ($p > .05$). In both groups, nape attacks often failed to solicit a response from the cagemate.

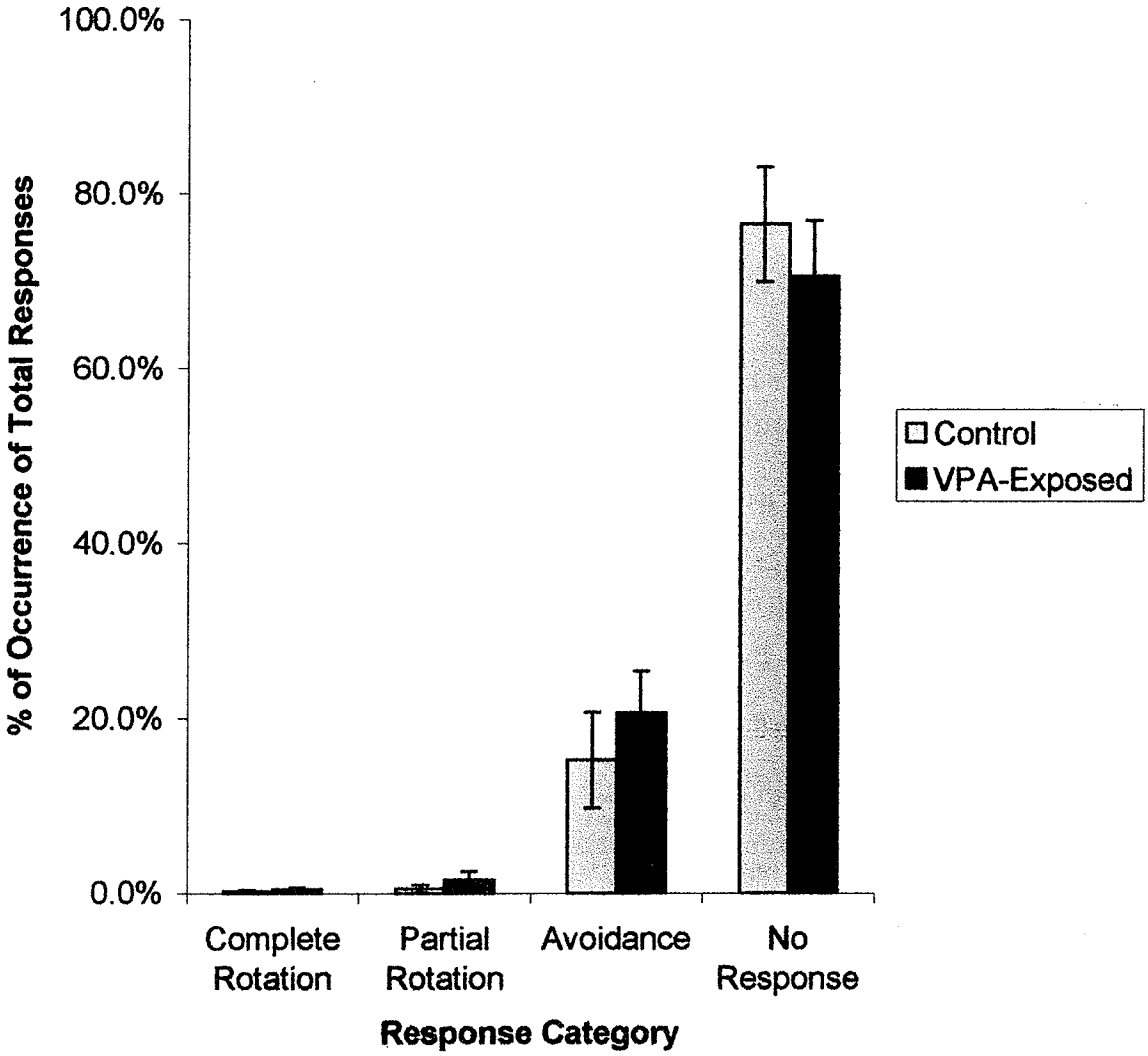

100.0%
80.0%
60.0%
40.0%
20.0%
0.0%
% of Occurrence of Total Responses
Control
VPA-Exposed
Complete Rotation
Partial Rotation
Avoidance
No Response
Response Category

Figure 3. Mean frequency of occurrence (+/-SEM) of sniffing behaviour directed to each of the bodily regions examined per pair of animals for VPA exposed (n = 14), and control (n = 13) groups over 40 mins. ($p>.05$).

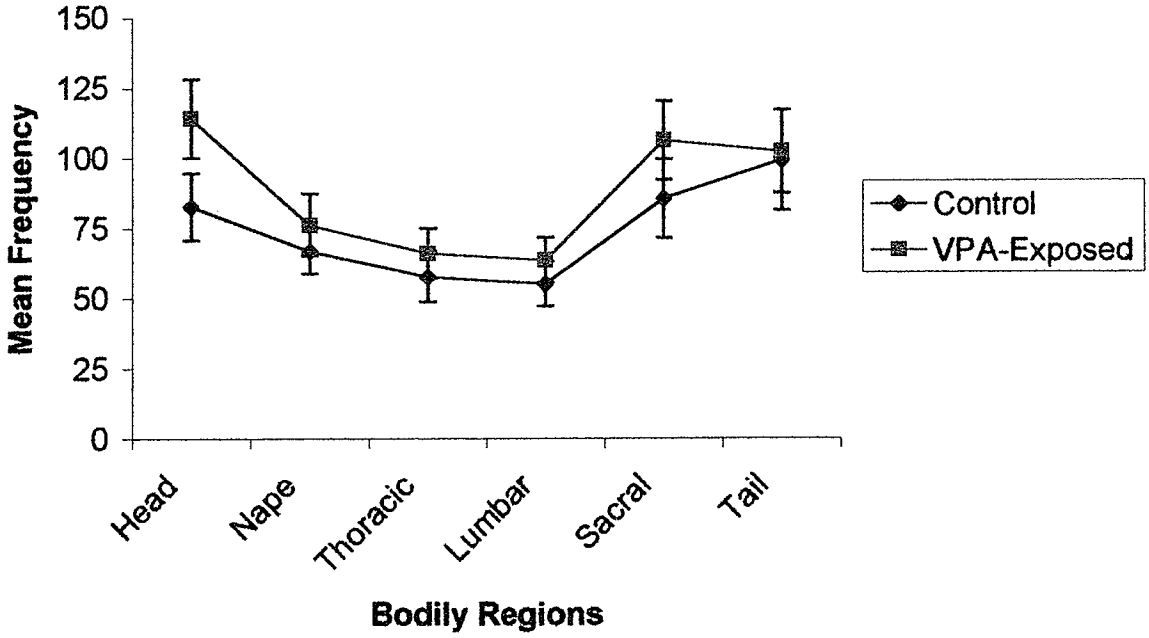

150
125
100
75
50
25
0
Mean Frequency
Head
Nape
Thoracic
Lumbar
Sacral
Tail
Bodily Regions
Control
VPA-Exposed

Figure 4. Mean frequency of occurrence (+/-SEM) of total sniffing behaviour exhibited per pair of animals for VPA exposed (n = 14), and control (n = 13) groups over 40 mins. ($p>.05$).

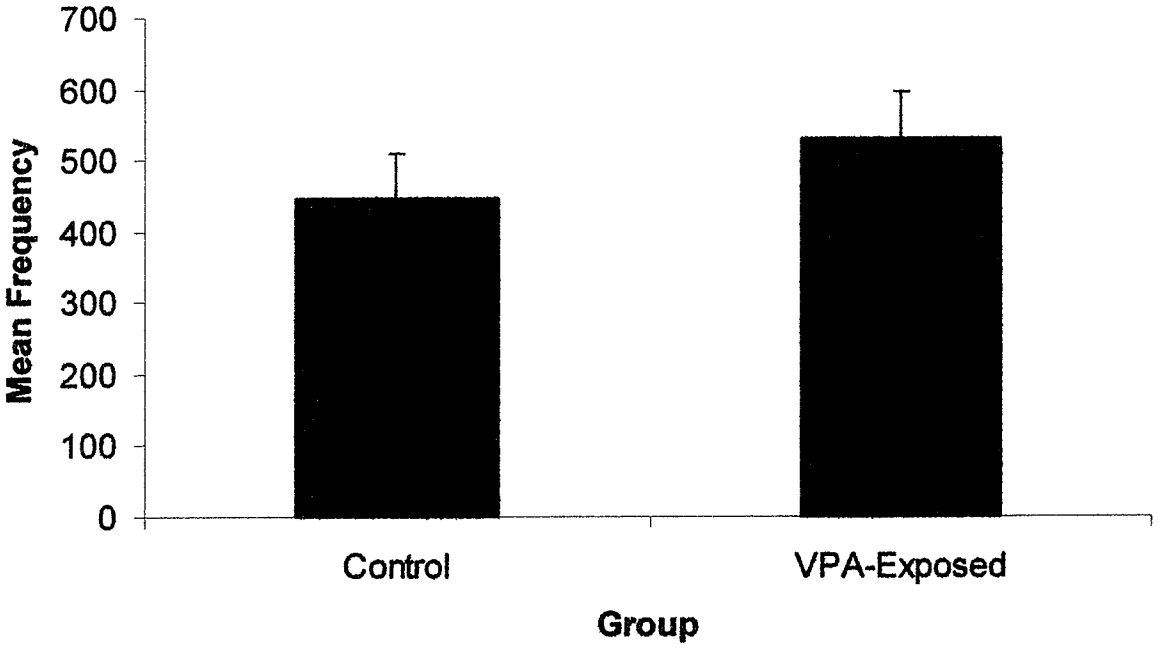

700
600
500
400
300
200
100
0
Mean Frequency
Control
VPA-Exposed
Group

Figure 5. Percentage of sniffs avoided (+/-SEM) at each bodily region per pair of animals for VPA exposed (n = 14), and control (n = 13) groups ($p > .05$). In both groups, sniffs to the head were avoided most frequently.

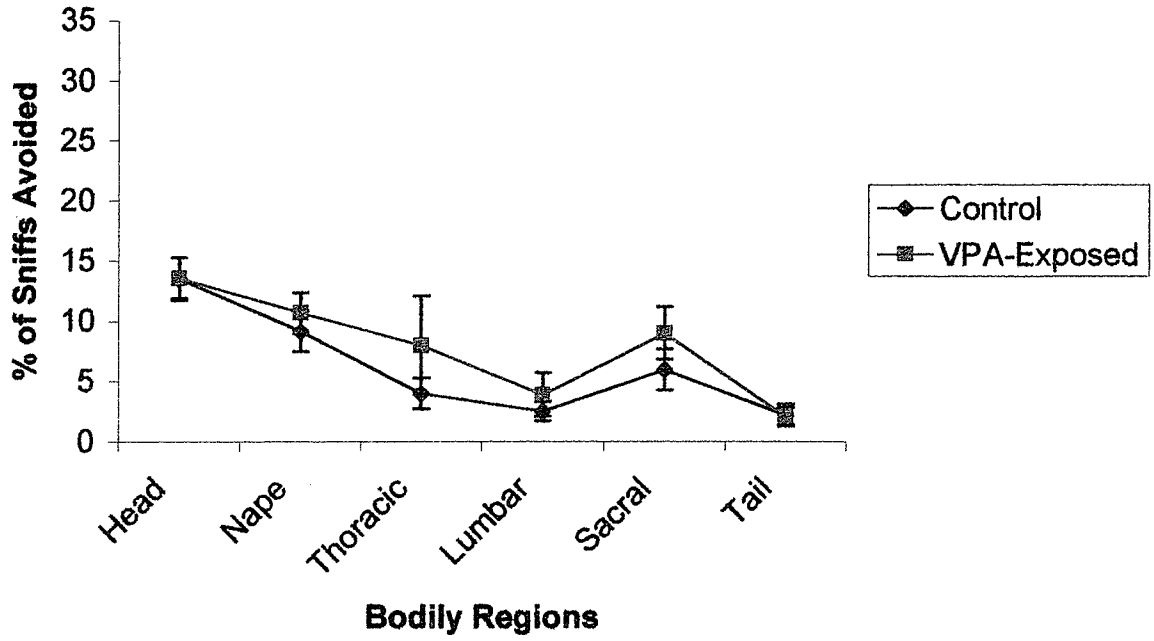

35
30
25
20
15
10
5
0
% of Sniffs Avoided
Head
Nape
Thoracic
Lumbar
Sacral
Tail
Bodily Regions
Control
VPA-Exposed

Figure 6. Percentage of sniffs avoided overall (+/-SEM) per pair of animals for VPA exposed (n = 14), and control (n = 13) groups. No significant difference was found between groups ($p > .05$).

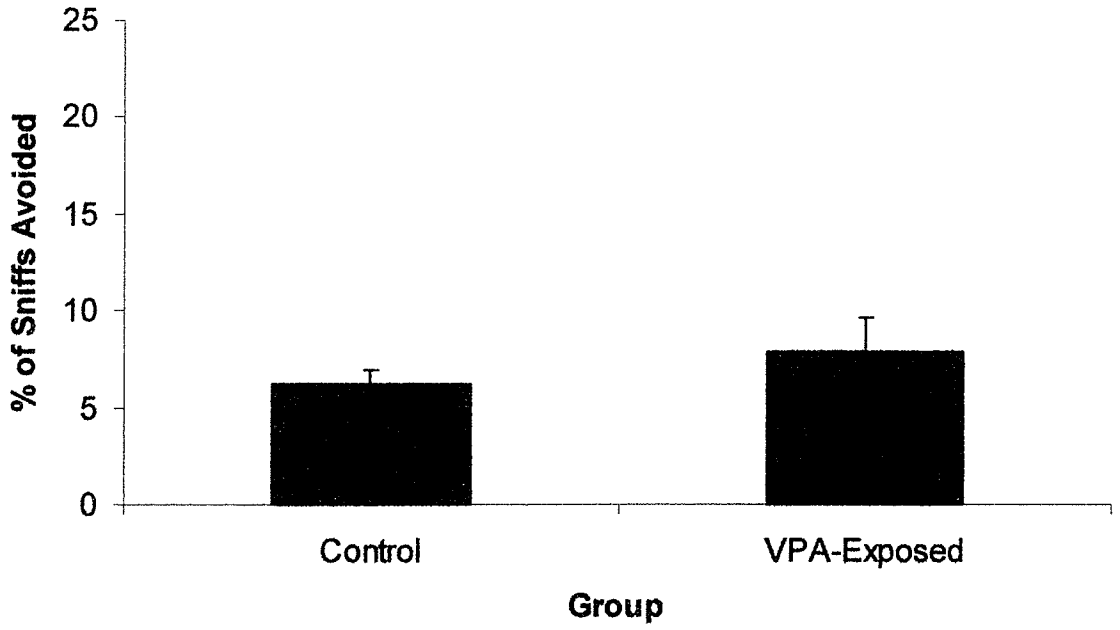

25
20
15
10
5
0
% of Sniffs Avoided
Control
VPA-Exposed
Group

Figure 7. Mean length (+/-SEM) of apical and basilar dendrites for both the VPA exposed (n = 9), and control (n = 9) groups. No significant difference was found between groups ($p > .05$).

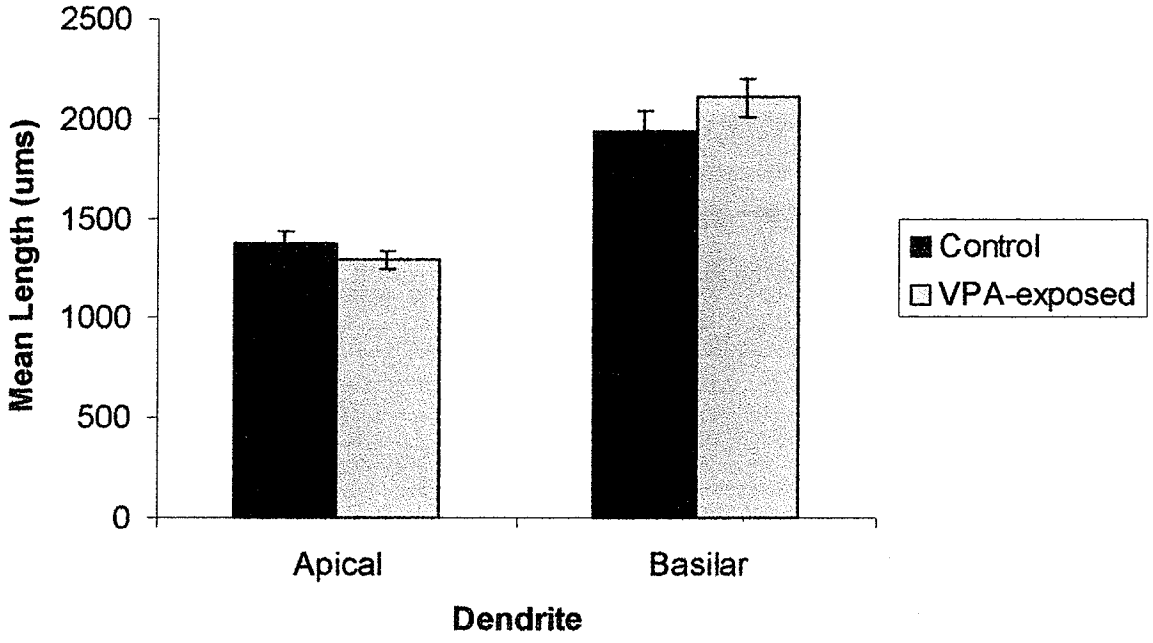

2500
2000
1500
1000
500
0
Mean Length (ums)
Apical
Basilar
Dendrite
Control
VPA-exposed

Figure 8. Mean length (+/-SEM) of dendrites in µms at each ring as a function of distance from the soma of basilar dendrites for the VPA exposed (n = 9), and control (n = 9) groups. Statistical tests were done on the length of dendritic material within the first 200 µms from the soma. Distances are in increments of 10 µms. No differences were found between the lengths at any of the distances from the cell body ($p>.05$).

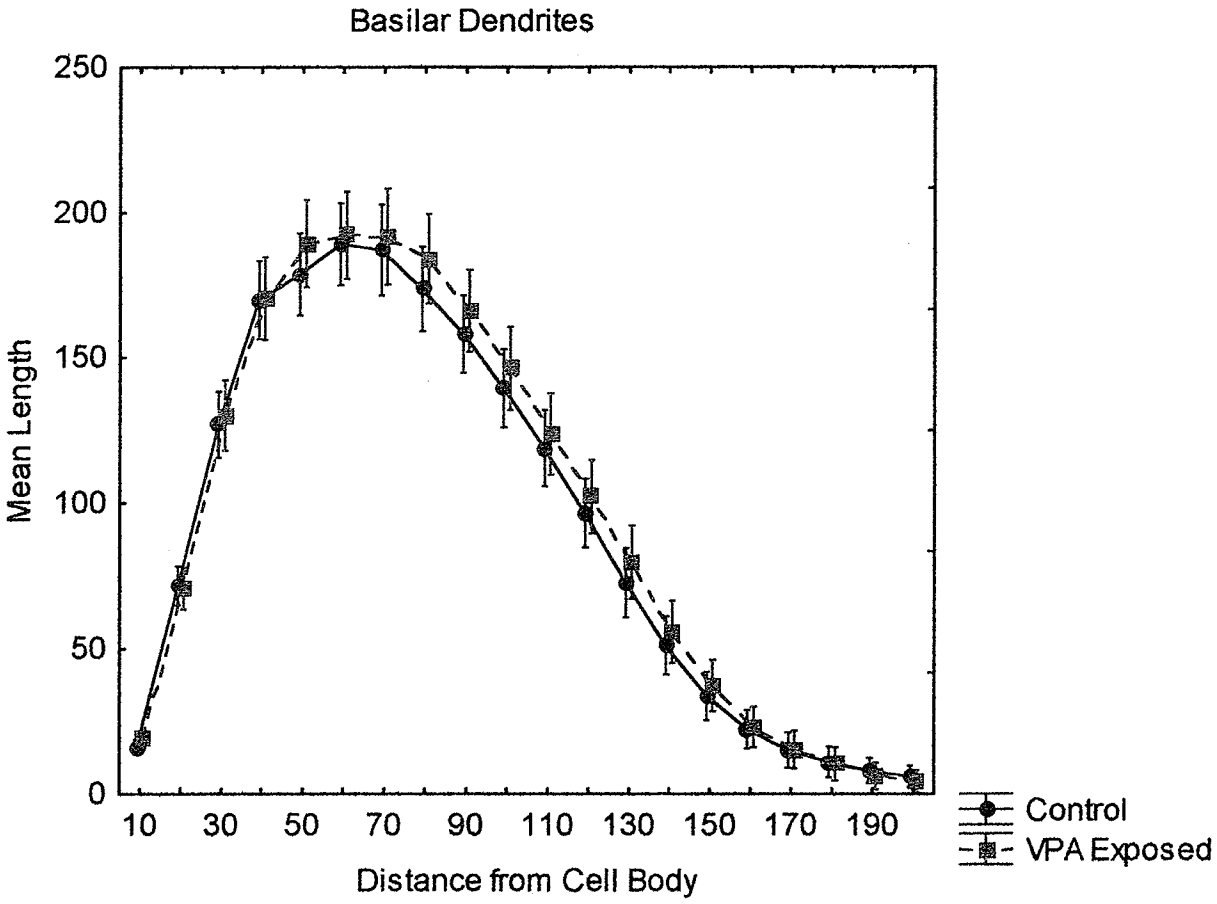
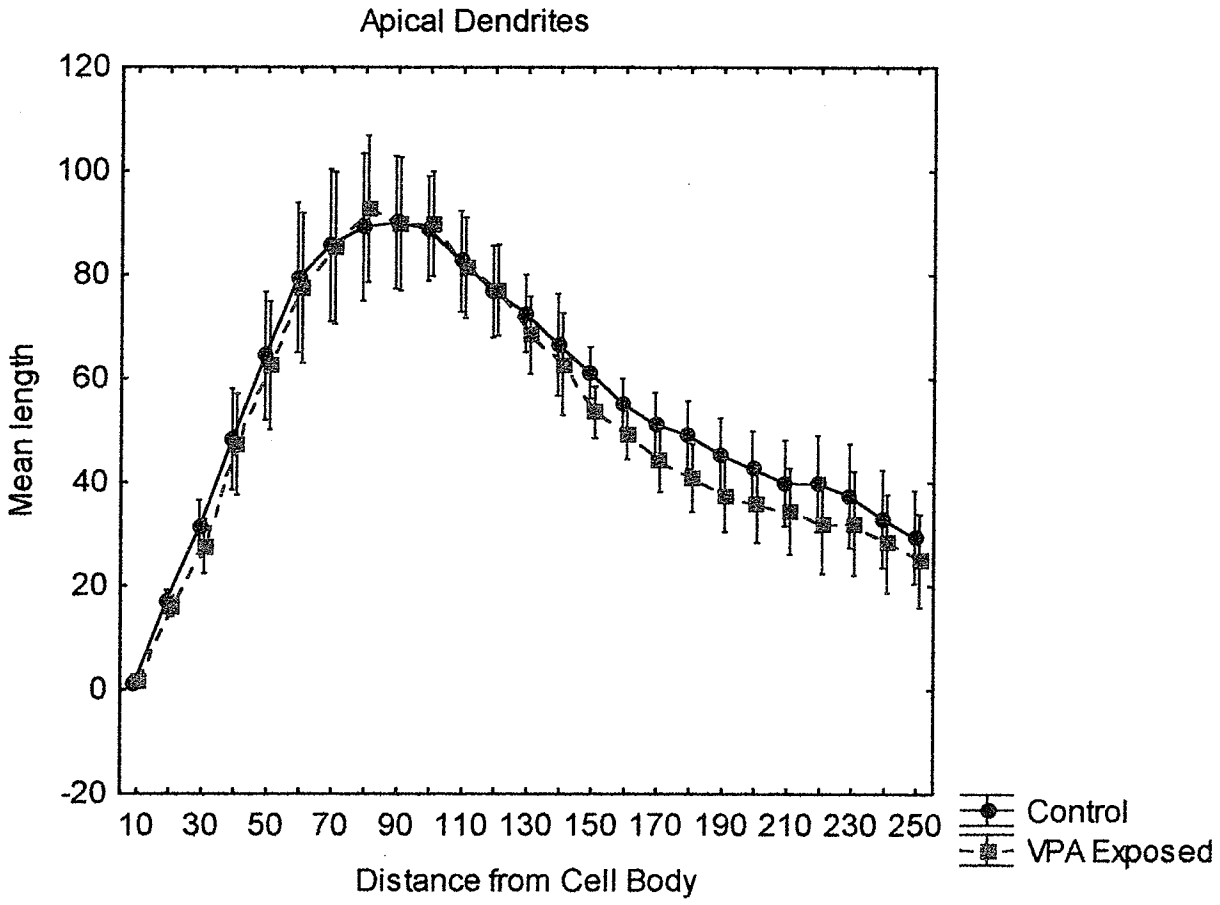

Basilar Dendrites
250
200
150
100
50
0
Mean Length
10
30
50
70
90
110
130
150
170
190
Distance from Cell Body
Control
VPA Exposed

Figure 9. Mean length (+/-SEM) of dendrites in µms at each ring as a function of distance from the soma of apical dendrites for the VPA exposed (n = 9), and control (n = 9) groups. Statistical tests were done on the length of dendritic material within the first 250 µms from the soma. Distances are in increments of 10 µms. No differences were found between the lengths at any of the distances from the cell body ($p>.05$).


Apical Dendrites
Mean length
120
100
80
60
40
20
0
-20
10 30 50 70 90 110 130 150 170 190 210 230 250
Distance from Cell Body
Control
VPA Exposed