Running head: BRAIN REORGANIZATION

Brain Reorganization of Fragile X Mice

Following Administration of Methylphenidate and Amphetamine

by

Maya Flat

A Thesis submitted to the Faculty of Graduate Studies
of

The University of Manitoba

in fulfilment of the requirements of the degree of

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THE UNIVERSITY OF MANITOBA

FACULTY OF GRADUATE STUDIES
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Dedication

This research is dedicated to all animals that have contributed to the advancement of

humankind.

Abstract

The brain's ability to change following experience is an adaptive process that occurs after environmental experiences, learning or damage. This adaptation, known as plasticity, can be measured by examining characteristics of neurons including changes in spine density and dendritic arborization. Examining what happens to the brain after drug use, such as with the use of psychostimulants, is a way of learning about brain reorganization and plasticity. Amphetamine and Cocaine trigger brain reorganization that can be long lasting. It is unclear whether Methylphenidate, a stimulant that is prescribed to children with Attention Deficit Hyperactivity Disorder (ADHD), may induce the same brain reorganization as seen with other stimulants. Methylphenidate is also prescribed to many children suffering from Fragile X Mental Retardation Syndrome (FXS) who have a high rate of comorbidity with ADHD. Stimulants induce a lesser therapeutic effect in children with retardation, including those with FXS, but there are no explanations about why this is the case. In order to examine whether Methylphenidate and Amphetamine induce reorganization in FXS, we conducted research on a mouse model of Fragile X which shows many similarities to humans suffering from FXS. We anticipated that the combined effect of mental retardation (FXS) and the use of Methylphenidate would initiate increased neuronal reorganization relative to control animals. Sholl analysis, a measure for estimating dendritic growth, showed significant interaction between group of mice and drug condition on apical measures of length. A significant interaction of group and drug condition in apical volume was also found. No significant interaction was found in dendritic complexity. Responses to stimulants between control and knockout mice are important when thinking about how stimulants might influence children with pathologically different brains. Future studies may explore changes found at the spine and/or synaptic level.

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Chapter 1

General Introduction

BRAIN PLASTICITY

Adaptation is a fundamental characteristic of all living things; it is necessary for the survival and the continued reproduction of species. Over generations, adaptation can fine-tune a species, increasing its chance of survival in its environment, whereas short-term adaptation can increase the chances of day-to-day survival. Brain plasticity is a crucial form of adaptation that is described as the brain's ability to change structure and function (Kolb & Whishaw, 1998). Throughout life, the brain will undergo several changes as the higher organism grows and interacts with the environment. The brain can alter its role following an experience in adaptive or maladaptive ways. Nelson (1999) reviewed three ways that the brain can change in response to experience. First, anatomical changes occur when, for example, the brain sprouts new axons or expands its dendritic surface of an existing cell. Second, neurochemical changes indicate the modification of activity (e.g. increasing synthesis and release of chemicals) of an existing synapse. Finally, metabolic changes signal the fluctuations in metabolic activity (e.g. the use of glucose or oxygen). In order to understand how these changes impact the organism, the role that they play in changing the brain as well as subsequent outcome will be explored.

At an anatomical level, the malleability of neuronal systems can be measured in a number of ways. A brief review of the literature suggests one of the most widely used measurements is dendritic arborization (for review see Kolb & Whishaw, 1998). Dendritic arborization is an important determinant of single-neuron function as well

as the circuitry among neurons. Dendritic trees undergo remodeling during development, aging, and many pathological conditions, with many of the morphological changes being confined to certain regions of the dendritic tree (Narayanan, Narayan, & Chattarji, 2005). The flexibility of neuronal systems and the ability of individual neurons to change is a fundamental building block of development. Throughout the process of development, the organism will grow and change, both in external appearance, and in the internal mechanisms that mediate its interaction with the world. Although the effects of experience on measures of synapse number are most profound in young animals, synaptogensis appears to occur throughout the lifespan and is not restricted to particular critical or sensitive periods of development (Greenough, Black, & Wallace, 1987; Grossman, Churchill, Bates, Kleim & Greenough, 2002). Discovery of the typical changes that follow from development have allowed researchers to manipulate environments in order to explore what impact certain changes can have on the organism.

Anatomical changes that occur in the brain can follow as a result of experience or injury to the brain and can produce a range of behavioral consequences. Several classical animal studies have allowed researchers to explore anatomical changes more in depth, all offering evidence to the plasticity of the brain. Providing animals with a complex, or enriched environment, training animals to perform specific types of motor tasks, and creating damage have all been useful tools for studying the reorganization of the brain and will be explored in greater detail. The mechanisms by which these changes occur provide valuable information for understanding the plastic brain.

Complex environments have been the subject of a great deal of investigation for the past few decades among researchers scrutinizing the anatomical changes that follow experience. In particular, research on the matter has sought to compare whether rats exposed to an enriched or modest environment would possess differing forms of cortical reorganization. Among the first to spark interest in this topic, Charles Darwin (1874) as cited by Bennett, Diamond, Krech, and Rosenzweig (1964) asserted that the brains of domestic rabbits were reduced in bulk compared to wild rabbits. Darwin believed that the difference in bulk followed from misuse of instincts over generations, and due to the confinement of the domesticated rabbit's intellectual capabilities. Over the following half-century, researchers fine-tuned this phenomenon into a replicable scientific experiment. Among the early pioneers in the field of Neuroscience, Donald Hebb (1947) raised a group of laboratory rats in his kitchen in order to test whether intelligence was influenced by experience. Allowing these rats to interact with people and roam around the home exposed them to a far more enriched environment than the laboratory raised rats. He then compared home raised rats to laboratory raised rats on an intelligence test called the Hebb-William maze and concluded that experience indeed influenced intelligence because the home raised rats performed far better on the task. In 1949, Hebb later used his data to help support his theory that synaptic plasticity underlies experience-dependent changes. These changes followed from a basic principle called the Hebb rule that is now commonly accepted: if one neuron sends many signals that excite another neuron, the connection between the two neurons are strengthened and the more active the two neurons are, the stronger the connection between them grows. Similarly, Bingham

and Griffiths (1952) established that rats reared in larger environments were superior in maze performance to animals reared in more restricted environments. A series of experimental manipulations followed from these discoveries, leading researchers to carefully craft three replicable environmental conditions.

The typical enriched environmental condition (EC) involved rats housed in a stimulating social and exploratory environment. This included a large and spacious environment for rats to roam around in with several other rats, along with access to varying types of exploratory "toys" that were replaced often with new toys. Rats housed in the standardized condition (SC) were kept alone and without toys. In many studies a third group was established that housed rats in an intermediate social condition (IC). In this condition, rats were placed in a medium sized cage with one or two other rats, but without toys (as per evidence cited by Hebb, 1947; Bingham & Griffiths, 1952; Forgays & Forgays, 1952; and Bennett et al., 1964). In 1952, Forgays and Forgays found that their EC rats were better problem solvers than the other groups they tested. Additionally, they concluded that the presence of stimulating toys apparently benefited the animal over and above having access to a large area. The observed behavioral changes in enriched rats were thought to parallel underlying changes in the central nervous system. In order to test this, the three environmental conditions created sought to isolate social and exploratory interaction. These discoveries paved the way for researchers to explore brain changes in response to the varying environmental conditions.

Among the first anatomical changes found following exposure to the differing environmental conditions was an increase in the total weight of the rat cerebral cortex

(Bennett et al., 1964), as well as in the wet weight of the occipital region of the

cerebral cortex (Bennett, Rosenzweig, & Diamond, 1969). Following this and similar

additional findings, the role of the occipital cortex in ensuing anatomical changes

began to receive a lot of attention. Volkmar and Greenough (1972) sought to

elucidate the types of anatomical changes that occurred in that region in rats exposed

to an enriched environment. They found that higher-order dendritic branching was

considerably greater in the occipital cortex of EC rats than in similar neurons in SC

rats. This increased branching suggested a greater capacity for information

processing in the brain of the animal reared in a more stimulating environment.

These findings were replicated by Greenough and Volkmar (1973). They found that

EC animals tended to have increased dendritic branching beyond the third and fourth

branches in the basal portion of the pyramidal cell dendritic tree in the occipital

cortex. This increased and complex dendritic branching could help account for the

increases in cortical weight and thickness. Occipital region changes in synaptic

number also followed from exposure to differing environments. When comparing the

ratio of synapses per neuron in layers I-IV of the occipital cortex of EC, IC or SC

rats, Turner and Greenough (1985) reported an overall increase in the number of

synapses per neuron of about 20 percent for EC animals. This increase in synapse

number presumably could account for the higher level of information processing

typically found in enriched animals.

   After the initial establishment of heavier and thicker cortices in

environmentally enriched animals, researchers began seeking answers for the

probable cause of the increased weight of the cortex. Black, Sirevaag and Greenough

(1987) examined how complex housing may affect cerebral vasculature, for example.

After rats were situated into their respective environments, the researcher reported

that EC rats had greater vascular capacity than did IC or SC animals. This finding

suggested that experience in a complex environment activated a late developmental

capacity for vessel production. The brain could improve vascular support by

generating new capillaries when neural plasticity generated long-lasting increases in

metabolic demand (Black et al., 1987). These changes could additionally support the

increase in cortical weight of EC rat brains. In 1991, Sirevaag and Greenough

examined the effects of rearing upon glial fibrillary acidic protein (GFAP)

immunoreactive astrocytes of the occipital cortex. They found that EC rats had

greater GFAP-immunoreactive astrocytic surface density than IC or SC rats after 30

and 67 days of environmental exposure, indicating a higher number of astrocytes.

More recent studies (e.g. Briones, Klintsova, & Greenough, 2004) had found that EC

rats have more synapses per neuron and that these changes tend to persist regardless

to age of exposure, perhaps requiring more astrocytic processes per neuron to

maintain a normal within body functioning. Astrocytes are beneficial in that they

play a functional role of maintaining an optimal ionic resting state and in general

playing a role in the aid of recovery (for review see Jones & Greenough, 1996). They

provide yet another way in which the EC rats may benefit from exposure to complex

environments. Moreover, this increase in astrocytic number could account for some

part of the greater cortical weight.

Chemical Changes

Following the discoveries made in the later mid-century, the typical environmental conditions established familiar anatomical brain changes. As early as 1960, researchers found that an enriched environment could have certain effects on brain chemistry (Krech, Rosenzweig, & Bennett, 1960). Krech et al. (1960) found that EC rats differed significantly from the SC group in their mean cortical-subcortical ratio, presumably due to differences in environmental stimulation and training. This ratio measured the cortical to subcortical enzyme cholinesterase (ChE) for each animal as a gauge of pattern change of ChE in the brain, with EC rats having a lower ratio. ChE along with acetylcholinesterase (AChE) are known to split up acetylcholine (ACh) in the brain (Rosenzweig, Krech, & Bennett, 1960), and higher levels of ACh have been found to be positively related to problem-solving ability in rats. Thus, the lower ratio of ChE in the EC group may be responsible for higher levels of AChE in the brain, and an increased ability of those rats to perform better on varying tasks. In order to determine whether reported differences of AChE activity play a part in reported behavioral differences between EC and SC rats, a range of eserine doses was administered after training on behavioral tasks. Eserine, a potent reversible inhibitor of AChE, has been implicated in the facilitation of memory through reducing attention to external interruptions (Greenough, Yuwiler & Dollinger, 1973). Post-trial administration of eserine in EC and SC rats suggested that it could facilitate memory formation, developing differently with differential rearing. In fact, EC rats benefited more from eserine, potentially because of their better-developed attentional mechanisms (Greenough et al., 1973).

Critical period and time frame

Speculation as to whether these types of changes are limited to younger rats in their critical period of learning have initiated an ongoing debate between researchers who hold differing opinions on the matter. In favor of all age groups having an equal chance of benefiting from complex environments, Bennett and colleagues (1964) concluded that the adult brain shows changes as readily as the younger brain. The authors supported the argument that the effects, rather than being consequences of accelerated early development, are residuals of experience. The debate continues, however, with researchers sitting on both sides of the fence on this issue. Ferchmin and Eterovic (1985) attempted to determine the shortest period of exposure to environmental complexity that would produce measurable changes in brain weight, or RNA content, and if it varied with age. They found that only periadolescent rats aged 30- to 40-days-old, had a total increase in total cortical weight and RNA content after 10 minutes of exposure a day for four days. Both young and old rats were eventually able to produce these same changes, although it required longer periods of exposure to the enriched environment. More recently, it has been found that the changes following environmental enrichment vary with age at the time of experience, details of the experience and the sex of the animal, and that only certain phases of each of these factors induce plastic changes (Kolb, Gibb, & Gorny, 2003). Additionally, Branchi, Francia, and Alleva (2004) reported that four days of exposure to an enriched environment appeared sufficient to produce brain changes in periadolescent rats, but not in adults. Although a variety of factors may influence the timeframe for

the ensuing changes in EC rats, it is clear that exposure to complex housing can enhance motor and cognitive skills and can produce reliable increases in brain weight, independent of body weight. These changes offer a wide spectrum of support for the capabilities of our plastic brain.

Motor Learning Tasks

Motor learning has been found to induce plastic changes in the brain as well. Like much of the research done on environmental enrichment, training rats to perform a motor task appears to evoke the same cascade of neurochemical events that cause plastic changes in the brain (eg Rosenzweig & Bennett, 1996). Much like the environmental enrichment task, the motor task is a learning task that allows researchers to control activity, and therefore manipulate the variable they chose with greater ease. Two types of motor learning tasks have been used, an acrobatic learning task and a reaching task. The typical acrobatic motor learning task consists of randomly allocating adult rats to an acrobatic condition (AC), a motor activity condition (MC), and occasionally, an inactive control group (IC). The AC animals are trained to traverse a complex series of obstacles, arranged similarly to an obstacle course. Each AC animal is pair-matched with an MC animal that is given access to a similar motor activity, like an exercise wheel, or is given the opportunity to traverse an alleyway equal in length to the runway the AC animal traverses, although obstacle-free (Kleim, Lussnig, Schwarz, Comery & Greenough, 1996). The inactive controls are maintained in standard cages without access to activities. These animals live in a standard environment without a running wheel or access to other motor activities. A second motor task, the motor reaching task, requires researchers to train rats to reach

for a treat with one of their arms and hands generally through a plexi-glass container

with a slot for them to reach through (e.g. VandenBerg, Hogg, Kleim, & Whishaw,

2002). These rats are pair-matched with an inactive control group that is given access

to the same treat, without requiring them to reach for it. Occasionally, a motor

activity control group trained to use their arms in an unskilled, but purposeful way is

also included, to control for changes related to motor activity. Both types of tasks

have produced some striking changes in the brain.

Following training rats to reach for bits of food with their preferred paw,

researchers sought to untangle whether an explicit hemispheric reorganization would

manifest itself in the brain. Indeed it was found that following training rats to reach

for bits of food in a tube, the hemisphere opposite the trained forepaw had a larger

apical dendritic field (Greenough, Larson & Withers, 1985). The authors looked at

the same tissue and found a lateralized effect of training on layer V pyramidal cells as

well as a nonspecific effect on the upper layer II/III forked apical pyramidal cells

(Withers & Greenough, 1989). Suggesting that at the higher layer pyramidal cells the

changes produced by the motor task were evenly and generally enhanced, whereas

layer V cells showed an enhanced effect only in one hemisphere. These findings

suggest that learning and practicing a motor skill is accompanied by an increased

efficacy of horizontal connections in the motor cortex.

In order to further isolate the specific areas in the brain being altered

following skilled learning, researchers compared rats trained on a skilled reaching

task with rats trained on a simple bar pressing task (Kleim, Barbay, & Nudo, 1998).

Microelectrode stimulation was employed to derive high resolution maps of the

forelimb and hindlimb representations within the motor cortex. Reaching trained reaching rats exhibited significantly higher mean areas of the wrist and digit representations, demonstrating that motor skill learning is associated with a reorganization of movement representations within the rodent motor cortex. Following this, and other similar findings, an ongoing debate ensued about whether motor activity in general was responsible for this forelimb reorganization, and not the specific motor learning task itself. In addressing this issue, Kleim and colleagues (2002) demonstrated an increase in synapse number, occurring in layer V following skilled forelimb training. Additionally, it was found that the functional reorganization of the forelimb motor cortex occurred in response to development of skilled forelimb movements and not simply to increased forelimb use. Thus, it was shown that motor activity alone was not responsible for the ensuing brain changes. Furthermore, (Luft, Buitrago, Ringer, Dichgans, & Schulz, 2004) found that skill learning is interrupted by protein synthesis inhibition (PSI). Protein synthesis is considered necessary in order for these changes to persist.

VandenBerg, Hogg, Kleim, and Whishaw (2002) explored the different cortical representations of the motor map of two breeds of rats. They found that topographic map size in the motor cortex conforms to a principle of proper mass; that is, more skilled movements are associated with larger maps. This finding reinforces the common notion that skilled learning in general is largely represented within the neocortex. Wawryko, Ward, Whishaw, and Ivanco (2004) suggest that the ensuing changes brought about were due to changes in efficacy and organization within the

different breeds of rats despite a great degree of physiological similarity in motor cortex.

Motor skill learning on complex acrobatic tasks provided similar results as on the reaching tasks. In 1990, Black, Isaacs, Anderson, Alcantara, and Greenough found that AC animals had greater number of synapses per Purkinje cell in the paramedian lobule (PML) then animals from either the MC or IC group. As previously noted, this suggests that increased movement alone is not responsible for the increase in Purkinje cell synapses, but rather that a degree of skilled learning must be present for the change to occur. In a similar study, Kleim, Vij, Ballard, and Greenough (1997) split a group of rats into either the AC or MC group and trained them for either 10, 28, or 38 days. All rats were sacrificed on the same day, when the last group had completed training. It was found that AC rats had significantly more synapses per Purkinje cell then the MC group irrespective of the number of days they were trained. This finding suggested that these changes could sustain themselves for at least 28 days post-training and that they did not require a continuous stream of AC training for them to occur. Kleim, Swain, Armstrong, Napper, Jones, and Greenough (1998) similarly found an increase in the number of synapses within the cerebellar cortex in the Purkinje cells using quantitative electron microscopy following a complex motor learning task. Despite the fact that the MC group traveled a far greater distance then the AC animals, they did not show an increase in synapses, reemphasizing that complex motor learning, and not merely an increase in activity level, was required for these changes to occur.

Purkinje cells, found in the cerebellar cortex, got a lot of attention in terms of the changes that are produced in the motor cortex. Similarly, stellate cells were also found to increase after motor learning. Kleim, Swain, Czerlanis, Kelly, Pipitone, and Greenough (1997) showed that following testing rats on either an AC or MC condition, AC animals had significantly greater cerebellar stellate cell dendritic arborizations than the activity controls. This indicates that motor learning is associated with an increase in the amount of postsynaptic space on these neurons. Marr (1969) has suggested that stellate cells may inhibit the Purkinje cell to prevent the overloading by parallel fiber excitation. Several studies have noted that acrobatic animals have significantly more parallel fiber to Purkinje cell synapses than control animals (Black et al., 1990), thus the increased number of stellate cells observed in motor studies may serve to balance the excitatory and inhibitory input to Purkinje cells. Indeed, many studies have repetitively found that complex motor skill learning is associated with structural plasticity in the cerebellar cortex, and surprisingly not in the lateral cerebellar nucleus, which is a primary output target of the cerebellar cortex (Kleim, Pipitone, Czerlanis, & Greenough, 1998). Additionally, researchers have found that rats participating in a motor learning task had increased formation of multiple synapses in the cerebellum as compared to the active or inactive controls (Federmeier, Kleim, & Greenough, 2002). The formation of these multiple synapses provides additional connection between a given parallel fiber and Purkinje cell, thereby enhancing particular pathways and constituting a fundamental mechanism of neural encoding. Therefore, the range of changes that follow from motor skill learning may prove to be advantageous.

Damage

Brain damage can provide an excellent means of studying the synaptic

reorganization of the brain. Through watching how an animal recovers from injury,

researchers have learned about the brain and its capabilities. Studying injury is

important for learning about the best way to increase chances of recovery as well as

supportive techniques that may improve recovery of function prior to injury. As

noted by Kleim, Jones and Schallert (2003), certain preventative measures, like

exercise before a focal ischemic injury, increase the defenses against cell death and

could maintain or expand motor representations in the brain. Many injury studies

involve producing some type of damage in an animal, then subsequently noting the

recovery outcome following training animals on a learning task. The literature

indicates an array of evidence as to the successive plasticity that follows brain injury

(Hebb, 1949; Will, Rosenzweig, Bennett, Hebert, & Morimoto, 1977). For example,

Rosenzweig and Bennett (1996) noted that at all parts of the life span, training and

enriched experience help in recovery from, or compensation for, the effects of brain

damage. Similar results have been reported in other studies. For example, experience

on a complex motor skills task following a unilateral lesion of the forelimb

representation region of the sensorimotor cortex (FLsmc) enhanced synaptic

structural changes in the cortex contralateral and homotopic to the lesions (Chu &

Jones, 2000). These findings suggest that behavioral training following cortical injury

facilitates structural plasticity in behaviorally relevant areas of the neocortex other

than the homotopic cortex contralateral to the lesion. Additionally, some researchers

believe that early after injury, an active or critical window opens where plasticity

mechanisms are at their highest and where early forced forelimb use or exercise has been shown to halt cell loss (Kleim et al., 2003). Recently Barbay et al. (2006) studied the neurophysiological consequences of delaying rehabilitation in squirrel monkey for one month following injury. The authors found that early retraining of a hand infarct resulted in regaining total hand, and especially digit, representations. With delayed training, however, digit representations do not appear to recover. Examining induction of plasticity following damage provides researchers and patients with valuable information about response capabilities following injury.

Damage to specific areas of the brain that correlate with certain behavioral deficits generally indicate a disruption in normal brain functioning. Cerebellar damage, for instance, has been increasingly linked to impairment in motor skill learning (Lincoln, McCormick & Thompson, 1982). Neuroscientists have, thus, taken up the task of identifying the types of damage that correspond with impairment, and the measures that produce the highest recovery. One such example has been found following infarcts in primates. Nudo, Wise, SiFuentes, and Milliken (1996) studied the reorganization of the motor cortex following a focal ischemic infarct in primates. Retraining of skilled hand use following an infarct resulted in the prevention of the loss of hand territory adjacent to the infarct. This finding suggests that rehabilitative training following damage to the motor cortex can shape subsequent reorganization in the adjacent intact cortex. These findings suggest that the plasticity of the brain can be partially reliant on selected positive actions of the individual.

Brain plasticity has been described as the brain's ability to alter its structure

and function following experience, learning, and injury. These changes have led to

both positive and negative impacts on the higher organism. Although many of the

changes we have explored up until now have elucidated some of the positive ways the

brain can change, it is equally if not more important to understand some of the

destructive changes that can occur in the brain. In general, studies of brain plasticity

reveal additional mechanisms active in the brain and gives further information as to

the complex manner in which it operates.

Drug Impact

Some of the most compelling long-term and socially relevant examples of

experience-dependent changes in behavior and psychological function are those that

occur with the development of addictions (Robinson & Kolb, 2004). Brain changes

that follow from drug abuse mimic experience-dependent changes and their effects

can be long-lasting (Robinson & Kolb, 1997). An ideal location for studying these

behavioral changes is via dendrites; one of the indicators of change within the central

nervous system (CNS), growing and retracting in response to various events.

In order to test the dendritic changes that follow from exposure to

psychostimulants, Robinson and Kolb (1997) conducted a study on rats in which they

injected half of a group of rats with amphetamine and the other half with saline. They

tested whether persistent behavioral sensitization would produce structural

modifications in the nervous system similar to those seen in association with other

forms of experience-dependent plasticity. One month after the last treatment with

amphetamine there was an increase in dendritic surface and in the number of

dendritic spines on medium spiny neurons located in the shell and core subregions of

the nucleus accumbens and on only the apical dendrites of layer III pyramidal cells in

the prefrontal cortex. These changes in dendritic structure are considered strong

evidence of changes in synaptic connectivity and provide evidence to the long lasting

changes that can follow from this type of drug exposure.

The notion that drug exposure could produce long lasting changes in dendritic

structure has sparked additional research on the topic. Dendritic changes have been

associated with increased drug sensitization. Paulson, Camp, and Robinson (1991)

found that behavioral sensitization was fully evident 2 weeks to 1 month after the last

drug treatment and could persist for at least 1 year. Robinson and Kolb (1999)

similarly found that following exposure to two psychostimulants, amphetamine and

cocaine, persistent changes in dendrites and dendritic spines were still evident 1

month after discontinuation of drug treatment. These findings imply that changes

following drug use can be long lasting. Additionally, Robinson and Kolb (1999)

considered the impact of increased motor activity and the administration of

psychostimulants on amplified sensitization and changes in dendrites. Although it

was found that both exercise and psychostimulants did produce changes on dendrites,

psychostimulants were found to increase the number of distal portion branches on the

dendritic tree and the density of dendritic spines on medium spiny neurons in the shell

of the nucleus accumbens, and on apical dendrites of layer V pyramidal cells in the

prefrontal cortex. Increased motor activity produced a decrease in dendritic

branching localized to branch orders 2 and 3 (Robinson & Kolb, 1999). These

findings subsequently suggest that motor activity influences different synaptic

changes than psychostimulant drugs, and that the effects of these psychostimulants are not just secondary to their ability to increase motor activity.

Kolb, Gorny, Li, Samaha and Robinson (2003) explored the possibility of amphetamine and cocaine limiting the ability of experience at a later time to promote structural plasticity in the neocortex and nucleus accumbens. Prior to housing rats in EC or SC groups for several months, they injected them with either amphetamine or cocaine. Prior treatment with amphetamine or cocaine did indeed interfere with the ability of experience in a complex environment to increase dendritic arborization and spine density. These findings suggest that psychostimulant use may hinder the ability of experience to produce changes within the brain, and thus make understanding their precise role so critical.

Beyond interfering with the beneficial aspects of complex environments, the use of psychostimulants have also been found to induce some troubling behaviors. Robinson and Becker (1986) found that one consequence of repeated amphetamine use is amphetamine psychosis, characterized by paranoid-schizophrenic-like symptoms. They found that amphetamine caused behavioral sensitization in which an enhanced sensitivity to amphetamine followed its repeated usage. Thus, exposure to psychostimulants mimics the experience-dependent changes that are produced in the plastic brain in day-to-day life, but the changes tend to be bigger, and can have long lasting impacts on both brain and behavior.

Overall, the findings that have been reviewed provide strong evidence of the plasticity of the brain. Plastic changes in the brain have been observed in response to novel situations, learning, damage, and in response to drugs. The prefrontal cortex

has been found to have an impressive ability to reorganize in response to experience

with drugs. Early research has focused on drug related changes in normal

populations. A timely change in focus of researchers has led to a growing body of

evidence on how drugs may influence the abnormal brain.

Chapter 2

Disorders and Drugs

<u>Fragile X</u>

Fragile X Mental Retardation Syndrome (FXS) results from an inherited genetic mutation preventing, or greatly reducing, Fragile X Mental Retardation Protein (FMRP) production in humans (Oberle et al., 1991). It is an X-linked genetic disorder with an incidence of 1/2,000 in males, and about half that in females (Brown, 1996). In addition, the disease phenotype includes moderate to severe mental retardation, developmental delay, machroorchidism, and pronounced facial features (Hagerman, 1996). Like humans, the mouse *Fmr1* gene is located on the X chromosome. In both the human and the mouse, this gene is responsible for the production of FMRP. Expression of this protein in the brain and body can be seen in similar locations and follows similar developmental time courses (Bakker & Oostra, 2003). To mimic FXS in the mouse, the mouse model was created by disrupting the murine *Fmr*1 gene, which inactivates it and leads to an absence of FMRP in the mouse. These *fmr*1 knockout mice then exhibit physical and behavioral abnormalities similar to human FXS patients (Dutch-Belgian Fragile X Consortium, 1994).

The mouse model of FXS must prove to be similar to the human model in order to ensure its use as such a model. Various studies have explored how the changes seen in the mouse model mimic those seen with humans. Of significant importance to their similarities is whether FMRP shows a comparable role in both species. Along with showing that the murine FMRP homologue shows a 97% similarity to the human form, the Dutch-Belgian Fragile X Consortium (1994) found

the mouse model to show macroorchidism and behavioral abnormalities that are similarly found in the human patient. Macroorchidism is one of the most common physical features in FXS patients. Knockout *fmr1* mice have likewise shown these features as a result of increased Sertoli cell proliferation during testicular development (Slegtenhorst-Eegdeman et al., 1998). This increase in Sertoli cell proliferation subsequently causes increased germ cell numbers and elevated testis weight. Additionally, Slegtenhorst-Eegdeman and colleagues found that knockout mice, along with showing common physical similarities to humans, show certain typical FXS characteristics, such as learning difficulties and hyperactivity.

The lack of FMRP expression in the knockout mice has been implicated in a variety of attributes of dendritic spines. Weiler et al. (1997) examined local translation of proteins in synapses and identified mRNA for FMRP. They observed increased expression of FMRP after mGluR1 stimulation, suggesting rapid FMRP production near synapses in response to activation may be important for normal maturation of synaptic connections. Irwin, Galvez and Greenough (2000) suggested that FMRP serves as an 'immediate early protein' at the synapse that orchestrates aspects of synaptic development and plasticity. They noted that cortical spine morphology appears to be immature in FXS both for humans and in the knockout mouse model. Spines appear to be long and thin rather than the more characteristic stubby and mushroom-shaped spines seen in normal development (Comery et al., 1997; Irwin et al., 2000; Irwin et al., 2001). Additionally, (Irwin et al., 2000, Irwin et al., 2001) found that Fragile X is often accompanied by a higher density of spines along dendrites, suggesting a possible failure to properly eliminate extraneous

synapses. These findings all corroborate the importance of FMRP in normal development and suggest a role in maturation and pruning.

Fragile X and Attention-deficit hyperactivity disorder

ADHD is seen in the majority of children with FXS (Hagerman et al., 2002b). The proportion of FXS boys with ADHD ranges from 70 to 100% depending on the study (Baumgardner, Reiss, Freund, & Abrams, 1995; Hagerman, 1996; Turk, 1992), whereas FXS girls with ADHD range from 30 to 50% (Freund, Reiss, & Abrams, 1993; Hagerman 1992). Farzin et al. (2006) recently reported that FXS children exhibiting symptoms of FXS had a 93% chance of displaying ADHD symptoms and that premutation carriers had a higher incidence of autism spectrum disorder (ASD) than controls, but did not exhibit a higher rate of ADHD compared with controls. Currently, there are no treatments directed at the underlying neuronal defect due to the absence of FMRP, thus, treatment strategies for FXS individuals are designed to maximize functioning. Given that the incidence of ADHD is so high among FXS children, stimulant medication, such as Methylphenidate, is widely prescribed to them (Berry-Kravis & Potanos, 2004; Hagerman, Murphy & Wittenberger, 1988). In some individuals with FXS, stimulants exacerbate anxiety, mood lability, or aggressive tendencies and must be discontinued. As FXS is often accompanied by problems in several symptom areas, careful supervision of medication is necessary as medications may help with some behaviors, but aggravate others (Berry-Kravis & Potanos, 2004).

In populations with nonspecific mental retardation, stimulants have been shown to be more effective in individuals with a higher intelligence quotient (IQ), whereas side effects are more problematic in those with a lower IQ (Aman, Marks,

Turbott, Wilsher, & Merry, 1991). These authors conclude that Methylphenidate

should be used sparingly and only as a second-line treatment in cognitively immature

children, such as in preschoolers and, especially, mentally retarded children with low

mental ages (i.e., below 4.5 years) and/or low IQs (i.e. less than or equal to 45). This

finding has implications for use of stimulants on children with FXS and may indicate

that children with FXS have a smaller chance of reacting well to them. Handen,

Feldman, Gosling, Breaux, and Mcauliffe (1991) found that the use of

Methylphenidate had adverse consequences in a large percentage of children with

lower IQs (in the range of 48 to 74). In their study, 27 children with low IQ and

hyperactivity were treated with Methylphenidate and their side effects were

monitored. They concluded that stimulant medication increased the risk for

developing motor tics and becoming socially withdrawn, especially in children with

mental retardation. This finding additionally suggests that FXS children may have a

reduced chance of reacting well to Methylphenidate, or other stimulant medication

prescribed to them.

Methylphenidate

Methylphenidate (Ritalin) is a stimulant drug similar to amphetamine that is

used to treat ADHD. Like other stimulants, Methylphenidate has been shown to have

long lasting social and behavioral effects in animals (McDougall, Collins, Karper,

Watson, & Crawford, 1999; Waite, Hess & Kolb, 2004). Currently, Methylphenidate,

a mild CNS stimulant used to modulate behavior of children with ADHD, has a mode

of action that is not clearly understood, but it is presumed to activate the brain stem

and cortex (Hewitt et al., 2005). Methylphenidate appears to be involved in

remedying the inadequate activity in the prefrontal lobe of individuals with ADHD during attentional tasks, revealed by neurofunctional imaging studies (Hynd et al., 1993). This activity in the prefrontal lobe seems to reflect a rapid depletion of dopamine, which is essential for executive functioning. The disruptions of attention may occur through excessive inputs leading into the prefrontal cortex or through decreased activity of inhibitory pathways exiting the prefrontal cortex (Hunt, Paguin, & Payton, 2001).

Methylphenidate, like amphetamine, is a psychostimulant and, as such, shares many characteristics with other well known stimulants. It is known, for example, that chronic exposure to Methylphenidate in animals can lead to tolerance and dependence (Waite et al., 2004), similar to that seen with other psychostimulants. The issue of whether tolerance truly exists after doses have been titered to account for body weight has been examined by several researchers. Safer and Allen (1989) aimed to clarify whether an initially effective dose of Methylphenidate needed to be increased with time to maintain its behaviorally beneficial effects in children. In order to explore this, they viewed case folders of hyperactive and inattentive students in long-term stimulant treatment. Safer and Allen (1989) found that the dose of Methylphenidate, corrected for body growth, showed no change over the course of treatment, and that the initial dose did not significantly differ from the final dose. This provided evidence that tolerance did not develop to Methylphenidate. Students that showed initial benefits from Methylphenidate, but lost their benefit within the first 3 years of treatment however, were not included in this study. Winsberg, Matinsky, Kupietz and Richardson (1987) suggested that the incidence of tolerance to Methylphenidate

is unknown because those children that develop tolerance early in the treatment are not eligible to participate in long-term studies on the effects of Methylphenidate. In the Winsberg et al. (1987) study on the effects of Methylphenidate on reading achievement, some evidence for the development of tolerance, in the form of increased hyperactivity in the later weeks of the study, was found. This suggests that tolerance may develop in chronic Methylphenidate treatment at higher doses. Greenhill and colleagues (2001) found that when testing low, medium and high doses of Methylphenidate on children that responses were evenly distributed and suggested that drugs need to modified for each individual. Several other studies have found that the effectiveness of Methylphenidate wears off over time when administered at high doses and that in order to achieve effectiveness again, patients must be switched to a different drug, typically dextroamphetamine, and then back to Methylphenidate when that drug begins to wears off (Eichlseder, 1985; Ross, Fischhoff & Davenport, 2002). Tolerance in these studies was defined as the failure to maintain a clinical response to the same dosage, and thus required that the drug dosage be increased.

Others have found that within a day, but not over long stretches of time, a tolerance to Methylphenidate develops in humans. Swanson et al. (1999) found that an acute tolerance developed in children receiving Methylphenidate in a flat sequence, which is similar to sustained release Methylphenidate that maintains a constant drug concentration throughout the day. Results from this study suggested that constant drug concentrations do not produce a constant behavioral effect and that within a day acute tolerance can develop in these cases but returns to normal the next day. More recently, Steele et al. (2006) reported that OROS- Methylphenidate, a

controlled once a day formula with an ascending pharmacokinetic profile designed to counteract acute tolerance typically found in the multiple release formula, was more effective for children as well as easier to implement. Taken together, these findings suggest that there is development of tolerance in some individuals, especially at higher doses and with the multiple releases dosing regime, but that it is not always considered a typical attribute of Methylphenidate usage. Additionally, the types of studies that measure these types of changes are very limited and often times are anecdotal findings. More systematic review of the effects of stimulants that can be measured objectively is necessary to drawing any firm conclusions about the development of tolerance.

Rationale

Regardless of the debate about tolerance and the addictive properties, the consequences following long term exposure to stimulants in the developing brain are still unknown. Given that Methylphenidate and amphetamine belong to the same class of drugs, similar dendritic reorganization is expected and reorganization of cortical neurons that has been shown in the rat is expected (eg amphetamine: Robinson & Kolb, 2004; Methylphenidate: Waite et al., 2004).

It is expected that the positive consequences of many drugs provided to children to alter their behavior may be related to the drug's ability to reorganize neurons. We, thus, hypothesized that exposure to stimulants (including Methylphenidate) would induce neuronal change. To make the problem more difficult, the consequences and potential side effects of Methylphenidate treatment when two or more disorders are present have not been well investigated. We

therefore proposed that the side effects of Methylphenidate would be amplified in children with mental retardation, including children with Fragile X Mental Retardation Syndrome, as a result of amplified neuronal reorganization that occurs with administration of the drug. This potential increase in neuronal growth would be expected to be even more dramatic after long-term exposure.

Little evidence is available on the response of the brain to stimulant treatment. Given that this study cannot be conducted in a human population, a simple evaluation of neuronal structure following exposure of Methylphenidate to an animal model of Fragile X may be informative. Thus, we studied the effects of Methylphenidate and another stimulant given to FXS children in a mouse model of Fragile X Mental Retardation Syndrome. The *fmr1* knockout mouse shares many similarities with the human disorder at the physical, behavioral and anatomical levels (Bakker & Oostra, 2003).

Hypothesis

We hypothesized that 1) exposure to Methylphenidate would induce neuronal reorganization similar to that seen with psychostimulant (amphetamine) exposure in rats (Robinson & Kolb, 2004); and, 2) Methylphenidate and amphetamine would produce more dramatic reorganization in the *fmr1* knockout mouse than in control mice.

Chapter 3

Materials and Methods

<u>Methods</u>

Subjects consisted of 16 *fmr1* knockout and 13 wildtype sibling mouse pups

from the Ivanco laboratory colony. Mice were previously purchased from Jackson

Laboratories and a homozygous knockout, heterozygous, and wildtype population of

sighted animals on the FVB strain was maintained at the University of Manitoba.

Male wildtype mice aged 21 days and male *fmr1* knockout mice were randomly

assigned to one of three experimental conditions: saline, amphetamine (3 mg/kg

Dexadrine), or Methylphenidate (10 mg/kg Ritalin) (consistent with Berridge, 2001).

The dose is within the normal range of human dosages (Pelham et al., 1999; Stein et

al., 2003) to accommodate for the faster metabolism of the mice, but higher than that

normally prescribed for Fragile X children (0.2-0.3 mg/kg) (Hagerman, 2002). Due

to the varying treatment conditions, there were six groups of animals: knockout

mouse with saline (n=6), knockout with amphetamine (n=5), knockout with

Methylphenidate (n=5), wildtype mouse with saline (n=4), wildtype with

amphetamine (n=5), and wildtype with Methylphenidate (n=4). The investigator was

blind to the genotype. Mice were familiarized to corn syrup for several days before

drug treatment, until they readily ingested it. Drugs were mixed into a drop of syrup

and put on individual glass slides for each mouse. Mice ingested drug and corn syrup

twice a day for five consecutive days, followed by two treatment-free days (to mimic

weekday ingestion of Ritalin and weekend free periods). This procedure was

repeated for three consecutive weeks.

Tissue Preparation

After three weeks, all animals were sacrificed with a lethal dose of sodium

pentobarbital (100 mg/kg). The animals were perfused with saline through the heart,

and brains extracted and immersed in Golgi-cox solution (as per Gibb & Kolb, 1998).

Immersion time was 15 days, after which time the tissue was transferred to a sucrose

solution for 3 days, and then changed into a fresh batch of sucrose where it remained

until tissue was sectioned. The tissue was sectioned at 150 μm with a vibratome,

sections were placed onto glass slides, and allowed to sit for 48 hours in a cold humid

chamber. Tissue sections were then processed using ammonium hydroxide, which

caused the formation of a precipitate within the cell. This precipitate fills the cell in

its entirety in 1-4% of cells allowing for visualization of the cell and its components

using light microscopy. Tissue slides were cover-slipped using Permount. Under the

microscope, tissue sections revealed clearly visible cell bodies, dendrites, and

dendritic spines.

Data Collection

Pyramidal cells were drawn from layer III of the prefrontal cortex of the mice.

Ten cells from each mouse were drawn with an attempt to obtain five cells from each

hemisphere. The experimenter was kept blind to the condition. Cells were judged

acceptable for drawing according to uniform staining of neuronal processes including

the cell body, apical and basal dendrites and spines, the absence of debris. An

attempt to pick cells with uniform thickness and natural tapering of dendritic branches

with no abrupt blunt ends was also made. These cells were traced using NeuroLucida

software, a program that utilizes a microscope and motorized stage. The

NeuroLucida software program allows for an three-dimensional sphere analysis of dendrites as well as analysis of volume and complexity of cells by analyzing three-dimensional reconstructions of traced neurons using NeuroExplorer (MicroBrightField, Inc.). NeuroExplorer is a three-dimensional and morphometric analysis program designed for viewing, analyzing and presenting data acquired using MicroBrightField's NeuroLucida program.

Comparison of differences in total dendritic length was accomplished using a three-dimensional Sholl analysis, which involves counting the number of dendrites that intersect each ring using a series of concentric, equidistant three-dimensional spheres. The cell was analyzed by counting the number of dendrites that intersect each sphere. Dendritic intersections were counted and multiplied by the distance between each ring, 10 μm, to provide an estimate of total dendritic length. The complexity of dendritic branching was determined using the NeuroExplorer software program. A branch order was assigned by the number of bifurcation points the segment was away from the apical shaft for apical dendrites or from the cell body for basilar dendrites. Dendritic branches arising from the cell body are first order branches until they split (or bifurcate) into second order segments, which branch into third order and so on.

Statistical Analysis

Analysis of variance (ANOVA), using the results from the NeuroLucida software was used to analyze the data. A 2 X 3 repeated measures ANOVA (genotype and drug type) was conducted in order to reveal any main effect of genotype and drug type on each measure of neuronal morphology. Tests of length,

volume, and cell complexity were conducted, independently for apical and basilar

dendrites.

Chapter 4

Results and Discussion

<u>Results</u>

The ANOVA conducted on length of apical dendrites showed no main effect of group for cells in layer III, $F(1, 23) = 0.4$, $p = 0.54$. There was no main effect of drug conditions, $F(2, 23) = 0.6$, $p = 0.535$. There was a statistically significant group x drug condition interaction, $F(2, 23) = 3.8$, $p = 0.039$ (see Figure 1). Tukey post hoc tests for unequal sample sizes showed no clear differences in the comparisons of interest.

Sholl analysis on basilar dendrites of layer III cells also revealed no effect of group $F(1, 23) = 0.7$, $p = 0.407$ or drug condition $F(2, 23) = 0.5$, $p = 0.625$. There was also no significant group x drug interaction $F(2, 23) = 0.4$, $p = 0.659$ (see Figure 2).

A test of apical branch order revealed no main effects for group, $F(1, 23) = 0.3$, $p = 0.567$, or drug condition, $F(2, 23) = 0.6$, $p = 0.557$. No group x drug interaction was revealed, $F(2, 23) = 2.6$, $p = 0.097$ (see Figure 3).

Basilar branch order analysis revealed no main effects for group, $F(1, 23) = 0.7$, $p = 0.403$, or drug condition, $p = 0.994$, and no significant group x drug condition interaction $F(2, 23) = 0.5$, $p = 0.593$ (see Figure 4).

Volume analysis of apicals revealed no main effect of group, $F(1, 23) = 0.167$, $p = 0.686$ or drug condition, $F(2, 23) = 0.729$, $p = 0.493$, but did reveal a significant group x drug interaction $F(2, 23) = 4.226$, $p = 0.027$ (see Figure 5). Tukey Post hoc

tests for unequal sample sizes revealed no clear differences in the comparisons of interest.

Volume analysis of basilar dendrites revealed no main effect of group, $F(1, 23) = 0.028$, $p = 0.869$, or drug condition $F(2, 23) = 0.205$, $p = 0.816$ and no group x drug condition interaction $F(2, 23) = 1.145$, $p = 0.336$.

Discussion

It was expected that the administration of Methylphenidate and amphetamine would induce neuronal growth in all the mice tested; particularly amplified neuronal reorganization in the *fmr1* mice. Depending on the type of changes that were found, these findings could have implications for drug administration protocols for children, as well as for evaluating side effects of drugs that may be producing unintended affects in many children.

Statistical analysis revealed several findings. ANOVA tests revealed a group x drug condition interaction in cell apical length. Tukey post hoc tests for unequal sample sizes showed no clear differences in the comparisons of interest. Apical volume also showed a group x drug interaction and, although post hocs concluded it was significant, further inspection indicated that this finding was coupled with high error variability within subjects and was, thus, considered to be non-significant. A larger sample would decrease the effect of this variability. Apical complexity showed a marginally significant group x drug interaction; this pattern showed a similar shape to the effect noted in apical length. This marginal finding was partially a result of high error variability (see Figure 3). Additionally, basilar volume showed a cell x

group interaction (see Figure 6) which indicated an unusually large volume in the

fifth cell drawn from all animals; this was a chance occurrence and not considered

significant. This unusual characteristic would be expected to wash out with other

cells added to the sample

Significance was noted in Sholl analysis and branch order, which can be

explained by a simple demonstration of how typical cells behave. When conducting

this type of analysis, both ring and branch order will always reveal significance

because of their nature; their distributions follow a normal distribution curve. Figure

7 is a drawing made by a student on the NeuroLucida system and it provides a visual

explanation for how these analyses follow a normal distribution curve. The image

shows what a typical cell might look like and how Sholl anaylsis is typically

conducted on cells. Sholl analysis is conducted by counting the number of dendrites

that intersect at each concentric ring. At each concentric ring, the number of

dendrites counted is then multiplied by the distance between each ring (10 μm) from

the cell body in order to give an approximation of total dendritic length. Given the

natural pattern of dendrites, such that the number of ring intersections as well as

branch orders originate from the cell body with few dendrites coming off the nucleus

at first and then gradually increase as the dendrites branch off and grow more

complex and eventually taper off at the outer edges of the rings, these graphs follow a

normal distribution curve.

No significant main or interactions were noted in any of the other measures

explored. All relevant findings will be discussed in relation to our expected results.

Interpreting the Results

Our first hypothesis was that exposure to Methylphenidate would induce neuronal reorganization similar to that seen with psychostimulant (amphetamine) exposure in rats (Robinson and Kolb, 2004). Results indicated a visual trend towards this finding upon inspecting the means in the wildtype mice, but overall were inconsistent with previous research that specifies a strong reorganization in response to stimulants. This trend was stronger when comparing wildtype mice in the saline versus amphetamine group then in the Methylphenidate group. The knockout mice showed no significant trends in the drug conditions.

When comparing means, a trend was also noted between wildtype and knockout mice in the saline control condition, indicating that knockout mice possess a larger dendritic field to begin with. Thus, the general downward trend noted in the graph for knockout mice may stem from the large dendrites in the knockout saline condition. This pattern can be noted in Figure 1.

Our second hypothesis was that Methylphenidate and amphetamine would produce more dramatic reorganization in the *fmr1* knockout mouse than in control mice. This was not observed in the data. *fmr1* knockout mice were expected to show increased dendritic length and branch complexity in the prefrontal cortex as compared to wildtype mice. The data suggests that no such effect was noted.

Overall, the differences we anticipated were not found. No differences in complexity were noted in either apical or basilars dendrites. No relevant differences in basilar volume as well as no differences in basilar length were noted. There are several possible explanations for why we did not note the trend we anticipated. To

begin with, studies showed that changes in dendritic length and complexity were noted in pyramidal cells both in the prefrontal cortex and the nucleus accumbens (Robinson & Kolb, 1997). Perhaps the changes in the prefrontal cortex were subtle and had we done a similar evaluation of cells in the nucleus accumbens, we would have detected the expected changes. In the knockout mice, we saw no trend towards our hypothesized effect suggesting that perhaps changes exist in other areas then observed. Additionally, studies on the effects of stimulants have generally been conducted in rats and not in mice. Although both animals tend to behave similarly overall, the effects of stimulants between the two groups may have differed. This may have contributed to the weaker trend towards reorganization in response to stimulants in the wildtype mice.

One result of interest is the marginally different effect of apical length between saline knockout and saline wildtype mice. These findings may suggest that pathologically different brains exhibit their differences in the physical length of their dendrites. Although this was not a finding in the literature, it in itself would be both surprising and interesting if it holds. Studying dendrites provides researchers with a useful tool for indirect measurement of available postsynaptic space, and is also suggestive of synapse number. As has been documented in several studies (Irwin et al., 2001, Irwin et al., 2002), FXS patients and *fmr*1 mice tend to show increased spine density that appear long and thin, generally as a result of lack of pruning following development. This suggests that knockout mice show an increased density of immature spines. Irwin and colleagues suggest (Irwin et al., 2000, Irwin et al., 2001, Irwin et al., 2002) *fmr*1 mice tend to show developmental delays in spine

pruning and these findings may serve to link spine pruning delays with additional delays in pruning of overall dendritic length. Thus, the increased apical length of knockout mice may have been the result of an increased dendritic length to match the increased spine density that was noted. Murphy and Magness, (1984) found that peak increases in spine density during development in rabbits paralleled increases in dendritic length. On a similar note, Duan, Wearne, Morrison, and Hof (2002) found that longer layer III pyramidal cells in the prefrontal cortex had a higher density of spines relative to shorter cells. In mice, Ruiz-Marcos and Valverde (1969) found this peak increase in spines occurred around day 20 followed by a steady decline until around day 48. This time course could serve to explain why control mice might have pruned their spines and/or dendrites by around day 42 when the mice were sacrificed, and could account for the lack of pruning of dendrites and potentially spines in knockout mice. This would suggest that control mice would have sufficient time to prune their spines, while developmentally delayed mice may still have been in the process of pruning. Caution should be extended to firmly drawing this type of conclusion from our data. The group composed of saline exposed knockout mice consisted of 6 animals, and 4 mice were present in the wildtype group, which increases the possibility that this effect could have been a result of variability within subjects.

It is possible that *fmr1* mice would require an increased timeframe of exposure to illicit a similar or an increased sensitization to the drugs. However, the graph shows a non-significant trend towards the opposite or no effect in the knockout mice. This suggests that pathologically different mice may be responding differently

to stimulant exposure. Although the wildtype mice show a marginal increase in dendritic size, no such apparent change is noted in the knockout mice. Stimulants may be producing a slight decline in dendritic size in the knockout mouse, or more probable, no effect at all. If replicated, these findings could help elucidate the mechanism by which these drugs work in the brain, especially in pathologically different populations. Further research would be necessary to confirm this theory and comment on any implications for these drugs in children.

<u>Summary</u>

This study investigates the effects of stimulant exposure on the dendritic circuitry of the brain. One drawback to this study was the limited sample size, which contributed to limited power. Interactions that were marginally significant might have been strengthened by more animals contributing to the data and decreasing the variability, a larger sample would provide greater confidence in this effect. Several studies have noted no change in the branches of *fmr1* mice, but have found that changes exist at different resolutions/locations such as at the spine and/or synaptic level (Irwin et al., 2002; Irwin et al., 2001). This suggests that more significant changes may have been noted in other areas, such as spine density/morphology, had we looked. However, one interaction was observed that offers support to the notion that stimulants do indeed create changes on the neuronal level in the brain. Although these results do not offer a clear message as to how stimulants affect the brain in children suffering from mental retardation, it does offer some insight into the fact that stimulants do impact the maturing brain. Additionally, the findings suggest that pathologically different brains respond in differing fashions to stimulant exposure.

It would be necessary to conduct further research on this topic in order to elucidate the nature of these changes with a larger sample size and differing dosages. For now, we cannot comment on whether the brain differences noted in this one interaction are good or bad. Further research would be necessary to making any decisions about drug prescriptions in populations of mentally challenged children.

Future Research

Future research may choose to explore different regions and resolutions, or other portions of the cell or brain chemistry that were not examined in this particular study. In this particular experiment we examined dendritic changes, whereas other studies could explore spine changes also found at the electron and light microscopy levels or even changes found at higher resolutions such as synaptic changes. Furthermore, use of a larger sample size would increase the confidence of the observed effect. Lastly, future researchers may want to investigate if these changes would persist at different dosages and different lengths of administration of the stimulant. It is vitally important to know how drugs prescribed to children impact their brain, both immediately and over the long run. Since it is not uncommon to prescribe children any type of medication, even at a young age, it is crucial to understand what changes they may be producing.

Group X Drug Interaction

Group X Drug Interaction

Group X Drug Interaction

Group X Drug Interaction

Group X Drug Interaction

Cells X Group
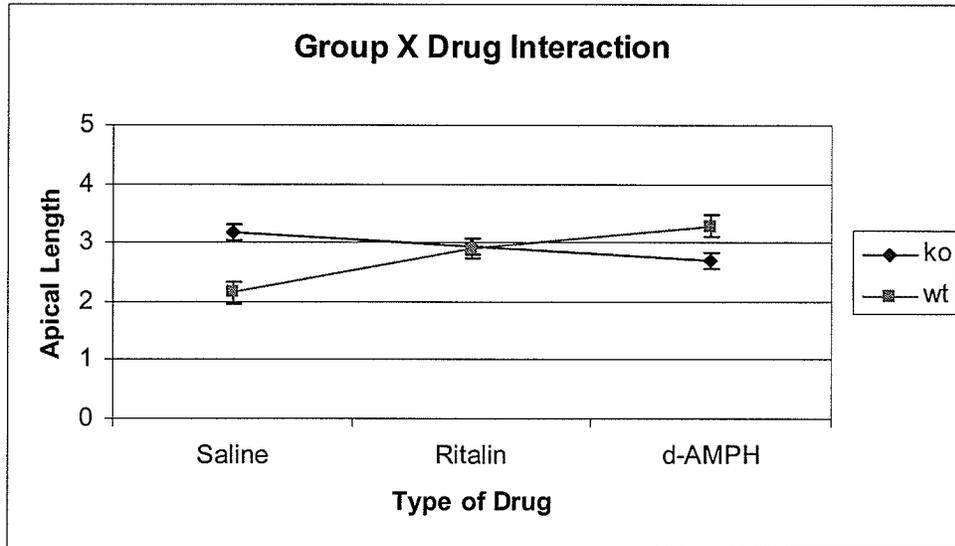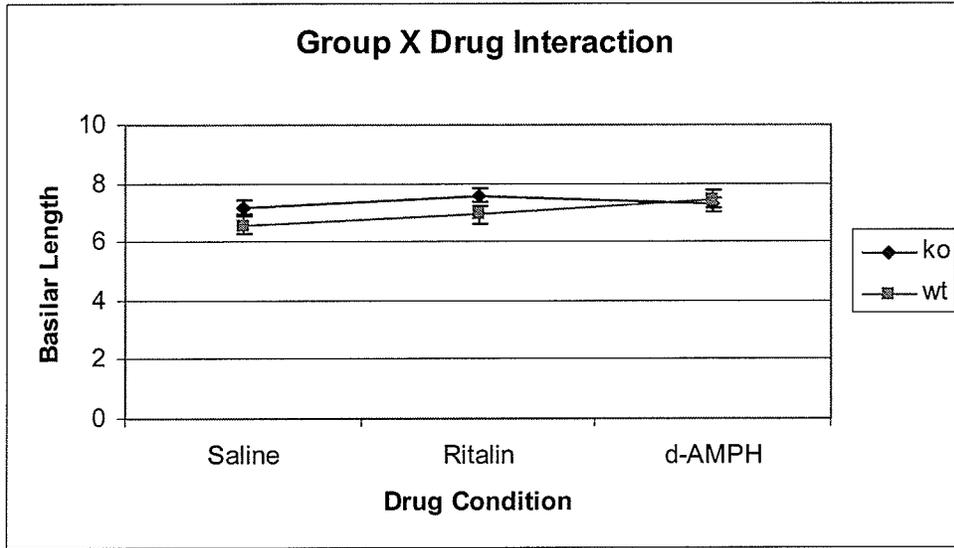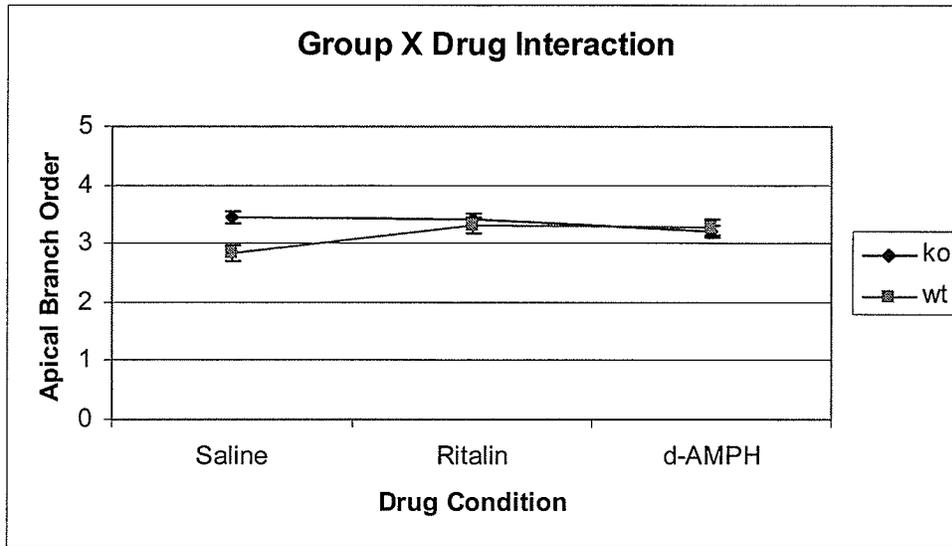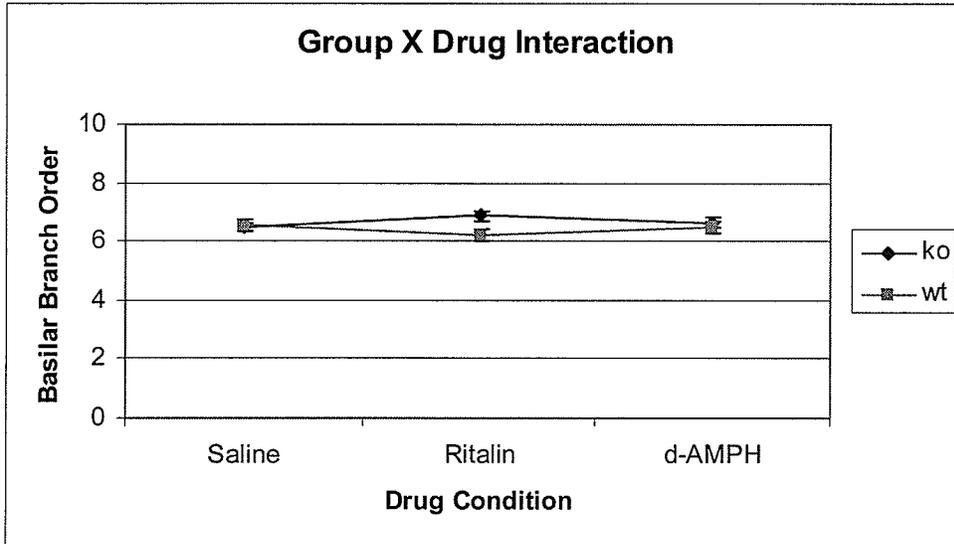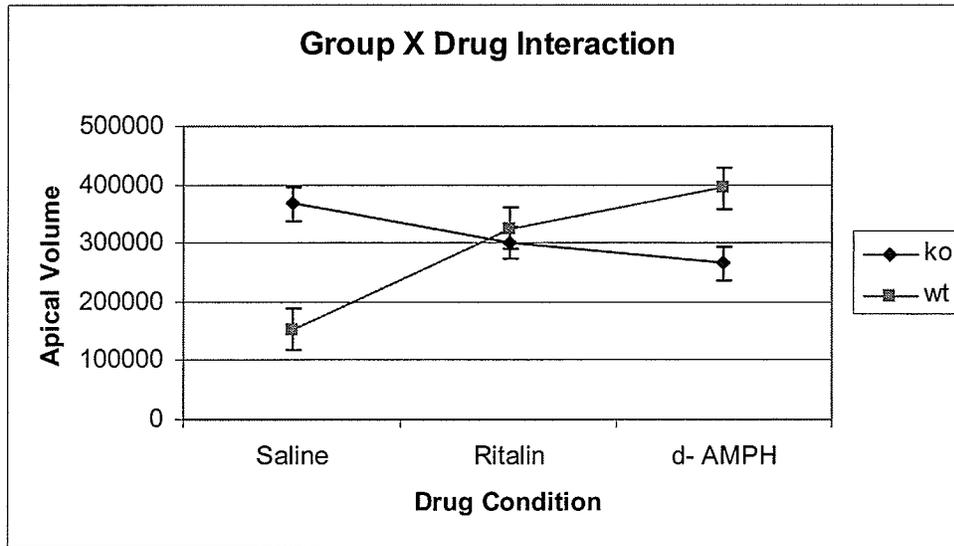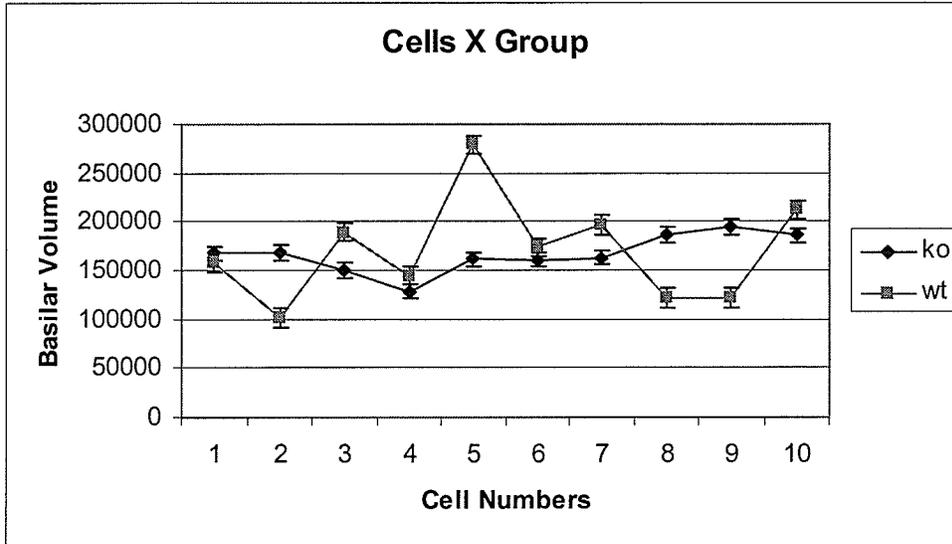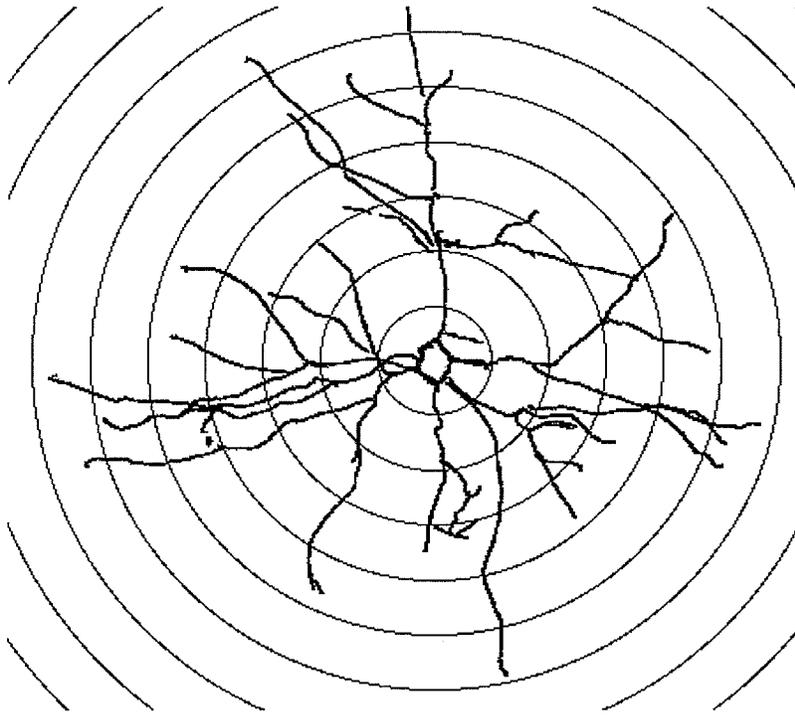
References

Aman, M. G., Marks, R. E., Turbott, S. H., Wilsher, C. P., and Merry, S. N. (1991).

Clinical effects of methylphenidate and thioridazine in intellectually

subaverage children. *Journal of the American Academy of Child and*

Adolescent Psychiatry, 30, 246-256.

Bakker, C. E., and Oostra, B. A. (2003). Understanding fragile X syndrome: insights

from animal models. *Cytogenetic Genome Research, 100,* 111-23.

Barbay, S., Plautz, E. J., Friel, K. M., Frost, S. B., Dancause, N., Stowe, A. M., Nudo

R. J. (2006). Behavioral and neurophysiological effects of delayed training

following a small ischemic infarct in primary motor cortex of squirrel

monkeys. *Experimental Brain Research, 169,* 106 – 16.

Baumgardner, T. L., Reiss, A. L., Freund, L. S., Abrams, M. T. (1995). Specification

of the neurobehavioral phenotype in males with fragile X syndrome.

Pediatrics, 95, 744 – 52.

Bennett, E.L., Diamond, M.C., Krech, D. and Rosenzweig, M.R. (1964). Chemical

and anatomical plasticity of brain. *Science, 164,* 610-619.

Bennett, E. L., Rosenzweig, M. R., and Diamond, M. C. (1969). Rat brain: Effects of

environmental enrichment on wet and dry weights. *Science, 163,* 825-6.

Berridge, C. W, (2001) Arousal-and-Attention-Related Actions of the Locus

Coeruleus-Noradrenergic System: Potential Target in the Therapeutic Actions

of Amphetamine-Like Stimulants. In Solanto, M.V; Arnsten, A. F. T., and

Castellanos, F. X. (Ed), Stimulant Drugs and ADHD Basic and Clinical Neuroscience (pp. 158-184). Oxford University Press.

Berry-Kravis, E., Potanos, K. (2004). Psychopharmacology in fragile X syndrome-present and future. *Mental Retardation Developmental and Disabilities Research Review, 10,* 42-8.

Bingham, W. E., and Griffiths, W. J. (1952). The effect of different environments during infancy on adult behavior in the rat. *Journal of Comparative and Physiological Psychology,45,* 307-12.

Black, J. E., Isaacs, K. R., Anderson, B. J., Alcantara, A. A., and Greenough, W. T. (1990). Learning causes synaptogenesis, whereas motor activity causes angiogenesis, in the cerebellar cortex of adult rats. *Neurobiology, 87,* 5568-5572.

Black, J. E., Sirevaag, A. M., and Greenough, W. T. (1987). Complex experience promotes capillary formation in young rat visual cortex. *Neuroscience Letters, 83,* 351-55.

Branchi, I., Francia, N. and Alleva, E. (2004). Epigenetic control of neurobehavioural plasticity: The role of neurotrophins. *Behavioural Pharmacology, 15,* 353-362.

Breton, J. J., Bergeron, L., Valla, J. P., Berthiaume, C., and Gaudet, N. (1999). Quebec child mental health survey: Prevalence of DSM-III-R mental health disorders. *Journal of Child Psychology and Psychiatry, 40,* 375-384.

Briones, T. L, Klintsova, A. Y., and Greenough, W. T. (2004). Stability of synaptic

    plasticity in the adult rat visual cortex induced by complex environment

    exposure. *Brain Research, 1018*, 130-5.

Brown, T. W (1996). The FRAXE syndrome: Is it time for routine screening?

    American Journal of Human Genetics, 58, 903-5.

Chu, C. J., and Jones, T. A (2000). Experience-dependent structural plasticity in

    cortex heterotopic to focal sensorimotor cortical damage. *Experimental*

    Neurology 166, 403–414.

Comery, T. A., Harris, J. B., Willems, P. J., Oostra, B. A., Irwin, S. A., Weiler, I. J.,

    and Greenough, W. T. (1997). Abnormal dendritic spines in Fragile X

    knockout mice: Maturation and pruning deficits. *Neurobiology, 94*, 5401-4.

Darwin , C. (1874). The Descent of Man. Chicago, Rand, McNally, p. 53.

Duan, H., Wearne, S. L., Morrison, J. H., and Hof, P. R. (2002). Quantitative

    analysis of the dendritic morphology of corticocortical projection neurons in

    the macaque monkey association cortex. *Neuroscience, 114*, 349 – 59.

Dutch-Belgian Fragile-X Consortium (1994). F*mr1* knockout mice: a model to study

    fragile X mental retardation. *Cell, 78*, 23-33.

Eichlseder, W. (1985). Ten years of experience with 1,000 hyperactive children in a

    private practice. *Pediatrics, 76*, 176-84.

Federmeier, K. D., Kleim, J. A., and Greenough, W. T. (2002). Learning-induced

    multiple synapse formation in the rat cerebellar cortex. *Neuroscience Letters,*

    332, 180-4.

Ferchmin, P. A., and Eterovic, V. A. (1985). Forty minutes of experience increase

the weight and RNA content of cerebral cortex in periadolescent rats.

Developmental Psychobiology, 19, 511-9.

Forgays, D. G., and Forgays, J. W. (1952). The nature of the effect of free-

environmental experience in the rat. *Journal of Comparative and

Physiological Psychology, 45,* 322-28.

Farzin, F., Perry, H., Hessl, D., Loesch, D., Cohen, J., Bacalman, S., Gane, L.,

Tassone, F., Hagerman, P., Hagerman, R. (2006). Autism spectrum

disorders and attention-deficit/hyperactivity disorder in boys with the

Fragile X premutation. *Journal of Developmental and Behavioral

Pediatrics*, 137 –144.

Freund, L. S., Reiss, A. L., Abrams, M. T. (1993). Psychiatric disorders

associated with fragile X in the young female. *Pediatrics, 91*, 321 – 9.

Gibb, R., & Kolb, B. (1998). A method for vibratome sectioning of Golgi-Cox

stained whole rat brain. *Journal of Neuroscience Methods, 79,* 1-4.

Greenhill, L. L., Swanson, J. M., Vitiello, B., Davies, M., Clevenger, W., Wu, M.

et al. (2001). Impairment and deportment responses to different

Methylphenidate doses in children with ADHD: The MTA titration trial.

Journal of American Academy Child Adolescence Psychiatry, 40, 180 –

87.

Greenough, W. T., Larson, J. R., and Withers, G. S. (1985). Effects of unilateral and

bilateral training in a reaching task on dendritic branching of neurons in the

rat motor-sensory forelimb cortex. *Behavioral and Neural Biology, 44*, 301-14.

Greenough, W. T., Black, J. E., and Wallace, C. S. (1987). Experience and brain development. *Child Development, 58*, 539 – 559.

Greenough, W. T. and Volkmar, F. R. (1973). Pattern of dendritic branching in occipital cortex of rats reared in complex environments. *Experimental Neurology, 40*, 491-504.

Greenough, W. T., Yuwiler, A., and Dollinger, M. (1973). Effects of posttrial eserine administration on learning in "enriched" and "impoverished" reared rats. *Behavioral Biology, 8*, 261-72.

Grossman, A. W., Churchill, J. D., Bates, K. E., Kleim, J. A., and Greenough, W. T. (2002). A brain adaptation view of plasticity: is synaptic plasticity an overly limited concept? *Progress in Brain Research, 138*, 91-108.

Hagerman, R. J. (1992). Annotation: fragile X syndrome: advances and controversy. *Journal of Child Psychological Psychiatry, 33*, 1127 – 39.

Hagerman R. J. (1996). Physical and behavioral phenotype. In: Hagerman RJ, and Cronister A, Editors. Fragile X Syndrome: Diagnosis, treatment, and research, 3rd Edition. The John Hopkins University Press: Boston. Pp3-87.

Hagerman R. J. (2002a). Physical and behavioral phenotype. In: Hagerman RJ, and Hagerman, PJ, Editors. Fragile X Syndrome: Diagnosis, treatment, and research, 2nd Edition. The John Hopkins University Press: Boston.   Pp3-109.

Hagerman R. J. (2002b). Medical follow-up and pharmacotherapy. In: Hagerman RJ, and Hagerman, PJ, Editors. Fragile X Syndrome: Diagnosis, treatment,

and research, 2nd Edition. The John Hopkins University Press: Boston.
Pp287-338.

Hagerman, R. J., Murphy, M. A., Wittenberger, M. D. (1988). A controlled trial of stimulant medication in children with the fragile X syndrome. *American Journal of Medical Genetics, 30,* 377 – 92.

Handen, B. L., Feldman, H., Gosling, A., Breaux, A. M., and Mcauliffe, S. (1991). Adverse side effects of methylphenidate among mentally retarded children with ADHD. *Journal of the American Academy Child and Adolescent Psychiatry, 30,* 241-45.

Hebb, D. O. (1947). The effects of early experience on problem solving at maturity. *American Psychologist, 2,* 306-307.

Hebb, D. O. (1949). The organization of behavior; a neuropsychological theory. New York, Wiley.

Hewitt, K. N., Shah, Y. B., Prior, M. J. W., Morris, P. G., Hollis, C. P., Fone, K. C. F., and Marsden, C. A. (2005). Behavioural and pharmacological magnetic resonance imaging assessment of the effects of methylphenidate in a potential new rat model of attention deficit hyperactivity disorder. *Psychopharmacology, 180,* 716-23.

Hunt, R. D., Paguin, A., and Payton, K. (2001). An update on assessment and treatment of complex attention-deficit hyperactivity disorder. *Pediatric Annals, 30,* 162-72.

Hynd, G. W., Hern, K. L., Novey, E. S., Eliopulos, D., Marshall, R., Gonzalez, J. J., and Voeller, K. K. (1993). Attention deficit-hyperactivity disorder and asymmetry of the caudate nucleus. *Journal of Child Neurology, 8*, 339-47.

Irwin, S. A., Galvez, R., and Greenough, W. T. (2000). Dendritic spine structural anomalies in Fragile-X mental retardation syndrome. *Cerebral Cortex, 10*, 1038- 44.

Irwin, S. A., Idupulapati, M., Gilbert, M. E., Harris, J. B., Chakravarti, A. B., Rogers, E. J., Crisostomo, R. A., Larsen, B. P., Mehta, A., Alcantara, C. J., Patel, B., Swain, R. A., Weiler, I. J., Oostra, B. A., and Greenough, W. T. (2002). Dendritic spine and dendritic field characteristics of layer V pyramidal neurons in the visual cortex of fragile-X knockout mice. *American Journal of Medical Genetics, 111*, 140–146.

Irwin, S. A., Patel, B., Idupulapati, M., Harris, J. B., Crisostomo, R. A., Larsen, B. P., Kooy, F., Willems, P. J., Cras, P., Kozlowski, P. B., Swain, R. A., Weiler, I. J., and Greenough, W. T. (2001). Abnormal dendritic spine characteristics in the temporal and visual cortices of patients with fragile-X syndrome: A quantitative examination. *American Journal of Medical Genetics, 98,* 161–67.

Jones, T. A., and Greenough, W. T. (1996). Ultrastructural evidence for increased contact between astrocytes and synapses in rats reared in a complex environment. *Neurobiology or Learning and Memory, 65*, 48 – 56.

Kleim, J. A., Barbay, S., Cooper, N. R., Hogg, T. M., Reidel, C. N., Remple, M. S., and Nudo, R. J. (2002). Motor learning-dependent synaptogenesis is localized

to functionally reorganized motor cortex. *Neurobiology of Learning and Memory, 77,* 63-77.

Kleim, J. A., Barbay, S., and Nudo, R. J. (1998). Functional reorganization of the rat motor cortex following motor skill learning. *Journal of Neurophysiology 80,* 3321–3325.

Kleim, J. A., Jones, T. A., and Schallert, T. (2003). Motor enrichment and the induction of plasticity before or after brain injury. *Neurochemical Research,* 28, 1757-69.

Kleim, J. A., Lussnig, E. L., Schwarz, E. R., Comery, T. A., and Greenough, W. T. (1996). Synaptogensis and Fos expression in the motor cortex of the adult rat after motor skill learning. *Journal of Neuroscience, 16,* 4529-4535.

Kleim, J. A., Pipitone, M. A., Czerlanis, C., and Greenough, W. T. (1998). Structural stability within the lateral cerebellar nucleus of the rat following complex motor learning. *Neurobiology of Learning and Memory, 69,* 290-306.

Kleim, J. A., Swain, R. A., Armstrong, K. A., Napper, R. M. A., Jones, T. A., and Greenough, W. T. (1998). Selective synaptic plasticity within the cerebellar cortex following complex motor skill learning. *Neurobiology of Learning and Memory, 69,* 274–289.

Kleim, J. A., Swain, R. A., Czerlanis, C. M., Kelly, J. L., Pipitone, M. A., and Greenough, W. T. (1997). Learning-dependent dendritic hypertrophy of cerebellar stellate cells: Plasticity of local circuit neurons. *Neurobiology of Learning and Memory, 67,* 29-33.

Kleim, J. A., Vij, K., Ballard, D. H., and Greenough, W. T. (1997). Learning-

dependent synaptic modifications in the cerebellar cortex of the adult rat

persist for at least four weeks. *The Journal of Neuroscience, 17*, 717-21.

Kolb, B., Gibb, R., and Gorny, G. (2003). Experience-dependent changes in dendritic

arbor and spine density in neocortex vary qualitatively with age and sex.

Neurobiology of Learning and Memory, 79, 1-10.

Kolb, B., Gibb, R., and Robinson, T. E. (2003). Brain plasticity and behavior.

Current Directions in Psychological Science, 12, 1-5.

Kolb, B., Whishaw, I. Q. (1998). Brain plasticity and behavior. *Annual Review of*

Psychology, 49, 43-64.

Kolb, B., Gorny, G., Li , Y., Samaha, A. N., Robinson, T. E. (2003). Amphetamine

or cocaine limits the ability of later experience to promote structural plasticity

in the neocortex and nucleus accumbens. *Proceedings of the National*

Academic of Sciences of the United States of America, 100, 10523 – 8.

Krech, D., Rosenzweig, M. R., and Bennett, E. L. (1960). Environmental effects on

brain chemistry. *Journal of Comparative and Physiological Psychology, 53,*

509-19.

Lincoln, J. S., McCormick, D. A., and Thompson, R. F. (1982). Ipsilateral cerebellar

lesions prevent learning of the classically conditioned nictitating

membrane/eyelid response. *Brain Research, 242*, 190-3.

Luft, A. R., Buitrago, M. M., Ringer, T., Dichgans, J., and Schulz, J. B. (2004).

Motor skill learning depends on protein synthesis in motor cortex after

training. *The Journal of Neuroscience, 24*, 6515- 20.

Marr, D. (1969). A theory of cerebellar cortex. *Journal of Physiology, 202*, 437-70.

McDougall, S. A., Collins, R. L., Karper, P. E., Watson, J. B., and Crawford, C. A. (1999). Effects of repeated methylphenidate treatment in the young rat: sensitization of both locomotor activity and stereotyped sniffing. *Experimental and Clinical Psychopharmacology, 7*, 208-18.

Miles, R., Toth, K., Gulyas, A. I., Hajos, N., and Freund, T. F. (1996). Differences between somatic and dendritic inhibition in the hippocampus. *Neuron, 16*, 815-23.

Murphy, E. H., Magness, R. (1984). Development of the rabbit visual cortex: a quantitative Golgi analysis. *Experimental Brain Research, 53*, 304-14.

Narayanan, R., Narayan, A., Chattarji, S. (2005). A probabilistic framework for region-specific remodeling of dendrites in three-dimensional neuronal reconstructions. *Neural Computation, 17*, 75-96.

Nelson, C. A. (1999). Neural plasticity and human development. *Current Directions in Psychological Science, 8*, 42-45.

Nudo, R. J., Wise, B. M. SiFuentes, F., and Miliken, G. W. (1996). Neural substrates for the effects of rehabilitative training on motor recovery after ischemic infarct. *Science, 272*, 1791-1794.

Oberle, I., Rosseau, F., Heitz, D., Kretz, C., Devys, D., Hanover, A., Boue, J., Bertheas, M., Mandel, J. (1991). Instability of a 550-base pair DNA segment and abnormal methylation in fragile X syndrome. *Science, 252*, 1097-1102.

Offord, D. R., Boyle, M. H., and Jones, B. R. (1987). Psychiatric disorder and poor

    school performance among welfare children in Ontario. *Canadian Journal of*

    Psychiatry, 32, 518 – 525.

Paulson, P. E., Camp, D. M., and Robinson, T. E. (1991). Time course of transient

    behavioral depression and persistent behavioral sensitization in relation to

    regional brain monoamine concentrations during amphetamine withdrawal in

    rats. *Psychopharmacology, 103,* 480-92.

Pelham, W. E., Aronoff, H. R., Midlam, J. K., Shapiro, C. J., Gnagy, E. M., Chronis,

    A. M., Onyango, A. N., Forehand, G., Nguyen, A., and Waxmonsky, J.

    (1999). A comparison of Ritalin and Adderall: efficacy and time-course in

    children with attention-deficit/hyperactivity disorder. *Pediatrics, 103,* 43-56.

Price, A.W. and Fowler, S. C. (1981). Deficits in contralateral and ipsilateral forepaw

    motor control following unilateral motor cortical ablations. *Brain Research,*

    205, 81–90.

Robinson, T. E., and Becker, J. B. (1986). Enduring changes in brain and behavior

    produced by chronic amphetamine administration: A review and evaluation of

    animal models of amphetamine psychosis. *Brain Research Reviews, 11,* 157-

    98.

Robinson, T. E. and Kolb, B. (1997). Persistent structural modifications in nucleus

    accumbens and prefrontal cortex neurons produced by previous experience

    with amphetamine. *The Journal of Neuroscience, 17,* 8491-8497.

Robinson, T. E. and Kolb, B. (1999). Alterations in the morphology of dendrites and

    dendritic spines in the nucleus accumbens and prefrontal cortex following

repeated treatment with amphetamine or cocaine. *European Journal of*

Neuroscience, 11, 1598-1604.

Robinson, T. E., and Kolb, B. (2004). Structural plasticity associated with exposure

to drugs of abuse. *Neuropharmacology, 47,* 33-46.

Rosenzweig, M. R., and Bennett, E. L. (1996). Psychobiology of plasticity: effects of

training and experience on brain and behavior. *Behavioural Brain Research,*

78, 57-65.

Ross, D. C., Fischohoff, J, and Davenport, B. (2002). Treatment of ADHD when

tolerance of Methylphenidate develops. *Psychiatric Services, 53,* 102.

Rosenzweig, M. R., Krech, D., Bennett, E. L. (1960). A search for relations between

brain chemistry and behavior. *Psychological Bulletin, 57,* 476 – 92.

Ruiz-Marcos, A., and Valverde, F. (1969). The temporal evolution of the distribution

of dendritic spines in the visual cortex of normal and dark raised mice.

Experimental Brain Research, 8, 284-94.

Safer, D. J., Allen, R. P. (1989). Absence of tolerance to the behavioral effects of

methylphenidate in hyperactive and inattentive children. *Journal of*

Pediatrics, 115, 1003 – 8.

Sirevaag, A. M., and Greenough, W. T. (1991). Plasticity of GFAP-immunoreactive

astrocyte size and number in visual cortex of rats reared in complex

environments. *Brain Research, 540,* 273-78.

Slegtenhorst-Eegdeman, K. E., de Rooij, D. J., Verhoef-Post, M., van de Kant, H. J.

G., Bakker, C. E., Oostra, B. A., Grootegoed, J. A., and Themmen, A. P. N.

(1998). Macroorchidism in FMR1 knockout mice is caused by increased

Sertoli cell proliferation during testicular development. *Endocrinology, 139*, 156-162.

Steele, M., Weiss, M., Swanson, J., Wang, J., Prinzo, R. S., and Binder, C. E., (2006). A randomized, controlled effectiveness trial of OROS-methylphenidate compared to usual care with immediate-release methylphenidate in ADHD. *Canadian Journal of Clinical Pharmacology, 13*, 50 – 62.

Stein, M. A., Sarampote, C. S., Waldman, I. D., Robb, A. S., Conlon, C., Pearl, P. L., Black, D. O., Seymour, K. E., and Newcorn, J. H. (2003). A dose-response study of OROS methylphenidate in children with attention-deficit/hyperactivity disorder. *Pediatrics, 112*, 404-13.

Swanson, J., Gupta, S., Guinta, D., Flynn, D., Agler, D., Lerner, M., Williams, L., Shoulson, I., and Wigal, S. (1999). Acute tolerance to methylphenidate in the treatment of attention deficit hyperactivity disorder in children. *Clinical Pharmacological Therapy, 66,* 295 – 305.

Tegenthoff, M., Cornelius, B., Pleger, B., Malin, J-P., Schwenkreis, P. (2003). Amphetamine enhances training-induced motor cortex plasticity. *Acta Neurologica Scandinavica, 109,* 330-336.

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

Turk, J. (1992). The fragile-X syndrome. On the way to a behavioural phenotype. *British Journal of Psychiatry, 160,* 24-35.

VandenBerg, P. M., Hogg, T. M., Kleim, J. A., and Whishaw, I. Q. (2002). Long-Evans rats have a larger cortical topographic representation of movement than Fischer- 344 rats: A microstimulation study of motor cortex in naïve and skilled reaching- trained rats. *Brain Research Bulletin, 6531*, 1-7.

Volkmar, F. R., and Greenough, W. T. (1972). Rearing complexity affects branching of dendrites in the visual cortex of the rat. *Science, 176*, 1445-7.

Waite, W. L., Hess, S., and Kolb, B. E. (2004). Methylphenidate produces sensitization, cross-sensitization, and social anomalies associated with structural changes. *Society for Neuroscience Abstracts*, 579.3.

Wawryko, P., Ward, N. L., Whishaw, I. Q., and Ivanco, T. L., (2004). Anesthetized Long Evans rats show similar protein expression and long-term potentiation as Fischer 344 rats but reduced short-term potentiation in motor cortex. *Brain Research, 1029*, 1 – 10.

Weiler, I. J., Irwin, S. A., Klintsova, A. Y., Spencer, C. M., Brazelton, A. D., Miyashiro, K., Comery, T. A., Patel, B., Eberwine, J., and Greenough, W. T. (1997). Fragile X mental retardation protein is translated near synapses in response to neurotransmitter activation. *Neurobiology, 94*, 5395 – 5400.

Withers, G. S., and Greenough, W. T. (1989). Reach training selectively alters dendritic branching in subpopulations of layer II-III pyramids in rat motor-somatosensory forelimb cortex. *Neuropsychologia, 27*, 61-9.

Will, B.E., Rosenzweig, M.R., Bennett, E.L, Hebert, M. and Morimoto, H. (1977). Relatively brief environmental enrichment aids recovery of learning capacity

and alters brain measures after postweaning brain lesions in rats. *Journal of*

Comprehensive Physiological Psychology, 91, 33 - 50.

Winsberg, B., Matinsky, S., Kupietz, S., and Richardson, E. (1987). Is there dose-

dependent tolerance associated with chronic methylphenidate therapy in

hyperactive children: Oral dose and plasma considerations.

Psychopharmacology, 23, 107 – 10.

Apical Sholl

	SS	df	dfError	MS	F	p
Animal Group	34	1	23	34.1	0.4	0.54
Drug Condition	113	2	23	56.6	0.6	0.535
Group X Drug	661	2	23	330.3	3.8	.039*
Cells	203	9	207	22.5	0.9	0.487
Cells X Group	89	9	207	9.9	0.4	0.927
Cells X Drug	480	18	207	26.7	1.1	0.337
Cells X Group X Drug	641	18	207	35.6	1.5	0.095
Rings	1.60E+04	19	437	841.2	221.9	0.000*
Rings X Group	89	19	437	4.7	1.2	0.223
Rings X Drug	149	38	437	3.9	1	0.416
Rings X Group X Drug	163	38	437	4.3	1.1	0.279
Cells X Rings	389	171	3933	2.3	1	0.497
Cells X Rings X Group	360	171	3933	2.1	0.9	0.751
Cells X Rings X Drug	743	342	3933	2.2	1	0.719
Cells X Rings X Group X Drug	978	342	3933	2.9	1.3	.002*

Basilars Sholl

	SS	df	dfError	MS	F	p
Animal Group	146	1	23	146	0.7	0.407
Drug Condition	196	2	23	98	0.5	0.625
Group X Drug	173	2	23	86	0.4	0.659
Cells	692	9	207	77	0.9	0.545
Cells X Group	1159	9	207	129	1.5	0.16
Cells X Drug	1599	18	207	89	1	0.444
Cells X Group X Drug	2165	18	207	120	1.4	0.146
Rings	7.33E+04	15	437	4890	579.9	0.000*
Rings X Group	34	15	437	2	0.3	0.997
Rings X Drug	372	30	437	12	1.5	0.056
Rings X Group X Drug	668	30	437	22	2.6	.000*
Cells X Rings	669	135	3933	5	0.9	0.727
Cells X Rings X Group	912	135	3933	7	1.3	.026*
Cells X Rings X Drug	1547	270	3933	6	1.1	0.228
Cells X Rings X Group X Drug	1680	270	3933	6	1.2	.046*

Apical volume

	SS	df	dfError	MS	F	p
Animal Group	2.97E+10	1	23	2.97E+10	0.167	0.686
Drug Condition	2.60E+11	2	23	1.30E+11	0.729	0.493
Group X Drug	1.50E+12	2	23	7.50E+11	4.226	.027*
Cells	5.60E+11	9	207	6.22E+10	1.225	0.281
Cells X Group	2.10E+11	9	207	2.32E+10	0.458	0.901
Cells X Drug	8.60E+11	18	207	4.78E+10	0.939	0.532
Cells X Group X Drug	9.50E+11	18	207	5.27E+10	1.037	0.42

Basilar volume

	SS	df	dfError	MS	F	p
Animal Group	7.63E+08	1	23	7.63E+08	0.028	0.869
Drug Condition	1.12E+10	2	23	5.63E+09	0.205	0.816
Group X Drug	6.29E+10	2	23	3.14E+10	1.145	0.336
Cells	1.80E+11	9	207	1.98E+10	1.479	0.157
Cells X Group	2.20E+11	9	207	2.49E+10	1.863	0.059
Cells X Drug	2.20E+11	18	207	1.22E+10	0.918	0.557
Cells X Group X Drug	1.90E+11	18	207	1.03E+10	0.776	0.727

Apical branch order

	SS	df	dfError	MS	F	p
Animal Group	13.3	1	23	13.31	1.48	0.236
Drug Condition	8.3	2	23	4.15	0.46	0.636
Group X Drug	24.1	2	23	12.07	1.34	0.281
Cells	35.8	9	207	3.98	0.85	0.569
Cells X Group	34.9	9	207	3.88	0.83	0.588
Cells X Drug	51.6	18	207	2.87	0.61	0.887
Cells X Group X Drug	90.3	18	207	5.02	1.07	0.38
Orders	282.9	3	437	94.31	54.72	0.000*
Orders X Group	4.6	3	437	1.53	0.89	0.451
Orders X Drug	15.3	6	437	2.55	1.48	0.198
Orders X Group X Drug	15.9	6	437	2.64	1.53	0.18
Cells X Orders	73.9	27	3933	2.74	1.44	0.07
Cells X Orders X Group	58.1	27	3933	2.15	1.13	0.292
Cells X Orders X Drug	86.5	54	3933	1.6	0.84	0.779
Cells X Orders X Group X Drug	97.1	54	3933	1.8	0.95	0.584

Basilar branch order

	SS	df	dfError	MS	F	p
Animal Group	23	1	23	23	0.7	0.403
Drug Condition	2	.	23		0	0.994
Group X Drug	34	2	23	17	0.5	0.593
Cells	233	9	207	26	1.5	0.156
Cells X Group	214	9	207	24	1.4	0.206
Cells X Drug	403	18	207	22	1.3	0.201
Cells X Group X Drug	452	18	207	25	1.4	0.116
Orders	1.24E+04	4	437	3107	413	0.000*
Orders X Group	13	4	437	3	0.4	0.793
Orders X Drug	37	8	437	5	0.6	0.77
Orders X Group X Drug	41	8	437	5	0.7	0.707
Cells X Orders	316	36	3933	9	1.4	.046*
Cells X Orders X Group	285	36	3933	8	1.3	0.113
Cells X Orders X Drug	409	72	3933	6	0.9	0.633
Cells X Orders X Group X Drug	501	72	3933	7	1.1	0.199