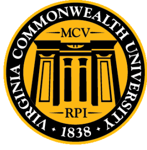

Virginia Commonwealth University
VCU Scholars Compass

Theses and Dissertations

Graduate School

2011

Prenatal Alcohol Exposure Reduces Dendritic Spine Density across Sensory Cortices

Francis Oppong
Virginia Commonwealth University

Follow this and additional works at: https://scholarscompass.vcu.edu/etd

Part of the Nervous System Commons

© The Author

Downloaded from
https://scholarscompass.vcu.edu/etd/2482

This Thesis is brought to you for free and open access by the Graduate School at VCU Scholars Compass. It has been accepted for inclusion in Theses and Dissertations by an authorized administrator of VCU Scholars Compass. For more information, please contact libcompass@vcu.edu.

© Francis Oppong 2011

All Rights Reserved

Prenatal Alcohol Exposure Reduces Dendritic Spine Density across Sensory Cortices

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science at Virginia Commonwealth University

by

Francis Oppong

University of Ghana

May, 2007

Major Director: M. Alex Meredith, PhD

Professor, Department of Anatomy & Neurobiology

Virginia Commonwealth University

Richmond, Virginia

May, 2011

ACKNOWLEDGEMENTS

I owe my deepest gratitude to Dr. M. Alex Meredith for allowing me the opportunity to pursue a master's degree in his lab. His encouragement, guidance and support from the initial to the final level enabled me to develop an understanding of the subject. In all sincerity, Dr. Meredith has done a great job in holding my feet to the fire to finish this thesis on time. I would also like to thank members of my committee, Dr. Alex Medina and Dr. Severn Churn for their guidance and insightful contributions to this project.

My appreciation also goes to members of Meredith labs, Dr. Leslie Keniston, Dr. Ruth Clemo, Alex Foxworthy and Alex Cojanu for their support.

Finally, I offer my regards to Dr. John Bigbee and all others who helped me during the completion of this thesis.

TABLE OF CONTENTS

Acknowledgements ii

List of Tables iv

List of Figures v

Abstract viii

Introduction 1

Methods 8

Alcohol Treatment

Animals and Histology

Data Collection

Data Analysis

Statistical Analysis

Results 13

- Total Spines counted
- Overall Dendritic Spine Density
- Spine density by cortical area
- Apical vs. Basilar spine density
- Pedunculated vs. Simple spine density
- Unisensory Cortices vs. Multisensory Spine density
- Apical vs. Basilar spine density from hierarchical cortical levels
- Pedunculated vs. Simple spine density from hierarchical cortical levels

Discussion 18

Conclusion 21

Figures 22

Tables 35

Literature Cited. 43

LIST OF TABLES

Table 1. Characteristics of animals used in the alcohol treatment and normal control studies. Control data were derived from Bajwa (2010, unpublished VCU Thesis)

Table 2. A summary of animals, neurons and dendrites sampled in the alcohol treatment and normal control studies. Control data were derived from Bajwa (2010, unpublished VCU Thesis)

Table 3. Average and standard error (SEM) of spine density measures at the different cortical regions in the alcohol treatment study.

Table 4. Average and standard error (SEM) of apical and basilar spine density measures by cortical region in the alcohol treatment study.

Table 5. Average and standard error (SEM) of spine density measures by dendritic location according to spine type in the alcohol treatment study.

Table 6. Average and standard error (SEM) of spine density measures for hierarchical levels and dendritic spine type (simple and pedunculated) in the alcohol treatment study.

Table 7. Values for dendritic spine density and standard error (SEM) from cortical areas A1, S1, PPr, and LRSS in normal, control ferrets. Data from Bajwa (unpublished VCU thesis, 2010).

LIST OF FIGURES

Figure 1. A schematic diagram of a dendritic spine synapse showing postsynaptic density and the various proteins involved in signal transduction. (Source: physiologyonline.physiology.org).

Figure 2. A dendritic segment (100x, oil) showing spine shapes examined in this study. Simple spines (marked with brown spots) and pedunculated spine (marked with read spots) Scale bar = 20μm.

Figure 3. Lateral view of ferret brain showing the different cortical regions. (Source: Bajwa, 2010. unpublished VCU Thesis)

Figure 4. Reconstructed representatives of Layer 2-3 pyramidal neurons (40x, with the aid of Neurolucida) showing the soma and the neuronal processes (branching dendrites and axon). Scale bar = 50 μm

Figure 5. For all neurons and dendritic segments measured in all cortical areas, the average value for spines/micron was 1.068 ± 0.014 (SEM) for alcohol treated animals and 1.249 ± 0.013 (SEM) for normal control animals. There was a significantly difference ('*'; $p<0.05$, t-test) between the overall spine density for the alcohol treated animals and that for the normal controls.

Figure 6. This bar graph compares the average (±SEM) of dendritic spines identified from neurons in A1 (primary auditory cortex); S1 (primary somatosensory cortex), PPr (rostral posterior parietal cortex), and the LRSS (lateral rostral suprasylvian sulcal cortex) between the alcohol treated animals and normal controls. There was a significance difference ('*'; $p<0.05$, ANOVA) between the spine densities at each cortical region.

Figure 7. Bar graph comparing the average (±SEM) of apical versus basilar dendritic spines identified from neurons in A1, S1, PPr, and the LRSS of the alcohol treated animals. Within each region, spine densities were not significantly different ('*'; $p<0.05$, t-test) between apical and basilar dendrites, except for those observed in A1.

Figure 8. This bar graph compares the average (±SEM) of simple versus pedunculated dendritic spines identified from neurons in A1, S1, PPr, and the LRSS of alcohol treated animals. Within each region, spine densities were significantly different ('*', p<0.05, t-test) for simple versus pedunculated spines except in the PPr.

Figure 9. This bar graph compares the average (±SEM) of dendritic spines measured from neurons in primary sensory cortices (A1 and S1) versus those identified in higher-level, multisensory cortices (PPr and LRSS) of alcohol treated animals. The total number of dendritic spines in primary sensory cortices were significantly greater than those observed in higher-level, multisensory regions ('*', p<0.05, t-test).

Figure 10. This bar graph compares the average (±SEM) of apical versus basilar dendritic spines identified from neurons in lower, primary sensory cortices (A1 and S1) versus those found in higher-level sensory cortices (PPr and LRSS) of alcohol treated animals. The density of apical spines was significantly greater ('*', p<0.05, t-test) than for basilar spines in the primary sensory cortices, but not in the higher-level multisensory cortices. .

Figure 11. . This bar graph compares the average (±SEM) of simple versus peduncular dendritic spines identified from neurons in lower, primary sensory cortices (A1 and S1) versus those found in higher-level sensory cortices (PPr and LRSS) of alcohol treated animals. Simple and pedunculated spine densities were determined to be significantly different ('*', p<0.05, t-test) for both primary and higher-level multisensory cortices.

Figure 12. This bar graph compares overall average apical and basilar spine density between alcohols treated animals and normal controls. There was a significant difference ('*'; p<0.05, ANOVA) in the overall apical and basilar spine density between the alcohol treatment and the normal control studies.

Figure 13. This bar graph compares overall average simple and pedunculated spine density between the alcohol treated animals and the normal controls. There was a significant difference

('*', $p<0.05$, ANOVA) in the overall simple and pedunculated spine density between the alcohol treatment and normal control studies.

ABSTRACT

PRENATAL ALCOHOL EXPOSURE INDUCES ALTERATION OF DENDRITIC SPINE DENSITY ACROSS SENSORY CORTICAL REGIONS

By Francis Oppong, B.S.

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science

at Virginia Commonwealth University

Virginia Commonwealth University, 2011

Major Director: M. Alex Meredith, PhD

Professor, Department of Anatomy & Neurobiology

Dendritic spines are the major site of excitatory synapses in cortex, and factors that reduce dendritic spine numbers will produce serious cortical processing deficits, such as has been demonstrated for mental retardation and other psychiatric disorders. Prenatal alcohol exposure also has detrimental effects on brain development that lead to Fetal Alcohol Spectrum Disorder (FASD), which results in reduction of dendritic spine numbers in the hippocampus, prefrontal cortex and somatosensory cortex. FASD also is associated with temporal processing disorders involving sequential auditory stimuli that would be processed in auditory cortical areas.

However, it is unknown if the reduction of spine density following prenatal alcohol exposure occurs at auditory cortex, or is generally reduced across the different sensory cortices. This present study examined that question. Young adult ferrets (176 days old, 1 male, 1 female), that were exposed to alcohol during the equivalent of third-trimester development, were used to prepare Golgi-Cox stained sections through primary auditory cortex (A1). Other cortical regions examined included primary somatosensory (S1), and higher-level multisensory cortices of lateral rostral suprasylvian (LRSS) and rostral posterior parietal (PPr) areas. Control values from normal animals (n=3) were derived from a previous study. The results of this present study demonstrated that, dendritic spine density was significantly (Student's t-test, $P < 0.05$) lower in the alcohol treated group than in normal controls in all the cortical regions examined. These data indicate that although reduced spine density in auditory cortex may underlie temporal processing disorders in FASD, pre-natal alcohol exposure has widespread consequences for sensory cortical processing in general.

INTRODUCTION

The central nervous system contains billions of cells of which the principal components are neurons and glial supporting cells. Neurons are electrically excitable cells which maintain voltage gradients across their membranes; changes in membrane potential are used to transmit signals within the neuron. Neurons are discrete, and communicate with groups of other neurons through complex and highly integrated circuits. Communication between individual neurons occurs chemically through a special junction called a synapse, or electrically through gap junctions. A synapse contains molecular machinery that allows rapid transmission of signals between neurons (Kandel, Schwartz and Jessel, 2000). Neurons consist of cell bodies and two major types of projections or processes of the cell body (Fiala et al., 1999): dendrites and an axon. Dendrites are branched projections of the cell body of neurons specialized for receiving and processing of synaptic inputs. Dendrites serve to increase the receptive surface of a neuron without excessively increasing the volume. The complexity of dendrites reflects the number of potential connections that a neuron can receive. Synapses can reside directly on the shaft of dendrites (shaft synapses) or on synaptic specializations such as dendritic spines.

Dendritic spines are membranous protrusions or enlargements of the dendrite and serve as the primary postsynaptic target for excitatory synapse as illustrated in Figure 1(Nimchinsky et al., 2002). Spines are the most common synaptic specialization of dendrites in the central nervous system and, like the dendrite on which they arise, function to increase the number of potential synaptic partners for neurons by extending the surface area while increasing brain volume only slightly. Spines range in volume from 0.01μm^3 for small spines to 0.08 μm^3 for large spines (Harris, 1999).

There is an enormous variety of different dendritic spine shapes. The most notable classes of spine shape include simple (sessile), pedunculated (mushroom) and filopodic (Fiala *et al*, 2002). Electron microscopy studies have further shown that there is a continuum of shapes between these categories (Stuart et al., 2008). Spines are also highly dynamic; they retract, extend, and change shape rapidly in response to synaptic activity. The variable spine shapes are thought to be correlated with the strength and maturity of each spine-synapse (Nimchinsky et al., 2002).

Spines are absent prior to the formation of synapses (Harris, 1999). However, all neurons exhibit dendritic filopodic spines transiently during development (Fiala et al., 1999). During the early stages of synaptogenesis, filopodic spines play a crucial role, often making early synaptic contacts. Majority of filopodic spines, however, remain synapseless. Filopodic spines are highly dynamic extending and retracting within a few minutes. According to a study (Harris, 1999), long filopodic spines, both in hippocampal cultures *in vitro* and after the first postnatal week *in vivo*, are rarely seen in adult brain as they diminish and are replaced by mature synapses.

In mature pyramidal cells, two main kinds of spines can be observed; simple (sessile) and pedunculated (Jones and Powell, 1969). The pedunculated spine is the classical dendritic spine of the cerebral cortex; a narrow pedicle of varying length is attached at one end to its parent dendrite that expands at the other end into cup-like or prism shaped bulb with a flattened side receiving an axonal terminal at a typical synaptic complex (Jones and Powell, 1969). The flattened side of the bulbous head of pedunculated spines contains a dense disc called postsynaptic membrane. The postsynaptic membrane contains receptors, structural proteins and signaling molecules whose functions underlie synaptic transmission and plasticity.

In contrast to pedunculated spines, a spine is regarded as simple if the pedicle is broad and there is little or no constriction at the junction with the parent dendrite as illustrated in Figure

2 (Jones and Powell, 1969). Simple spines are normally no more than 2μm in height (Fiala et al., 1999), and contain postsynaptic density (PSD) at the end of the pedicle where they form synapse with axon boutons.

The volume of spine head strongly correlates the size of PSD, as well as with the number of presynaptic vesicles (Harris and Stevens, 1989). These studies (Nusser et al., 1989: Racca et al., 2002) reported that the density of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA) and N-Methyl-D-aspartate (NMDA) receptors is constant within the PSD, and thus the number of receptors per synapse is proportional to PSD area and spine volume. Thus in terms of postsynaptic activity, large spines form relatively stronger synapses than simple spines (Nimchinsky et al., 2002). Another study (El-Husseini, 2000) corroborated the above observation by Nimchinsky (2002): manipulations that modulate spine size also appear to change measures of synaptic strength, including the amplitude of miniature synaptic currents.

Dendrites of a single neuron contain hundreds to thousands of spines. However, spine density (number of spines per micron of dendritic length) varies for same neuron type across brain regions: Hippocampal CA1 pyramidal neurons are very spiny, with an average spine density of 2.5 spines per micron while pyramidal cell of the visual cortex are much less spiny, averaging 1.5 spines per micron (Fiala et al., 1999). Similarly, a study by Bajwa (2010, unpublished VCU Thesis), reported different average dendritic spine density for layer 2-3 pyramidal neurons at different sensory cortices of the ferret brain examined; primary auditory cortex, A1; primary somatosensory cortex,_S1; rostral posterior parietal cortex, PPr; and lateral rostral suprasylvian sulcal cortex, LRSS. These sensory areas are illustrated in Figure 3 (lateral view of ferret cortex). Spine density can also differ significantly for apical and basilar dendrites

of a given neuron (Fiala et al., 1998). In addition, though dendritic spines are distributed throughout the dendritic tree, there are few spines at the initial segment relative to the distal segment of apical dendrites of neocortical pyramidal neurons (Nimchinsky et al., 2002). This observation suggests a variation in spine density along the length of a given dendrite, with the proximal segments showing relatively lower spine density than the distal segments. The proximal segments of dendrites often receive inhibitory inputs in the form of shaft synapse, and so show less spine density (García-López et al., 2006). Finally, dendrites of pyramidal neurons may also show different spine density for the different spine shapes. Some dendritic segments show a higher spine density for pedunculated spines than simple spines and vice versa (García-López et al., 2006).

In mammalian brain, virtually all excitatory input on cortical pyramidal neurons contacts dendritic spines. Subtle changes in spine numbers can have marked effects on neuronal circuitry. It was once assumed that, once formed, dendritic spines remain in place, as a synaptic unit with their presynaptic partners for the lifetime of the neuron. However, it is now known that, spines are prone to structural distortions and destruction by a variety of insults (Nimchinsky et al., 2002). Destruction and structural distortion of spines have also been observed in normal aging and in disease states. Generally, two categories of spine pathologies are observed following adverse events or conditions; pathologies of spine distribution and pathologies of spine ultrastructure (Fiala et al., 2002).

Disease-specific disruptions in spine shape, size or number accompany a large number of brain disorders. This suggests that spine number may serve as a common neuro-anatomical substrate of pathogenesis for a number of neuropsychiatric disorders including autism spectrum disorder (ASU), schizophrenia and Alzheimer's disease. The above mentioned disorders involve

deficits in information processing necessary for complex cognitive process (Penzes et al., 2011). The results of a study (Glantz et al., 2000) indicate a 23% decrease in spine density on deep layer 3 pyramidal neurons from dorsolateral prefrontal cortex (DLPFC) in schizophrenic subjects compared with normal controls (Penzes et al., 2011). Reduction in dendritic spine number has also been implicated in a number of neuro-developmental disorders such as mental retardation. A study (Purpura, 1974) used the Golgi method to compare spine density and morphology of post-mortem human cerebral cortex neurons in persons with mental retardation and normal controls. This study reported a significant reduction (15%) in spine density (spine dysgenesis) on neurons from the hippocampus and neocortex of children with mental retardation. This indicates a disturbance of the development of dendritic spines on cerebral cortex neurons. Subsequent Golgi studies (Huttenlocher 1990; Kaufmann and Moser 2000) on mental retardation (Fragile X syndrome) indicated a 13% reduction in dendritic spine density for pyramidal neurons from the cerebral cortex and hippocampus compared to normal controls. These observations confirmed an association between mental retardation and abnormalities in the morphology and density of dendritic spines.

Spine formation, plasticity, and maintenance depend on synaptic activity and can be modulated by sensory experience. Spines are maintained by optimal activation; extremely high or low neuronal excitability induces changes in both dendritic complexity and spine density. A study (Jiang et al., 1998) to examine the effect of high neuronal excitability on dendritic spine number reported a 35% and 20% decrease in apical and basilar spine numbers respectively for hippocampal CA3 pyramidal neurons in adult rats that became epileptic after recurrent seizures in infancy. Similarly, sensory stimuli also influence dendritic spine numbers on neurons at primary sensory cortices: A study by Parnavelas et al., (1973) counted dendritic spines along the

apical and basilar dendrites of layer V pyramidal neurons at the visual cortex in animal models exposed to continuous illumination from birth to 35 days. Spine density was found to be significantly higher (10%) in experimental animals than in controls. The results from these studies further suggest that neurons homeostatically regulate input through spine density. Another related study by Valverde (1967) on sensory deprivation showed that spine density in portions of layer V pyramidal neurons in the primary visual cortex was significantly decreased (15%) in mice raised from birth in total darkness compared with controls. These studies suggest that spine numbers increase with sensory exposure, but decrease with sensory loss or deprivation.

In addition, prenatal or postnatal exposure to alcohol can cause alteration of spine morphology and number on cortical neurons. Prenatal alcohol exposure leads to disorders collectively called Fetal Alcohol Spectrum Disorder. FASD describes a continuum of permanent birth defects and intellectual disability that is caused by maternal consumption of alcohol during pregnancy (Astley, 2004). There are 0.8 to 6.0 cases of FASD per 1,000 live births according to studies conducted by the Centers for Disease Control and Prevention (2005) in the United States. There are three distinct diagnosis of FASD according to Canadian guidelines (Chudley et al., 2005); Fetal Alcohol Syndrome (FAS), Partial Fetal Alcohol Syndrome (pFAS), and Alcohol-Related Neurodevelopmental Disorder (ARND). Fetal alcohol syndrome (FAS) is characterized by prenatal and postnatal growth retardation, facial dysmorphologies, and a host of neurobehavioral impairments. Neurobehavioral effects in FASD include poor learning and memory, attentional deficits, and motor dysfunction (Berman and Hannigan, 2000). Children with this condition also exhibit temporal processing disorder, meaning that if given a sequence of numbers, events, or words, they tend to forget the middle ones. For this reason, it is suspected

that their auditory cortical circuitry is different from normal. A possible mechanism that could underlie this temporal processing deficit would be the reduction of dendritic spines in auditory cortices that would reduce the amount of information processed there. Although alterations of spine number following prenatal alcohol exposure have been documented, it is unknown whether this effect occurs specifically within auditory cortex or is a more generalized effect that occurs broadly across the sensory cortices. This project was initiated to examine the potential dendritic spine loss in auditory and other sensory cortices of ferrets exposed to alcohol prenatally. The specific cortical areas examined are illustrated in Figure 3 (lateral view of ferret cortex).

METHODS

All procedures were carried out according to the National Research Council's Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research (2003) and with the approval of Virginia Commonwealth University's Animal Care and Use Committee.

Alcohol treatment

All alcohol treatments were conducted by the laboratory of Dr. Alexandre Medina. The ferrets received a series of alcohol injections (3.5 g/kg, 25% in saline, intraperitoneal) every other day for a period of 21 days beginning at postnatal day 10 (P10). Blood alcohol levels were measured at specific times after injection, but importantly blood alcohol levels >250 mg/dl were observed 1-5 hr post-injection. Following treatment, the animals were raised with their littermates until weaned, and then into adulthood in individual cages.

Animals and Tissue preparation

Adult male and female ferrets, with attributes summarized in Table 1, were anesthetized intraperitoneally with sodium pentobarbital (60 mg/kg). On becoming areflexive, the ferrets were perfused transcardially with 0.9% saline followed by 0.4% paraformaldehyde. In situ, the brain of each ferret was stereotaxically blocked in coronal plane, and removed from the cranium. The brains were then post-fixed in 0.4% paraformaldehyde for 24 hours. Following post-fixation, 7-10 mm thick blocks of cortex were taken from each hemisphere of the brain for

Golgi-Cox staining using the FD Rapid GolgiStain Kit (FD NeuroTechnologies, Ellicott City, MD, USA).

The tissue blocks were rinsed briefly in double-distilled water and then immersed in a Golgi-Cox solution (5% potassium dichromate, 5% mercuric chloride and 5% potassium chromate). This mixture was refreshed and replaced after 24 hours, and then stored at room temperature in the dark for 14 days. The tissue blocks were then transferred to Solution "C" (FD Rapid Golgi Stain Kit), and incubated in the dark for 7 days. Using a vibratome, the tissue blocks were sectioned serially at 100µm thickness. The tissue sections were mounted on gelatin-coated glass microscope slides, and moistened with Solution "C. These mounted sections were then left to dry in a humidor chamber overnight at room temperature. The dried mounted sections were rinsed with double distilled water for two minutes, and then reacted in equal parts of "Solution D" (FD Rapid Golgi Stain Kit) and "Solution E" (FD Rapid Golgi Stain Kit) in the dark for 10 minutes. These sections were further rinsed in double distilled water for 4 minutes, and dehydrated with and ascending alcohol series of baths (50%, 75% and 100%). Following dehydration, the sections were cleared in xylene and finally coverslipped with Permount (Fisher Scientific, Fair Lawn, NJ). The finished slides were archived for later analysis.

Data Collection

A light microscope (Nikon Eclipse E600) was used to examine the Golgi-Cox stained tissue sections. Tissue sections from each of four different cortical regions (A1, S1, PPr, and LRSS) were selected and the entire outline of the section plotted with a light microscope under low magnification (4x) using Neuroludica (MBF MicroBrightfield, Willston VT, USA) software. In each cortical region, only layer 2-3 pyramidal neurons with complete soma-dendritic filling

and dendrites that were continuous with the soma were selected for tracing, reconstruction and spine measurement. On selecting a neuron for reconstruction, the soma and the neuronal processes (branching dendrites and axon) were plotted under a magnification of 40x using Neurolucida as shown in Figure 4. Next, segments of the apical and basilar dendrites (≥50μm) containing spines were traced under a high magnification (100x, oil). Subsequently, the location of each simple or pedunculated spine occurring on the traced dendritic segment was marked and counted. Spines that were not visible (i.e., behind the dendrite shaft) were not sought or plotted. Spine classification was based on the criteria of Jones and Powell (1969). Pedunculated spines exhibited a narrow pedicle of varying length attached at one end to its parent dendrite and expanded at the other end into cup-like or prism shaped bulb. Simple spines had a broad pedicle without a constriction at the junction with the parent dendrite and were short (<2 μm). Filopodic spines were not marked or counted. This process was repeated for at least 2 apical and 3 basilar dendrites for each neuron and for at least 6 neurons per area as detailed in Table 2. For documentation, segments of the traced neurons were photographed using a light microscope (Nikon Eclipse E600).

Data Analysis

Using software (NeuroExplorer; MBF MicroBrightfield, Willston VT, USA) plots of dendritic segments marked with spines (simple and pedunculated) were analyzed. For each plot of dendritic segment, the following parameters were determined; length of the segment, simple spines counted and pedunculated spines counted. The soma area for all the neurons from the different cortical regions traced was also determined using NeuroExplorer (MBF

MicroBrightfield, Willston VT, USA) software. These data were tabulated by dendritic location (apical versus basilar), by cortical location (A1, S1, PPr, and LRSS) for comparison and statistical analysis.

Statistical Analysis

The data from all the neurons examined in this study was collated on a spreadsheet from which the following measures were calculated. First, the average spine density (and standard deviation) for all spines from neurons from each selected cortical region was determined as shown in Table 3. Then, the data was progressively refined by examining selected features. The average spine density for apical or basilar dendrites was calculated for each cortical region as shown in Table 4. These features were further examined by determining the density of simple versus pedunculated spines for the variables of areal or dendritic location as shown in Table 5. Next, these features of spine density were grouped according to hierarchical order of the cortical location. Neurons reconstructed from A1 and S1 were from lower-level, primary sensory cortices. Neurons reconstructed from PPr and LRSS were from higher-level, associational and multisensory cortices. Thus, values of average spine density, average apical versus basilar spine density, and average simple versus pedunculated spine density (and standard deviation) were calculated for neurons from primary sensory versus associational areas as detailed in Table 6. For all tests, statistical comparisons between different groups were compared using a t-test ($p<0.05$ = significant). Data and statistical treatments were graphically displayed using Excel (Microsoft).

Control, normal ferrets.

Dendritic spine densities were determined for control adult ferrets (n=3; males, 132 days of age). The identical staining, measuring and analytical methods were used to determine dendritic spine counts from the same ferret cortical areas (A1, S1, LRSS, PPr). All these procedures were carried out by Moazzum Bawja.

RESULTS

Alcohol-treated animals:

Using Golgi-Cox stained tissue from cortex of 2 young, adult ferrets with fetal alcohol exposure, 48 layer 2-3 pyramidal neurons were identified. Only neurons with complete soma-dendritic filling and dendrites that were continuous with the soma were reconstructed. This allowed us to identify the lamina from which each neuron was taken. Each selected neuron was mapped using Neurolucida to quantify dendritic branching features and measure dendritic dimensions. Once a neuron and its branching patterns were reconstructed, selected segments of the apical and basilar dendrites were examined for the presence and identification of dendritic spines.

For each neuron, dendritic spines were counted on segments of at least 2 apical and 3 basilar dendrites (or vice versa) that were at least 50µm long (to avoid small-measure artifacts).

<u>Total Spines counted.</u> Dendritic spines were identified as either simple (short <2µm, with no neck or constriction) or pedunculated (with neck/constriction on stalk and an expanded head). A total of 9162 simple and 7180 pedunculated spines were marked, making a total of 16,342 dendritic spines examined as summarized in Figure 5.

<u>Overall Dendritic Spine Density.</u> When the length of dendrite was included from which the spines were identified, an overall average density (i.e., spine count per unit of length) of 1.070 ± 0.014 spines/micron (±SEM) was calculated for alcohol treated group.

Spine density by cortical area. To determine if the spine density varied between different cortical areas, the data was separated by cortical area; primary auditory cortex (A1; 1.185 spines/µm ± 0.027 SEM), primary somatosensory cortex (S1; 1.084 spines/µm ± 0.038 SEM), rostral posterior parietal cortex (PPr; 1.045 spines/µm ± 0.022 SEM), and lateral rostral suprasylvian sulcal cortex (LRSS; 0.964 spines/µm ± 0.0.018 SEM). Using analysis of variance (ANOVA) there was no significant difference ($p<0.05$, t-test) in dendritic spine density between each of the different cortical areas measured in the alcohol treatment study. These effects are summarized in Figure 6.

Apical vs. Basilar spine density. To determine if the spine density varied for apical and basilar dendrites of neurons from the different cortical areas, a t-test was used to evaluate these measures. From Figure 7, there was no significant difference ($p<0.05$, t-test) in spine density between apical and basilar dendrites within each of the areas except for A1 (avg. apical spine density = 1.253 spines/µm ± 0.039 SEM; basilar spine density = 1.123 spines/µm ± 0.0.036 SEM).

Pedunculated vs. Simple spine density. To determine if there is difference in spine density for simple and pedunculated spines, t-tests were used to compare these measures within the same area. Figure 8 shows a significant difference ($p<0.05$, t-test) between simple and pedunculated spines for each of the cortical areas except for PPr (avg. simple spine density = 0.517 spines/µm ± 0.017 SEM and avg. pedunculated spine density = 0.527 spines/µm ± 0.028 SEM).

Unisensory Cortices vs. Multisensory Spine density. Finally, to determine if measures of spine density varied for neurons from different hierarchical cortical levels, combined data from S1 and A1 cortical regions (representing lower level/primary sensory cortices) was compared with

combined data from PPr and LRSS (representing higher-level multisensory cortices). Figure 9 shows a significant difference ($p<0.05$, t-test) between S1+A1 (avg. spine density = 1.135 spines/μm ± 0.0231 SEM) and PPr + LRSS (avg. spine density = 1.003 spines/μm ± 0.015 SEM).

Apical vs. Basilar spine density from hierarchical cortical levels. The hierarchical data was further analyzed to determine if spine density varied for apical and basilar dendrites of pyramidal neurons from lower level primary sensory cortices (A1+ S1) and higher-level multisensory cortices (PPr + LRSS). From figure 10, there is a significantly higher ($p<0.05$, t-test) apical spine density (avg. 1.205 spines/μm ± 0.034 SEM) than for basilar spine density (avg. = 1.073 spines/μm ± 0.033 SEM) within lower level/primary sensory cortices. However, there is no significant difference ($p<0.05$, t-test) in these same measures for the higher-level multisensory cortices (apical dendrites avg. spine density = 1.025 spines/μm ± 0.023 SEM and basilar dendrites avg. spine density = 0.986 spines/μm ± 0.019 SEM).

Pedunculated vs. Simple spine density from hierarchical cortical levels. Lastly, the hierarchical data was analyzed to determine if there is difference in spine density for simple and pedunculated spines. T-tests were used to compare these measures within lower level primary sensory cortices (A1+ S1) and higher-level multisensory cortices (PPr + LRSS). From figure 11, there is significantly higher ($p<0.05$, t-test) pedunculated spine density (avg. 0.684 spines/μm ± 0.020 SEM) than simple spine density (avg. 0.452 spines/μm ± 0.011 SEM) within lower level primary sensory cortices (A1+ S1). Similarly, pedunculated spine density (avg. 0.522 spines/μm ± 0.016 SEM) is significantly higher ($p<0.05$, t-test) than simple spine density (avg. 0.482 spines/μm ± 0.012 SEM) within the higher-level multisensory cortices (PPr + LRSS).

Normal controls:

For normal ferrets, a study (Bajwa 2010, unpublished VCU Thesis) made observations of dendritic spine density in which the overall average spine density from the same areas of sensory cortex was calculated. This study used young adult male ferrets (average age = 132 days). For layer 2-3 pyramidal neurons from all measured sensory cortices (A1, S1, PPr, LRSS), the overall average spine density was 1.249 spines/micron ± 0.013 SEM. In these normal animals, a significantly different average dendritic spine density was observed between each of the cortical regions. Also, for normal ferrets, there were significantly higher measures of dendritic spine density for primary sensory areas (A1, S1) than observed in association/multisensory areas (LRSS, PPr). However, while there was no significant difference between apical and basilar average spine density within each of the cortical regions, the simple and pedunculated spine density did vary significantly at different sensory cortical regions, where average pedunculated spine density was significantly higher than average simple spine density in all sensory regions (primary and association) sampled.

Comparison of Alcohol-treatment and controls:

In comparing the data from alcohol treated ferret cortex with that of normal ferret cortex (Bajwa (2010, unpublished VCU Thesis), there was an overall reduction of 14.33% in average spine density for the alcohol treatment sensory cortical neurons. As stated above, alcohol treated animals showed an average of 1.070 spines/micron ± 0.014 SEM, while normal ferrets showed 1.249 spines/micron ± 0.013 SEM, and this difference was statistically significant ($p<0.05$, ANOVA). Similarly, there is a significant reduction of spine density for each of the separate sensory cortical regions for the alcohol treated animals. There was a marked reduction in spine

density by 12%, 17%, 16% and 12% for layer 2-3 pyramidal neurons in A1(1.185 ± 0.027 SEM vs. 1.343 ± 0.027 SEM), S1(1.084 ± 0.038 SEM vs. 1.309 ± 0.026 SEM), PPr (1.045 ± 0.022 SEM vs. 1.242 ± 0.021 SEM) and LRSS (0.963 ± 0.018 SEM ± vs. 1.099 sd ± 0.022 SEM) cortical regions, from the alcohol treated and normal animals, respectively. These observations indicate that prenatal alcohol exposure affects dendritic spine density across different sensory areas and processing levels.

Regarding the overall density of apical versus basilar dendritic spines per micron, a comparison of the data from alcohol treated ferrets with that of normal ferrets (Bajwa, 2010, unpublished VCU Thesis) indicated a significant reduction of 12% and 16% for neurons across all measured sensory cortical areas, respectively. The average apical spine density in the alcohol treated animals was lower than the average apical spine density normal controls: (1.114 ± 0.017 SEM vs. 1.261 ± 0.012 SEM). Similarly the average basilar spine density in the alcohol treated animals versus normal controls was 1.028 ± 0.0.012 SEM vs. 1.237 ± 0.018 SEM, respectively as summarized in Figure 12. These measures indicate that dendritic spine location is affected by prenatal alcohol exposure.

In both the alcohol treated and normal ferret cortex, there was a significantly higher pedunculated spine density than simple spine density in almost all the sensory cortical regions sampled. However, there was a 42% reduction in average pedunculated spine density for sensory cortical neurons from the alcohol treated animals; (avg. pedunculated spine density for the alcohol treated animal vs. avg pedunculated spine density for normal: 0.601± 0.014 SEM vs. 1.033 ± 0.018 SEM) with a corresponding increase of 116% in average simple spine density (0.467 ± 0.008 SEM vs. 0.216 ± 0.005 SEM) as summarized in Figure 13.

DISCUSSION

Because FASD is accompanied by auditory processing disorders, the present results are consistent with the possibility that a reduction of dendritic spines in the auditory cortex of the alcohol treated animals could be responsible for those deficits. However, because the results from the other regions of the brain, including somatosensory and higher-level multisensory areas, also showed reductions of spine densities in the alcohol treatment study; it seems that prenatal alcohol exposure has widespread consequences for sensory cortical processing in general. To date, studies on prenatal alcohol exposure have reported similar findings: Prenatal exposure to alcohol induces significant reduction of dendritic spine density on pyramidal neurons in, hippocampal, somatosensory and motor cortical areas. In a study (Zhan-Jun et al., 2010) reported that spine density on layer V pyramidal neurons in the visual cortex of mice (at postnatal day of zero) in the treatment groups was significantly lower (0.13 spines/µm in the 4g/kg/day alcohol group and 0.18 spines/µm in the 2g/kg/day alcohol group) than observed in normal controls (0.21 spines/µm). The significant reduction of spine density in other brain regions could be associated with some of the clinical features of FASD subjects, who exhibit poor learning and memory, attentional deficits, and intellectual disability.

The results of the present study also showed significant decreases in average apical and basilar spine density by 12% and 16%, respectively, for neurons across all measured sensory cortical areas. This is consistent with the results of other studies on prenatal alcohol exposure. In a study (Abel et al., 1983), spine number was counted for hippocampal CA1 pyramidal neurons of 90-day-old rats following prenatal alcohol exposure via intragastric intubation (6 g/kg/day) throughout gestation. This study found significant 27% and 31% decreases in spines density on apical and basilar dendritic branches. Another related study (Hamilton et al., 2010) reported a

similar observation: 27%-47% decreases in apical and basilar spine density respectively for CA1pyramidal neurons in adult rats that were prenatally exposed to alcohol (6g/kg/day). This indicates that the reduction of spine density following prenatal alcohol exposure occurs on both apical and basilar segments of the dendritic arbor. In addition, the reduction of spine density following prenatal exposure to alcohol was relatively higher for spines on basilar dendrites than those on apical dendrites. This observation is consistent with that reported by both Abel et al., (1983) and Hamilton et al., (2010), indicating that alcohol has more impart on basilar dendritic spines than apical dendritic spines in a developing brain. Thus spine location on dendritic arbor is affected by prenatal alcohol exposure.

From the results of the present study, there was a 42% decrease in the overall pedunculated spine density. However, the overall simple spine density increased markedly by 116%. This observation may possibly be the result of spine dynamism triggered by prenatal alcohol exposure. Dendritic spines are highly dynamic: retracting or extending their length and/or head volume that cause them to change from one spine type to another. Filopodic spines are the longest among spines, and are normally seen on dendrites during the early stages of synaptogenesis. However, during development, filopodic spines can retract completely to leave shaft synapse in some cases or simple spines in other cases (Fiala et al., 2002). Simple spines also changes to pedunculated (mature spines) during development. In addition, synapse functional integrity also influences spine dynamism. Depending on the functional integrity of simple spine synapses, a simple spine can either change to filopodic or pedunculated spines. Long term potentiation (LTP) and long term depression (LTD) also can influence the dynamic nature of spines. For instance, LTD can cause a pedunculated spine to change to simple spine. Since animals used in both the alcohol treatment and normal control studies were approximately

of the same age and were raised in a similar environment, the observed alteration of simple and pedunculated spine density could be attributed to the prenatal alcohol exposure. Thus alcohol is one of the factors that trigger dynamism in dendritic spine.

CONCLUSION

The results of the present study have confirmed that the density of dendritic spine is reduced broadly across sensory cortex in alcohol treated animals. The significant reduction in spine density in primary auditory cortex and in the associative cortices examined in the present study could underlie the impaired temporal processing disorder involving sequential processing of auditory stimuli in FASD patients. Other neurological impairments associated with FASD also could be attributed to the general reduction in spine density across sensory cortical areas.

Figure Legends

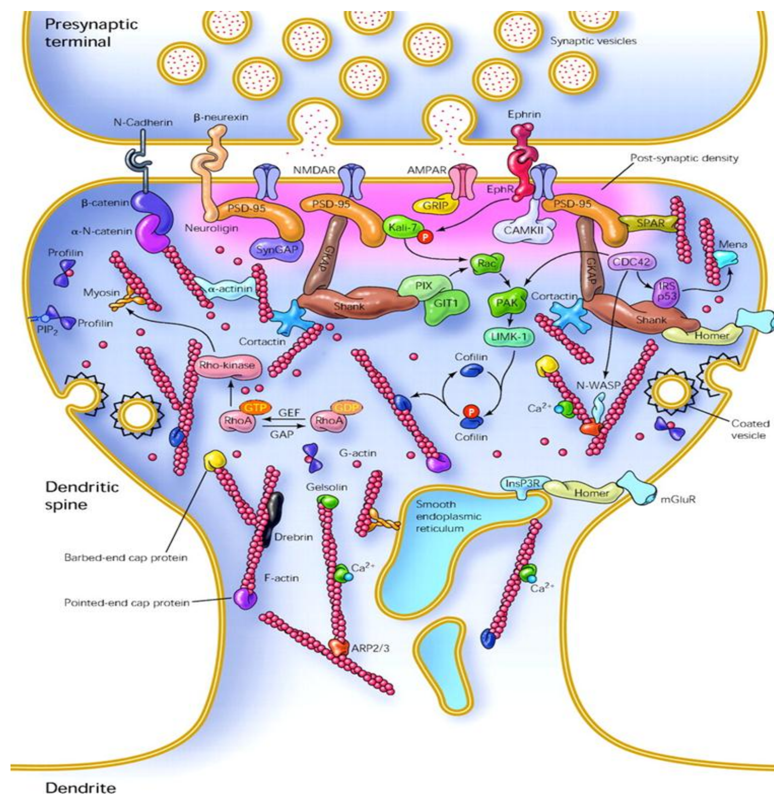

Figure 1: A schematic diagram of a dendritic spine synapse showing postsynaptic density and the various proteins involved in signal transduction.

(Source: physiologyonline.physiology.org)

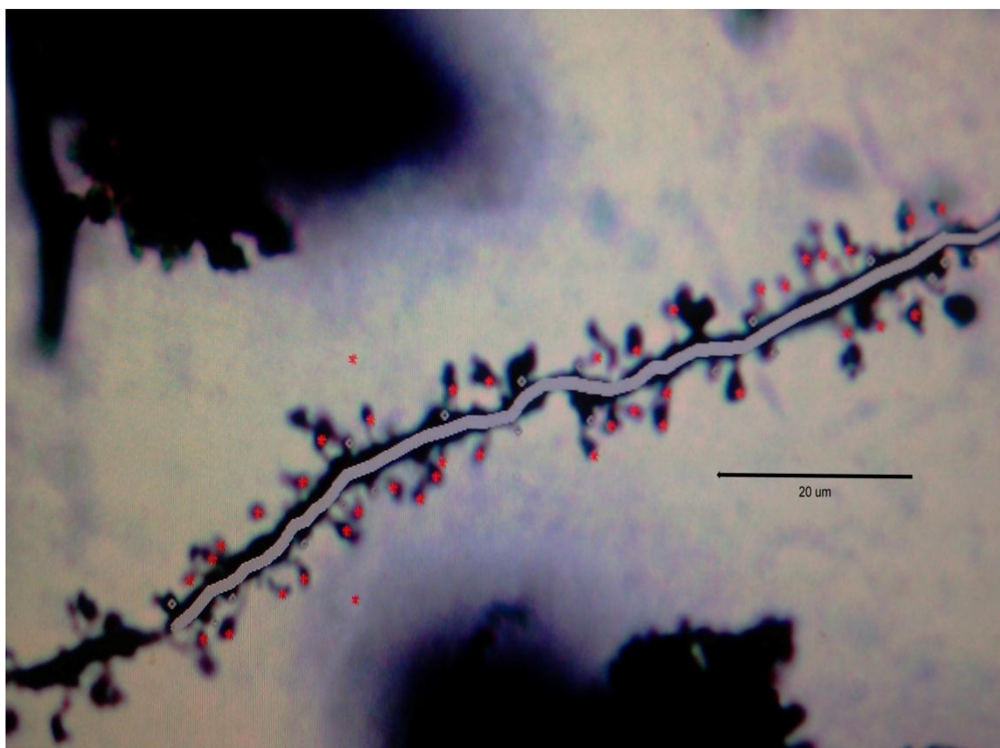

Figure 2. A dendritic segment (100x, oil) showing spine types examined in this study. Simple spines (marked with brown spots) and pedunculated spine (marked with read spots) Scale bar = 20µm.

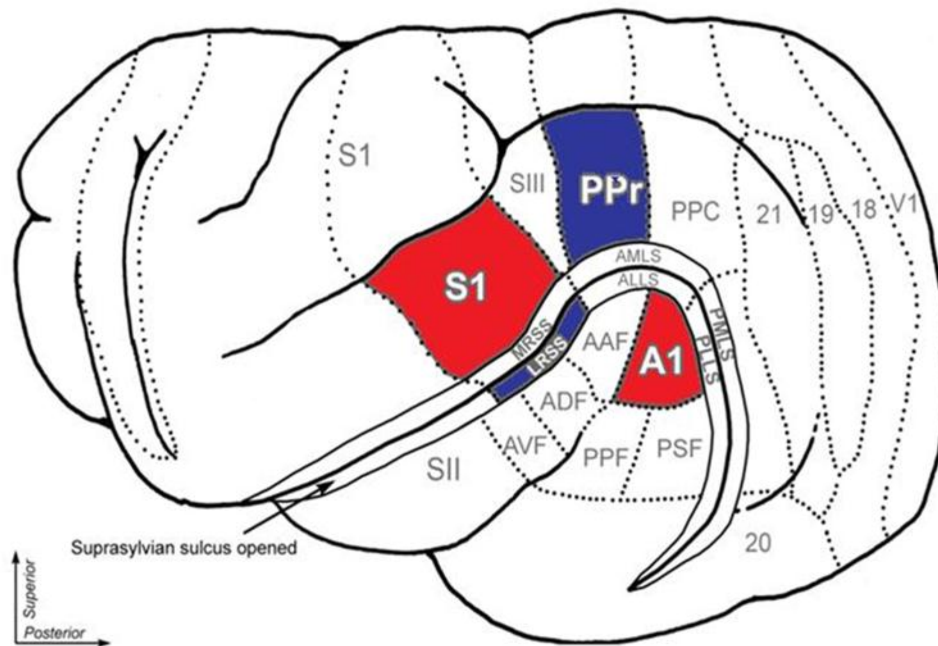

Figure 3. Lateral view of ferret brain showing the different cortical regions. With color marking showing the regions examined in this study; primary cortical sensory regions S1 and A1 (colored red); multisensory cortical regions PPr and LRSS (colored blue).

(Source: Bajwa, 2010. unpublished VCU Thesis)

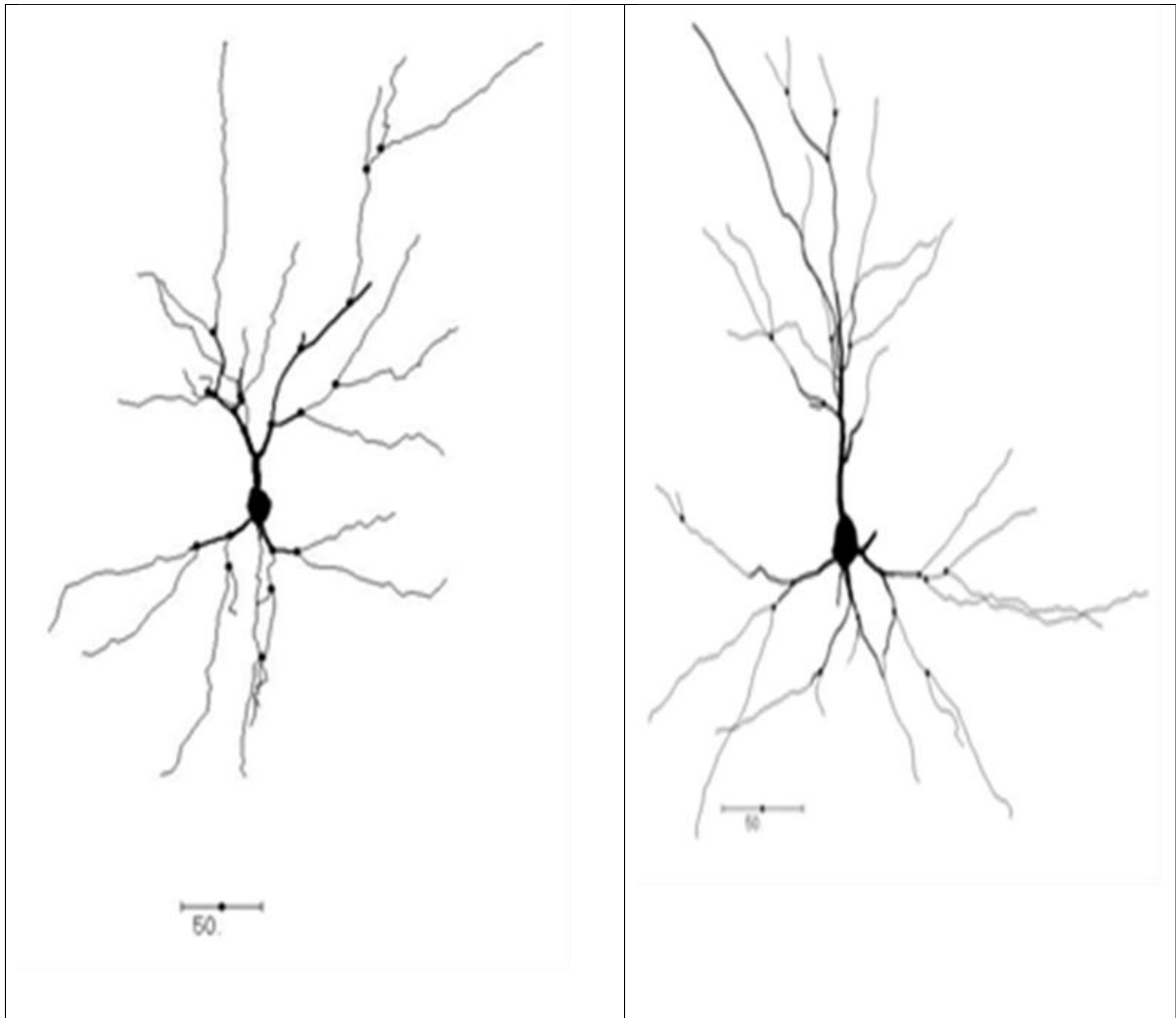

Figure 4. Reconstructed representatives of Layer 2-3 pyramidal neurons (40x, with the aid of Neurolucida) showing the soma and the neuronal processes (branching dendrites and axon). Scale bar = 50 µm

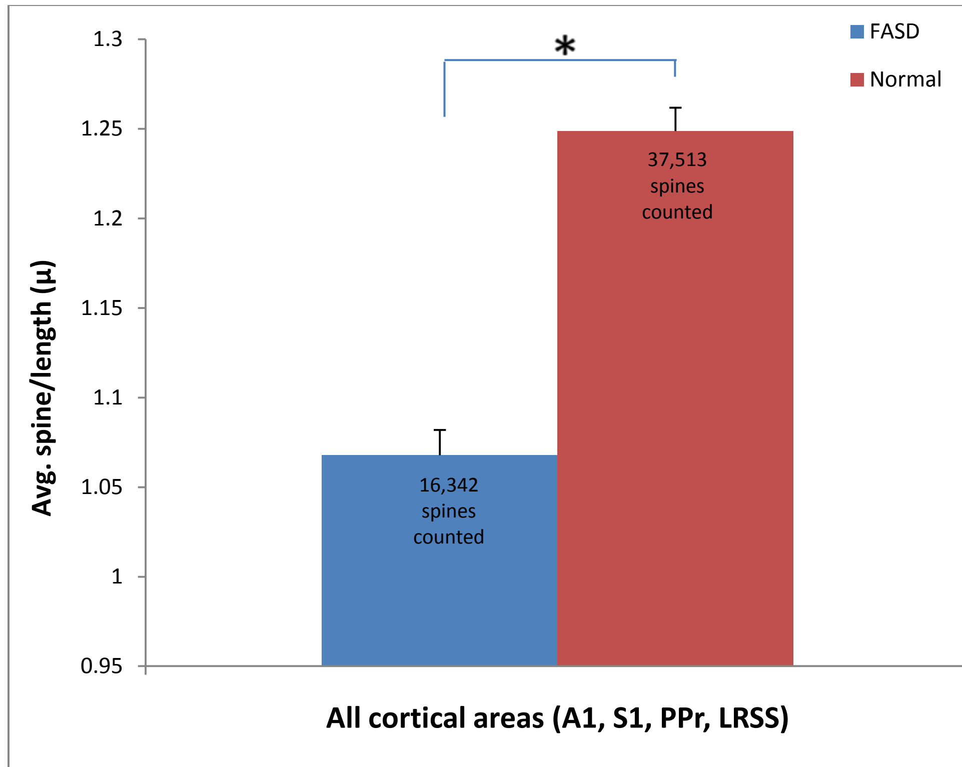

Figure 5. For all neurons and dendritic segments measured in all cortical areas, the average value for spines/micron was 1.068 ± 0.014 (SEM) for the alcohol treated animals and 1.249 ± 0.013 (SEM) for normal control animals. There was a significantly difference ('*'; $p<0.05$, t-test) between the overall spine density for the alcohol treated animals and that for normal controls.

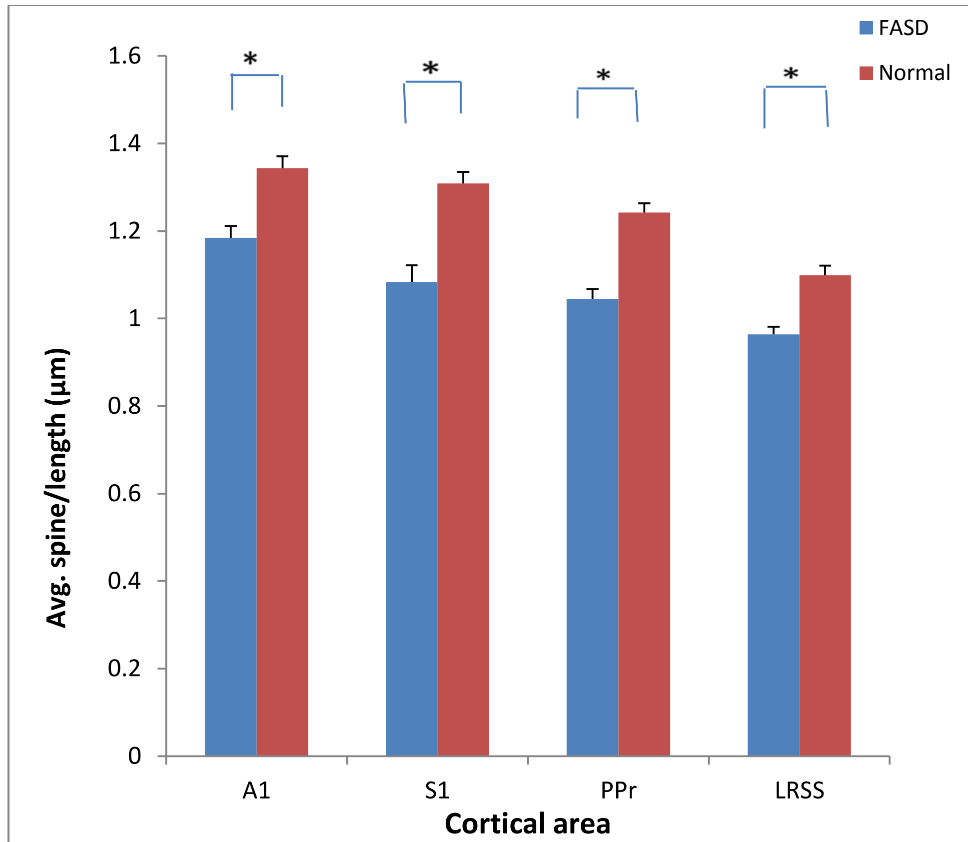

Figure 6. This bar graph compares the average (±SEM) of dendritic spines identified from neurons in A1 (primary auditory cortex); S1 (primary somatosensory cortex), PPr (rostral posterior parietal cortex), and the LRSS (lateral rostral suprasylvian sulcal cortex) between and the alcohol treated and normal control animals. There was a significance difference ('*'; $p<0.05$, ANOVA) between spine density for the alcohol treated animals and that for normal control at each cortical region.

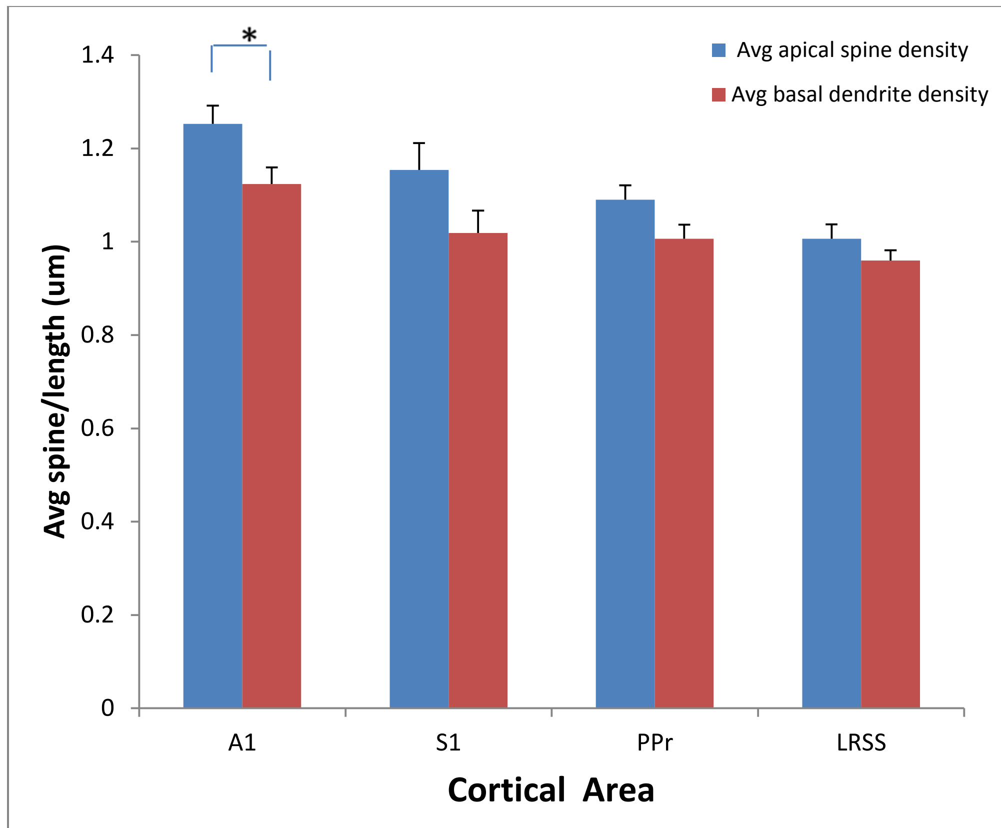

Figure 7. This bar graph compares the average (±SEM) of apical versus basilar dendritic spines identified from neurons in A1, S1, PPr, and the LRSS of the alcohol treated animals. Within each region, spine densities were not significantly different ('*'; $p<0.05$, t-test) between apical and basilar dendrites, except for those observed in A1.

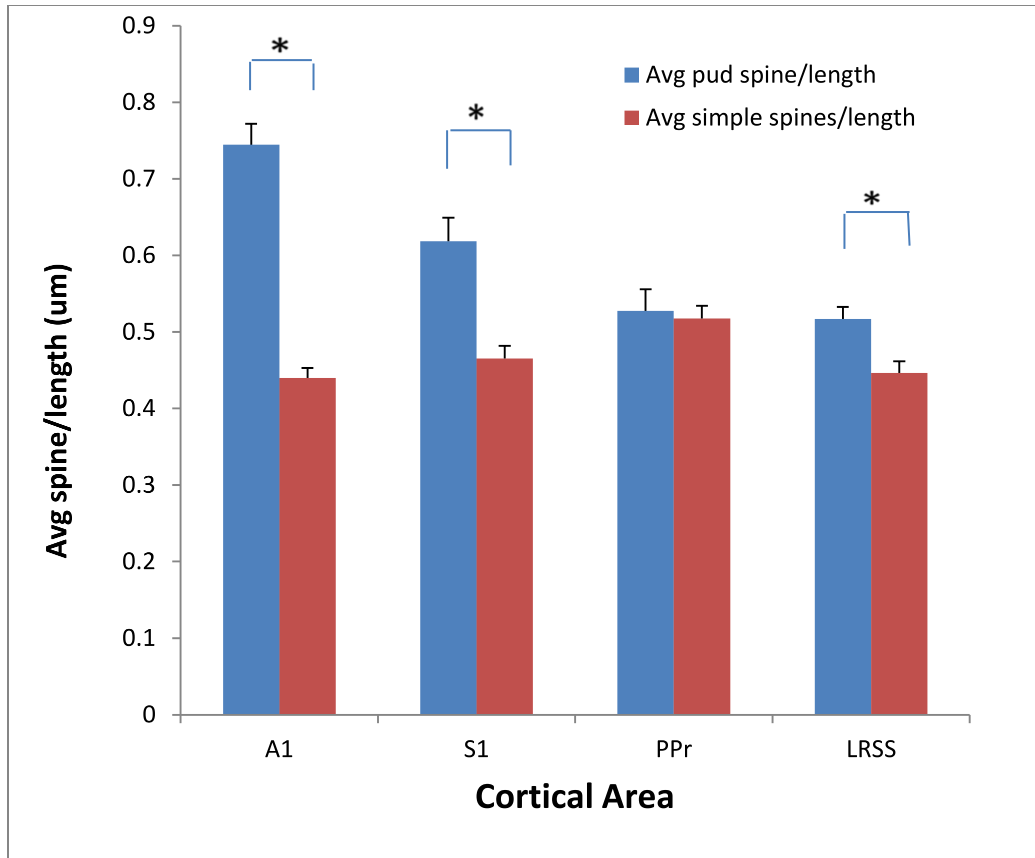

Figure 8. This bar graph compares the average (±SEM) of simple versus pedunculated dendritic spines identified from neurons in A1, S1, PPr, and the LRSS of the alcohol treated animals. Within each region, spine densities were significantly different (‘*’, $p<0.05$, t-test) for simple versus pedunculated spines except in the PPr.

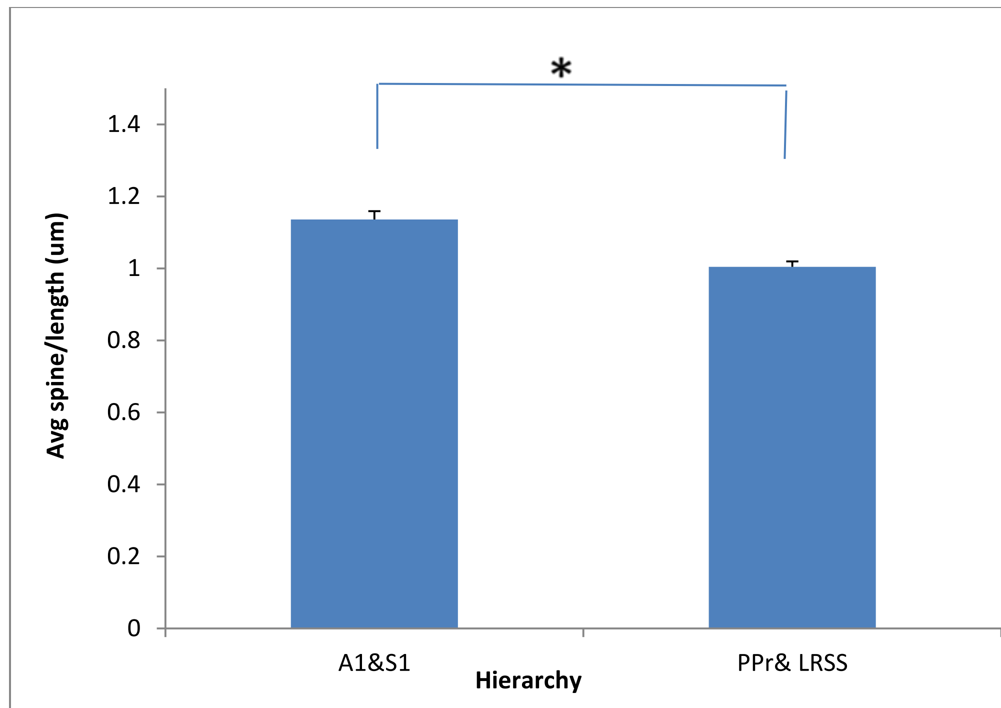

Figure 9. This bar graph compares the average (±SEM) of dendritic spines measured from neurons in primary sensory cortices (A1 and S1) versus those identified in higher-level, multisensory cortices (PPr and LRSS) of the alcohol treated animals. The total number of dendritic spines in primary sensory cortices were significantly greater than those observed in higher-level, multisensory regions ('*', $p<0.05$, t-test).

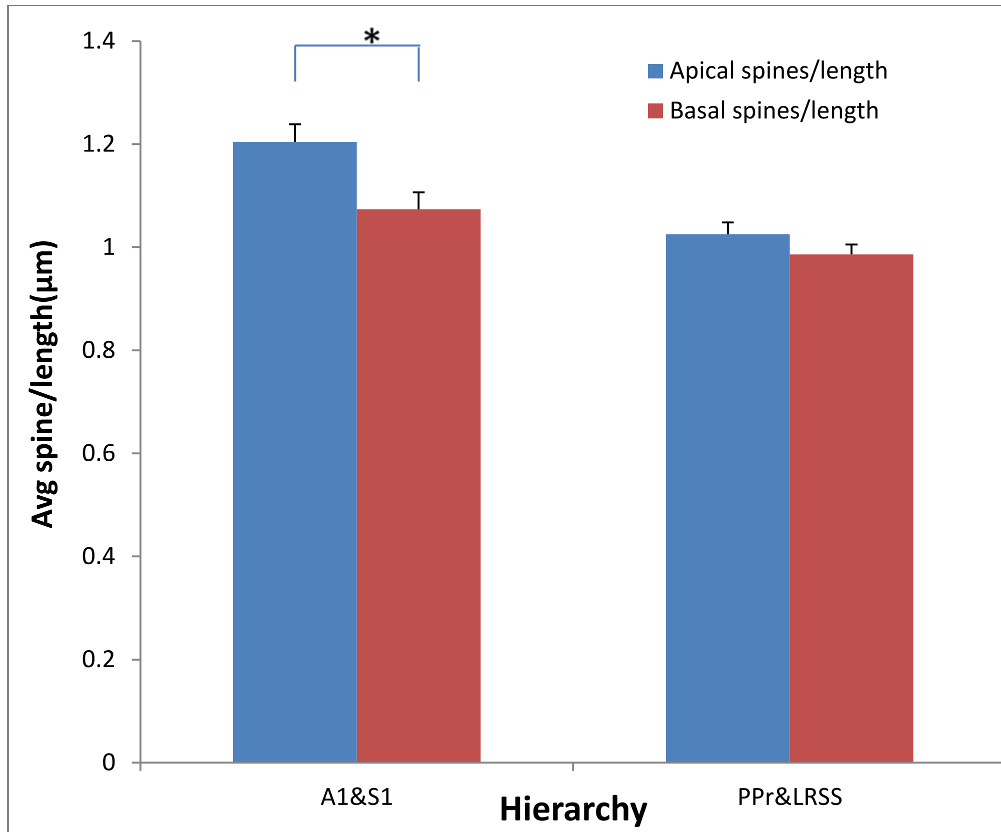

Figure 10. This bar graph compares the average (±SEM) of apical versus basilar dendritic spines identified from neurons in lower, primary sensory cortices (A1 and S1) versus those found in higher-level sensory cortices (PPr and LRSS) of the alcohol treated animals. The density of apical spines was significantly greater ('*', $p<0.05$, t-test) than for basilar spines in the primary sensory cortices, but not in the higher-level multisensory cortices.

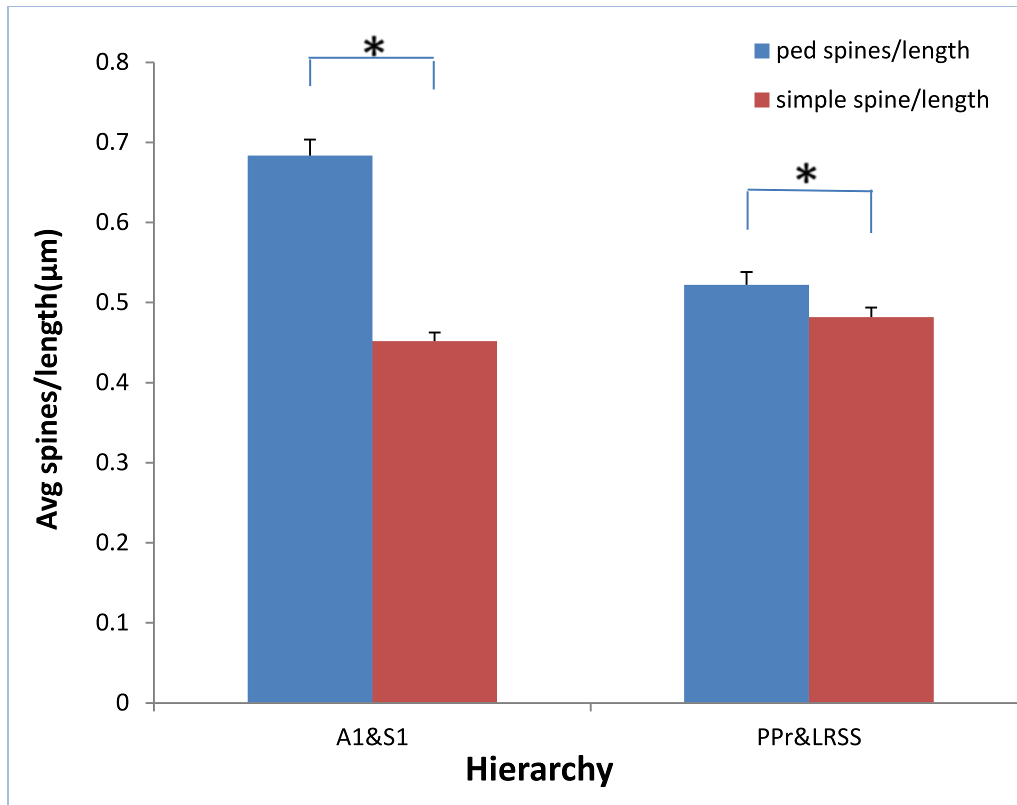

Figure 11. This bar graph compares the average (±SEM) of simple versus peduncular dendritic spines identified from neurons in lower, primary sensory cortices (A1 and S1) versus those found in higher-level sensory cortices (PPr and LRSS) of the alcohol treated animals. Simple and pedunculated spine densities were determined to be significantly different ('*', $p<0.05$, t-test) for both primary and higher-level multisensory cortices.

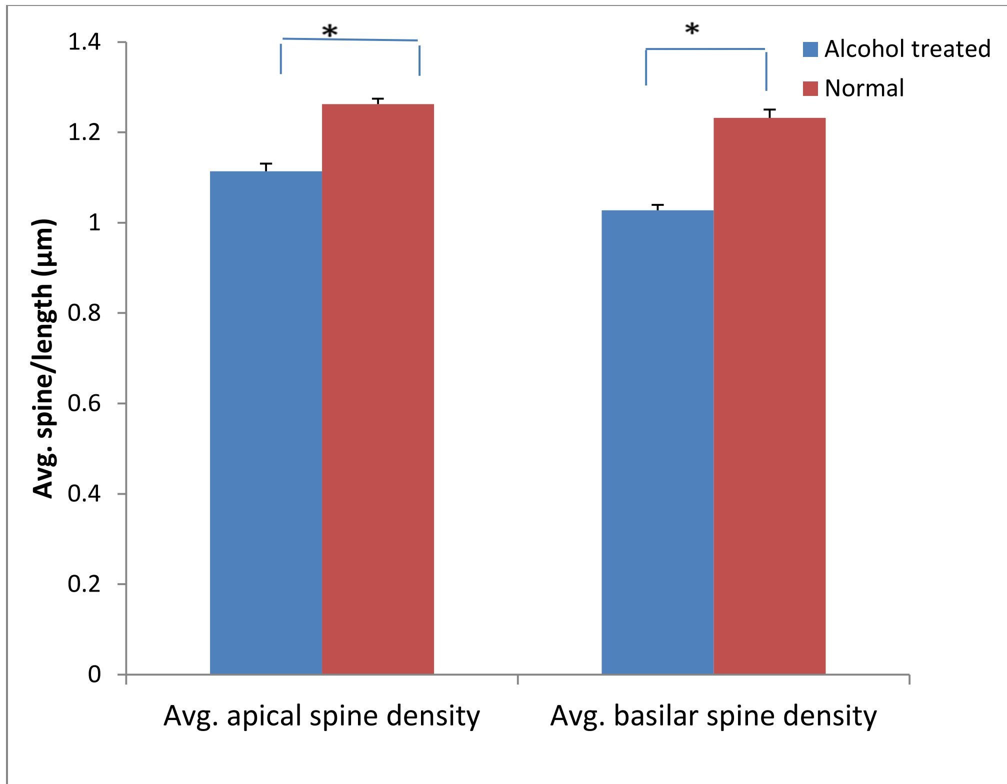

Figure 12. This bar graph compares overall average apical and basilar spine density between alcohols treated animals and normal controls. There was a significant difference ('*'; $p<0.05$, ANOVA) in the overall apical and basilar spine density between the alcohol treatment and the normal control studies.

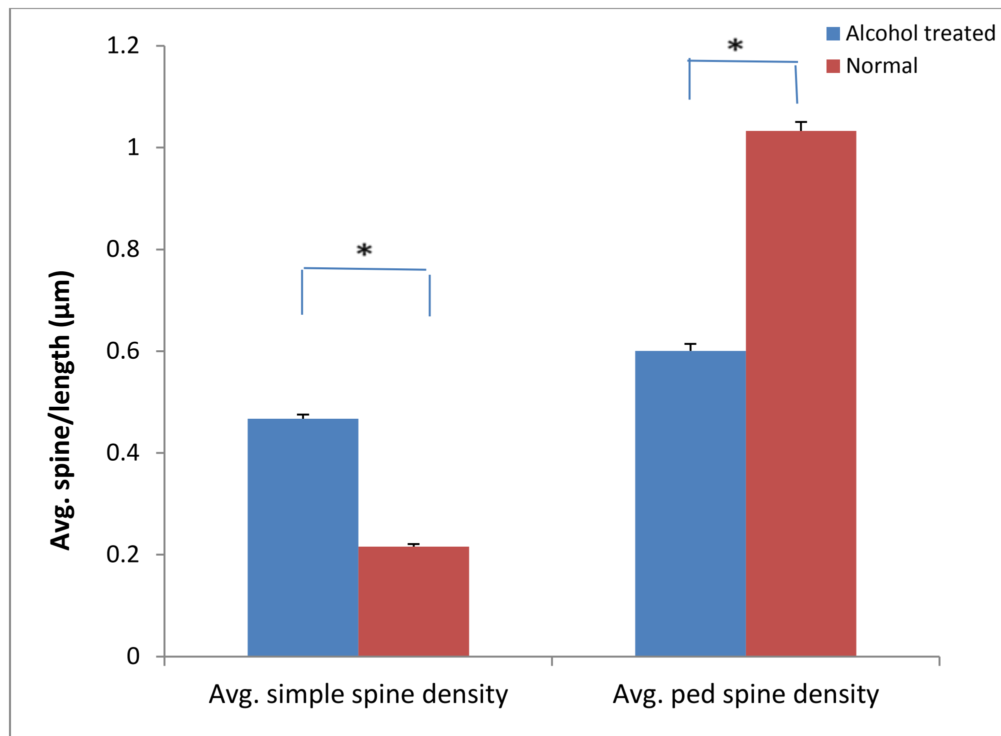

Figure 13. This bar graph compares overall average simple and pedunculated spine density between the alcohol treated animals and the normal controls. There was a significant difference (‘*’, $p<0.05$, ANOVA) in the overall simple and pedunculated spine density between the alcohol treatment and normal control studies.

TABLE

Ferret ID	Age (days)	Weight (kg)	Sex
F10-012	176	0.75	Female
F10-013	176	1.6	Male
F09-016 (Control)*	135	1.3	Male
F09-017 (Control)*	126	1.3	Male
F09-018 (Control)*	134	1.6	Male

Table 1. Characteristics of animals used in both the alcohol treatment and the normal control studies. Control /*/ data were derived from Bajwa (2010, unpublished VCU Thesis)

Cortical Region	Cases	Neurons	Dendrites (apical and basilar)
S1	2	12	60
A1	2	12	56
PPr	2	12	60
LRSS	2	12	58
S1* (Control)	3	17	93
A1* (Control	3	18	100
PPr* (Control	3	18	94
LRSS* (Control)	3	17	97

Table 2. A summary of animals, neurons and dendrites sampled in the alcohol treatment and normal control studies. Control /*/ data were derived from Bajwa (2010, unpublished VCU Thesis)

Cortical area	**Average spine density (spines/µm ± SEM)**
A1	**1.185 ± 0.027**
S1	**1.084 ± 0.038**
PPr	**1.045 ± 0.022**
LRSS	**0.964 ± 0.018**
ALL	**1.068 ± 0.014**

Table 3. Average and standard error (SEM) of spine density measures at the different cortical regions in the alcohol treatment study.

Cortical region	Average apical spine density (spine/µm ± SEM)	Average basal dendrite density (spine/µm ± SEM)
A1	**1.253 ± 0.039**	**1.124 ± 0.036**
S1	**1.154 ± 0.058**	**1.019 ± 0.048**
PPr	**1.090 ± 0.031**	**1.007 ± 0.030**
LRSS	**0.960 ± 0.031**	**0.966 ± 0.022**
ALL	**1.114 ± 0.017**	**1.028 ± 0.012**

Table 4. Average and standard error (SEM) of apical and basilar spine density measures by cortical region in the alcohol treatment study.

Cortical region	**Average pedunculated spine density (spine/µm ± SEM)**	**Average simple spine density (spine/µm ± SEM)**
A1	**0.745 ± 0.176**	**0.440 ± 0.104**
S1	**0.618 ± 0.211**	**0.465 ± 0.119**
PPr	**0.527 ± 0.198**	**0.518 ± 0.121**
LRSS	**0.517 ± 0.118**	**0.447 ± 0.110**

Table 5. Average and standard error (SEM) of spine density measures by dendritic location according to spine type in the alcohol treatment study.

	Hierarchical Level	
	A1&S1	**PPr& LRSS**
Avg spine density (spine/µm ± sd)	**1.136 ± 0.231**	**1.004 ± 0.148**
Avg. simple spine density(spine/µm±sd)	**0.452 ± 0.112**	**0.482 ± 0.121**
Avg. ped spine density(spine/µm±sd)	**0.684 ± 0.204**	**0.522 ± 0.161**
Avg. apical spine density (spine/µm ± sd)	**1.205 ± 0.234**	**1.025 ± 0.157**
Avg. basilar spine density (spine/µm ± sd)	**1.073 ± 0.213**	**0.986 ± 0.139**

Table 6. Average and standard error (SEM) of spine density measures for hierarchical levels and dendritic spine type (simple and pedunculated) in alcohol treatment study.

Cortical area (Normal controls)	Average spine density (spines/µm ± SEM)
A1	**1.344 ± 0.027**
S1	**1.309 ± 0.026**
PPr	**1.242 ± 0.021**
LRSS	**1.099 ± 0.022**
ALL	**1.249 ± 0.013**

Table 7. Values for dendritic spine density and standard error (SEM) from cortical areas A1, S1, PPr, and LRSS in normal, control ferrets.

Source: Data from Bajwa (unpublished VCU thesis, 2010).

LITERATURE CITED

Abel EL, Jacobson S, Sherwin BT. 1983. In utero alcohol exposure: functional and structural brain damage. Neurohbehav Toxicol Teratol 5:363–366.

Astley SJ, (2004). Diagnostic Guide for Fetal Alcohol Spectrum Disorders: The 4-Digit Diagnostic Code. Seattle: University of Washington

Bajwa M, (2010, unpublished VCU Thesis). Dendritic Spine Density Varies Between Unisensory and Multisensory Cortical Regions

Berman RF, and Hannigan JH (2000). Effects of Prenatal Alcohol Exposure on the Hippocampus: Spatial Behavior, Electrophysiology, and Neuroanatomy. Hippocampus 2000;10:94–110. Wiley-Liss, Inc.

Center for Disease Control (2005). Annual Report. www.cdc.gov/ncbddd/fas

Chudley A, Conry J, Cook J, et al. (2005). Fetal alcohol spectrum disorder: Canadian guidelines for diagnosis. CMAJ 172 (5 Suppl): S1–S21.

El-Husseini AE, Schnell E, Chetkovich DM, Nicoll RA, Bredt DS (2000). PSD-95 Involvement in Maturation of Excitatory Synapses. Science 290:1364–68

J.C. Fiala, K.M. Harris, Dendrite structure, in: G. Stuart, Spruston, M. Ha¨usser (Eds.), Dendrites, Oxford University Press, pp. 1–34.

Fiala JC, Spacek J, Harris KM (2002). Dendritic Spine Pathology: Cause or Consequence of Neurological Disorders? Brain Research Reviews 39 (2002) 29–54

García-López P, García-Marín V, Freire M (2006). Three-dimensional reconstruction and quantitative study of a pyramidal cell of a Cajal histological preparation. J. Neurosci. 26 (44): 11249–52.

Glantz LA, Lewis DA. (2000). Decreased dendritic spine density on prefrontal cortical pyramidal neurons in schizophrenia. Arch. Gen. Psychiatry 57:65–73

Hamilton GF, Whitcher LT, and Klintsova AY (2010). Postnatal Binge-Like Alcohol Exposure Decreases Dendritic Complexity While Increasing the Density of Mature Spines in mPFC Layer II/III Pyramidal Neurons. SYNAPSE 64:127–135. VVC 2010 Wiley-Liss, Inc.

Harris K M. (1999) Structure, development, and plasticity of dendritic spines. Current opinion in neurobiology 1999;9(3):343-8.

Harris KM, Stevens JK. 1989. Dendritic spines of CA1 pyramidal cells in the rat hippocampus: serial electron microscopy with reference to their biophysical characteristics. J. Neurosci. 9:2982–97

Huttenlocher, P.R. (1990) Morphometric study of human cerebral cortex development. Neuropsychologia 28, 517–527.

Jiang M, Lee CL, Smith KL, and Swann JW (1998). Spine Loss and Other Persistent Alterations of Hippocampal Pyramidal Cell Dendrites in a Model of Early-Onset Epilepsy. J. Neurosci. 18(20):8356–8368

Jones EG and Powell TPS (1969). Morphological Variation in the Dendritic Spines of the Neocortex. J. Cell Sci. s, 509-529 (1969) 509

Kandel E.R., Schwartz J.H., Jessell T.M. (2000). Principles of Neural Science, 4th ed. P 26-31, McGraw-Hill, New York.

Kaufmann, W. & Moser, H.W. (2000). Dendritic anomalies in disorders associated with mental retardation. Cerebral Cortex 10, 981–991.

Nimchinsky EA, Sabatini BL, Svoboda K. (2002) Structure and function of dendritic spines. Annual review of physiology. 2002;64:313-53.

Nusser Z, Lujan R, Laube G, Roberts JD, Molnar E, Somogyi P. (1998). Cell type and pathway dependence of synaptic AMPA receptor number and variability in the hippocampus. Neuron 21:545–59

Parnavelas JG, Lynch G, Brecha N, Cotman CW (1974). Spine loss and regrowth in hippocampus following deafferentiation. Nature 248:71–73

Penzes P, Cahill ME, Jones KA, VanLeeuwen J, and Woolfrey KM (March, 2011). Dendritic spine pathology in neuropsychiatric disorders. Nature neuroscience. 14: 3 285-293

Purpura D., Dendritic Spine "Dysgenesis" and Mental Retardation (20 December 1974) Science 186 (4169), 1126.

Racca C, Stephenson FA, Streit P, Roberts JD, Somogyi P. (2000). NMDA receptor content of synapses in stratum radiatum of the hippocampal CA1 area. J. Neurosci. 20:25 12–22

Stuart G., Spruston N., Hausser M. (2008). Dendrites. Oxford University Press, pp. 43–54.

Valverde, F. (1967). Apical dendritic spines of the visual cortex and light deprivation in the mouse, Experimental Brain Research., 3: 337-352.

Zhan-Jun Cui, Kai-Bing Zhao, Hui-Jie Zhao, Dong-Ming Yu, Yan-Li Niu, Jun-Shi Zhang and Jin-Bo Deng (2010). Prenatal Alcohol Exposure Induces Long-Term Changes in Dendritic Spines and Synapses in the Mouse Visual Cortex. Alcohol & Alcoholism Vol. 45, No. 4, pp. 312–319.