MORPHOLOGICAL CHANGES IN SCHIZOPHRENIA: FACT OR NEUROLEPTIC EXPOSURE

by

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Abstract

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ABSTRACT

There are converging lines of evidence suggesting significant morphological changes in the prefrontal cortex in schizophrenia. There are consistent findings of increased neuronal density without a concomitant change in the total number of neurons, suggesting possible changes in the cortical neuropil. A contentious issue surrounding these findings in the prefrontal cortex is the confounding effects of neuroleptic exposure. Neuroleptics are known to cause changes in the ultrastructure and synaptic elements in the cortical neuropil and as such might confound the reported alterations in schizophrenia. This study employed MAP2 and Neurogranin Immunohistochemistry, Nissl staining and Golgi impregnation to analyze the pyramidal cells and their structures in layer III and V in areas 9 and 17 in a cohort of Huntington's brains and compared changes to that observed in subjects with schizophrenia and in controls. Both MAP2 and Neurogranin area fraction analysis in both layer III and V in area 9 showed a significant decrease in Schizophrenic brains compared to controls and Huntington brains. In the Huntington's brains, MAP2 area fraction was lower than the control brains but not different in layer III; similarly, the Neurogranin area fraction was not different from controls in both layers. The cell density measurements for both pyramidal cells and total neuronal cells showed no significant differences comparing Huntington, schizophrenia and controls in both layers. There was a schizophrenia-associated decrease in basal dendrites and spines in both layers III and V

in area 9 and no significant difference in Huntington's brains compared to controls. There were no significant changes seen in Area 17 for all of the parameters measured in the three groups. The data support the hypothesis suggesting that antipsychotic medication might not be responsible for the neuroanatomical changes observed in the neuropil of the prefrontal cortex in schizophrenia. Furthermore, these observations provide additional insightful information for both schizophrenia and Huntington Chorea.

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Ya Devi Stuyate Nityam Vibhuhairvedaparagaih SaMe Vasatu Jihvagre Brahmarupa Saraswati.

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CHAPTER 1

Introduction

The term schizophrenia, literally means a fragmenting of the mind, was coined by the Swiss psychiatrist Bleuler in 1911. Previous to Bleuler's nomenclature, Kraeplin in 1898 named the illness "dementia praecox" where he emphasized the fragmentation of the mind as the fundamental abnormality of the disease. Thus, both Kraeplin and Bleuler were credited as 'founding fathers' of the disease. At the clinical level, schizophrenia is characterized by an array of psychological dysfunction with a multiplicity of symptoms that reflect several mental processes, e.g. language, emotion, reasoning, motor activity and perception (For review see Andreasen 2000). There are three classes of symptoms in schizophrenia: positive, negative, and cognitive symptoms. The positive symptoms are characterized by hallucinations, or abnormalities in perception; delusions, having false beliefs of control or danger; disorganized speech, or abnormalities in language and disorganized behavior, or abnormalities in behavioral monitoring and control. The negative symptoms are characterized by disturbances of mental functions such as: alogia, a decrease in the fluency of thought and speech; affect blunting, a decrease in the ability to express emotions; avolition, a decrease in the ability to initiate goal-directed behavior; and anhedonia, a decrease in the ability to seek out and experience pleasurable activities. Positive symptoms represent an excess or distortion of normal function; whereas, negative symptoms represent a loss of normal function. The cognitive symptoms are characterized as disturbances in basic cognitive functions, such as attention, executive functions and specific forms of memory, particularly working memory (For review see Andreasen 2000). These various symptoms are expressed in patients with schizophrenia in patterns where there may be no overlap.

Several neuroimaging studies attempted to show a relationship between neuroanatomy and neurofunctioning in schizophrenia. Liddle et al., (1992) using positron emission tomography (PET) showed increased cerebral blood flow in the left mesiotemporal structures in patients with hallucinations and delusions as well as increased flow in the right anterior cingulate cortex, left superior temporal gyrus and dorsomedial thalamus. On the other hand, a decreased blood flow was seen in the left prefrontal and parietal cortex in patients exhibiting negative symptoms of psychomotor poverty. Additionally, several PET studies have linked hypofrontality of the frontal lobe with increased negative symptoms in schizophrenics (Volkow et al., 1987; Andreasen et al., 1992, 1994; Wolkin et al., 1992; Schroder et al., 1995). Similarly, Taminga et al., (1992) showed decreased cerebral metabolism in frontal and parietal cortex as well as thalamic areas associated with the severity of negative symptoms. It was suggested that negative and cognitive symptoms like volition and planning are associated with prefrontal lobe dysfunction and may be related to increased neuronal density (Selemon et al., 1998). However, relating the symptoms of schizophrenia to anatomical areas of the brain is a very difficult and complex task. There is some consensus among researchers that the dysfunctions are in brain circuitry (Andreasen et al., 1997) rather than in a few localized brain areas (See Figure 1.1). There are several consistent reports relating to the epidemiology of schizophrenia (Black el al., 1999). These include the following: a 1% lifetime prevalence of the disorder throughout the world; the onset of the disease is in young adult life; males are more frequently and severely affected; the illness tends to run in families; and despite the fact that most schizophrenics do not marry or have children, the disease persists in the human population. An array of etiological factors with both genetic and non-genetic influences has been implicated in the illness (Andreasen, 2000). They range from genes coded within DNA to exposure to toxins or drugs, viruses and

other pathogens, injuries to the brain during or after delivery, nutrition, and psychological experiences from exposure to stress.

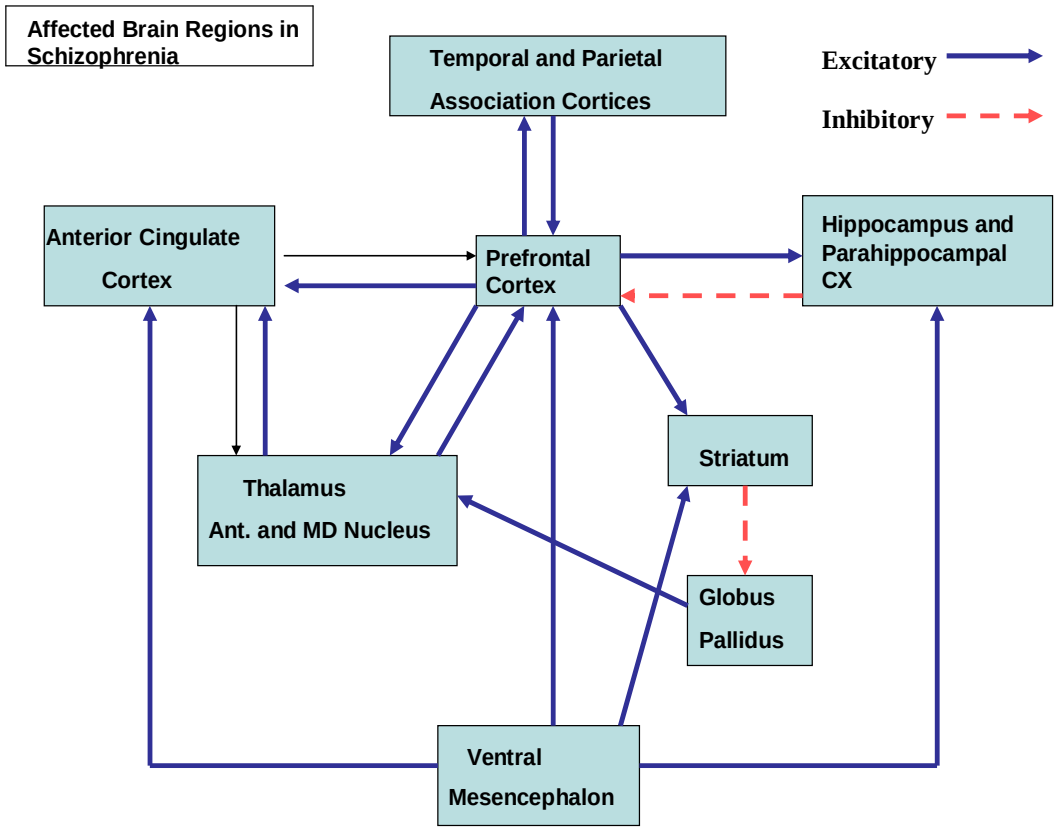

Figure 1.1: Diagram showing the interconnections between the prefrontal cortex and other affected areas in schizophrenia.

Neuropathological research in schizophrenia has progressed significantly within the last three decades; however, the central etiological mechanisms of this debilitating disease remain a mystery. The first significant report was by Johnstone et al., (1976); using computed tomographic (CT) scans which reported dilation of the lateral ventricles in a small group of chronic schizophrenic patients. Following this study, numerous more

sophisticated neuroimaging studies using techniques such as magnetic resonance imaging (MRI) and positron emission tomography (PET) scans, consistently showed ventricular enlargement, sulcal widening and cortical atrophy in schizophrenia (Reveley et al., 1982; Andreasen et al., 1988, 1990, 1994; Lawrie and Abukmeil 1998; Van Horn and McManus 1992). Lateral ventricle studies showed a 20-75% increase in the ventricular to brain ratio (Daniel et al., 1991; Van Horn and McManus 1992) and a median 40% increase in volume using MRI (Lawrie and Abukmeil, 1998). Additionally, some of these volumetric studies also showed an 8% decrease in the overall temporal lobe and 4-12 % decrease in volume of medial temporal structures, such as the hippocampus, parahippocampus and amygdala (Lawrie and Abukmeil 1998). Of significant importance are imaging studies of monozygotic twins discordant for schizophrenia. In all pairs studied the affected twin had the larger ventricles (Reveley et al., 1982; Suddath et al., 1990) and smaller cortical and hippocampal size (Noga et al., 1996). These studies were supported by data from family studies, which showed that the affected relatives had larger ventricles and smaller brain volume (Honer et al., 1994; Sharma et al., 1998; Silverman et al., 1998).

The frontal lobe, an area that has been shown to be involved in schizophrenia given the symptomatic and functional evidence implicating this area, showed an overall median volume reduction of about 5% (For a review see Harrison and Roberts, 2000). Buchanan et al., (1998), in an effort to identify reductions in specific subregions of the frontal lobe, found a 13% decrease in the inferior prefrontal grey matter compared with an average 5% decrease in other frontal regions. MRI studies of subcortical structures showed small decreases in the thalamic volume of schizophrenics (Andreasen et al., 1994; Buchsbaum et al., 1996; Byne et al., 2001, 2002; Jones 1997; Popken et al., 2000; Young et al., 2000; Brickman et al., 2004). Basal ganglia imaging studies of the caudate, putamen and globus pallidus showed no clear picture regarding size differences in

schizophrenia. However, there seems to be some correlation with volume increases and neuroleptic drugs exposure (For a review see Harrison and Roberts 2000).

Structural imaging findings and macroscopic changes in the brain provided the impetus for more stereomorphometric and immunocytochemical investigations of the cytoarchitecture of cortical and subcortical structures of post-mortem brains. These types of studies can probe the microscopic and molecular features of the areas implicated by the neuroimaging studies. One particular area of major interest has been the prefrontal cortex.

Prefrontal Cortex

The prefrontal cortex (PFC) is located in the frontal lobe (See Figure 1.2). It is rostral to premotor and primary motor areas. The PFC is the prominent cortical projection of the medial dorsal (MD) nucleus of the thalamus (Uylings et al., 1990). It also receives reciprocal connections from areas of the diencephalon, mesencephalon and limbic system as well as cortical afferents of visual, auditory and somatic origin. It is one of several association areas in the brain and is concerned with cognitive behavior and motor planning.

The prefrontal cortex can be divided into several subregions; however, there are two main regions: the prefrontal association cortex proper, located on the dorsolateral surface of the frontal lobes, and the orbitofrontal cortex, located on the medial and ventral portions of the frontal lobe (Leonard 1972). In primates, the mid-dorsolateral PFC is targeted as a locus for working memory processes, and it encompasses the region within and above the principal sulcus (Broadmann's areas 46 and 9).

In recent years, many studies have focused on the prefrontal cortex as a site of perturbation in schizophrenia (Benes et al., 1991; Shapiro, 1993; Davis and Lewis, 1995; Perone-Bizzozero et al, 1996; Beasley et al., 1997; Glantz and Lewis, 1997, Honer et al,

1997; Garey et al, 1998; Thompson et al, 1998; for review, see Harrison, 1999; Selemon and Goldman-Rakic, 1999; Kalus et al, 2000; Lewis et al, 2001; Pierri et al, 2001; Broadbelt et al, 2002; Buxhoeveden et al, Jones et al, 2002; Kindermann et al., 2004). Functionally, the prefrontal cortex is involved with attention, memory, orderly thinking and planning (Goldberg 1995), cognitive functions which have been shown to be impaired in schizophrenia and patients with damage to the prefrontal cortex (Weinberger et al., 1988). Studies of identical twins discordant for schizophrenia showed decreased blood flow in the prefrontal cortex of the affected twin while they were asked to perform the Wisconsin Card Sort Test, a measurement of set-switching ability (Weinberger et al., 1992). Additionally, patients with frontal lobe lesions show cognitive abnormalities similar to those seen in schizophrenia (Levin 1984; Weinberger et al., 1994). Both groups are similarly impaired on the Continuous Performance Task (Buchsbaum et al., 1990); on tests of categorization and flexibility, such as the Wisconsin Card Sort Test (Weinberger et al., 1986), the Stroop Test, a color naming task as a paradigmatic measure of selective/controlled attention (Schooler et al., 1997), and the Tower of London task, which measure planning ability (Shallice 1982).

Much evidence points specifically to the dorsolateral prefrontal cortex (DLPFC) as a site for dysfunction in schizophrenia (Weinberger et al., 1986; Buchsbaum et al., 1990; Benes 1991; Lewis 1993; Pakkenberg 1993; Goldman-Rakic and Selemon 1995, 1997; Harrison 1999; Andreasen 2000; Thune et al., 2001; Jones et al., 2002; Broadbelt et al., 2002; Eastwood and Harrison 2005). Schizophrenics perform poorly on tasks that require the use of working memory (Baddeley 1986). The intricate nature of working memory was first identified in studies of human cognition (e.g., Norman 1970, Baddeley 1986). Working memory is active and relevant for a few short seconds. For example a newly read phone number is stored until it is dialed and after it is immediately forgotten.

Much evidence has demonstrated that the prefrontal cortex has a preeminent role in working memory (For a review see Goldman-Rakic 1995). Morphological post-mortem studies in the DLPFC in schizophrenia showed an increase in neuronal density (Benes et al., 1991; Selemon et al., 1995) without a change in the number of neurons (Pakkenberg 1993; Thune et al., 2001). Both Benes et al., (1991) and Selemon et al., (1995) hypothesized that increases in neuronal density without a change in the number of neurons would imply a change in the DLPFC neuropil, which includes the axon terminals, dendrites and dendritic spines that are the site for most cortical synapses. This was corroborated by several studies that showed a decrease in the synapse-associated protein synaptophysin (Karson et al., 1996; Perrone-Bizzozero et al. 1996; Glantz and Lewis 1997). A study by Buxhoeveden et al., (2000) reported reduced neuropil space in area 9 of schizophrenics. There are consistent findings of reduced spine density in layer III pyramidal neurons of the temporal cortex, BA 22 and 38, and frontal cortex, BA 10 and 46 (Garey et al., 1993, 1998; Glantz and Lewis 2000) in schizophrenia. Spine density is a marker of the number of excitatory inputs to pyramidal neurons (Mates and Lund 1983). Glutamate and dopamine afferents terminate on dendritic spines whereas, inhibitory GABA terminals are often found on dendritic shafts and cell bodies (Levitt et al., 1993). Moreover, several hypotheses implicate one or more of these neurotransmitter systems in the pathophysiology of schizophrenia (Weickert et al., 1998; Haroutunian et al., 2003; Bergson et al., 2003). Layer III pyramidal neurons are the corticocortical projections (Lund et al., 1975); they play a critical role in information processing such as working memory. Layer V pyramidal neurons are the main projection cells from the cortex to other subcortical and cortical areas (Lewis 1997); therefore, changes in information in one cortical area could affect many brain regions. Understanding the significance of these alterations requires an understanding of which elements of the DLPFC circuitry are disturbed.

Functional maturation of the DLPFC circuitry in monkeys and humans seems to be uniquely protracted. It does not become functionally mature until after puberty (for a review see Lewis 1997). Human PET studies by Chugani et al., (1987) showed cerebral blood flow in the frontal cortex does not reach adult levels until 15 to 19 years of age. This seems to correspond with the appearance of clinical symptoms during late adolescence in schizophrenia. Additionally, adult levels of performance on some cognitive tasks, like delayed-response tasks, subserved by the DLPFC are not achieved until after puberty in both monkeys and humans (Fuster 1989). The pyramidal neurons, are the primary cortical projection neurons, are of major interest in this regard. Their axon collaterals extend for considerable distances horizontally through the gray matter and give rise to clusters of axon terminals in the superficial layers, which are organized as a series of stripes 2 µm wide and 1.8 mm long (Levitt et al 1993). There are reciprocal connections among these stripes and over 90% of the synapses furnished by these collaterals target the dendritic spines of other pyramidal cells (Metchitzky et al., 1995). It was suggested that these connections could provide the substrate for a reverberating cortical circuit that coordinates and maintains the activity of spatially segregated, but functionally-related populations of DLPFC pyramidal neurons during the delay phase of the delayed-response task (Lewis and Anderson 1995)

Very few studies have examined the morphology of pyramidal cells in the prefrontal cortex. As mentioned above, two studies showed a decrease in soma size and others decreased spine density in schizophrenia (Garey et al., 1993, 1998; Glantz and Lewis 2000). Soma size is directly proportional to dendritic and axonal arborization (van Ooyan et al., 1995; van Pelt et al., 1996); therefore, a decrease in soma size, as seen in schizophrenics, might lead to decreases in dendritic arborization. The studies on spine density, Garey et al., (1998), examined the prefrontal cortex in general and not specific brain areas whereas, Glantz and Lewis (2000) examined areas 46 and 17. Recently,

prefrontal cortical studies of pyramidal cells by Jones et al., (2002) showed a decrease in microtubule associated protein 2 (MAP2), a protein found in dendrites and cell bodies, in layers III and V of areas 9 and 32 of the prefrontal cortex in schizophrenia. In a second study they reported decreases in the primary and secondary basilar dendrites in area 32 of the prefrontal cortex (Broadbelt et al., 2002). More recently, they reported decreases in neurogranin, a protein found in dendrites and spines (paper submitted).

A major issue surrounding these morphological deficits in the prefrontal cortex is whether they are disease specific or are effects of chronic neuroleptic drug exposure. Neuroleptic drugs are known to cause changes in volume and the synaptic ultrastructure in the brain (Benes et al., 1985, Klinzova et al., 1989, Meshul et al., 1992, Dorph-Petersen et al., 2005). They have the ability to bind to neurotransmitter receptors, such as dopamine (D_1-D_5) serotonin (Harrison 1999), which are coupled to second messengers that regulate the activity of kinases and phosphatases. These enzymes have the ability to phosphorylate protein such as MAP2; phosphorylation of MAP2 affects microtubule polymerization and as such cytoskeletal stability (Lidow et al., 2001). It is possible this could have major effects on processes of the cell such as dendrites, spines and synapses (Lidow et al., 2001). All of the studies mentioned above, that report deficits in the DLPFC, were performed on tissue from schizophrenics treated with neuroleptics. Controlling for neuroleptic exposure has been a frustrating issue for researchers since all schizophrenics take neuroleptics.

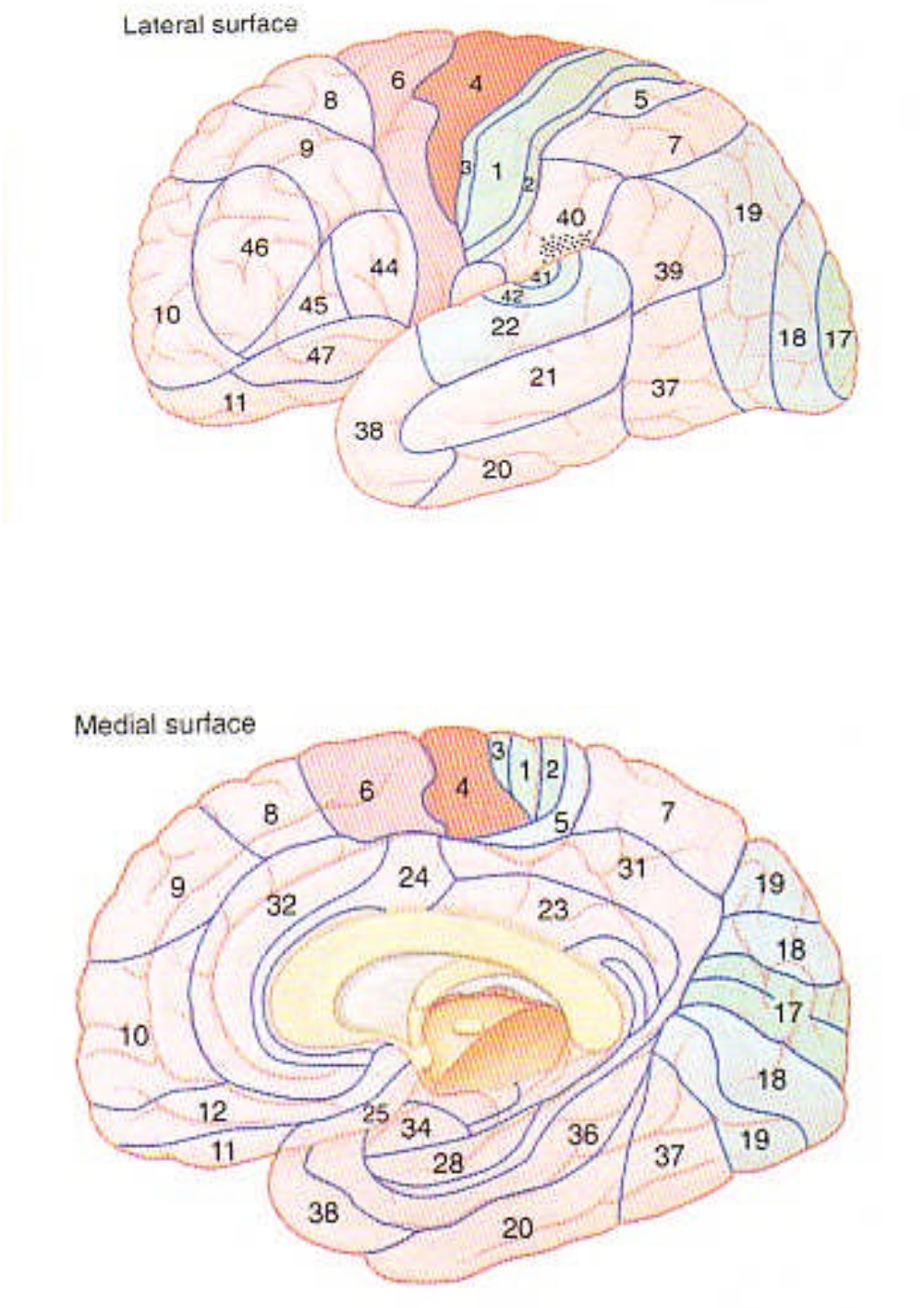

Figure 1.2: Broadmann's map of the brain showing areas 9 and 17 both from the lateral and medial surfaces of the brain (Fitzgerald MJT, 2002. Clinical Neuroanatomy. p244)

Neuropathological effects of antipsychotic drugs

Since their introduction in 1959, neuroleptic drugs have been used extensively in the treatment of schizophrenia and other neuropsychiatric diseases, such as bipolar disease, depression and schizoaffective disease (Harrison et al., 2000). Almost all of the patients in recent neuropathological studies in schizophrenia have received neuroleptic medication. In recent years a battery of treatments have become available that treat the symptoms of schizophrenia and attempt to improve the quality of life of patients. The conventional or older typical antipsychotic medication (phentothiazines, butyrophenones, and thioxanthenes) e.g., chlorpromazine, haloperidol, fluphenazine and molindone, are used to reduce the positive symptoms of schizophrenia and have a strong affinity for dopamine and serotonin receptors (Hirsch and Weinberger 2003). The recently developed medications e.g., clozapine, risperidone, olanzapine, quetiapine and sertindole, are more effective against the negative symptoms of schizophrenia (Hirsch and Weinberger 2003). The newer medications, often called atypical because they have a different mechanism of action than the older medications, show fewer side effects, and are effective against treatment-resistant patients. The therapeutic effects of the major neuroleptics be it typical or atypical, are based on their ability to bind neurotransmitter receptors (Harrison 1999a).

There are five classes of dopamine receptors D_1-D_5; all are seven transmembrane domain G protein-coupled receptors linked to adenyl cyclase (Harrison 1999a). The serotonin 5-HT receptors are divided in seven branches 5-HT_{1-7}. The 5-HT_3 is an ion channel and all the others are coupled to G proteins linked to adenyl cyclase or the phosphor-inositol system. The 5-HT_{2a} is of particular relevance in schizophrenia because of it affinity for atypical neuroleptics (Harrison 1999a). D_2 blockade has been central to the antipsychotic activity of typical neuroleptics. The atypical neuroleptics such as clozapine bind to D_1, D_3, D_4 and D_5 as well as 5-HT_{2a} and all of the noradrenergic receptors; however,

D_4 shows the strongest affinity (Harrison 1999a). Recently, the D_4 and 5-HT_{2a} receptors are of particular importance in schizophrenia due to their binding mechanisms with atypical neuroleptics. The exact mechanisms of how these interactions operate are still under investigation.

Although they are treasured for their therapeutic significance, neuroleptics have been shown to produce structural brain changes in areas such as the striatum, where they have been shown to cause increases in the number of symmetric and axodendritic synapses relative to asymmetric and axospinous synapses (Benes et al., 1985, Klinzova et al., 1989, Meshul et al., 1992). This suggests that antipsychotic favors inhibitory synapses, since asymmetric and axospinous synapses are mostly glutamatergic and as such excitatory (Benes et al., 1985). Some reports suggest a correlation of antipsychotic dosage and increased brain atrophy (Madsen et al., 1998) and decreased thalamic volume (Gur et al., 1998). These macroscopic studies suggest that neuroleptic exposure is a potential confounding variable. Several studies have suggested that long-term treatment with antipsychotics might cause the morphological changes observed in schizophrenia (Benes et al., 1985; Klinzova et al., 1989; Meshul et al., 1992). Although these studies were done in rodents with normal brains, together the data provide good evidence that chronic antipsychotic treatment induces synaptic plasticity and alters the synaptic ultrastructure.

Recently, a study on rhesus monkeys demonstrated long-term haloperidol exposure can increase phosphorylation of MAP2 and downregulate spinophilin, a dendritic spine associated protein (Lidow et al., 2001). This is different from what is shown for MAP2 in schizophrenia; therefore, animal studies may not be the best indicator of neuroleptic effect. Additionally, animal studies are based on normal neural networks so the neuroleptic drugs may not have the same effect on altered neural networks.

Because antipsychotic drugs can affect many neurotransmitter systems (Harrison et al., 2000), they have the ability to regulate the activity of kinases and phosphatases via second messengers (Lidow et al., 2001). These enzymes regulate phosphorylation states of many proteins, one such is MAP2 (Diaz-Nido et al., 1990). MAP2 is found in dendrites and cell bodies and is an important protein involved in the formation and stabilization of microtubules (Matus 1988). Phosphorylation of MAP2 can destabilize dendritic microtubules because it is a sensitive cross-linker and adjustable spacer in the polymerization of tubulin in microtubules, and as such the cytoskeletal processes of the cell (Boyne et al., 1995).

In order to correctly interpret the morphological data it is important to know what alterations are due to neuroleptics and which are not. A comparison of drug-naïve and treated subjects in contemporary postmortem studies is not feasible since nearly all patients with schizophrenia use neuroleptics. The use of animals to study the effects of antipsychotics has some attraction; however, there are problems when extrapolating results between species. First, the cerebral cortex in animals and humans vary in terms of size and the distribution of neurotransmitter receptors. For example, rodent's cerebrum is a thousand times smaller than humans (Harrison et al., 2000). Secondly, there are marked differences in organization of the PFC. For example, in primate PFC there is a distinct layer IV which is absent in rat (Harrison et al., 2000). Lastly, rodents metabolize antipsychotics differently than humans, and might respond neuropathologically in different ways; moreover, animal brains are normal (Harrison et al., 2000). Thus, researchers have devised other means to control for neuroleptic exposure. The use of a non-schizophrenic group treated with antipsychotics, such as bipolar disease, schizoaffective and depression often produced mixed results (Harrison et al., 2000). This may be attributed to the fact that a significant number of patients who were first diagnosed as schizoaffective or for

depression are later diagnosed with schizophrenia, which suggest that they share intrinsic pathological features with schizophrenia (Harrison et al., 2000). Therefore, it is foreseeable why when used as controls for neuroleptic exposure the results are overlapping.

This study used a cohort of Huntington Chorea brains to determine if neuroleptic drugs can cause morphological changes in the brain and confound the results reported in schizophrenia. Although there are reports of cortical changes in Huntington Chorea (Harrison et al., 2000), the main deficit is a loss of neurons in the striatum (Harrison et al., 2000). Huntington's patients experience some psychiatric symptoms, like hallucinations and delusions, similarly to schizophrenics; therefore, many are given neuroleptic drugs to control those symptoms. A comparison of schizophrenic and Huntington Chorea will be a more meaningful assessment in determining if neuroleptic drugs could be responsible for some of the morphological changes reported in schizophrenia.

Huntington Chorea

Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder with midlife onset characterized by motor, cognitive and psychiatric symptoms (For review see Petersen et al., 1999). The first definitive description of the disease was given by George Huntington in 1872 which has subsequently borne his name. The symptoms of the disease can be related to its neuropathology, which is characterized by a loss of neuronal populations in many parts of the brain (For review see Petersen et al., 1999). The most striking feature is the loss of γ-aminobutyric acid (GABA)ergic medium spiny neurons expressing D1- and D2-receptors in the neostriatum (Vonsattel et al., 1985). The cause of loss of this specific population of neurons is still under investigation. Recent evidence points to a defect in energy metabolism which may lead or contribute to free radical

formation, excitotoxicity, mitochondrial dysfunction, decreased mitochondrial membrane potential, a lower threshold for apoptosis, and alterations in the haemodynamic responsivity of the cerebral vessels (Schapira 1997). This loss of neurons in the striatum results in an enlargement of the lateral ventricles in Huntington's brains as well as a decrease in the cerebral hemispheres, the diencephalon, the cerebellum, the brainstem and the spinal cord (Forno and Norville 1979). The severity of HD pathology is divided into five grades (0-4) based mainly on the extent of damage in the striatum (Vonsattel et al., 1985 and Myers et al., 1988). Grade 0 cases show a strong clinical and familial history suggesting HD but no detectable histological neuropathology at autopsy. Grade 1 cases show neuropathological changes microscopically with as much as 50 per cent loss of striatal neurons but no gross atrophy. In grades 2-4 there are progressively more pronounced gross atrophy, neuronal loss and gliosis. In the most severe grade 4 cases more than 90 per cent of the striatal neurons are lost (Vonsattel et al., 1985 and Myers et al., 1988).

HD strikes all races but occurs with the highest prevalence, about 1 in 10,000 individuals, in populations of Western European ancestry (Petersen et al., 1999). The genetic basis of the disease is due to a mutation of the IT14 gene on chromosome 4 resulting in a CAG trinucleotide expansion. This trinucleotide expansion codes for an unstable glutamine expansion on the Huntingtin protein that form cytoplasmic and nuclear aggregations (Huntington's Disease Collaborative Research Group, 1993). These aggregations, which are cleaved protein fragments, result in neuronal degeneration primarily in the striatum and cortex (Huntington's Disease Collaborative Research Group, 1993). As a result, the patients show disturbances in motor functions and experience psychotic symptoms such as hallucinations and delusions (Petersen et al., 1999). Huntington's patients, because they experience psychotic symptoms, take neuroleptic

drugs similar to schizophrenics. Thus, several studies on schizophrenia have used brains from Huntington Chorea patients to control for neuroleptic exposure (Pakkenberg B. 1990, Selemon et al., 1995, Rajkowska et al., 1998). These studies were done in the thalamus and prefrontal cortex looking for changes in neuronal number, density and size. Taken together these studies showed no effect of neuroleptic exposure using the Huntington's brains as controls. Long-term exposure to neuroleptics is defined as two years of continuous use with drug dosages in chlorpromazine equivalents (Gur et al., 1998). Both schizophrenic and Huntington Chorea patients receive comparable dosages, about 407 chlorpromazine equivalents/day (Gur et al., 1998).

MAP2

MAPs are proteins that promote tubulin capacity to self-associate into microtubule polymers (Herzog and Weber 1978). Microtubule-associated proteins can also interact with actin filaments and with components of the intermediate filament proteins thus, pointing to their functional role in the regulation of the functional organization of the cytoskeletal network of neurons (For review see Maccioni and Cambiazo 1995). The family of neuronal MAPs includes high-molecular-mass components, namely MAP-1A, MAP-1B, MAP-1C, MAP-2A, and MAP-2B; the neuronal MAP-3; MAP-4 is found in both neuronal and nonneuronal cells; and intermediate-size polypeptides such as tau; and the small 70-kDa MAP-2C. MAPs have been found to be compartmentalized in neurons, with MAP1 being widely distributed, while MAP2 is essentially a dendritic protein and tau an axonal component. The majority of MAPs have a rather widespread distribution among different cell types and even tissues, but certain MAPs have been found localized in specific cells and not in others (For review see Maccioni and Cambiazo 1995).

MAP2 was first described by Murphy and Boresy (1975). It is an abundant protein in brain tissues which copolymerizes with brain microtubules in vitro and promotes the polymerization of tubulin. High levels of MAP2 can be found in the somatic and dendritic compartments, but not axons (Matus and Bernhardt 1986). In dendrites MAP2 is associated with microtubules and has an active role in the development and maintenance of dendritic processes by promoting polymerization of tubulin to form microtubules (Hirokawa et al., 1988). Because of its role in microtubule assembly and its selective association with dendrites, MAP2 has been implicated as playing a major role in the molecular mechanisms regulating dendritic growth and stabilization (Matus and Bernhardt 1986). Therefore, assembly and stability of microtubules are regulated by MAP2. MAP2 is a sensitive cross-linker and adjustable spacer in dendritic architecture. The phosphorylation state of MAP2 modulates its interaction with microtubules. In low-phosphorylation states MAP2 binds to microtubules and increases microtubule assembly and/or stability. Increased phosphorylation decreases these effects (Audesirk et al., 1997). Hely et al., (2001) proposed a model which suggests that dephosphorylated MAP2 favors elongation by promoting microtubule polymerization and bundling; whereas, MAP2 phosphorylation which increases microtubule spacing could cause dendritic branching. This is through the action of CAMKII being activated by elevated calcium concentrations, which is regulated upstream by calmodulin and neurogranin. Dendritic branching is due to changes in the cytoskeleton through the interaction of microtubules and actin filaments. Any factor that can alter microtubule dynamics will affect the dendritic archicecture. The MAP family of proteins is known to regulate many factors of microtubule dynamics such as, depolymerization, bundling, spacing, and interaction with actin filaments (for review see Maccioni and Cambiazo 1995)

Neurogranin

Neurogranin (RC3), a postsynaptic calpacitin, was first identified in a hybridization study designed to isolate mRNAs enriched in the rat forebrain but absent in the cerebellum. As the name indicated, it was rat cortex-enriched cDNA clone number 3 (Watson et al., 1990). Neurogranin was independently purified by Baudier et al., (1991) from brain based on its affinity for calmodulin (CaM) and as a substrate for protein kinase C (PKC). Neurogranin is only 78 amino acids long and has sequence similarity to neuromodulin, a protein associated with axonal growth cone development and maturation (Baudier et al., 1991). Interestingly, both neurogranin and neuromodulin share a 20 amino acid sequence, AAAAKIQASFRGHMARKKIK, designated as the IQ motif (Apel and Storm 1992). This sequence contains a binding domain for CaM and a PKC phosphorylation site (Baudier et al., 1991). Neurogranin however, is found abundantly in neuronal cell bodies, dendrites and dendritic spines. In areas such as the frontal parietal cortex, granular cells of the dentate gyrus, apical dendrites of pyramidal cells of the CA1 and CA3 regions of the hippocampus, and the striatal cortex (Chicurel et al., 1993, Neuner-Jehle et al., 1996). Immunoelectron microscopic studies in the cerebral cortex, hippocampus and neostriatum in rats showed that neurogranin exists in the perinuclear and dendritic cytosol. It concentrates in dendritic spines in close proximity with postsynaptic densities and subsynaptic membranes (Watson et al 1992, Neuner-Jehle et al., 1996). Neurogranin is a PKC substrate that interacts with CaM and both PKC and CaM are required for the induction of long term potentiation (Gerendasy and Sutcliffe 1997). Much research suggest that neurogranin might be involved in Ca^{2+}/CaM and PKC-dependent cascades that guide dendritic spine development and remodeling, as well as long-term potentiation (LTP) and long-term depression (LTD). Gerendasy and Sutcliffe (1997) postulated that neurogranin regulates Ca^{2+} fluxes in dendritic spines by releasing CaM to bind Ca^{2+}. The size and duration of Ca^{2+} fluxes determine which Ca^{2+}-dependent enzymes are stimulated and

ultimately, which second messenger cascades are activated for LTP or LTD. Enzymes such as CaM kinase II and adenylate clyclase favours LTP; whereas, calcineurin and cyclic nucleotide phosphodiesterase, favor LTD (For review see Gerendasy and Sutcliffe 1997). The binding of calmodulin by neurogranin is abrogated by phosphorylation by PKC, oxidation by nitric oxide or large concentration of Ca^{2+} (Ho Pak et al., 2000 and Prichard et al., 1999).

Immunohistochemical studies in rats and mice showed that peak expression of neurogranin postnatally coincides with developmental periods of rapid dendritic growth and the formation of 80% of cortical synapses (Alvarez-Bolado et al., 1996, Uylings et al., 1990). Suggesting therefore, an increase in neurogranin concentration coincides with the onset of synaptogenesis. The number and size of dendritic spines is mediated by calcium-dependent mechanisms that are initiated by glutamate receptor-mediated influx of Ca^{++} ions (Gerendasy and Sutcliffe 1997). Proteins involved in Ca^{++} signaling, such as neurogranin, therefore may play a major role in spine morphology and number and as such cell signaling.

This study will attempt to address the issue of neuroleptic exposure by examining area 9 of the DLPFC in schizophrenics, Huntington and controls. Area 9 is one area in the prefrontal cortex that has consistently showed deficits in schizophrenia and is connected to the MD nucleus of the thalamus implicated in the disease. Ideally, it would be of great value to compare medicated patients to non medicated patients with schizophrenia. However, since nearly all patients with schizophrenia take neuroleptics it makes this impossible. Therefore, we have decided to select a cohort of Huntington Chorea patients that take neuroleptics similarly to schizophrenics and compare them to cohorts of schizophrenics and controls. Although, some studies in Huntington Chorea have shown changes in parts of the cerebrum (Pakkenberg B. 1990, Selemon et al., 1995, Rajkowska et al., 1998) there are no studies to date that have shown changes in area 9 of the PFC.

Thus, an analysis can be made by comparing the changes observed in the two groups with controls. Changes seen in schizophrenia and not in Huntington and control will suggest that it is disease related and not an effect of neuroleptics. On the other hand, similar changes seen in both Huntington and schizophrenia compared to controls will suggest possible effects of neuroleptic exposure. The brains were matched for age, sex and postmortem interval. The focus of this study are the pyramidal cells in layers III and V, since they are the main cells involved in information processing. Measurements of the pyramidal cells include the following: pyramidal cell density, total neuron density, basal dendrite and spine counts, and immunocytochemical measurements for two proteins, MAP2 and neurogranin. These are proteins that contribute to dendritic structure and spine morphology that our lab have been studying and have been shown to be decreased in this part of the brain in schizophrenia. Area 17, being the control area for the study, was analyzed similarly to area 9 for all of the above mentioned parameters. The neuropathlogical findings in HD and schizophrenia continue to be unveiled and this study provides additional insightful information about the cytoarchitecture in this part of the brain in both diseases.

CHAPTER 2

A comparative study of MAP2 alterations in areas 9 and 17 in schizophrenia and Huntington Chorea and the role of neuroleptic exposure

Abstract

Increasing evidence suggests that there may be significant morphological changes in the neuropil of the dorsolateral prefrontal cortex in schizophrenia (Weinberger et al., 1986; Buchsbaum et al., 1990; Benes 1991; Lewis 1993; Pakkenberg 1993; Goldman-Rakic and Selemon 1995, 1997; Harrison 1999; Andreasen 2000; Thune et al., 2001; Jones et al., 2002; Broadbelt et al., 2002). There are consistent findings of decreases in the synapse-associated protein synaptophysin (Karson et al., 1996; Perrone-Bizzozero et al. 1996; Glantz and Lewis 1997); spine density (Garey et al., 1993, 1998; Glantz and Lewis 2000); neuropil space (Buxhoeveden et al., 2000); MAP2 (Jones et al., 2002) and dendrites (Broadbelt 2002). A controversial issue surrounding these deficits in the cortical neuropil is the confounding effects of antipsychotic (neuroleptic) medication. We employed MAP2 Immunohistochemistry and Nissl staining to analyze eight matched groups of Huntington, schizophrenia and control, in areas 9 and 17 layers III and V of the PFC. Huntington's patients take neuroleptics similar to schizophrenics; therefore, by comparing the two groups to controls we can begin to determine if neuroleptics play a role in the deficits reported in schizophrenia. Our results showed a significant decrease in MAP2 in schizophrenia compared to controls. The Huntington's brains showed a significant difference only in layer V compared to controls. There were no significant differences seen in area 17 for both schizophrenia and Huntington Chorea compared to controls. This observation of MAP2 suggests that antipsychotic medication might not be responsible for the morphological changes observed in the neuropil of the PFC in schizophrenia.

1. Introduction

Much evidence points to the prefrontal cortex (PFC) as a major site for perturbation in schizophrenia (Benes et al., 1996; Goldman-Rakic and Selemon, 1997; Harrison, 1999; Selemon et al., 1999; Broadbelt et al., 2002; Jones et al., 2002; For review, see Shapiro, 1993). Post mortem studies in specific areas of the PFC by Benes et al., (1991) and Selemon et al., (1995) showed increases in neuronal density; whereas, studies by Pakkenberg (1993) and Thune et al., (2001) reported no change in the number of neurons. Together these studies suggest that there might be changes in neuropil content as was hypothesized by both Benes et al., (1991) and Selemon et al., (1995). The neuropil includes axon terminals, dendrites and dendritic spines (Harrison and Roberts 2000). There are reports of decrease spine density in the PFC (Garey et la., 1998) and specifically in area 9 (Glantz and Lewis 2000). More recently, studies by Jones et al., (2002) showed alterations in MAP2, a marker for dendrites and dendritic development in vitro (Bernhardt and Matus, 1984; Decamille et al., 1984; Fischer et al., 1987; Crandell et al., 1989), in areas 9 and 32 of the PFC. Additionally, they also showed decreases in primary and secondary dendrites of pyramidal cells in areas 9 (personal communication) and 32 of the PFC (Broadbelt et al., 2002). A major issue surrounding these reported deficits in the PFC is the effect of neuroleptic medication. Several studies suggested that long-term treatment with antipsychotics might produce morphological changes in synaptic elements in the brain (Benes et al., 1985; Klinzova et al., 1989; Meshul et al., 1992). Imaging studies showed evidence of striatal enlargement in drug treated patients (Keshavan et al., 1994; Chakos et al., 1994, 1995; Doraiswamy 1995) and rats (Chakos et al., 1998). There are reports of progressive brain atrophy (Madsen et al., 1998) and increasing thalamic volume (Gur et al., 1998). Most recently, a study on rhesus monkeys demonstrated long-term haloperidol exposure can increase phosphorylation of MAP2 and downregulate spinophilin (Lidow et al., 2001). Antipsychotic drugs, by binding to

dopaminergic and serotonergic neurotransmitter receptors, have the ability to regulate the activity of kinases and phosphatases via second messengers (Lidow et al., 2001). These enzymes can regulate phosphorylation states of proteins like MAP2 (Diaz-Nido et al., 1990). Phosphorylation of MAP2 can destabilize dendritic microtubules and as such the cytoskeletal processes of the cell (Boyne et al., 1995). Therefore, morphological studies examining dendritic alterations in the cortex must take into consideration the confounding effects of neuroleptic exposure.

To begin to address the possibility of neuroleptic induced morphological alterations in schizophrenia, we used immunocytochemistry to examine the expression of MAP2 in a cohort of Huntington Chorea brains and compare it to that of a matched cohort of schizophrenics and controls. Many Huntington Chorea patients take similar neuroleptic drugs to schizophrenics; therefore, by comparing the two groups' one can begin to see if neuroleptics play a role in the changes observed in schizophrenia.

2. Methods

2.1. Subjects and tissue

Postmortem brain tissues from 8 subjects diagnosed with Huntington Chorea, 8 subjects diagnosed with schizophrenia and 8 controls were collected from Harvard Brain Tissue Resource Center (Table 2.1). The three groups of tissues were all matched for age, sex and postmortem interval. All tissues were collected at the time of autopsy in accordance with an approved Institutional Review Board protocol. Diagnoses confirmed to DSM-IV criteria and were established retrospectively by reviewing medical records and interviewing knowledgeable individuals. Tissue was not included in the study if there was evidence of neuropathology (e.g. Alzheimer's disease) or substance dependence as

determined by the HBTRC. The brain bank coded the tissues and the codes were maintained by one of the authors who did not conduct quantitative assessment (L.J.) until quantitative procedures had been completed.

Blocks of 1-cm thick sections were dissected in the coronal plane by a qualified neuropathologist at the HBTRC. The sections were formalin-fixed in a consistent manner for shipment. The accuracy of the dissections was confirmed by examining thionin-stained sections prepared from each block. Specimens from area 9 were taken from its dorsolateral extent, which is founded on the middle third of the superior frontal gyrus (Rajowska and Goldman-Rakic, 1995a). Compared with the medial region, the lateral region of area 9 is more differentiated. In addition to having a distinguishable layer IV, it has a Layer II with a distinct upper margin and the supragranular cell density is conspicuously higher than the infragranular cell density (Barbas and Pandya, 1989).

Inclusion criteria: Brains were not selected on a racial basis. Approximately equal numbers of male and female brains were obtained. Schizophrenics and Huntington patients had a DSM IV diagnosis and range in age from 34 to 80 years of age. Nonpsychiatric controls came from nursing homes and hospitals; have no psychiatric diagnosis established by the DEAD scale. Controls from nursing homes had resided there for at least five years prior to death.

Exclusion criteria: No brains with evidence of traumatic injury, infarction or Alzheimer's type changes were accepted into the study.

Brain #	*Distributive Diagnosis*	*Medication*	*Age*	*Sex*	*PMI*	*Storage Time*
4227	Huntington	No med. report Grade 3	58	M	18.25	18 months
5047	Schizophrenia	Clozaril(50mg) Haldol(5 mg)	63	M	20.1	11 months
4872	Control	None	58	M	20.8	12 months
5107	Huntington	Rescripine Descipramine (0.5mg)	66	M	12.4	9 months
3833	Schizophrenia	No med. report	67	M	21.3	13 months
4729	Control	None	66	M	17.8	15 months
2634	Huntington	No med. report	68	F	1.2	8 months
3742	Schizophrenia	Thorarine (30mg)	71	M	19	7 months
3875	Control	None	68	F	14	12 months
4449	Huntington	Xarax (0.25 mg) Prozali (20 mg)	67	F	16.3	18 months
3557	Schizophrenia	Oxazepur (15 mg)	66	F	16.7	13 months
3619	Control	None	74	F	19.8	11 months
4441	Huntington	Haloperidol (2 mg)	72	M	3.6	23 months
3634	Schizophrenia	Thorazine	71	M	18	15 months
3626	Control	None	65	F	16.6	15 months
3041	Huntington	No treatment	38	M	11.8	10 months
3915	Schizophrenia	Resperidol (200 mg)	34	M	17.4	10 months
3932	Control	None	38	M	21.9	10 months
3702	Huntington	No med. report Grade 4*	62	M	21.5	12 months
3546	Schizophrenia	No med. report	69	M	17.8	13 months
3625	Control	None	70	F	18.2	11 months
2926	Huntington	Chlortrimeton	53	M	18.5	10 months
3673	Schizophrenia	No med. report	43	M	20.8	10 months
3748	Control	None	44	M	23	8 months

Table 2.1: Demographic data for the matched brains used in the MAP2 and cell density projects. * All Grade 4 Huntington brains had both behavioral and movement disorders, therefore they took neuroleptics at some point in their life.

2.2. Histological procedures

Coronal serial sections were cut at 50µm on a freezing sledge sliding microtome. Every section was collected in phosphate buffered saline (PBS) to be processed for either immunocytochemistry or for histological staining. Even number sections were stored in a cryoprotectant that allows tissue storage at –70°C until they were processed for immunocytochemistry. Odd numbered serial sections were processed for thionin staining. Every tenth frozen section was then used for MAP2 immunocytochemistry. All of the materials were processed in triplets to minimize variability on a given day.

2.3. Nissl Staining

Coronal serial sections (50µm) through the cortex were cut on a freezing sledge microtome. Every section was collected in phosphate buffered saline (PBS) to be processed for either immunocytochemistry or for histological staining. Even number sections were stored in a cryoprotectant that allows tissue storage at –70°C until they were processed for immunocytochemistry. Odd numbered serial sections were processed for thionin staining. The sections for histological staining were mounted onto subbed slides and dried at room temperature. The sections were then dehydrated in graded alcohol starting with 70% ethanol to 100% ethanol. After dehydration in alcohol the sections were then defatted in xylene for 10 minutes and dehydrated in alcohol for a second time in reverse starting with 100% ethanol and finally with 70% ethanol. After dehydration the sections were placed in deionized water for one minute and then in thionin stain for 20 seconds. After staining the sections were rinsed in water and dehydrated in 95% and 100% alcohol. After dehydration the sections were then defatted for a second time in xylene. Finally, the sections on the slides were coverslipped with permount solution.

2.4. MAP immunocytochemistry

A free-floating method of immunocytochemistry was employed to maximize the impregnation and exposure to the MAP2 antibody (Figure 2.6 & 2.7). MAP2 is a cytoskeletal protein specific for dendrites and cell bodies. An antibody from Chemicon specific for the MAP2a and MAP2b, which correspond to the two high molecular weight forms found in the adult brain was used. The appropriate tissue sections were removed from the cryoprotectant, washed in PBS and then pretreated with 3% H_2O_2 for ten minutes to remove any endogenous peroxides. The sections were washed in PBS and incubated in 4% instant milk in PBS (BLOTTO) with 0.2% Tritonx-100 for one hour and then incubated for 48 hours at room temperature in the primary antibody at 1:300 dilution. All incubations were performed on an orbital shaker. On the third day the sections were washed in PBS three times for 10 minutes. The sections were then incubated in a secondary antibody for 30 minutes. The primary antibody was made in mouse; therefore, a biotinylated anti-mouse secondary at 1:100 dilution (Vector Laboratories) was used. The avidin-biotin complex was then used according to Vectastain's protocol. This procedure is followed by a standard DAB (diaminobenzidine) reaction to view the staining. The sections were mounted on to glass slides and after drying they were dehydrated in graded alcohol starting at 70%, defatted in xylene and coverslipped with permount solution.

2.5. Quantification of pyramidal cell density

Pyramidal cells were counted in three evenly spaced Nissl sections with a random start. Contours were traced for each layer, and a counting grid was superimposed on the contour. Every third intersection with a random start was marked for counting so that 30% of the intersections were marked. A counting box of 200/200/27 µm with a volume of 0.00000108 cm^3 (a buffer zone of 5 µm on either surface was employed so as not to

include the cut surfaces) was placed inside the marked intersections. Counting was done using a 20X Plano Apo objective, which give a final screen magnification of 1000. Pyramidal cells were distinguished based upon their morphology. They had to have a distinguishable apical surface oriented toward the pial surface in order to be counted. Every pyramidal cell inside the box with a visible nucleolus that did not touch the exclusion line was counted. Pyramidal cell density was determined by dividing the average number of cells per box by the volume of the counting box.

2.6. Quantification of total neuronal cell density

All neurons, pyramidal and non-pyramidal, were counted in three evenly spaced Nissl sections with a random start. Contours were traced for each layer, and a counting grid was superimposed on the contour. Every third intersection with a random start was marked for counting so that 30% of the intersections were marked. A counting box of 200/200/27 µm with a volume of 0.00000108 cm^3 (a buffer zone of 5 µm on either surface was employed so as not to include the cut surfaces) was placed inside the marked intersections. Counting was done using a 20X Plano Apo objective, which gave a final screen magnification of 1000. Neurons were distinguished based upon their morphology. Every neuron cell body inside the box with a visible nucleolus that did not touch the exclusion line was counted. Neuron density was determined by dividing the average number of cells per box by the volume of the counting box.

2.7. Quantification of the MAP2 material

MAP2 Immunostaining for layers III and V was quantified by area fraction analysis. Thionin sections were employed to identify layers III and V. Within a defined sampling box,

area fraction refers to the ratio of the area occupied by MAP2-positive profiles vs. the total area of the box. Area fraction was measured from sections using a Bioquant Image Analysis system interfaced with an Olympus AX70 microscope and a Sony 3-chip color camera which relayed the microscopic images to a video monitor. The sampling box was a square with each side equal to the width of the lamina. Bioquant software was employed to select pixels within the sampling box that matched threshold criteria for the MAP2 positive profiles set by the investigator. Threshold was set at the level that selected the lightest stained cell bodies and dendrites without selecting background staining. The setting for illumination was kept constant throughout the analysis. The computer then outlined and summed the thresholded areas and determined the ratio of stained area to the total area of the sampling box. Every section stained with MAP2 was used in the data analysis. An average of eight sections per brain was measured. For each section a total of eight measurements were made for both layer III and V. The measurements were made along the straight sides of a gyrus so as to have as little distortion of the lamina as possible. The eight sampling boxes were taken consecutively as long as there were no histological artifacts in the tissue and the lamina could be determined. If there were artifacts in the tissue, then the box was placed at the next straight edge after the artifact.

2.8. Statistical analysis

Standard descriptive statistics were used in all analysis. Calculations and data analysis were carried out using StatView (V5.0) for Windows (Abacus Concepts Software/SAS). MAP2 area fraction, pyramidal cell density and total neuronal density data were taken from layers III and V of areas 9 and 17 and were expressed as mean for each group, Huntington, schizophrenia and control. A one-way analysis of variance (ANOVA) was used to test for differences between the 3 groups at 95 % confidence limit. Fisher's

post-hoc tests were used to compare the 3 groups. All of the tissues were prepared and analyzed in matched groups; therefore, we believe that the ANOVA is a more sensitive test for intergroup comparison. Additionally, a power analysis of the spine data showed that a minimum sample size of five was enough to yield levels of significance with a 95 % confidence limit (See Figure 2.1). Similar studies by Garey et al., (1998) for spine density measurements used a sample size of four schizophrenics and a MAP2 study by Arnold et al., (1991) used only six brains.

Sample Size

Difference in Means	Error Std Dev	Alpha
214	40	0.050

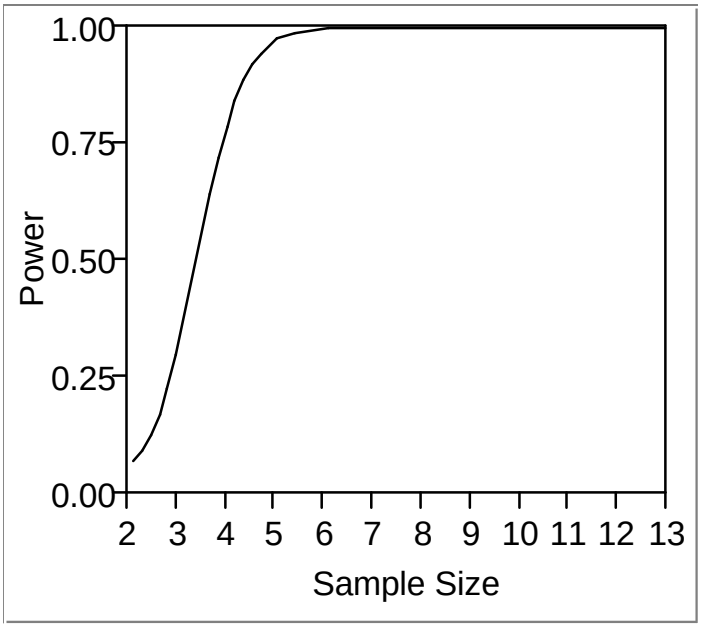

Figure 2.1: Power Analysis of the spine data for Schizophrenia vs Control.

3. Results

3.1 Pyramidal cell density in area 9

Pyramidal cell density was completed on the same 8 Huntington, 8 schizophrenic and 8 controls employed in the area fraction analysis (Figure 2.2 & 2.3). Compared to controls, schizophrenics exhibited a small increase in both layers III and V. On the other hand, the Huntington brains exhibited a very slight increase in layer III only (Table 2.2).

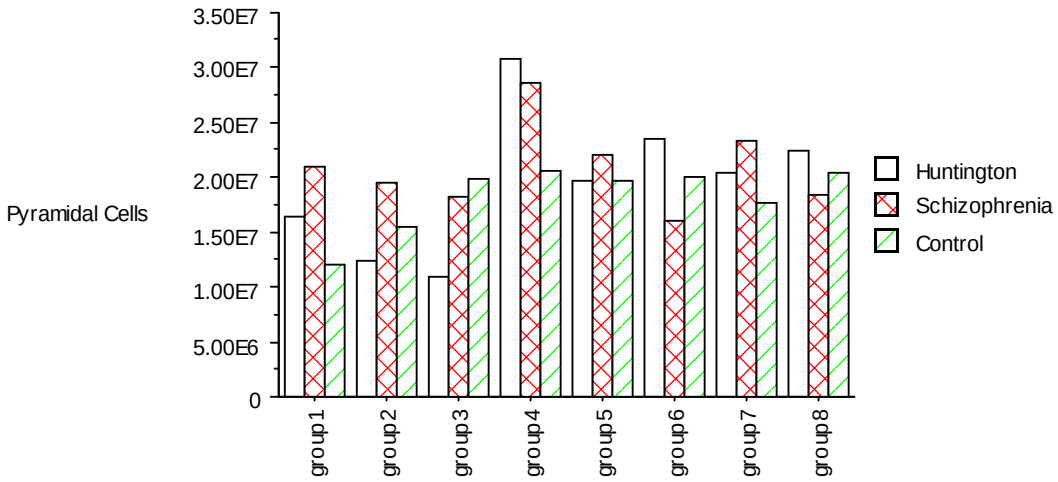

Figure 2.2: Raw data showing the mean pyramidal cell density for the eight groups in Layer III Area 9.

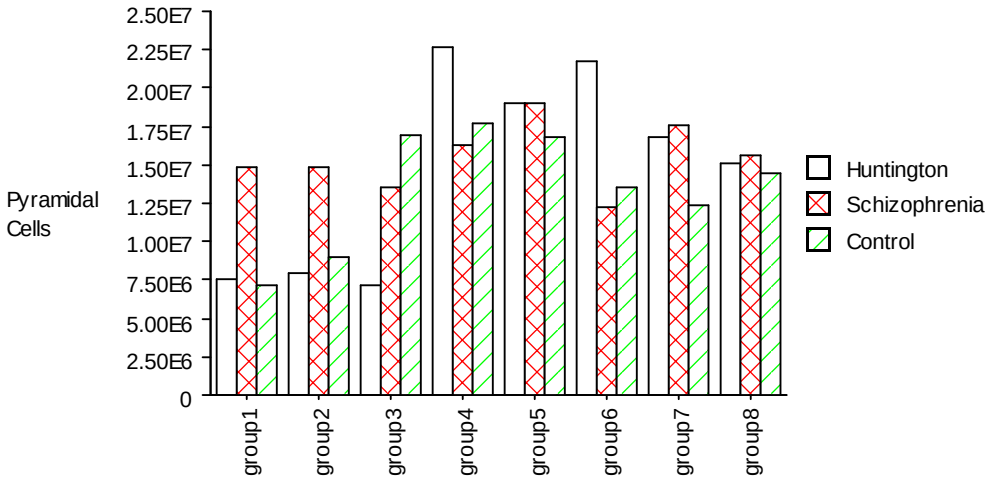

Figure 2.3: Raw data showing the mean pyramidal cell density for the eight groups in Layer V Area 9.

DIAGNOSIS	MEANS (10^6)/cm^3		P-value	
	III	V	III	V
Huntington	19.6	14.0	0.57	0.84
Schizophrenia	20.9	15.5	0.26	0.44
Control	18.2	13.5	1.0	1.0

Table 2.2: Pyramidal cell density showing means and P-value for layers III and V in area 9 (n=8).

3.2 Total neuronal density in area 9

Total neuronal density measurements were made on 8 Huntington, 8 schizophrenics and 8 controls (Figure 2.4 & 2.5). The total neuronal density in both layers III and V showed no statistical difference in schizophrenia and Huntington Chorea compared to controls (Table 2.3).

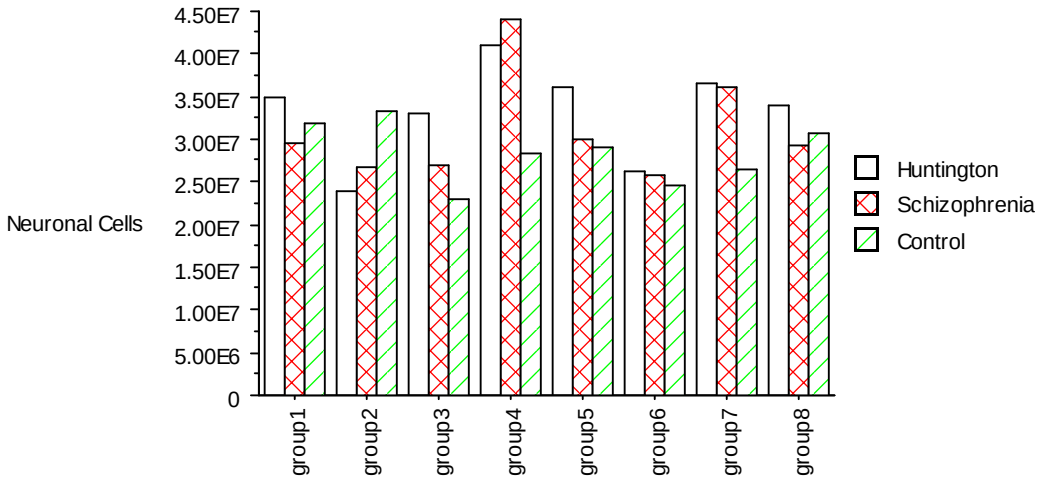

Figure 2.4: Raw data showing the mean neuronal cell density for the eight groups in Layer III Area 9.

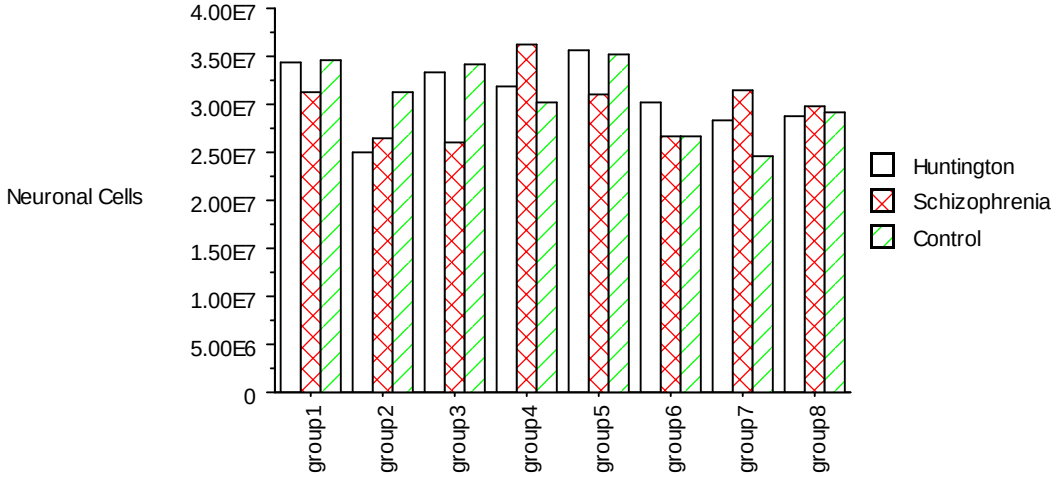

Figure 2.5: Raw data showing the mean neuronal cell density for the eight groups in Layer V Area 9.

DIAGNOSIS	MEANS (10^6)/cm^3		Std. Dev. (10^6)		P-value	
	III	V	III	V	III	V
Huntington	33.2	30.9	5.6	3.5	0.8	0.89
Schizophrenia	31.1	29.9	6.1	3.5	0.32	0.64
Control	28.5	30.7	3.6	3.8	1.0	1.0

Table 2.3: Total neuronal density showing means, standard deviation and P-value for layers III and V in area 9 (n=8).

3.3 MAP2 in Area 9

Area fraction analysis was completed on 8 Huntington, 8 schizophrenic and 8 controls (Figure 2.8 &2.9). Compared to controls, schizophrenics exhibited a 39% decrease (P-value 0.001, SD ± 0.03) in layer III and a 38 % decrease (P-value 0.004, SD ± 0.06) in layer V (Table 2.4). There was no significant decrease in MAP2 in layer III in Huntington compared to controls. There was a significant 29% decrease (p-value 0.02, SD ± 0.01) in layer V in Huntington (Table 2.4). This was the only significant result in Huntington compared to control.

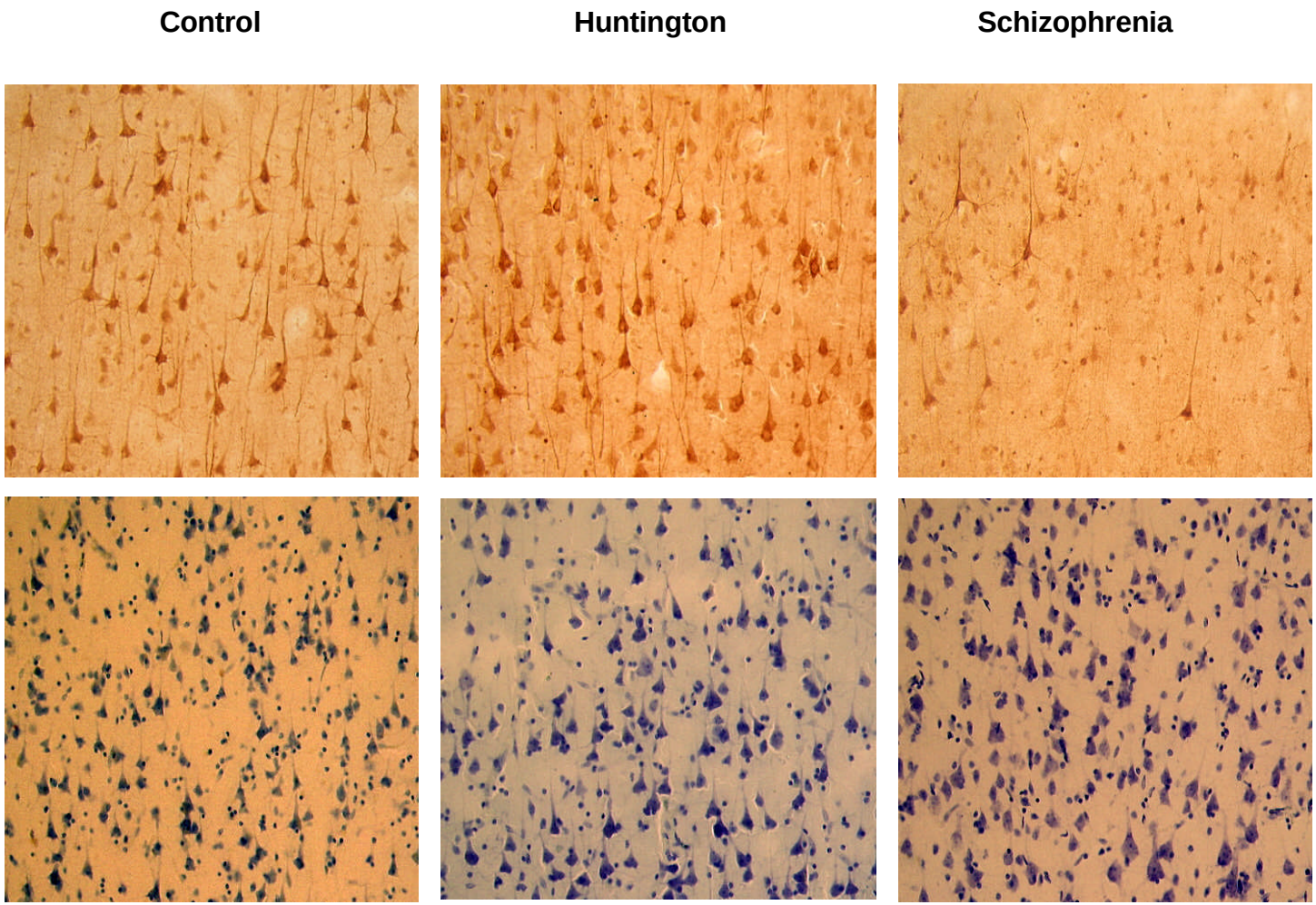

Figure 2.6: Photomicrographs showing MAP 2 (above) and Nissl Staining (below) in Layer III Area 9 (20X)

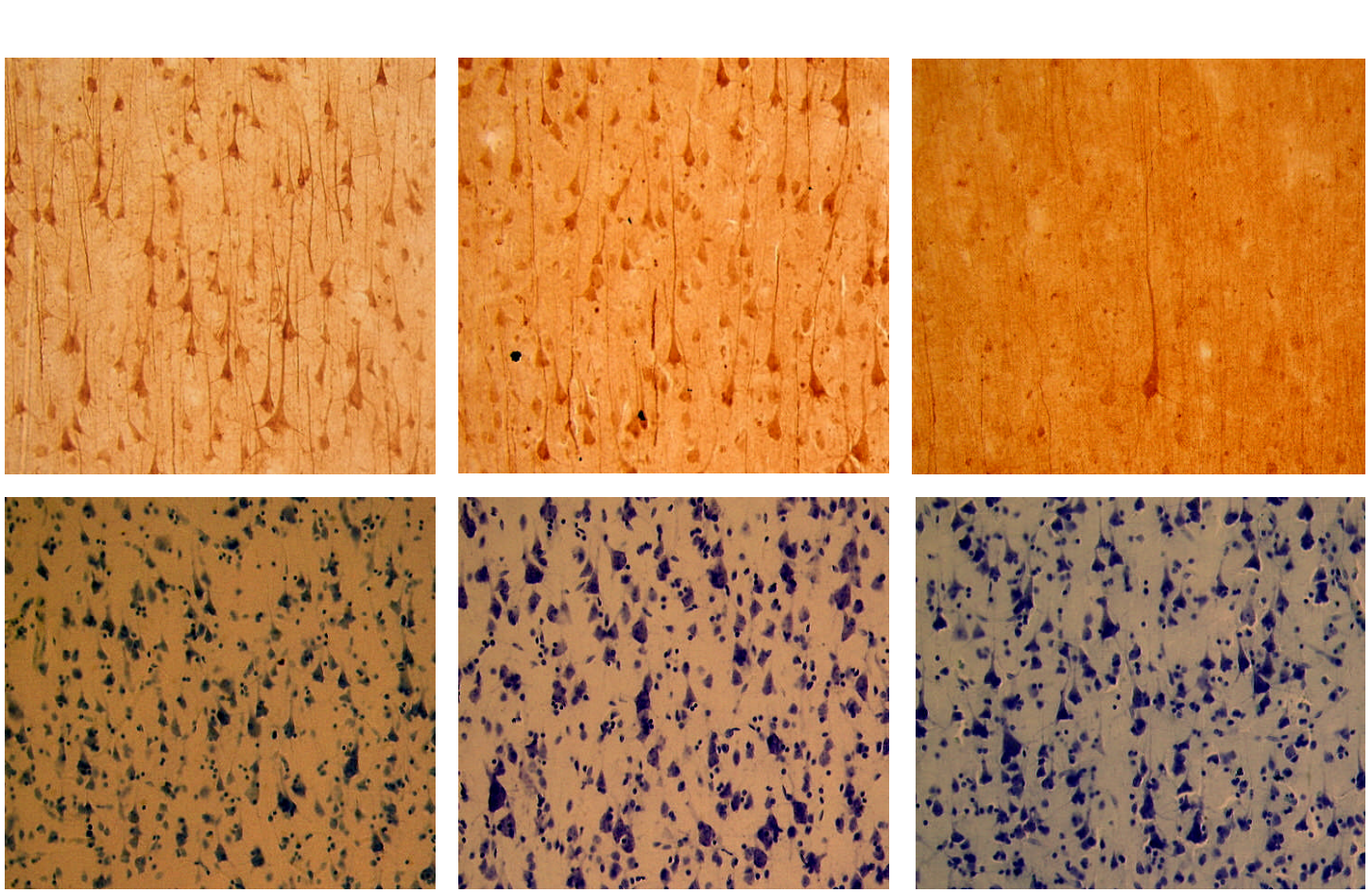

Figure 2.7: Photomicrographs showing Map 2 (above) and Nissl Staining (below) in Layer V Area 9 (20X)

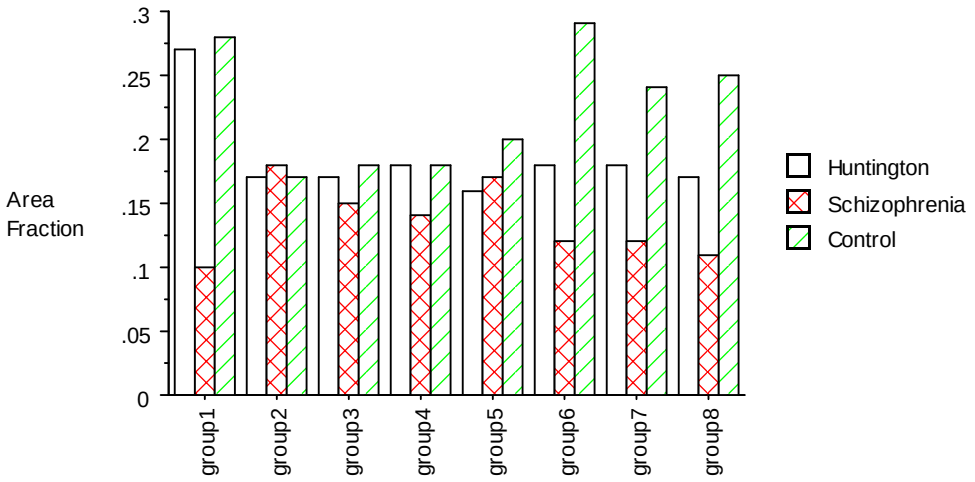

Figure 2.8: Raw data showing MAP2 area fraction means for the eight groups in Layer III in Area 9.

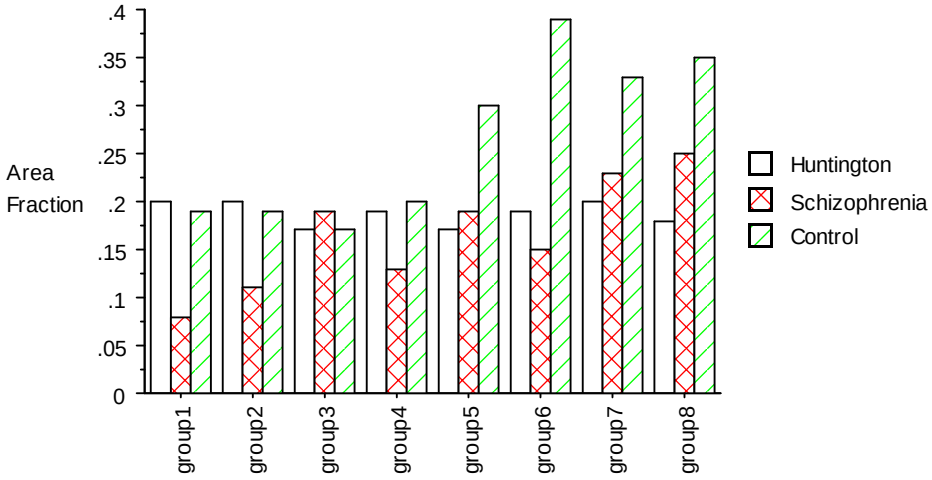

Figure 2.9: Raw data showing MAP2 area fraction means for the eight groups in Layer V in Area 9.

DIAGNOSIS	MEANS		P-value	
	III	V	III	V
Huntington	0.185	0.188 * ±0.013	0.0536	0.0191 *
Schizophrenia	0.136 * ± 0.029	0.166 * ± 0.059	0.0001 *	0.0040 *
Control	0.224	0.265	1.0	1.0

Table 2.4: MAP2 data showing means and P-value for layers III and V in area 9 (n=8).

3.4 Pyramidal cell density in control (area 17).

Pyramidal cell density means for the eight groups of brains in area 17 layers III and V showed no significant difference when comparing Huntington, schizophrenia and controls (Table 2.5).

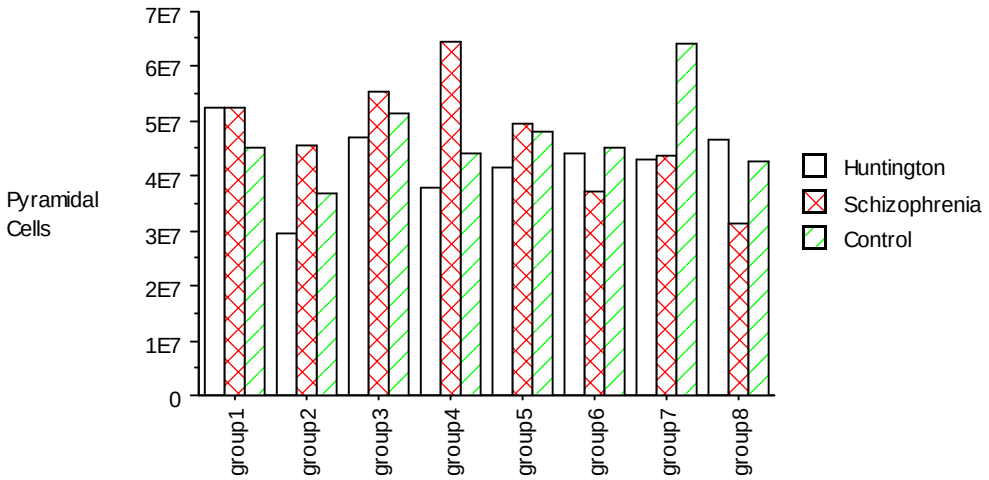

Figure 2.10: Raw data showing pyramidal cell density for the five groups in layer III in control.

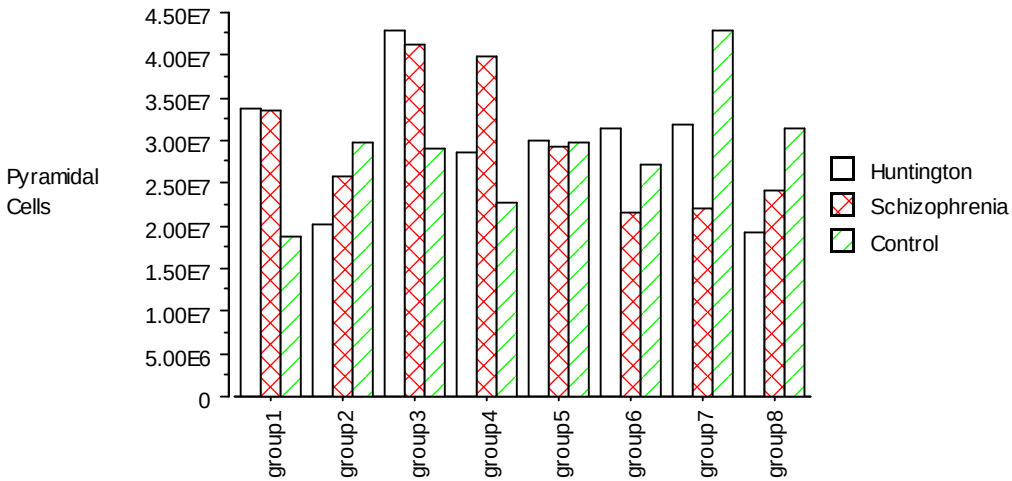

Figure 2.11: Raw data showing pyramidal cell density for the five groups in layer V in control.

DIAGNOSIS	MEANS (10^6)/cm^3		Std. Dev. (10^6)		P-value	
	III	V	III	V	III	V
Huntington	42.7	29.7	6.8	7.5	0.3	0.8
Schizophrenia	47.5	29.7	10.4	7.7	0.9	0.8
Control	47.2	28.9	8.0	7.1	1.0	1.0

Table 2.5: Pyramidal cell density showing means, standard deviation and P-value for layers III and V in control (n=8).

3.5 Neuronal density in control (area 17)

Total neuronal density means for the eight groups of brains in area 17 layers III and V showed no significant difference when comparing Huntington, schizophrenia and control (Table 2.6).

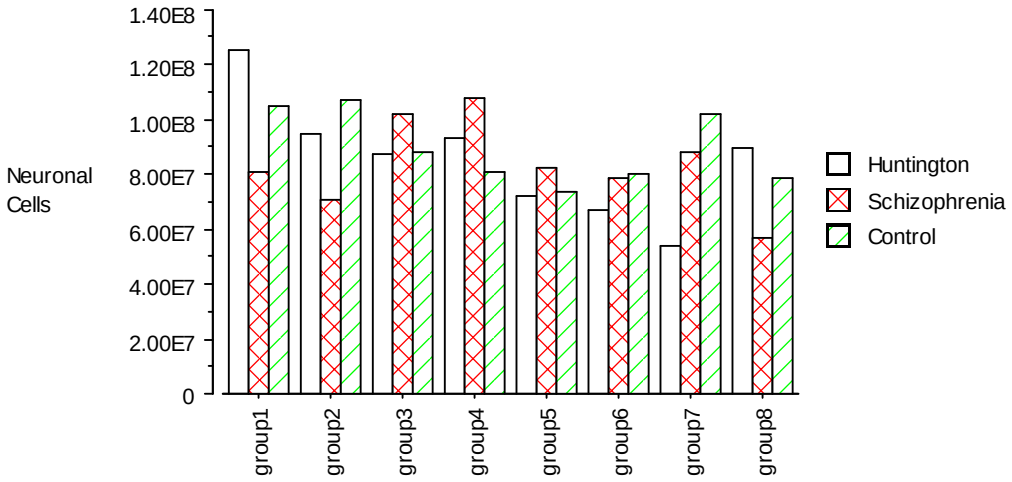

Figure 2.12: Raw data showing total neuronal density for the five groups in layer III in control.

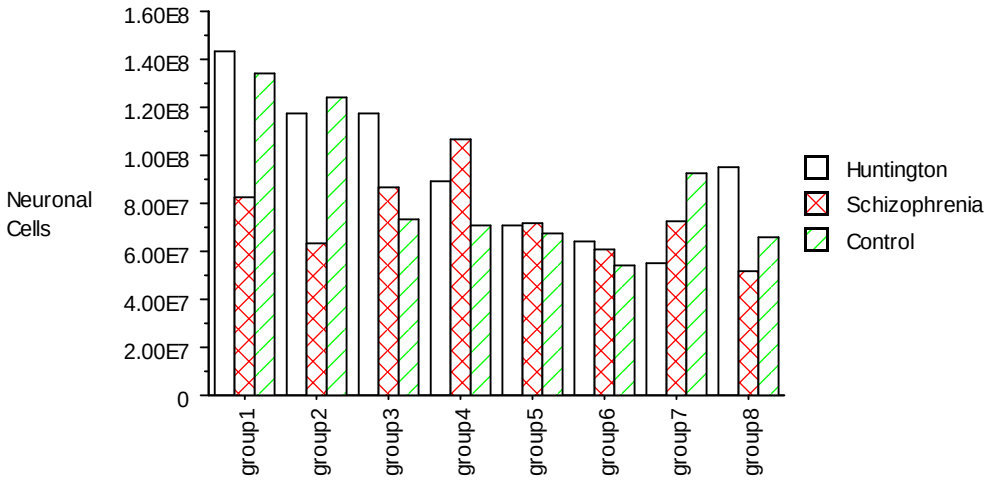

Figure 2.13: Raw data showing total neuronal cell density for the five groups in layer V in control.

DIAGNOSIS	**MEANS (10^6)/cm^3**		**Std. Dev. (10^6)**		**P-value**	
	III	**V**	**III**	**V**	**III**	**V**
Huntington	85.5	94.1	21.7	30.4	0.65	0.51
Schizophrenia	83.5	74.5	16.3	17.1	0.50	0.42
Control	89.5	85.3	13.5	29.2	1.0	1.0

Table 2.6: Total neuronal density showing means, standard deviation and P-value for layers III and V in control (n=8).

3.6 MAP2 in control (area 17)

MAP2 area fraction means for the five groups of brains in area 17 layers III and V showed no significant difference when comparing Huntington, schizophrenia and control (Table 2.7).

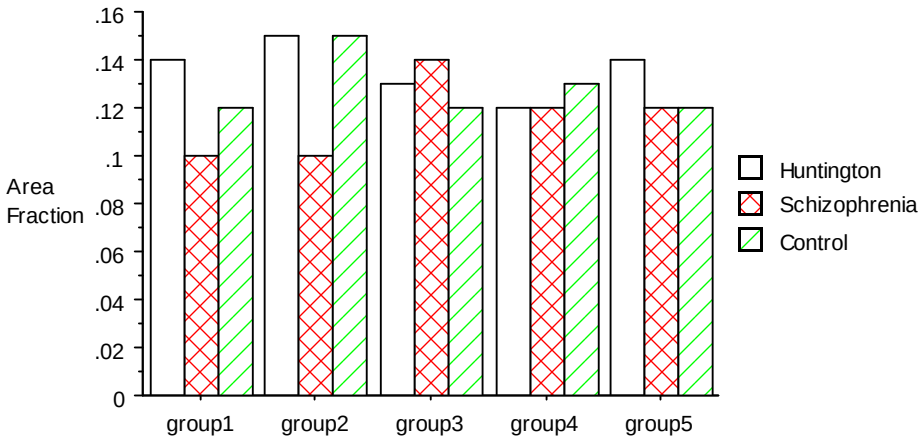

Figure 2.14: Raw data showing MAP2 area fraction for the five groups in layer III control.

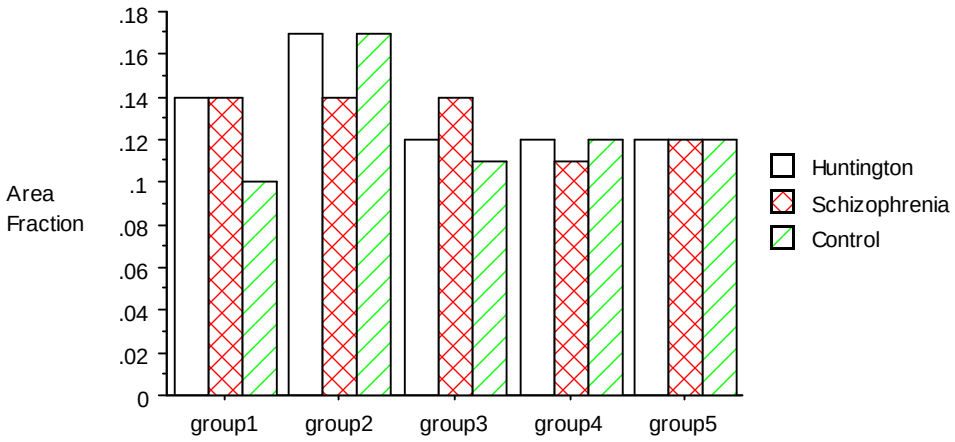

Figure 2.15: Raw data showing MAP2 area fraction for the five groups in layer V in control.

DIAGNOSIS	MEANS		Std. Dev.		P-value	
	III	V	III	V	III	V
Huntington	0.136	0.134	0.011	0.022	0.38	0.48
Schizophrenia	0.116	0.130	0.017	0.014	0.20	0.67
Control	0.128	0.124	0.013	0.027	1.0	1.0

Table 2.7: MAP2 data showing means, standard deviation and P-value for layers III and V in control (n=5).

Discussion

The present data shows significant decreases in MAP2 in both layers III and V in schizophrenia as compared to controls which confirms previous findings in our laboratory (Jones et al., 2002). When comparing Huntington Chorea to controls a trend towards a decrease was seen in MAP2 in layer III; however, it was not significant. However, there was a significant decrease in MAP2 in layer V when comparing Huntington Chorea to

controls. This observation of MAP2 in Huntington Chorea can be explained in two ways. First, this decrease may be due to neuroleptic exposure since both Huntington and schizophrenic brains showed decreases in MAP2. It is foreseeable that neuroleptic drugs because they have the ability to bind to dopamine and serotonin receptors can phosphorylate MAP2 through the action of second messenger activation of phosphatases (Lidow 2001). Phosphorylation of MAP2 will result in altered dendritic surface area, therefore decrease MAP2 (Audesirk et al., 1997; Hely et al., 2001). Secondly, this decrease may be due to the disease and not an effect of neuroleptic exposure. The latter seems more plausible because layer III area fraction in area 9 and both layers III and V in area 17 in Huntington Chorea showed no significant decrease as compared to controls. We believe that neuroleptic drugs would affect all areas equally; although, schizophrenics take neuroleptics for longer periods than Huntington's patients both groups showed long term exposure. Therefore, we believe what we are seeing in schizophrenia and Huntington Chorea is significant and real. The decrease in MAP2 in Huntington Chorea maybe attributed to the close association of the abnormal Huntingtin protein with microtubules, thus MAP2. Furthermore, it suggests that the structural integrity of the pyramidal cells might be compromised, since MAP2 functions as a stabilizing agent in the assembly of microtubules and as such the cytoskeletal processes of the cell. It further suggests that the dendritic elements of the pyramidal cells would also be compromised as was seen in area 32 in a previous study by our lab (Broadbelt 2002). The dendritic elements are the scaffolding for 90% of the synaptic input to the pyramidal cells in the PFC. Together, this suggest a loss of input in this part of the brain which would result in disturbances in information processing as seen in schizophrenia. Furthermore, both the Huntington and schizophrenic brains showed no significant changes in pyramidal cell or neuronal density in both layer III and V in area 9 as compared to controls. The data did show a small increase in pyramidal cell density in layer III in schizophrenics. In area 17, there were no

differences seen in cell density or MAP2 measurements in Huntington, schizophrenia or control in both layers III and V. This study support what has been previously reported by our lab and other researchers. Because there were no significant differences in layer III MAP2 and pyramidal cell density in Huntington Chorea as seen in schizophrenia suggest that neuroleptic drugs are not responsible for the changes observed in schizophrenia and as such not a confounding variable.

CHAPTER 3

Dendritic and spine alterations in areas 9 and 17 in schizophrenia and Huntington Chorea and the role of neuroleptic exposure

Abstract

Recent morphological studies in schizophrenia suggest atrophic changes in the neuropil of the prefrontal cortex (Beasley et al., 1997; Glantz and Lewis, 1997, Honer et al, 1997; Garey et al, 1998; Harrison, 1999; Selemon and Goldman-Rakic, 1999; 2000; Bertolino et al, 1999; 2000; Kalus et al, 2000; Lewis et al, 2001; Pierri et al, 2001; Reynolds and Beasley, 2001; Jones, 2001; Broadbelt et al, 2002; Buxhoeveden et al, Jones et al, 2002; Kindermann et al., 2004). Most recently, we showed a schizophrenia-associated decrease in MAP2 in schizophrenia (Jones 2002), which we believed is not due to neuroleptic exposure. MAP2 is a very important protein in the assembly of microtubule in neurons; therefore, it plays a major role in neuronal processes like dendrites, spines and synapses. Additionally, recent studies from our lab showed decreases in dendrites in area 32 (Broadbelt 2002) and area 9 (personal communication). In this study we examined the dendrites and spines in area 9 and 17 to determine if neuroleptic drugs play a role. We employed Golgi silver impregnation to analyze five matched groups of Huntington, schizophrenia and control. Huntington's patients take neuroleptics similar to schizophrenics; therefore, by comparing the two groups to controls we can determine if neuroleptics play a role in the deficits reported in schizophrenia. Our results showed a significant decrease in both basal dendrites and spines for both layers III and V in area 9 in schizophrenia compared to controls. The Huntington's brains showed no significant difference compared to controls. In area 17, there was also no significant difference when comparing the three groups. The data suggest that neuroleptic drugs may not be responsible for the changes observed in schizophrenia.

1. Introduction

The neuroanatomical basis of schizophrenia is still a mystery to researchers. However, much research over past few decades was designed to examine blood flow in the brain; differential neuronal counts and density; neuronal size and volume; dendritic architecture; spine counts; transmitter systems; and several structural and synaptic proteins in various cortical areas. (Weinberger et al.,1986; Buchsbaum et al., 1990; Benes 1991; 1993; Pakkenberg 1993; Goldman-Rakic and Selemon 1995, 1997; Beasley et al., 1997, Glantz and Lewis 1997, Garey et al., 1998; Harrison 1999; Selemon and Goldman-Rakic 1999;; Buxhoeveden et al., 2000; Glantz et al 2000; Andreasen 2000; Thune et al., 2001; 2001; Jones et al., 2002; Broadbelt et al., 2002) As a result, much is known about the cortex of the brain. There are two types of neurons in the cortex, pyramidal and non-pyramidal neurons. The pyramidal neurons constitute approximately 70% of the cortical neurons and the non-pyramidal neurons account for about 25% (Powell 1981). The pyramidal neurons are named for their pyramidal shape cell body; they have an apical dendrite extending towards the pial layer as well as basal dendrites on the base of the cell body (see Figure 3.2). Spines are protrusions of the neuronal membrane consisting of a head connected to the neuron by a thin spine neck (see figure 3.7). They can be found on the dendrites, the soma and on the axon hillock (Mates and Lund 1983). Spines are the site of synaptic transmission and about 90% of the synapses on spines are excitatory (Mates and Lund 1983). There are three types of spines: mushroom, stubby and filopodium (Peters and Kaiserman 1970). In the DLPFC in schizophrenia, structural imaging studies suggest a decrease in volume in this part of the brain (Daviss and Lewis 1995, Selemon et al., 1995); whereas, most neuron density measurements showed an increase in neuronal density without a change in the total number of neurons (Benes 1991,

Selemon et al., 1995). Thus, researchers theorized that there are changes within the neuropil. Loss of spines dendrites and axons would strongly suggest altered connectivity in patients with schizophrenia. Previously we reported a decrease in MAP2 in area 9 and 32 in schizophrenics, which we believe is not due to neuroleptic exposure (Jones et al., 2002). We believe that this decrease in MAP2 in schizophrenia will result in consequences reflected in the cytoskeletal processes of the pyramidal cells, like the dendrites and spines. Our lab had previously reported decreases in dendrites in area 32 of the prefrontal cortex (Broadbelt 2002) and the issue of neuroleptic exposure remains unanswered.

Several imaging studies showed evidence of striatal enlargement in drug treated patients (Keshavan et al., 1994; Chakos et al., 1994, 1995; Doraiswamy 1995) and rats (Chakos et al., 1998). Madsen et al., (1998) reported progressive brain atrophy; whereas, Gur et al., (1998) reported increasing thalamic volume. Most recently, a study on rhesus monkeys demonstrated long-term haloperidol exposure can increase phosphorylation of MAP2 (Lidow et al., 2001). Antipsychotic drugs because of their affinity to bind neurotransmitters receptors have the ability via second messenger systems to phosphorylate proteins like MAP2. Because MAP2 is associated with microtubule assembly, it can affect the cytoskeletal processes of neurons like dendrites and spines (Lidow 2001).

This study employs Golgi silver Impregnation to analyze the dendrites and spines of the pyramidal cells in area 9 of the PFC. We employed a modify Golgi-Cox method by Armstrong and Parker and counted basal dendrites and spines on pyramidal cells in area 9 layers III and V in cohorts of Huntington Chorea, schizophrenia and control. Both Huntington's patients and Schizophrenics take similar neuroleptic drugs; therefore, by

comparing the two groups' one can begin to see if neuroleptics play a role in the changes observed in schizophrenia.

2. Methods

2.1. Subjects and tissue

Postmortem brain tissues from five subjects diagnosed with Huntington Chorea, five subjects diagnosed with schizophrenia and five controls were collected from Harvard Brain Tissue Resource Center (HBTRC) at McLean Hospital (Table 3.1). The three groups of tissues were all matched for age, sex and postmortem interval. All tissues were collected at the time of autopsy in accordance with an approved Institutional Review Board protocol. Diagnoses confirmed to DSM-IV criteria and were established retrospectively by reviewing medical records and interviewing knowledgeable individuals. Tissue was not included in the study if there was evidence of neuropathology (e.g. Alzheimer's disease) or substance dependence as determined by the HBTRC. The brain bank coded the tissues and the codes were maintained by one of the authors who did not conduct quantitative assessment (L.J.) until quantitative procedures had been completed.

Inclusion criteria: Brains were not selected on a racial basis. Approximately equal numbers of male and female brains were obtained. Schizophrenics and Huntington patients had a DSM IV diagnosis and range in age from 34 to 80 years of age. Nonpsychiatric controls came from nursing homes and hospitals, had no psychiatric diagnosis established by the DEAD scale. Controls from nursing homes had resided at the home for at least five years prior to death.

Exclusion criteria: No brains with evidence of traumatic injury, infarction or Alzheimer's type changes were accepted into the study.

2.2. Golgi Silver Impregnation

The brains were processed in groups (control and schizophrenia or Huntington). The tissue was cut into 2mm slabs then wrapped in gauze. All of the glassware used in this experiment was acid washed. The tissue was first placed in a solution containing 100mls dH_2O, 3g potassium dichromate, 12.5g sucrose, 5ml 37% formalin, and 7.5ml of 3% H_2O_2 and incubated in an oven at 55°C for 6 hours. The tissue was removed from the oven and placed in fresh solution and left at room temperature overnight. The following day the tissue was placed in a second solution containing 100ml dH_2O, 3g potassium dichromate and 12.5g sucrose and incubated in an oven at 55°C for 6 hours. Following the incubation, the tissue was placed in fresh solution and left again at room temperature overnight. The tissue was then rinsed in 0.75% silver nitrate and placed in fresh 0.75% silver nitrate solution. Because silver nitrate is light sensitive, the jars were wrapped in aluminum foil and left at room temperature for 5 days. Finally the tissue was serially sectioned on a vibratome into 150μm thick sections and collected in 70% alcohol. The sections were dehydrated in graded alcohol starting at 70%, defatted in xylene, mounted onto slides and coverslipped with permount solution.

2.3 Quantification of dendrites and spines

The material was analyzed using a Bioquant Image Analysis System interfaced with an Olympus AX70 microscope connected to a Ludl Motorized stage and a Sony 3-chip camera. Three measurements were performed on Golgi material, 1) number of primary basilary dendrites, 2) number of secondary basilary dendrites and 3) spine density along the primary and secondary basilary branches (Figure 3.2 & 3.7). Fifty cells per brain were

chosen randomly to be analyzed for dendritic measurements and ten cells per brain for the spine measurements. The cells must meet the following criteria to be analyzed: The cell body must be easily seen with no secondary branches being obscured by background or by another cell and the apical dendrite must be visible from the soma to the pial surface. All measurements were made using stereologic methods. The Bioquant system comes with a stereologic package that was used to make measurements. The total area, that contains cells, was traced. A 100/100 grid was placed within the contour. The computer randomly marked grids to be measured, a counting box with a set z range was placed inside the marked grids and the cells within the grid which meet criteria were measured. Two different measurements were made for each cell at one time. The numbers of primary and secondary basilar branches were counted and an average for each brain was determined. The counting of spines along the primary and secondary basilary dendrites was done at 100X under oil. Ten cells per brain were chosen randomly. Dendrites whose cell meets criteria were measured for their length in microns at 60X under oil and have their spines counted manually at 100X. By moving through the focal plane we counted all of the spines visible along the dendrite using a hand counter. In addition, the numbers of spines counted were entered manually into the Bioquant to keep track of the number of spines counted. The data was expressed as average number of spines per millimeter of dendrite in each lamina in the different brain regions.

2.4. Statistical analysis

Standard descriptive statistics were used in all analysis. Calculations and data analysis were carried out using StatView (V5.0) for Windows (Abacus Concepts Software/SAS). Dendritic and spine data were taken from layers III and V of areas 9 and 17 and were expressed as means for each of the Huntington, schizophrenia and control

groups. A one-way analysis of variance (ANOVA) was used to test for differences between groups at 95 % confidence limit. All of the tissues were prepared and analyzed in matched groups; therefore, we believe that the ANOVA is a more sensitive test for intergroup comparison. Additionally, a power analysis of the spine data showed that a minimum sample size of five was enough to yield levels of significance with a 95 % confidence limit (See Figure 3.1). Similar studies by Garey et al., (1998) for spine density measurements used a sample size of four schizophrenics and a MAP2 study by Arnold et al., (1991) used only six brains.

Sample Size

Difference in Means	Error Std Dev	Alpha
214	40	0.050

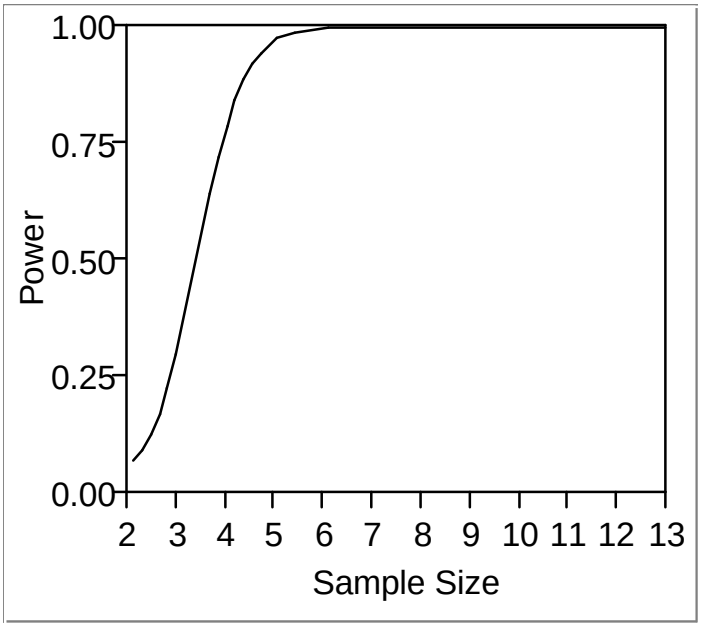

Figure 3.1: Power Analysis for Schizophrenia vs Control of Primary Spines of Layer III

Brain #	*Distributive Diagnosis*	*Medication*	*Age*	*Sex*	*PMI*	*Storage Time*
5039	Huntington	Haldol (.5 mg)	54	F	14	29 months
5662	Schizophrenia	Thioridazine (100mg)	52	F	16	15 months
5734	Control	None	NR	NR	NR	NR
5344	Huntington	No med. report Grade 4*	69	F	26	17 months
5656	Schizophrenia	No psych. Med.	73	F	29	15 months
5805	Control	None	NR	NR	NR	NR
5464	Huntington	No med. report Grade 4	61	M	21	20 months
5785	Schizophrenia	Depakote(750 mg), Zyprexa (5 mg), Neurontin (200 mg)	63	M	15	9 months
5715	Control	None	NR	NR	NR	NR
2161	Huntington	Haldol (10 mg)	59	F	14	109 months
4942	Schizophrenia	Zyprexa (15 mg), Luvox (50 mg), Humibid (1200 mg)	61	F	14	16 months
4810	Control	None	62	M	16	12 months
4292	Huntington	No med. report Grade 4	56	M	5	38 months
3013	Schizophrenia	Chlorpromazine (100 mg), Fluphenzarine (10 mg),	62	M	6	86 months
4932	Control	None	67	M	22	8 months

Table 3.1: Demographic data for the matched brainsused in the Golgi projects.

NR = No medical report

***** All Grade 4 Huntington brains had both behavioral and movement disorders, therefore they took neuroleptics at some point in their life.

3. Results

3.1 Dendrites and spines in area 9

The number of dendrites and spines were obtained from 5 Huntington, 5 schizophrenic and 5 controls in decrease (P-value 0.01, SD ± 0.67) in layer V (Table 3.2). There were no significant changes seen in the Huntington's brains compared to controls. For secondary dendrites, both layers showed a trend towards a decrease in schizophrenics compared to controls (Figure 3.5 & 3.6). There were no significant differences seen in Huntington's brains compared to controls (Table3.3).

The spine data is expressed as number of spines per millimeter of dendrite (Figure 3.8 & 3.9). Compared to controls the primary spines exhibited a 37% decrease (P-value 0.0001, SD ± 32) in layer III and a 38% decrease (P-value, SD ± 31) in layer V in area 9. The Huntington brains showed no significant changes in both layers compared to controls (Table 3.4). Compared to controls the secondary spines exhibited a 37% decrease (P-value, SD ± 39) in layer III and a 37% decrease (P-value, SD ± 58) in layer V (Figure 3.10 & 3.11). In the Huntington brains there were no significant changes in both layers compared to controls (Table 3.5).

3.2 Dendrites and spines in control (Area 17).

The number of primary and secondary basilary dendrites was obtained from five Huntington, five schizophrenic and five controls in both layer III and V in area 17 (Figure 3.14 & 3.15). The data is expressed as number of dendrites per brain. In both layers III and V there were no significant difference for primary and secondary dendrites in Huntington and schizophrenia compared to controls (Table 3.6). Similarly, there was no

significant difference in primary and secondary spines in both layers for Huntington and schizophrenia compared to controls (Table 3.8 and 3.9)

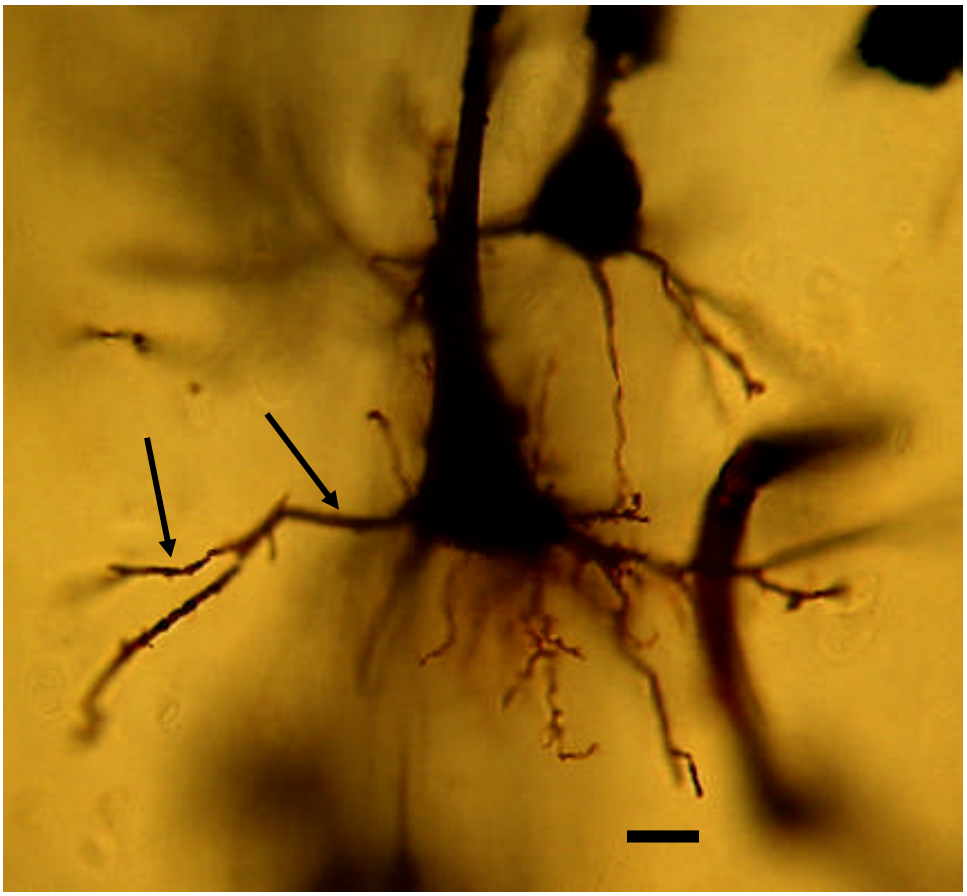

Figure 3.2: Photomicrograph of a pyramidal cell showing primary and secondary basilary dendrites. Primary basilary dendrites come off the cell body directly and secondary basilary dendrite comes off the primary basilary dendrite. Scale bar = 0.01 mm.

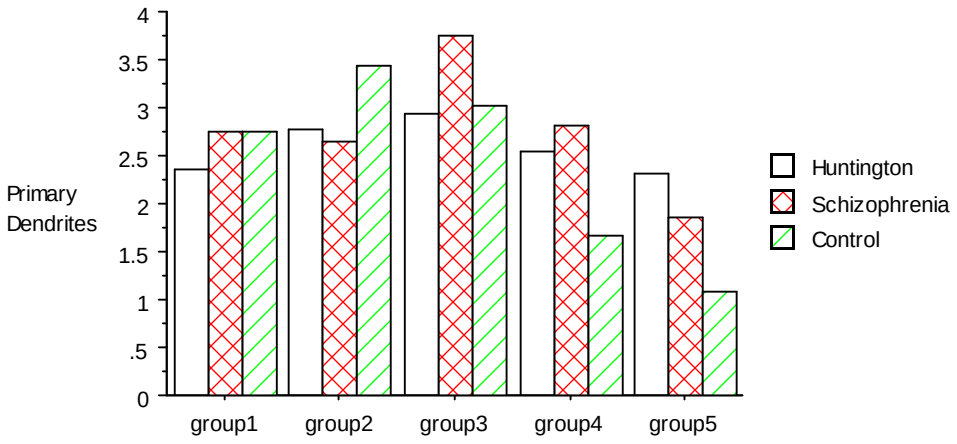

Figure 3.3: Raw data showing primary dendrites for the five groups in layer III area 9

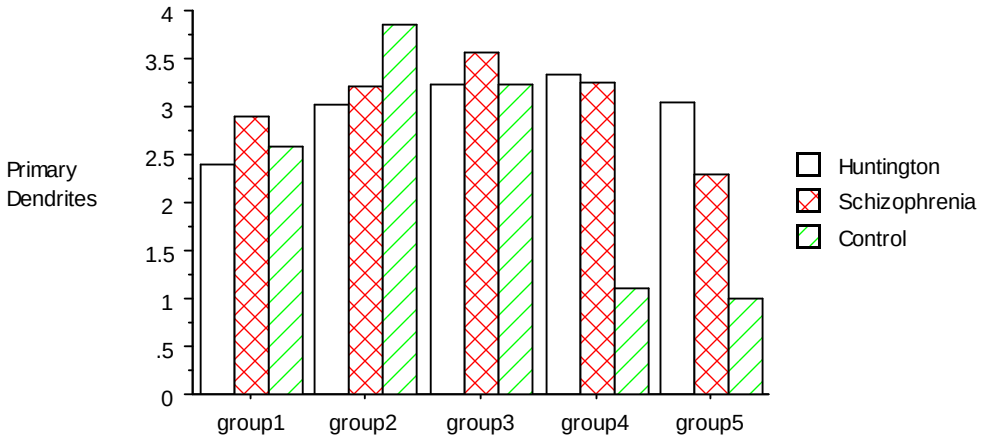

Figure 3.4: Raw data showing primary dendrites for the five groups in layer V area 9

DIAGNOSIS	MEANS		P-value	
	III	V	III	V
Huntington	2.6	3.0	0.68	0.89
Schizophrenia	1.8 * ± 0.5	2.1* ± 0.67	0.007*	0.01*
Control	2.7	3.1	1.0	1.0

Table 3.2: Golgi data showing means and P-value for primary dendrites in layers III and V in area 9 (n=5).

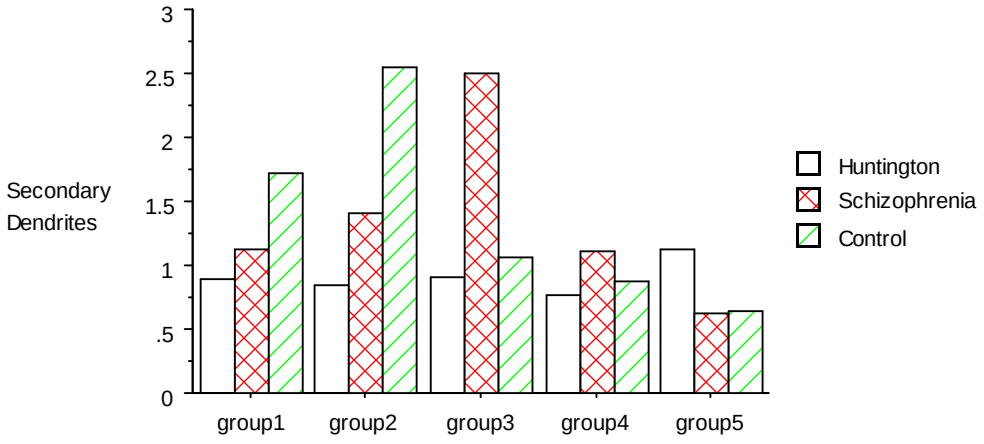

Figure 3.5: Raw data showing secondary dendrites for the five groups in layer III area 9.

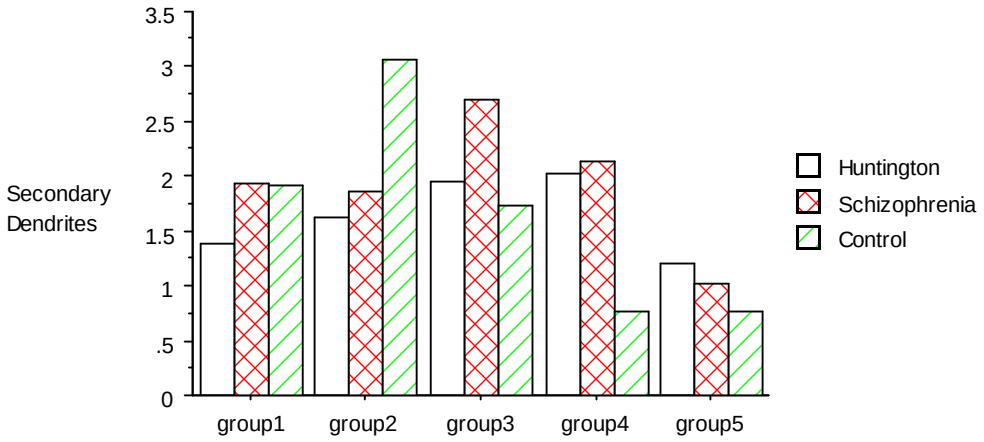

Figure 3.6: Raw data showing secondary dendrites for the five groups in layer V area 9.

DIAGNOSIS	**MEANS**		**Std. Dev.**	
	III	**V**	**III**	**V**
Huntington	1.5	1.6	0.86	0.35
Schizophrenia	0.9	1.3	0.29	0.41
Control	1.5	1.8	0.63	0.69

Table 3.3: Golgi data showing means, standard deviation and P-value for secondary dendrites in layer III and V in area 9 (n=5).

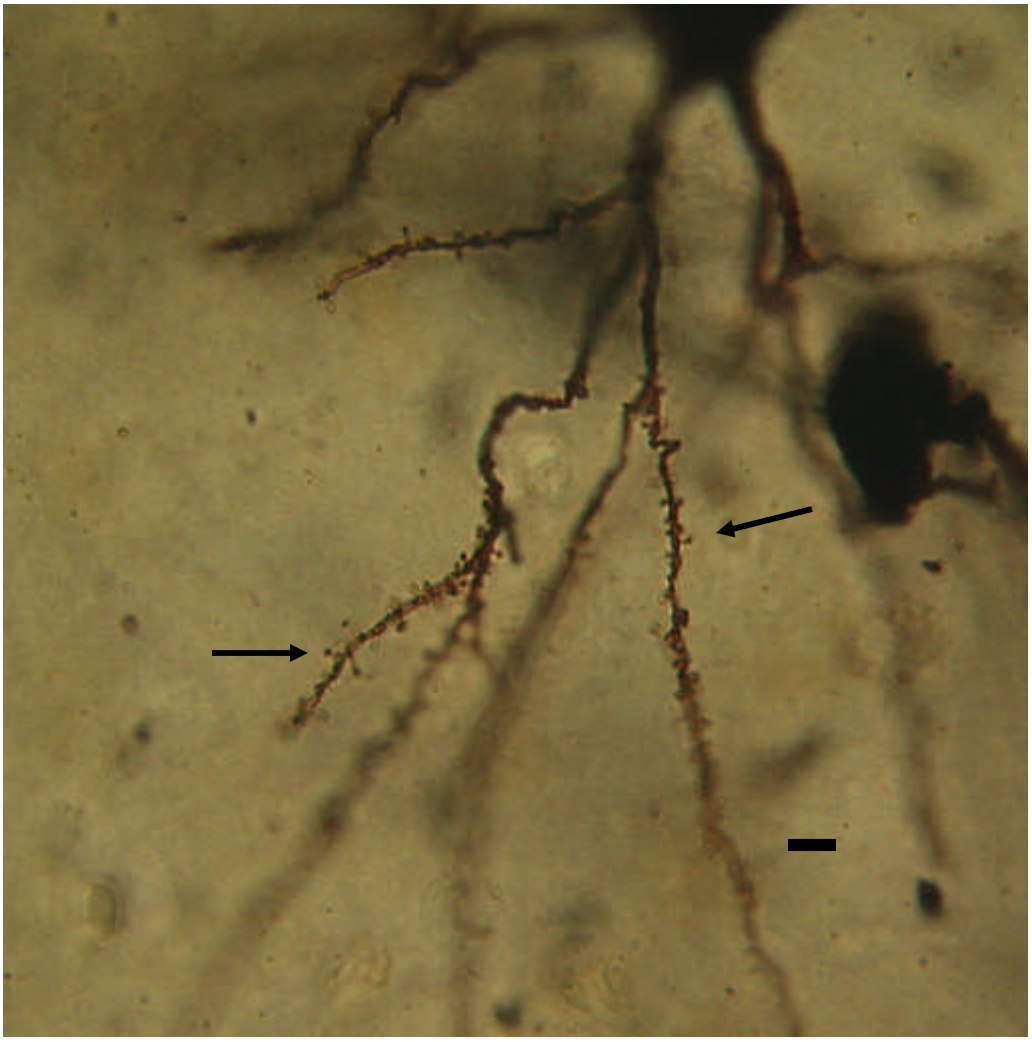

Figure 3.7: Micrograph showing spines on the basal dendrites of a pyramidal cell. Scale bar = 0.01 mm.

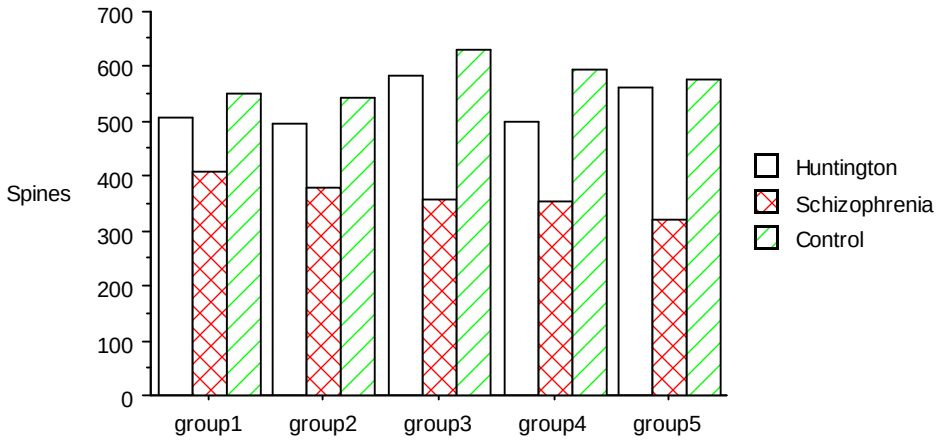

Figure 3.8: Raw data showing the mean primary spines/mm dendrite for the five groups in Layer III Area 9.

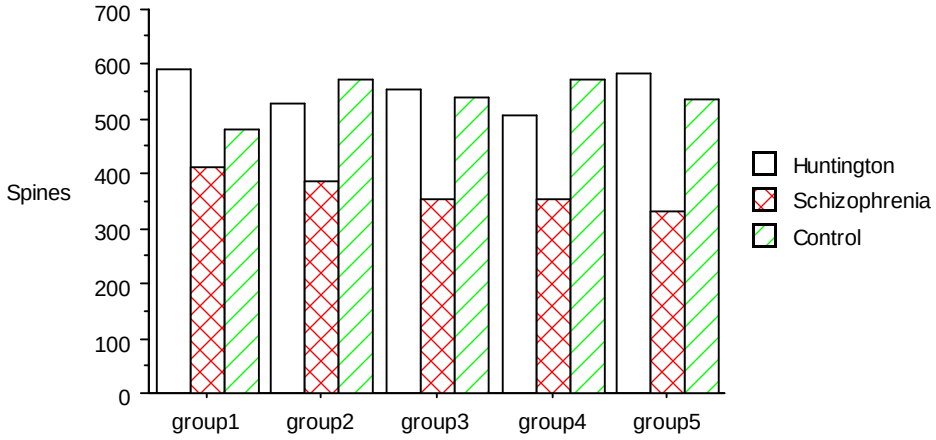

Figure 3.9: Raw data showing the mean primary spines/mm dendrite for the five groups in Layer V Area 9.

DIAGNOSIS	MEANS		P-value	
	Counts/mm dendrite		III	V
	III	V		
Huntington	529	553	0.516	0.558
Schizophrenia	363 * ± 0.32	367 * ± 0.32	<0.0001*	<0.0001*
Control	578	540	1.0	1.0

Table 3.4: Spine counts showing means and P-value for primary spine counts per millimeter of dendrite in layers III and V in area 9 (n=5).

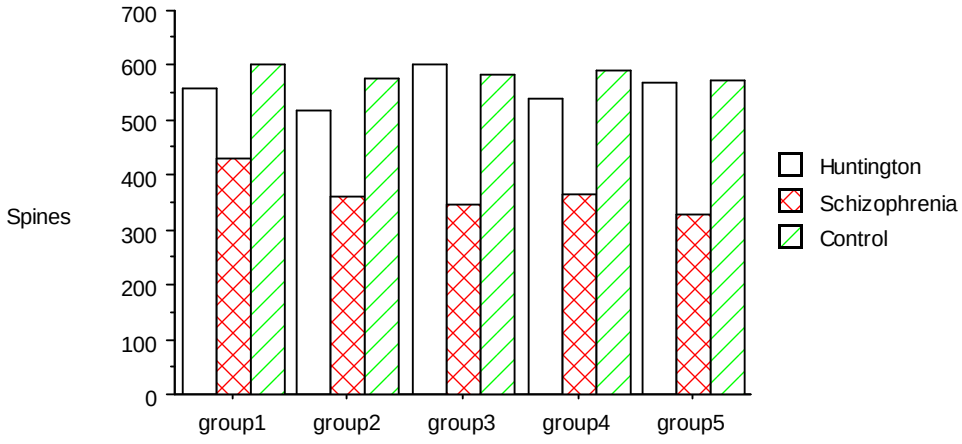

Figure 3.10: Raw data showing mean secondary spines/mm dendrite for the five groups in Layer III Area 9.

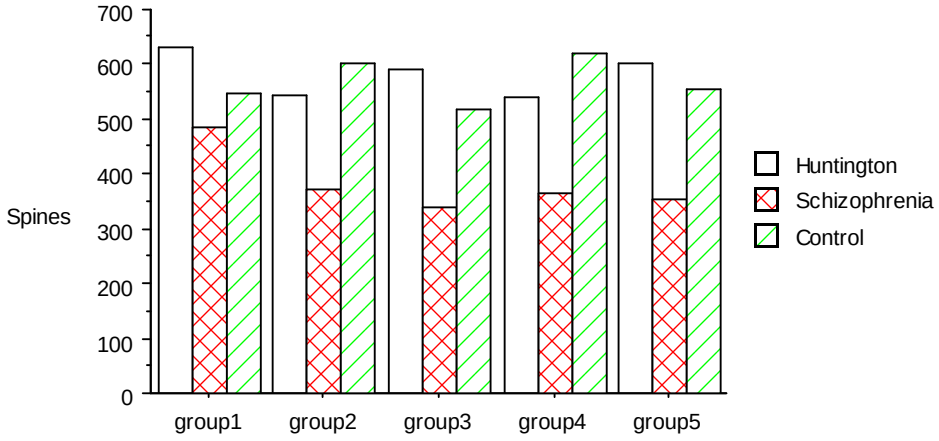

Figure 3.11: Raw data showing mean secondary spines/mm dendrite for the five groups in Layer V Area 9.

DIAGNOSIS	**MEANS** Counts/mm dendrite		**P-value**	
	III	**V**	**III**	**V**
Huntington	557	582	0.16	0.621
Schizophrenia	366 * ±0.39	382 * ±0.58	<0.0001*	<0.0001*
Control	585	567	1.0	1.0

Table 3.5: Spine data showing means and P-value for secondary spines counts/mm dendrite in layers III and V in area 9 (n=5).

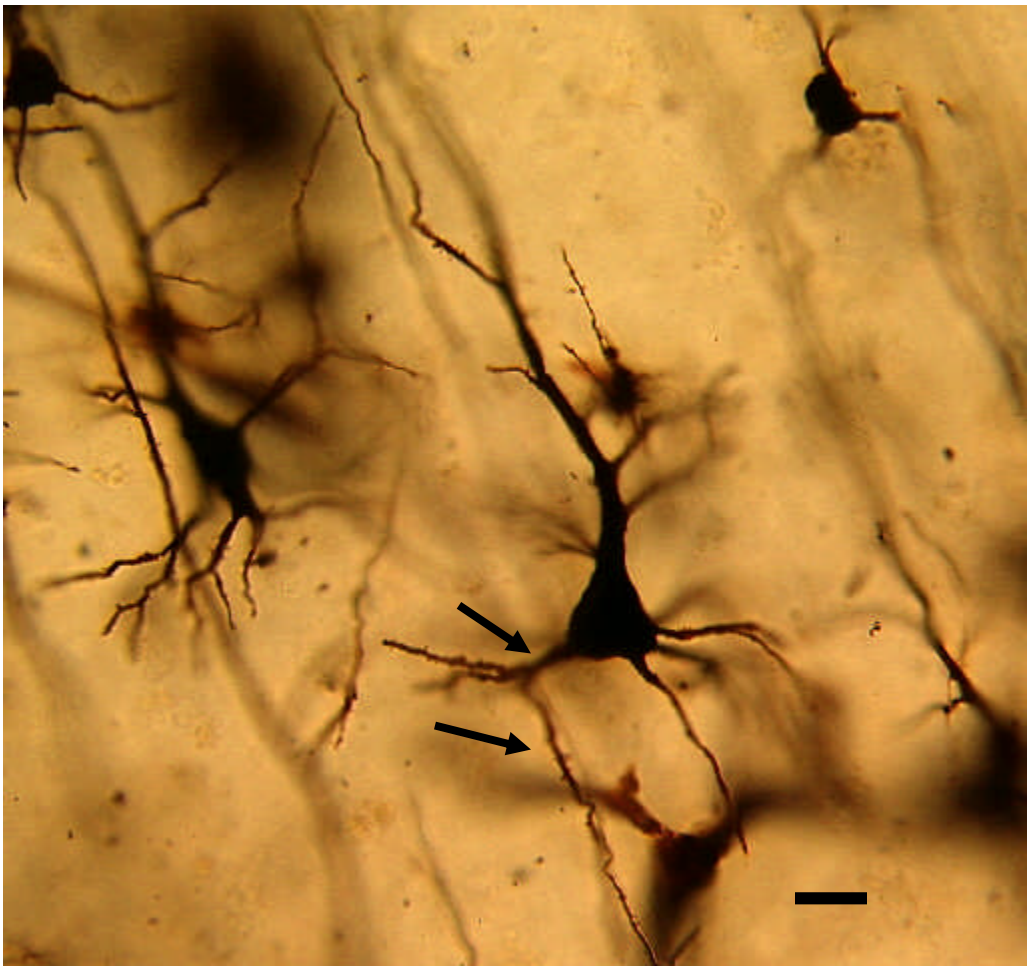

Figure 3.12: Micrograph of a pyramidal cell in Layer III in Area 17 showing primary and secondary dendrites. Scale Bar = 0.01 mm.

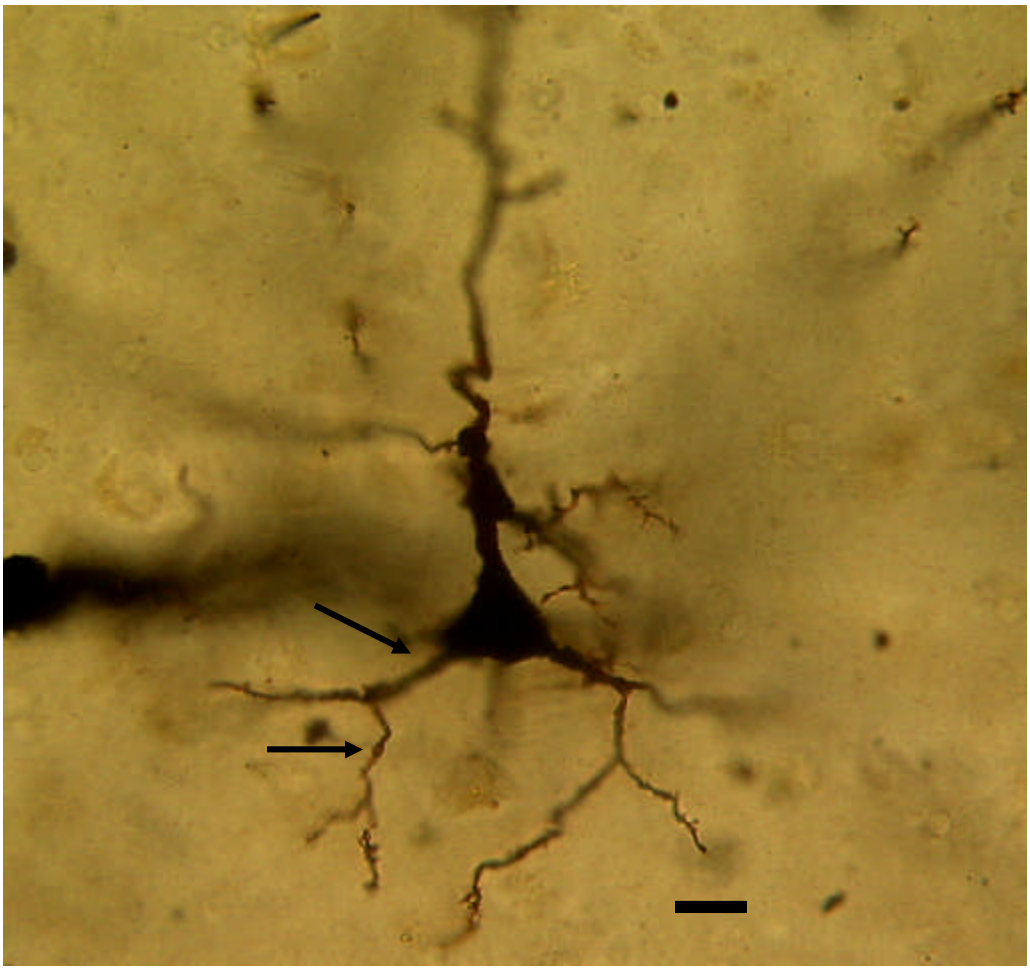

Figure 3.13: Micrograph of a pyramidal cell in Layer V in Area 17showing primary and secondary dendrites. Scale Bar = 0.01 mm.

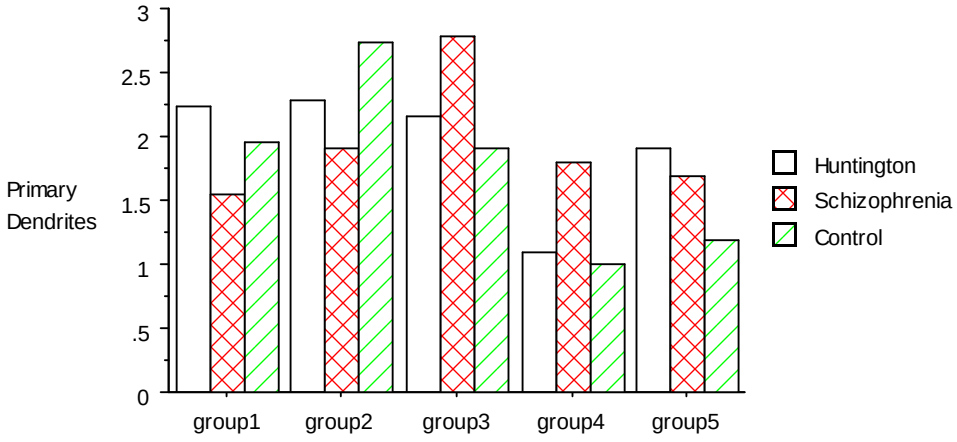

Figure 3.14: Raw data showing primary basilary dendrite for the five groups in layer III in control.

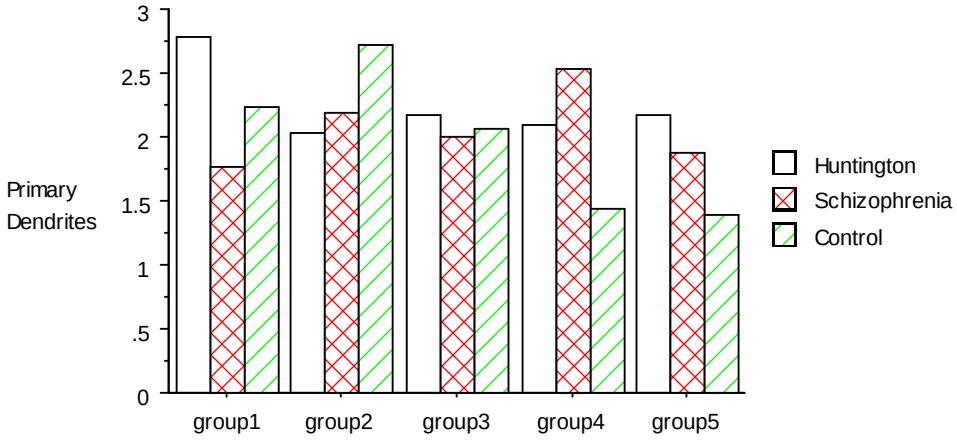

Figure 3.15: Raw data showing primary basilary dendrite for the five groups in layer V in control.

DIAGNOSIS	MEANS		Std. Dev.	
	III	V	III	V
Huntington	1.9	2.0	0.49	0.30
Schizophrenia	1.9	2.1	0.49	0.29
Control	1.8	2.0	0.69	0.56

Table 3.6: Golgi data showing means and standard deviation for primary dendrites in layers III and V in control (n=5).

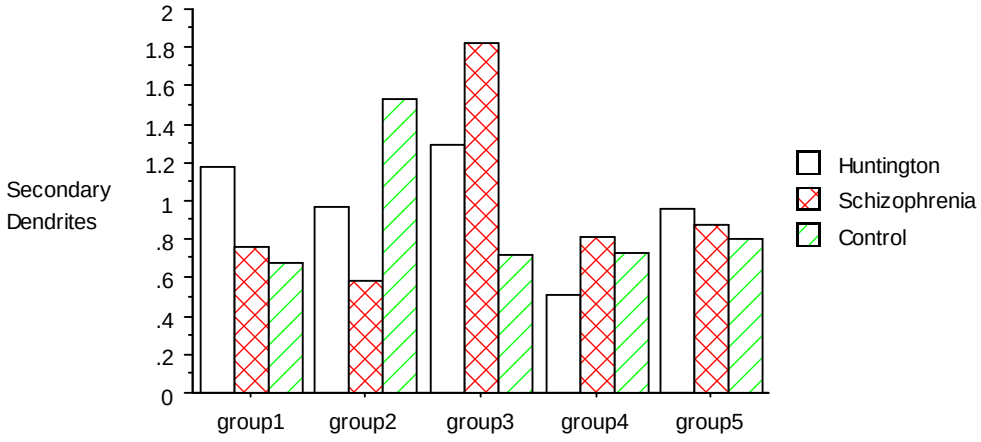

Figure 3.16: Raw data showing secondary basilary dendrite for the five groups in layer III in control.

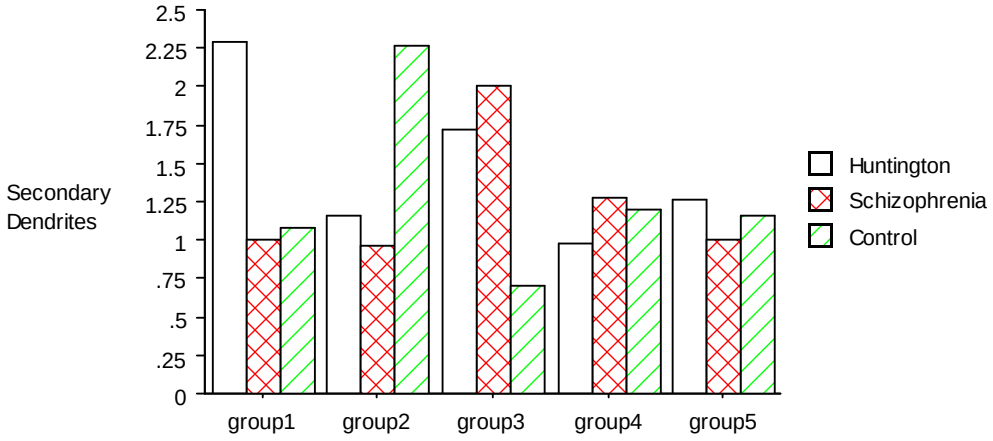

Figure 3.17: Raw data showing secondary basilary dendrite for the five groups in layer V in control.

DIAGNOSIS	MEANS		Std. Dev.	
	III	V	III	V
Huntington	0.98	1.4	0.29	0.52
Schizophrenia	0.97	1.2	0.48	0.43
Control	0.90	1.3	0.36	0.58

Table 3.7: Golgi data showing means and standard deviation for secondary dendrites in layers III and V in control (n=5).

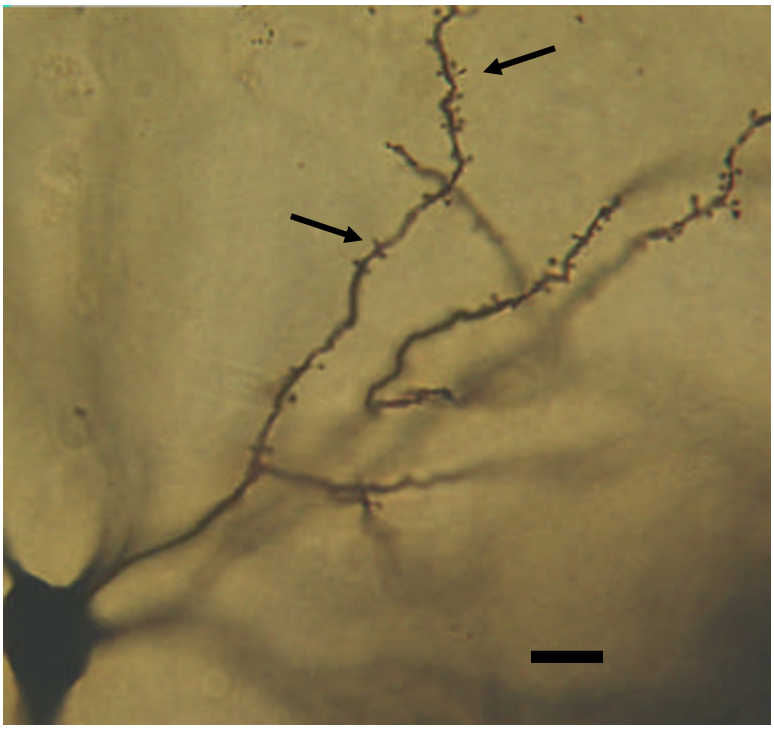

Figure 3.18: Micrograph showing spines on basal dendrites of a pyramidal cell with 100x oil. Scale Bar = 0.01mm.

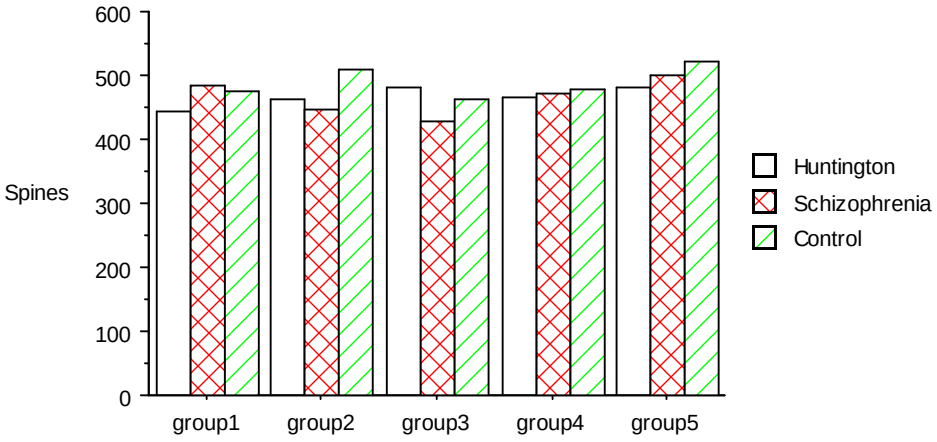

Figure 3.19: Raw data showing primary basilary spine for the five groups in layer III in control.

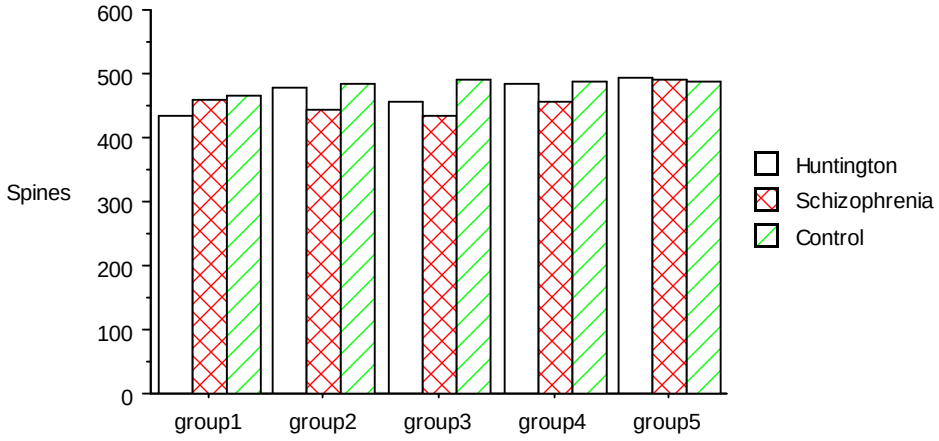

Figure 3.20: Raw data showing primary basilary spine for the five groups in layer V in control.

DIAGNOSIS	MEANS		Std. Dev.	
	III	V	III	V
Huntington	467	468	15	23
Schizophrenia	466	456	29	21
Control	489	483	24	9

Table 3.8: Spine data showing means and standard deviation for primary spine counts/mm dendrite in layers III and V in control (n=5).

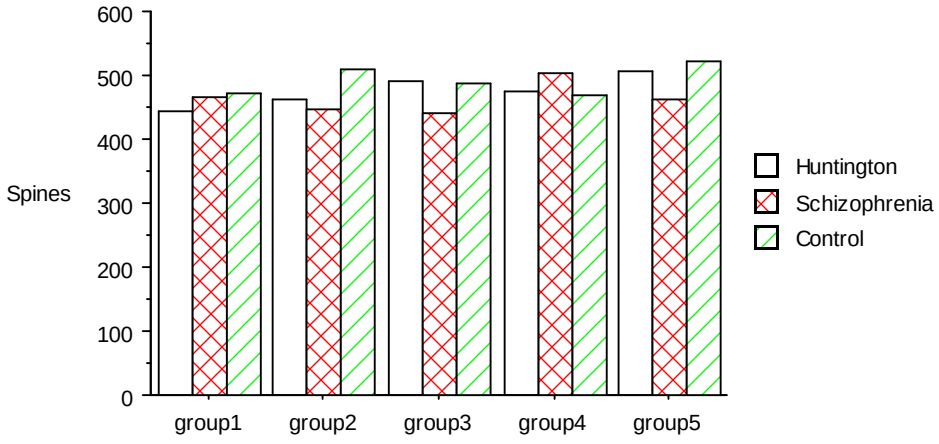

Figure 3.21: Raw data showing secondary basilary spine for the five groups in layer III control.

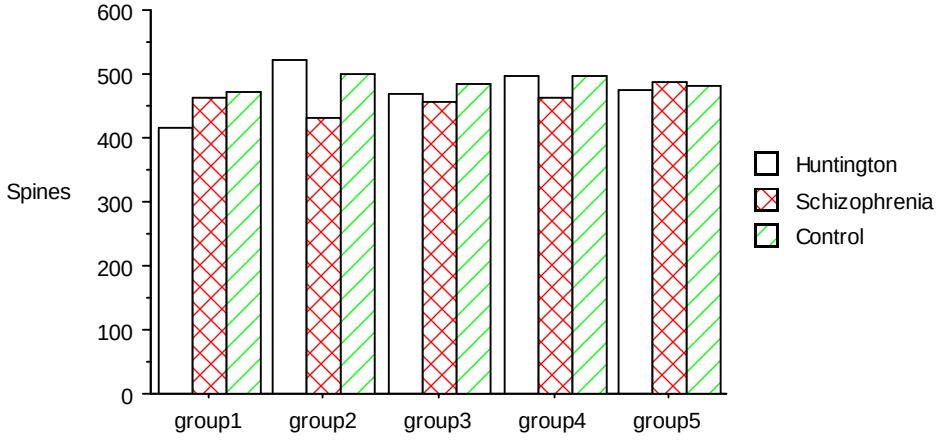

Figure 3.22: Raw data showing secondary basilary spine for the five groups in layer V control.

DIAGNOSIS	MEANS		Std. Dev.	
	III	V	III	V
Huntington	475	475	24	39
Schizophrenia	463	460	23	21
Control	491	486	23	11

Table 3.9: Spine data showing means and standard deviation for secondary spine counts/mm dendrite in layers III and V in control (n=5).

4. Discussion

The primary dendritic data in area 9 in both layers III and V showed a significant decrease in schizophrenia compared to controls; whereas, there was a trend towards a decrease in the secondary basilary dendrites. We believe that these data support what was reported in area 32, where a decrease in primary and secondary basilary dendrites was reported (Jones et al., 2002). Furthermore, this decrease in dendrites directly correlates with the decrease in MAP2. Because MAP2 is involved in the cytoskeletal processes of the cell, a decrease will most likely impact the dendritic processes of the pyramidal cells. The Huntington's brains on the other hand, did not show any significant difference in their dendritic processes compared to the control brains. Similarly, the spine data suggest that there is a schizophrenia-associated decrease in spines in area 9, which support what was reported by Garey et al., (1998) and Glantz and Lewis (2000). There

was also no significant difference in spine counts in the Huntington brains compared to controls. Together, because we are not seeing any difference between Huntington and controls suggest that neuroleptic drugs might not be responsible for the dendritic and spine alterations seen in schizophrenia.

Neuroleptics drugs, because of their mode of action on metabotropic neurotransmitter receptors, can phosphorylate proteins like MAP2 (Lidow 2001). Thus, they have the ability to alter neuronal processes such as dendrites and spines. Both Huntington and Schizophrenic patients take comparable dosages of about 407 chlorpromazine equivalents per day and for at least two years of continuous use, which is consider long term usage (Gur et al., 1998). We believe that this data support the hypothesis that neuroleptic drugs are not responsible for the dendritic and spine alterations seen in schizophrenia and that they are due to the disease.

The loss of dendritic spines on pyramidal cells in schizophrenia has several implications. The pyramidal cells in the PFC are glutamatergic and they project between various cortical regions; whereas, layer V pyramidal cells project to various cortical areas as well as subcortical areas. A loss of spines reflects a loss of excitatory input to these neurons; therefore, it is expected that cognitive information processing might be disturbed as seen in schizophrenia. This is consistent with previous reports of decrease synaptophysin, a 38-kd integral membrane protein of small synaptic vesicles, which is important in calcium-dependent synaptic transmission (Glantz and Lewis 1997). Moreover, the spines of basal dendrites receive both dopamine and glutamate afferents (Smiley et al., 1992); thus, this give credence to possible disturbances in these transmitter systems in schizophrenia. Additionally, the basal dendrites are the site for afferents from the MD nucleus of the thalamus, an area of the brain that has consistently shown neuroanatomical deficits in schizophrenia (Andreasen et al., 1994; Buchsbaum et al., 1996; Byne et al.,

1997, 2001, 2002; Jones 1997, Popken et al., 2000; Young et al., 2000). The data further support the hypothesis that neuroleptic drugs might not be responsible for the decrease in spines seen in schizophrenia since there were no significant differences in the Huntington spine counts and the control spine counts. Moreover, in area 17, there were no changes seen in dendritic or spine measurements in Huntington, schizophrenia compared to control in both layers III or V. Together, these data provide additional insightful information for both Huntington's disease and schizophrenia.

CHAPTER 4

A comparative study of neurogranin alterations in areas 9 and 17 in schizophrenia and Huntington Chorea and the role of neuroleptic exposure

Abstract

The confounding effects of neuroleptic medication on neurochemical studies in schizophrenia are well documented (Harrison 1999a). Neuroleptic drugs by binding to neurotransmitter receptors can activate enzymes which can phosphorylate proteins like MAP2 (Lidow 2001). Recent studies from our lab (submitted for publication) showed a schizophrenia-associated decrease in neurogranin in area 9, a protein found in dendritic and spine compartments in neurons. Neurogranin is involved in the Ca^{++} signaling pathway where it acts as an upstream regulator of calcium by binding calmodulin. Its functional role in this pathway results in the activation of several enzymes which are involved in longterm potentiation (LTP) and longterm depression (LTD) (Gerendasy and Sutcliffe 1997). This study employed immunohistochemistry to analyze three cohorts of brains Huntington, schizophrenia and controls to determine if neuroleptic drugs can cause the observed changes in previous neurogranin studies in schizophrenia. Huntington patients take neuroleptics similar to schizophrenics; therefore, by comparing the two we will be able to determine if neuroleptics play a role. The data showed a schizophrenia-associated decrease in neurogranin compared to controls. The Huntington brains showed no significant difference in neurogranin compared to controls. In area 17 the data showed no significant differences when comparing the groups Huntington, schizophrenia and control. The data suggest that neuroleptic drugs are not probably playing a role in the schizophrenia-associated decrease in neurogranin.

1. Introduction

The reduced neuropil hypothesis of schizophrenia postulated by Selemon and Goldman-Rakic (1999) has triggered an array of studies examining the structural elements of the pyramidal cells in the PFC. Previously we showed a schizophrenia-associated decrease in dendrites and spines in schizophrenics, which we believe are not an effect of neuroleptic drug treatment. Additionally, a recent study from our lab had suggested a loss of basilary dendrites in areas 9 and 32 of the PFC (Broadbelt et al., 2000); whereas, several studies had consistently shown a schizophrenia-associated spine decrease (Garey et al., 1998 and Glantz and Lewis 2000) of pyramidal cells in the PFC. It is known that spine morphology is intimately mediated by glutamatergic neurotransmission and the Ca^{++} signaling pathway. Neurogranin is a protein that is involved in this pathway. Like MAP2 it is localized to the cell body, dendrite and spine compartments of neurons. Neurogranin is a calmodulin (CaM) binding protein except when it is phosphorylated by protein kinase C (PKC). Functionally, neurogranin acts as an upstream regulator of calcium, by binding calmodulin. Release of CaM to bind calcium (CaM/Ca^{++}) results in the activation of several enzymes which are involved in LTP and LTD. Additionally, neurogranin expression has been shown to coincide with spine density in synaptogenesis suggesting that it may play a role in spine formation (For review see Gerendasy and Sutcliffe 1997). Moreover, CaMKinase II activation which is involved in LTP can regulate the expression of proteins such as MAP2 (For review see Gerendasy and Sutcliffe 1997). This suggests therefore, that decreases in neurogranin may result in both structural and functional changes in neurons which can impact both dendritic structure and spine morphology. Preliminary studies by Dr. Jones suggest a significant decrease in neurogranin expression in the prefrontal cortex in schizophrenia (personal communication). However, it is not known if these changes are due to the disease or to

neuroleptic exposure. This study will attempt to determine if neuroleptic drugs can cause the decreases in neurogranin we reported in schizophrenia. Neuroleptics by binding to receptors have the ability to activate phosphatases which can phosphorylate proteins like MAP2 (Lidow 2001). MAP2 phosphorylation will consequently result in cytoskeletal changes in neurons affecting dendritic and spine morphology. Neurogranin because it is localized in the dendritic and spine compartments of neurons, can therefore be affected. This study employs neurogranin immunocytochemistry to analyze layers III and V in area 9 in a cohort of Huntington brains and compare it to that of a cohort of schizophrenics and controls. Studies into possible alterations in the expression of neurogranin by neuroleptic drugs will add to the understanding of the growing body of data that suggests structural alterations of pyramidal cells in the prefrontal cortex.

2. Methods

2.1. Subjects and tissue

Postmortem brain tissues from five subjects diagnosed with Huntington Chorea, five subjects diagnosed with schizophrenia and five controls were collected from Harvard Brain Tissue Resource Center (HBTRC) at McLean Hospital (Table 4.1). The three groups of tissues were all matched for age, sex and postmortem interval. All tissues were collected at the time of autopsy in accordance with an approved Institutional Review Board protocol. Diagnoses confirmed to DSM-IV criteria and were established retrospectively by reviewing medical records and interviewing knowledgeable individuals. Tissue was not included in the study if there was evidence of neuropathology (e.g. Alzheimer's disease) or substance dependence as determined by the HBTRC. The brain bank coded the tissues and the codes were maintained by one of the authors who did not conduct quantitative assessment (L.J.) until quantitative procedures had been completed.

Blocks of 1-cm thick sections were dissected in the coronal plane by a qualified neuropathologist at the HI from its dorsolateral extent, which is founded on the middle third of the superior frontal gyrus (Rajowska and Goldman-Rakic, 1995a). Compared with the medial region, the lateral region of area 9 is more differentiated. In addition to having a distinguishable layer IV, it has a Layer II with a distinct upper margin and the supragranular cell density is conspicuously higher than the infragranular cell density (Barbas and Pandya, 1989).

Inclusion criteria: Brains were not selected on a racial basis. Approximately equal numbers of male and female brains were obtained. Schizophrenics and Huntington patients had a DSM IV diagnosis and range in age from 34 to 80 years of age. Nonpsychiatric controls came from nursing homes and hospitals, had no psychiatric diagnosis established by the DEAD scale. Controls from nursing homes had resided at the home for at least five years prior to death.

Exclusion criteria: No brains with evidence of traumatic injury, infarction or Alzheimer's type changes were accepted into the study.

2.2. Immunocytochemistry for Neurogranin

A free-floating method of immunocytochemistry was employed to maximize impregnation and exposure to the antibody. Appropriate sections were removed from cryoprotectant, washed in phosphate buffered saline (PBS), and pretreated with 0.3% H_2O_2 for ten minutes to remove any endogenous peroxidases. After pretreatment, sections were then washed in PBS and incubated in 4% instant milk in PBS (BLOTTO) with 0.2% TritonX-100 for 1 hour. Sections were then incubated in neurogranin (Chemicon) diluted 1:500 in BLOTTO overnight at room temperature on an orbital shaker. The following day sections were washed in PBS 3 times for 10 minutes. After the final wash, the sections were incubated in a biotinylated anti-mouse secondary (Vector Laboratories) at 1:100 for 1

hour followed by three 10 minutes washes. Sections were then incubated in HRP-avidin/biotin complex according to the manufacturer's protocol followed by a standard diaminobenzadine reaction to view the immunohistochemical staining. All incubations were done in 12 well plates. Sections were mounted on to glass slides and after drying they were dehydrated in graded alcohol starting at 70%, defatted in xylene and coverslipped with permount solution.

3.3. Quantification of Neurogranin

Neurogranin Immunostaining for layers III and V was quantified by area fraction analysis. Thionin sections were employed to identify layers III and V. Within a defined sampling box, area fraction refers to the ratio of the area occupied by MAP2-positive profiles vs. the total area of the box. Area fraction was measured from sections using a Bioquant Image Analysis system interfaced with an Olympus AX70 microscope and a Sony 3-chip color camera which relayed the microscopic images to a video monitor. The sampling box was a square with each side equal to the width of the lamina. Bioquant software was employed to select pixels within the sampling box that matched threshold criteria for the neurogranin positive profiles set by the investigator. Threshold was set at the level that selected the lightest stained cell bodies and dendrites without selecting background staining. The setting for illumination was kept constant throughout the analysis. The computer then outlined and summed the thresholded areas and determined the ratio of stained area to the total area of the sampling box. Every section stained with neurogranin was used in the data analysis. An average of eight sections per brain was measured. For each section a total of eight measurements were made for both layer III and V. The measurements were made along the straight sides of a gyrus so as to have as little distortion of the lamina as possible. The eight sampling boxes were taken

consecutively as long as there were no histological artifacts in the tissue and the lamina could be determined. If there were artifacts in the tissue, then the box was placed at the next straight edge after the artifact.

Due to variation in the thickness of the original tissue slab the number of sections available for analysis varied among specimens. All sections, however, were used in the data analysis. Sixteen sampling boxes were examined per section (8 within layer III and 8 within layer V). Area fraction was averaged for all sampling boxes within a particular lamina for each specimen block.

2.4. Statistical analysis

Standard descriptive statistics were used in all analysis. Calculations and data analysis were carried out using StatView (V5.0) for Windows (Abacus Concepts Software/SAS). Neurogranin data were taken from layers III and V of areas 9 and 17 and were expressed as mean for each group, Huntington, schizophrenia and control. A one-way analysis of variance (ANOVA) was used to test for differences between groups at 95 % confidence limit. Fisher's post-hoc tests were used to compare the 3 groups. All of the tissues were prepared and analyzed in matched groups; therefore, we believe that the ANOVA is a more sensitive test for intergroup comparison. Additionally, a power analysis of the spine data showed that a minimum sample size of five was enough to yield levels of significance with a 95 % confidence limit (See Figure 4.1). Similar studies by Garey et al., (1998) for spine density measurements used a sample size of four schizophrenics and a MAP2 study by Arnold et al., (1991) used only six brains.

Sample Size

Difference in Means	Error Std Dev	Alpha
214	40	0.050

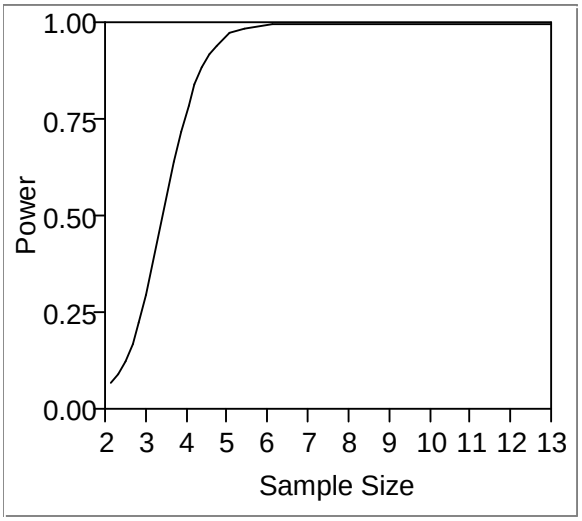

Figure 4.1: Power Analysis for Schizophrenia vs Control of Primary Spines of Layer III

Brain #	*Distributive Diagnosis*	*Medication*	*Age*	*Sex*	*PMI*	*Storage Time*
5039	Huntington	Haldol (5 mg)	54	F	14	29 months

5662	Schizophrenia	Thioridazine (100mg)	52	F	16	15 months
5734	Control	None	NR	NR	NR	NR
5344	Huntington	No med. report Grade 4*	69	F	26	17 months
5656	Schizophrenia	No psych. Med.	73	F	29	15 months
5805	Control	None	NR	NR	NR	NR
5464	Huntington	No med. report Grade 4	61	M	21	20 months
5785	Schizophrenia	Depakote(750 mg), Zyprexa (5 mg), Neurontin (200 mg)	63	M	15	9 months
5715	Control	None	NR	NR	NR	NR
2161	Huntington	Haldol (10 mg)	59	F	14	109 months
4942	Schizophrenia	Zypiexa (15 mg), Luvox (50 mg), Humibid (1200 mg)	61	F	14	16 months
4810	Control	None	62	M	16	12 months
4292	Huntington	No med. report Grade 4	56	M	5	38 months
3013	Schizophrenia	Chlorpromazine (100 mg), Fluphenzarine (10 mg),	62	M	6	86 months
4932	Control	None	67	M	22	8 months

Table 4.1: Demographic data for the matched brains used in the neurogranin project.

NR = No medical report

***** All Grade 4 Huntington brains had both behavioral and movement disorders, therefore they took neuroleptics at some point in their life.

3. Results

3.1 Neurogranin in area 9

Area fraction means for neurogranin were collected from five Huntington, five schizophrenics and five controls in both layers III and V in area 9 (Figure 4.3 & 4.4). The data show a significant schizophrenia-associated decrease in neurogranin. Compared to control there were a 35% (P-value 0.015, SD ± 0.015) decrease in neurogranin in layer III and 33% decrease (P-value 0.01, SD ± 0.015) in layer V in schizophrenia. The Huntington's brains showed a trend toward an increase in both layers when compared to controls; however, it was not significant (Table 4.2).

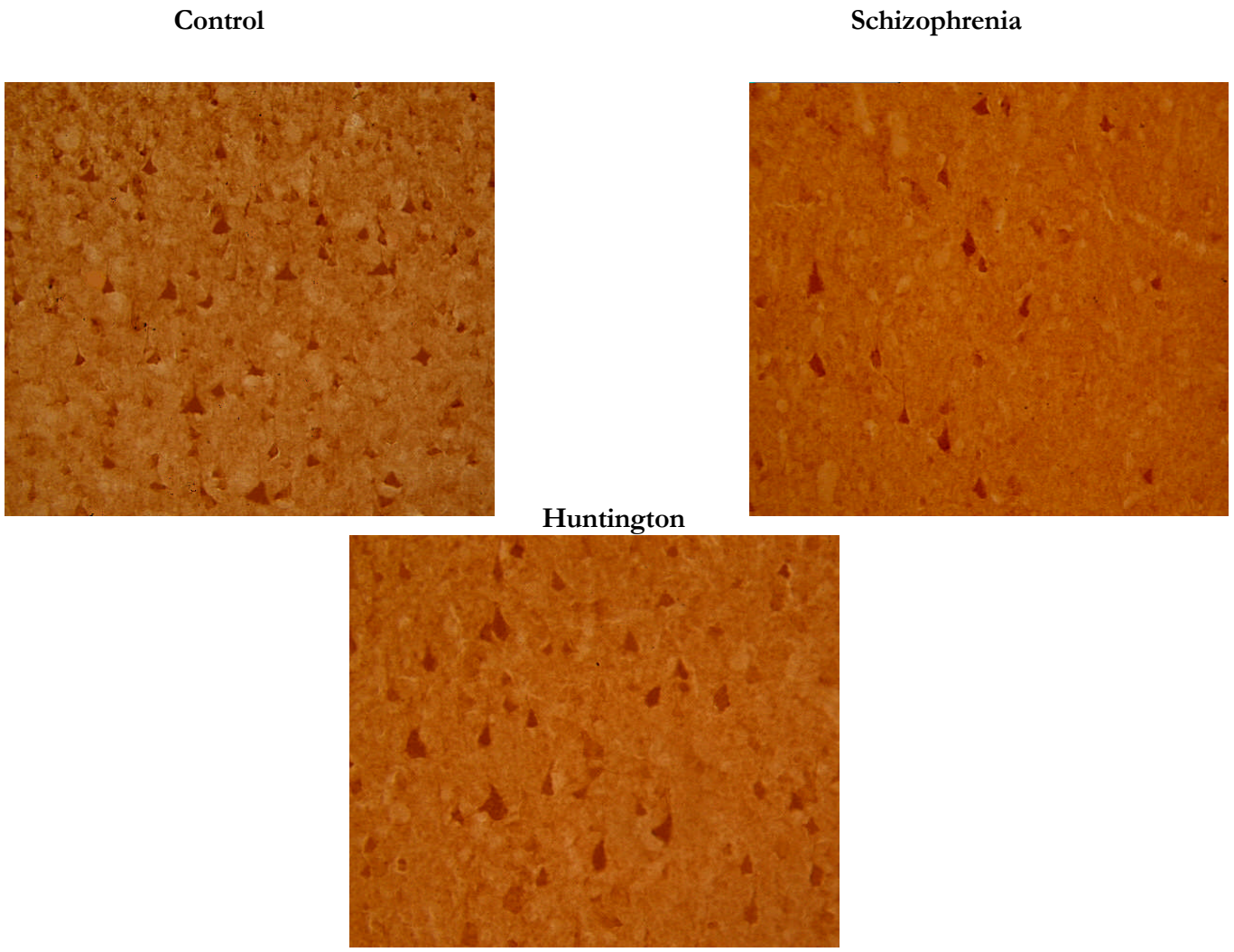

Figure 4.2: Photomicrographs showing neurogranin staining in Layer III Area 9 (20X).

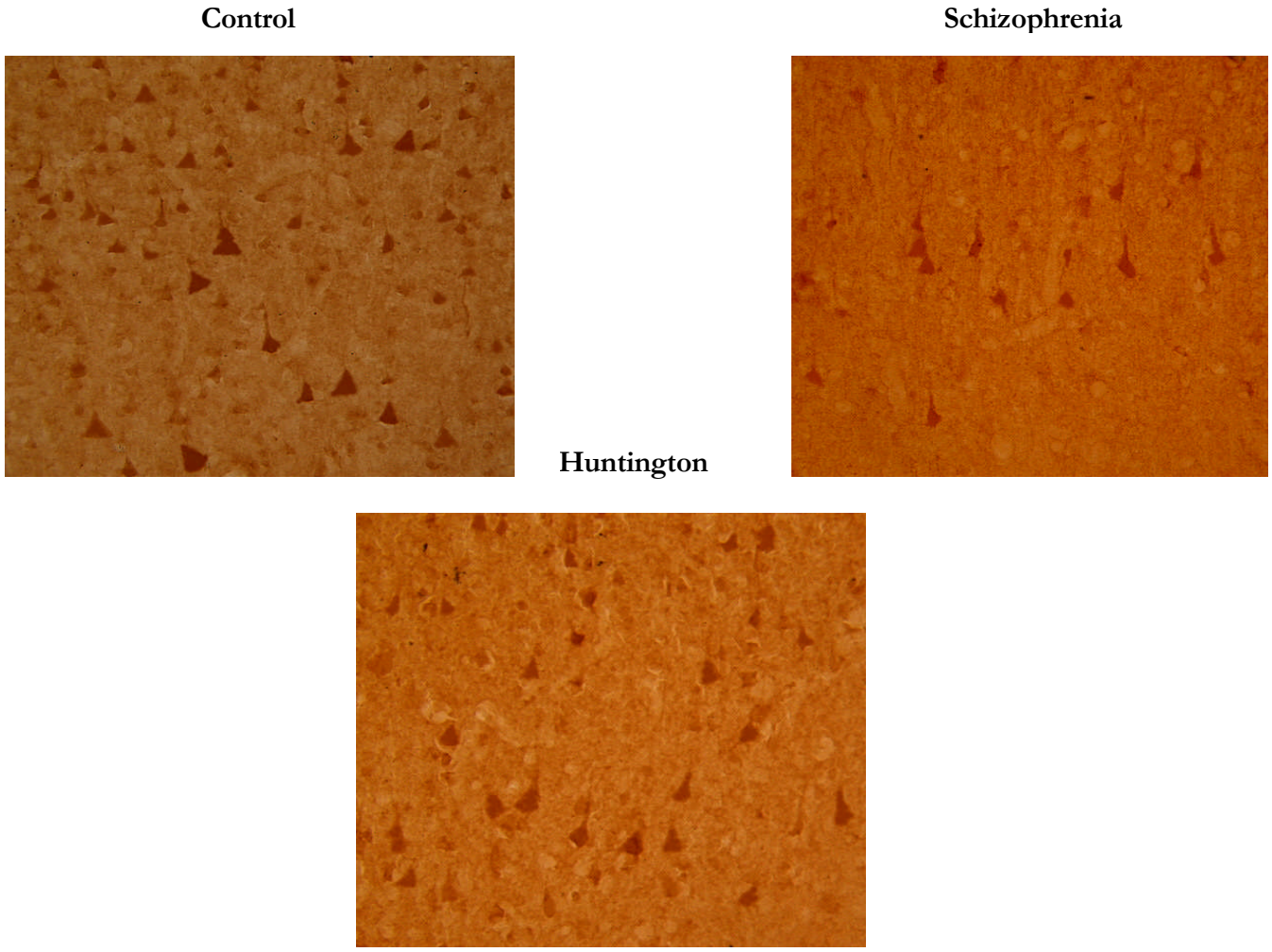

Figure 4.3: Photomicrographs showing neurogranin staining in Layer V Area 9 (20X)

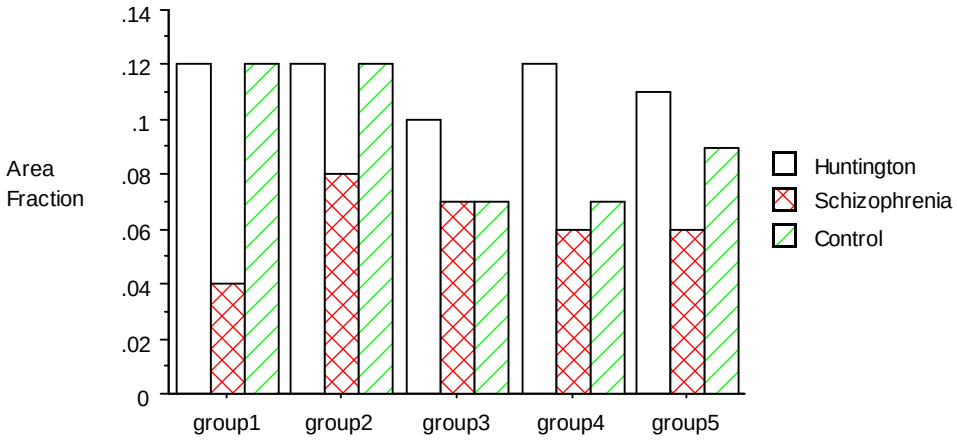

Figure 4.4: Raw data showing neurogranin area fraction means comparing the five groups in Area 9 Layer III.

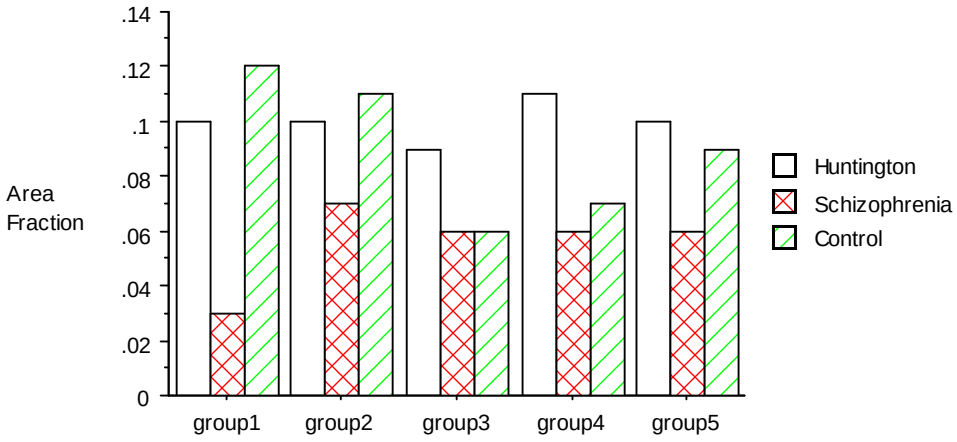

Figure 4.5: Raw data showing neurogranin area fraction means comparing the five groups in Area 9 Layer V.

DIAGNOSIS	MEANS		P-value	
	III	V	III	V
Huntington	0.114	0.1	0.098	0.39
Schizophrenia	0.062 * ± 0.015	0.06 * ± 0.015	0.014 *	0.01 *
Control	0.094	0.09	1.0	1.0

Table 4.2: Neurogranin area fraction showing means and P-values for layers III and V in area 9 (n=5)

3.2 Neurogranin in control.

Neurogranin area fraction means for the five groups of brains in area 17 layers III and V showed no significant difference when comparing Huntington, schizophrenia and control (Table 4.3).

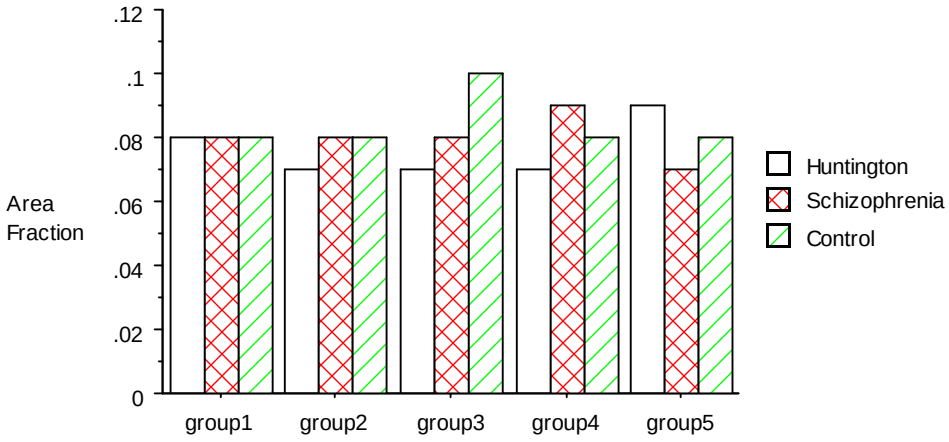

Figure 4.6: Raw data showing neurogranin area fraction means comparing the five groups in Layer III in control.

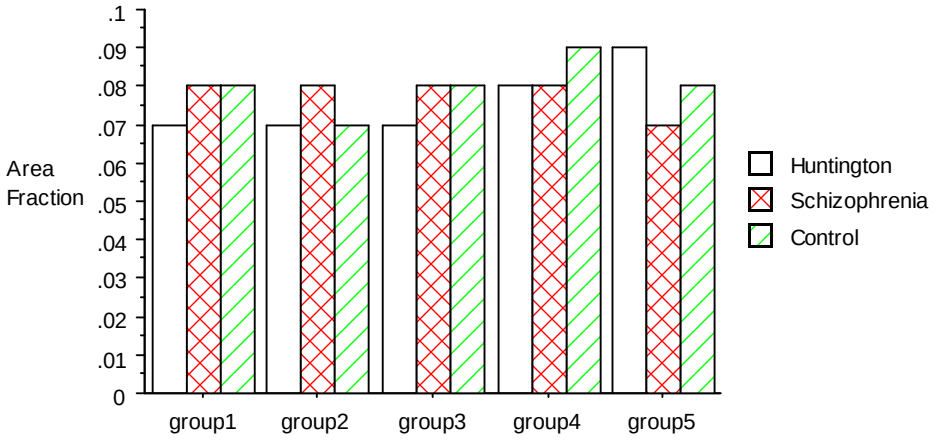

Figure 4.7 : Raw data showing neurogranin area fraction means comparing the five groups in Layer V in control.

DIAGNOSIS	**MEANS**		**Std. Dev.**		**P-value**	
	III	**V**	**III**	**V**	**III**	**V**
Huntington	0.076	0.076	0.009	0.009	0.15	0.39
Schizophrenia	0.08	0.078	0.007	0.004	0.46	0.66
Control	0.08	0.08	0.009	0.007	1.0	1.0

Table 4.3: Neurogranin area fraction showing means, standard deviation and P-value for layers III and V in control (n=5).

4. Discussion

The present data shows a schizophrenia-associated decrease in neurogranin in both layers III and V as compared to controls which confirms previous findings in our laboratory. On the other hand, when comparing Huntington Chorea to controls there were no significant differences seen in both layers III and V in area 9. Because there was a significant decrease in neurogranin in the schizophrenic brains and not in the Huntington's brains, suggest that this decrease seen in schizophrenia is probably disease specific and not and effect of neuroleptic drugs. We believe that because both patient groups show longterm exposure to antipsychotic medication, that the drug would affect both groups the same way. This is not the case in this study, only the schizophrenics show a significant reduction in neurogranin. This schizophrenia-associated decrease in neurogranin has several implications. First, because neurogranin is localized in the dendritic compartments and is concentrated in dendritic spines, suggest detrimental effects on dendritic spine formation and synaptogenesis; thus; there may be neurodevelopmental implications as was hypothesized in schizophrenia. Secondly, a loss of neurogranin could alter the calcium-calmodulin signal transduction pathway by allowing excess calmodulin to be available for binding by free calcium. Therefore, CaM/Ca^{++} activation of enzymes like CaMKinase II in the signaling pathway will be inappropriately regulated. Activation of this pathway incorrectly will affect how information is processed by the pyramidal cells which could lead to disturbances in behavior as seen in schizophrenia. Lastly, CaMkinase II can regulate the expression of MAP2. Inappropriate regulation of MAP2 will affect microtubule polymerization and as such dendritic growth and stabilization. Both MAP2 and dendritic deficits of pyramidal cells in the PFC have been reported in schizophrenia. It is fair to warn however, that the order of events is difficult to determine whether decreased neurogranin leads to dendritic and or spine loss or alternatively, decreased dendrites and or spines

cause a loss of neurogranin. Moreover, in area 17 there were no differences seen in dendritic or spine measurements in Huntington, schizophrenia or controls in both layers III or V. This further support our hypothesis that neuroleptic drugs are not responsible for the morphological changes observed in the PFC. Together these results provide further insightful information for both schizophrenia and Huntington Chorea.

CHAPTER 5

Conclusion

There is consensual support that area 9 of the PFC is a major site perturbed in schizophrenia. Functionally it is an area involved in cognitive tasks, such as working memory, which has been shown to be disturbed in schizophrenia. Both imaging and morphological studies suggest deficits in the ultrastructure of the PFC (Weinberger et al.,1986; Buchsbaum et al., 1990; Benes 1991; 1993; Pakkenberg 1993; Goldman-Rakic and Selemon 1995, 1997; Beasley et al., 1997, Glantz and Lewis 1997, Garey et al., 1998; Harrison 1999; Selemon and Goldman-Rakic 1999;; Buxhoeveden et al., 2000; Glantz et al 2000; Andreasen 2000; Thune et al., 2001; 2001; Jones et al., 2002; Broadbelt et al., 2002). Additionally; the PFC is reciprocally connected to the MD nucleus of the thalamus, an area that has consistently shown to be disturbed in schizophrenia (Andreasen et al., 1994; Buchsbaum et al., 1996; Byne et al., 1997, 2001, 2002; Jones 1997, Popken et al., 2000; Young et al., 2000; Brickman et al., 2004). Recently, studies from our lab showed a schizophrenia-associated decrease in MAP2 in both areas 9 and 32 (Jones 2002) and a decrease in basilary dendrites in area 32 (Broadbelt 2002). A contentious issue surrounding these deficits in the PFC is the effects of neuroleptic drugs. The confounding effects of neuroleptic medication on neurochemical investigations are well known (Harrison 1999a). Some reports suggest a correlation of antipsychotic dosage and increased brain atrophy (Madsen et al., 1998) and decreased thalamic volume (Gur et al., 1998). These macroscopic studies suggest that neuroleptic exposure is a potential confounding variable in most morphological and neurochemical findings reported in schizophrenia.

Several studies have suggested that long-term treatment with antipsychotics might cause the morphological changes observed in schizophrenia (Benes et al., 1985; Klinzova et al., 1989; Meshul et al., 1992). Although these studies were done in rodents with normal brains, together the data provide good evidence that chronic antipsychotic treatment induces synaptic plasticity and alters the synaptic ultrastructure. Because neuroleptics are known to bind dopamine, serotonin and glutamate receptors, they have the ability to regulate phosphatases via second messenger systems. This in turn can result in the phosphorylation of many proteins like MAP2 (Lidow 2001). MAP2 being an important protein in the cytoskeletal scaffolding of neurons, would affect normal dendritic, spine and synapse formation. Thus, several researchers have suggested that neuroleptic drugs can cause the morphological changes observed in schizophrenia (Harrison et al., 2000). Thus, we believed that a detailed analysis of the pyramidal cells and its structural components in area 9 was warranted to identify the structures compromised and what role neuroleptic drugs play if any.

The pyramidal cells are the main projection neurons ipsilaterally and contralaterally as well as to subcortical structures; therefore, they play an important role in information processing. Our pyramidal cell density measurements showed no significant difference when comparing Huntington, schizophrenia and control in both areas 9 and 17. This further corroborates what was reported previously by our lab and other researchers and because we see no significant differences between the groups (Huntington, schizophrenia and control) the data suggests that there is no loss of pyramidal cells in both layers III and V in areas 9 and 17. However, there was a small increase in pyramidal cell density in layers III and V in the schizophrenic group in area 9. This is similar to what was reported by Selemon (1995). Not seeing any loss of pyramidal cell does not imply that there are not changes in non-pyramidal cells which make up about 25% of the neurons in the PFC. We

did total neuronal density measurements in areas 9 and 17 to see if there are changes in the total neuronal density. Our data in both areas 9 and 17 showed no significant changes in total neuronal density in both layers III and V. However, these cell density measurements are not designed to detect small changes in cell density which might be a possibility in schizophrenia. Additionally, because there are no changes in cell density does not suggest that there are not changes in the structural components of the pyramidal cells which are the focus of this study.

Previously our lab reported a significant decrease in MAP2, a protein that is a marker for dendritic lesions in neurons, in areas 9 and 32 of the PFC in schizophrenia (Jones 2002). In the present study we analyzed eight matched groups (Huntington, schizophrenia and control) of brains to determine if neuroleptic drugs can cause the changes observed in our previous study. This study also showed a significant decrease in MAP2 in schizophrenia in both layers III and V in area 9. There were no decreases seen in area 17 for both layers. In the Huntington brains, layer III showed a trend toward a decrease; however, it was not significant. In layer V there was a significant decrease in the Huntington's brains compared to controls. We believe that these changes are probably not an effect of neuroleptic exposure since no changes were seen in Area 17. In the Huntington's brains this is supported by the fact that MAP2 and the Huntingtin protein are closely associated with microtubules, thus possible atrophic interactions. Additionally, some layer V pyramidal cells project to subcortical areas such as the striatum where there is gross loss of neurons in Huntington's disease (Vonsattel et al., 1985).

In schizophrenia, there are several ways to explain the decrease in MAP2. It can be caused by a decrease in dendritic elements which contain the protein or it can be caused by a decrease in the expression of the protein or both. Because we see a decrease in both dendrites and MAP2 suggest that it is probably due to a loss of dendrites.

MAP2 is protein that promotes microtubule polymerization and bundling which forms the cytoskeletal processes of the cell. Therefore, a decrease of this protein would suggest abnormal dendritic processes as seen in our recent study in area 32. We therefore decided to test this hypothesis in area 9 of the PFC.

The dendritic tree of a neuron functions as the scaffolding for synapses. We now know that about 95% of a cell synapses are on the dendrites (Levitt et al., 1993), and the dendritic spines are the location of about 90% of the excitatory synapses (Levitt et al., 1993). Therefore, analyzing dendrites and spines provide a good measure for synaptic density and neuronal connectivity. The present study showed a significant decrease in primary and secondary basal dendrites in area 9, layers III and V, in Schizophrenia compared to controls. This corroborates what our lab had previously reported in area 32. Additionally, this study showed no significant difference in primary and secondary basal dendrites in the Huntington's brains compared to controls in area 9. Suggesting, that the loss of dendrites in area 9 in schizophrenia is real and not an effect of neuroleptic exposure. Similarly, like the MAP2 study we did not see any difference in primary and secondary basal dendrites in the groups (Huntington, schizophrenia and control) in area 17. Spine counts were done on the primary and secondary basal dendrites in both layer III and V in areas 9 and 17. We saw a significant decrease in spines in area 9 on both the primary and secondary basal dendrites in schizophrenia compared to controls. There was no significant loss of spines in the Huntington's brains compared to controls. In area 17, we saw no significant difference in spine counts on the basal dendrites in both layers. The loss of spines was seen only in schizophrenia and not Huntington, strongly suggesting that this is due to the disease process and not an effect of neuroleptic drug exposure. In addition, we believe that there is a relationship between the loss of dendrites and spines, and the loss of MAP2. Moreover, a loss of dendrites and spines on these pyramidal cells

suggest a loss of excitatory input to the cell and information processing causing the type of problems in working memory as seen in schizophrenia.

It is known that spine morphology is intimately mediated by glutamatergic neurotransmission and the Ca^{++} signaling pathway. Neurogranin is a protein that is involved in this pathway. Like MAP2 it is localized to the cell body, dendrite and spine compartments of neurons. Previously our lab showed that there is a significant decrease of neurogranin in schizophrenia. Neurogranin because of its role in the Ca^{++} signaling pathway makes it an important link as a functional protein that can effect the structural organization of spines. In this study, we also showed a significant decrease in neurogranin in schizophrenia in area 9 in both layers III and V. The Huntington's brains on the other hand, showed no significant differences compared to controls. Suggesting again, that these changes seen in schizophrenia are real and not an effect of neuroleptic exposure. This is further supported by the area 17 data, where no differences were seen when comparing Huntington, schizophrenia and controls.

In summary, this study was designed to determine if microscopic changes observed in schizophrenia is due neuroleptic drugs exposure or to neuropsychiatric diseases. We have analyzed and compared cohorts of Huntington, schizophrenia and controls in areas 9 and 17. We have analyzed and compared pyramidal cell density, total neuronal density, dendrites, spines, MAP2 and neurogranin. The data showed significant decreases in dendrites, spines, MAP2 and neurogranin in schizophrenia in area 9. No significant differences were seen when comparing density measurements for pyramidal cells and total neuronal cells in area 9. In Huntington Chorea there were no significant differences for any of the above parameters measured except MAP2. Similarly, in area 17 there were no significant differences for all the parameters in both Huntington and schizophrenia compared to controls. Both schizophrenics and Huntington's patients had long term exposure to neuroleptic medication. Because all of the tissues in this study were

matched for age, sex and postmortem interval, we believe that neuroleptic drugs will have similar effects on both groups. In this study this is not the case for the Neurogranin, dendrites and spines where a decrease was seen in schizophrenia and not Huntington Chorea. Therefore, we believe that these decreases seen in schizophrenia are most likely due to the disease and not an effect of neuroleptic exposure. Furthermore, we believe that these results are compelling and further support the reduced neuropil hypothesis of regional cortical pathology in schizophrenia (Figure 6.1). That there is an increase in the packing density of the neurons and a loss of neuropil structures like dendrites and spines. Together, these results provide further insightful information for both schizophrenia and Huntington Chorea.

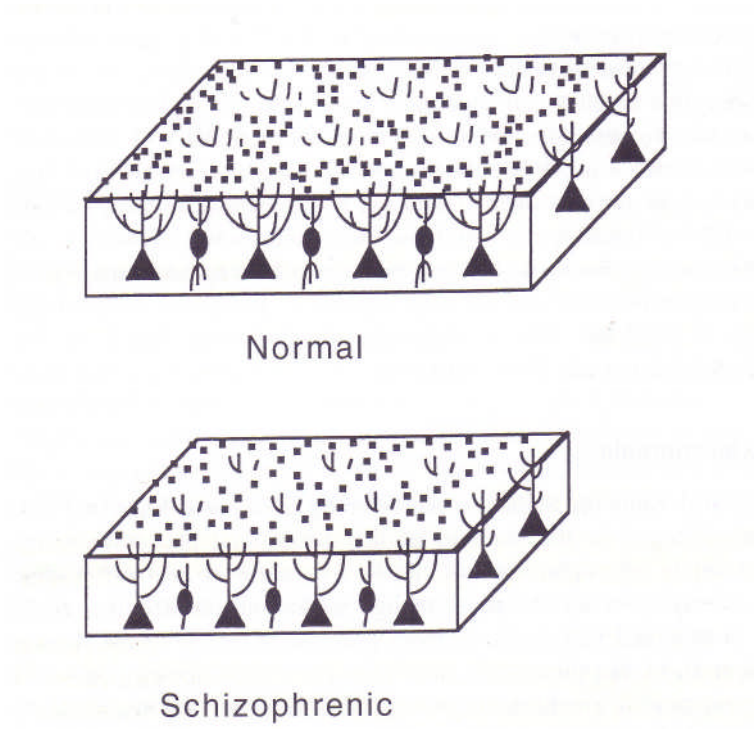

Figure 6.1: A diagram depicting the 'reduced neuropil' hypothesis of schizophrenia. The diagram shows a block of normal (upper) and schizophrenia (lower) dorsolateral prefrontal cortex. (Selemon and Goldman-Rakic, 1999)

Literature Cited:

Akbarian S, Kim JJ, Tafazzolli A, Hagman JO, Potkin SG, Bunney WE Jr., Jones EG (1993b) Distorted distribution of nicotinamide-adenine dinucleotide phosphate-diphorase neurons in temporal lobe of schizophrenics implies anomalous cortical development. Archives of Gen Psychiatry 50:178-187.

Akbarian S, Sucher NJ, Bradley D, Tafazzolli A, Trinh D, Hetrick WP, Potkin SG, Sandman CA, Bunney WE, Jones EG (1994) Selective alterations in gene expression NMDA receptor subunits in prefrontal cortex of schizophrenics. J of Neuroscience 16:19-30.

Alvarez-Bolado G, Rodrigues-Sanchez P, Tejero-Diez P, Fairen A, Diez-Guerra F (1996) Neurogranin in the development of the rat telencephalon. Neuroscience 73:565-580.

Andreasen NC, Ehrhardt JC, Swayze VW, Alliger RJ, Yuh WTC, Cohen G, Ziebell S (1990) MRI of the brain in schizophrenia: The pathological significance of structural abnormalities. Arch Gen Psychiatry 47:35-44.

Andreasen NC, Swayze N, Flaum M, Yates WR, Amdt S, McChesney C (1990) Ventricular enlargement in schizophrenia evaluated with CT scanning: effects of gender, age and stage of illness. Arch Gen Psychiatry 47:1054-9.

Andreasen NC, Rezai K, Alliger R (1992) Hypofrontality in neuroleptic-naïve and in patients with chronic schizophrenia: assessment with xenon-133 single-photon emission computed tomography and the Tower of London. Archives of General Psychiatry 49:943-958.

Andreasen NC, Flashman L, Flaum M, Arndt S, Swayze V, O'Leary DS, Ehrhardt JC, Yuh WTC (1994) Regional brain abnormalities in schizophrenia measured with MRI. JAMA 272:1763-69.

Andreasen NC, Arndt S, Swayze V 2nd, Cizadlo T, Flaum M, O'Leary D (1994) Thalamic abnormalities in schizophrenia visualized through magnetic resonance image averaging. Science 266:294-8.

Andreasen NC, O'Leary DS, Cizadlo T, Arndt S, Rezai K, Ponto LL, Watkins GL, Hichwa RD (1996) Schizophrenia and cognitive dysmitria: A PET study of dysfunctional prefrontal-thalamic-cerebellar circuitry. Proc Natl Acad Sci USA 93(18):9985-90.

Andreasen NC (1997) The role of the thalamus in schizophrenia. Can J Psychiatry 42:27-33.

Andreasen NC (1988) Brain Imaging: Applications in psychiatry. Science 239:1381-88.

Andreasen NC (2000) Schizophrenia: the fundamental questions. Brain Research Rev 31:106-112.

Apel E D, Storm D R (1992) Functional domains of neuromodulin (GAP)-43. Perspectives Development. Neurobiol. 1:3-11.

Arnold SE, Hyman BT, Van Hoesen GW, Damasio AR (1991) Some cytoarchitectural abnormalities of the entorhinal cortex in schizophrenia. Archives of Gen Psychiatry 48:625-32.

Audesirk G, Cabell L, Kern M (1997) Modulation of neurite branching by protein phosphorylation in cultured rat hippocampal neurons. Developmental Brain Research 102: 247-260.

Baddeley A (1986) Working Memory, Oxford University Press.

Barta PE, Pearlson GD, Powers RE, Richards SS, Tune LE (1990) Auditory hallucinations and smaller superior temporal gyrus volume in schizophrenia. Am Journal of Psychiatry 147:1457-62.

Baudier J, Delouime J C, Van Dorsselaer A, Black D and Matthes H W (1991) Purification and characterization of a brain-specific protein kinase C substrate, neurogranin (p17). Identification of a consus amino acid sequence between neurogranin and neuromodulin (GAP43) that corresponds to the protein kinase C phosphorylation site and the calmodulin-binding domain. J. Biol. Chem. 266:229-237.

Beasley CL, Reynolds GP (1997) Parvalbumin-immunoreactive neurons are reduced in the prefrontal cortex of schizophrenics. Schizophr. Res. 24:349-355.

Benes FM, Paskevich PA, Davidson J, Domesik VB (1985) Synaptic rearrangements in medial prefrontal cortex of haloperidol-treated rats. Brain Res 348:15-20.

Benes FM (1989) Myelination of cortical hippocampal relays during late adolescence. Schizophr Bull 15:585-93.

Benes FM (1991) Toward a neurodevelopmental understanding of schizophrenia and other psychiatric disorders. In Cicchetti D, Toth SL (eds), Models and Integrations: Rochester Symposium on Developmental Psychopathology, Vol 3, Rochester, NY, Univ of Rochester Press, pp 161-184.

Bergson C, Levenson R, Goldman-Rakic P, Lidow MS (2003) Dopamine receptor-interacting proteins: the calcium connection in dopamine signaling. Trends in pharmacological sciences 24:486-492.

Bernhardt R, Matus A (1994) Light and electron microscopic studies on the distribution for MAP2 in rat brain: a difference between dendritic and axonal cytoskeletons. Journal of Comp Neurol 226:203-221.

Black DW, Andreasen NC (1999) Schizophrenia, Schizophreniform, and Delusional (Paranoid) Disorders, in: The American Psychiatric Association Textbook of Psychiatry, Washington. pp. 425-78.

Blennow K, Davidsson P, Gottfries CG, Ekman R, Heilig M (1996) Synaptic degeneration in thalamus in schizophrenia. Lancet 384:692-3.

Bolz J, Kossel A, Bagnard D (1995) The specificity of interactions between the cortex and the thalamus. Ciba Found Symp 193:173-91.

Boyne LJ, Martin K, Hockfield S, Fischer I (1995) Expression and distribution of phosphorylated MAP1b in growing axons of cultured hippocampal neurons. Journal of Neurosci Res 40:439-450.

Brickman AM, Bushbaum MS, Shihabuddin L, Byne W, Newmark R, Brand J, Ahmed S, Mitelman SA, Hazlett EA (2004) Thalamus size and outcome in schizophrenia. Schizophrenia Research 71:473-484.

Broadbelt K, Byne WB, Jones LB (2002) Evidence for a decrease in primary and secondary basilar dendrites on pyramidal cells in area 32 of schizophrenic prefrontal cortex. Schizophrenia Res. 58:75-81.

Buchanan RW, Vladar K, Barta PE, and Pearlson GD (1998) Structural evaluation of the prefrontal cortex in schizophrenia. Am J Psychiatry 155:1049-1055.

Buchsbaum MS, Nuechterlein KH, Haier RJ, WU J, Sicotte N, Hazlett E, Asarnow R, Potkin S, Guich S (1990) Glucose metabolic rate in normals and schizophrenics during the Continuous Performance Test assessed by PET. British Journal of Psychiatry 156:216-27.

Buchsbaum MS, Haier RJ, Potkin SO, and others (1992) Frontostriatal disorder of cerebral metabolism in never-medicated schizophrenics. Arch Gen Psychiatry 49:935-42.

Buchsbaum MS, Someya T, Teng CY, Abel L, Chin S, Najafi A (1996) PET and MRI of the thalamus in never-medicated patients with schizophrenia. Am J Psychiatry 153:191-9.

Buxhoeveden D, Ror E, Switala A (2000) Reduced interneuronal space in schizophrenia. Biological Psychiatry 47(7):681.

Byne W, Jones L, Kemether E, Haroutunian V, Davis KL (1997) Towards localization of thalamic pathology in schizophrenia. APA Abstract NR544.

Byne W, Buchsbaum MS, Kemether E, Hazlett EA, Shinwari A, Mitropoulou V, Siever LJ (2001) Magnetic resonance imaging of the thalamic mediodorsal nucleus and pulvinar in schizophrenia and schizotypal personality disorder. Arch Gen Psychiatry 58:133-140.

Byne W, Buchsbaum MS, Mattiace LA, Hazlett EA, Kemether E, Elhakem SL, Purohit DP, Haroutunian V, and Jones L (2002) Postmortem assessment of thalamic nuclear volumes in subjects with schizophrenia. Am J of Psychiatry 159:59-65.

Chakos MH, Lieberman JA, Bilder RM, Borenstein M, Lerner G, Bogerts B, Wu H, Kinon B, Ashtari M (1994) Increase in caudate nucleus volumes of first episode schizophrenic patients taking antipsychotic drugs. Am J Psychiatry 151:1430-36.

Chakos MH, Lieberman JA, Alvir J, Bilder RM, Ashtari M (1995) Caudate nuclei volumes in schizophrenic patients treated with antipsychotics or clozapine. Lancet 345:456-57.

Chakos MH, Shirakaw O, Lieberman J, Lee H, Bilder R, Tamminga CA (1998) Striatal enlargement in rats chronically treated with neuroleptics. Biol Psychiatry 44:675-84.

Chicurel M E, Terrian D M, Potter H (1993) mRNA at the synapse: analysis of a synaptosomal preparation enriched in hippocampal dendritic spines. J. Neurosci. 13:4054-4063.

Chugani HT, Phelps ME, Mazziotta JC (1987) Positron emission tomography study of human brain functional development. Ann Neurol 22:487-497.

Crandell J, Tobet SA, Fisher I, Fox TO (1989) Age dependent expression of MAP2 in ventromedial nucleus of the hypothalamus. Brain Research Bulletin 22:571-574.

Creese I, Burt DR, Snyder SH (1976) Dopamine receptor binding predicts clinical and pharmacological potencies of antischizophrenic drugs. Science 192:481-83.

Daniel DG, Goldberg TE, Gibbons RD, Weinberger DR (1991) Lack of a bimodal distribution of ventricular size in schizophrenia: a Gaussian mixture of 1056 cases and controls. Biol Psychiatry 30:887-903.

Daviss SR, Lewis DA (1995) Local circuit neurons of the prefrontal cortex in schizophrenia: Selective increase in the density of calbindin-immunoreactive neurons. Psychiatr Res 59:81-96.

Decamilli P, Miller P, Navove F, Theurkauf WE, Vallee RB (1984) Distribution of MAP2 in the nervous system of the rat studied by immunoflouresence. Neuroscience 11:819-846.

Diaz-Nido J, Serrano L, Hernandez MA, Avila J (1990) Phosphorylation of microtubule proteins in rat brain at different development stages: Comparison with that found in neuronal cultures. Journal of Neurochemistry 54:211-222.

Doraiswamy PM, Tupler LA, Krishnan KRR (1995) Neuroleptic treatment and plasticity. Lancet 345:734-35.

Dorph-Petersen KA, Pierri JN, Perel JM, Sampson AR, Lewis DA (2005) The influence of chronic exposure to antipsychotic medication on brain size before and after tissue fixation: A comparison of Haloperidol and Olanzepine in Macaque Monkeys. Neuropsychopharmacology (submitted).

Eastwood SL and Harrison PJ (2005) Decreased expression of vesicular glutamate transporter1 and complexin II mRNAs in schizophrenia: further evidence for synaptic pathology affecting glutamate neurons (submitted).

Feinberg I, (1982) Schizophrenia: caused by a fault in programmed synaptic lamination during adolescence? J Psychiatr Res 17:319-34.

Fisher I, Kosik KS, Sapirstein VS (1987) Heterogeneity of MAP2 in vertebrate brain. Brain Research 436:39-48.

Forno L, Norville R (1979) Ultrastructure of the neostriatum in Huntington's and Parkinson's disease. Advances in Neurology 23:123-35.

Friede RL (1989) Developmental neuropathology. Berlin: Springer Verlag.

Fuster JM (1989) The prefrontal cortex: Anatomy, Physiology and Neurophysiology of the Frontal lobe. New York, Raven Press.

Garey LJ, Ong WY, Barnes TRE (1993) Dendritic spine loss on pyramidal cells of temporal cortex of schizophrenia. Neuropathol Appl Neurobiol 19:186-7.

Garey LJ, Ong WY, Patel TS, Kanani M, Davis A, Mortimer AM (1998) Reduced dendritic spine density on cerebral cortical pyramidal neurons in schizophrenia. J Neurol Neurosurgery Psychiatry. 65:446-53.

Gerendasy D, Sutcilffe G (1997) RC3/Neurogranin, a Postsynaptic Calpacitin for setting the response threshold to calcium influxes. Mol. Neurobiology 15:131-163.

Giguere M, Goldman-Rakic PS (1988) MD nucleus area laminar and tangential distribution of afferents and efferents in the frontal lobe of rhesus monkey. J Comp Neurol 277:195-213.

Glantz LA, Lewis DA (1997) Reduction of synaptophysin immunoreactivity in the prefrontal cortex of subjects with schizophrenia. Arch Gen Psychiatry 54:943-52.

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

Goldberg TE, Gold JM (1995) Neurocognitive deficits in schizophrenia. In Hirsch SR, Weinberger DR (eds): Schizophrenia. Oxford, Blackwell Science Ltd. pp 146-162.

Goldman-Rakic PS, Porrino LJ (1985) The primate mediodorsal nucleus and its projection to the frontal lobe. J Comp Neurol 242:535-60.

Goldman-Rakic PS (1995) Dissolution of cerebral cortical mechanisms in subjects with schizophrenia. In: Watson SJ. Ed. Biology of Schizophrenia and Affective Disease. Washington, DC: American Psychiatric Press pp. 113-27.

Goldman- Rakic PS, Selemon LD (1997) Functional and anatomical aspects of prefrontal pathology in schizophrenia. Schizophr Bull 23(3):437-458.

Gur RE, Maany V, Mozley PD, Swanson C, Bilker W, Gur RC (1998) Subcortical MRI volumes in neuroleptic-naïve and treated patients with schizophrenia. Am J Psychiatry 155:1711-17.

Haroutunian V, Dracheva S, Davis K (2003) Neurobiology of glutamatergic abnormalities in schizophrenia. 3:67-76.

Harrison PJ (1999) The neuropathology of schizophrenia. A critical review of the data and their interpretation. Brain 122:593-624.

Harrison PJ (1999a) Neurochemical alterations in schizophrenia affecting the putative receptor targets of atypical antipsychotics. British J of Psychiatry. 174 (Suppl. 38), 12-22.

Harrison PJ, Roberts GW (2000) The neuropathology of schizophrenia. Progress and interpretations. Oxford University Press pp 150-185.

Herzog W. and Weber K.(1978) Fractionation of brain microtubule-associated proteins. Isolation of two different proteins which stimulate tubulin polymerization in Vitro. Eur. J. Biochemistry. 92:1-8.

Hirokawa N, Hisanaga S, Shiomura Y (1988) MAP2 is a component of cross bridges between microtubules and neurofilament in the neuronal cytoskeleton. J. Neuroscience 8: 2769-2779.

Hirsch and Weinberger (2003) Schizophrenia 2nd Edition. Blackwell Publishing.

Honer WG, Bassett AS, Smith GN, Lapointe JS, Kalkai P (1994) Temporal lobe abnormalities in multigenerational families with schizophrenia. Biol Psychiatry 36:737-43.

Honer, W.G., Falkai, P., Young, C., Wang, T., Xie, J., Bonner, J., Hu, L, Boulianne, G.L., Luo, Z., Trimble, W.S., (1997) Cingulate cortex synaptic terminal proteins and neural cell adhesive molecule in schizophrenia. Neuroscience. 78: 99-110.

Ho Pak, J., Huang, F.L., Li, J., Balschun, D., Reymann, K.G., Westphal, H., Huang, K-P. 2000. Involvement of neurogranin in the modulation of calcium/calmodulin-dependent protein kinase II, synaptic plasticity, and spatial learning: a study with knockout mice. PNAS. 97:11232-11237.

Huntington's Disease Collaborative Research Group (1993) A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. Cell 72:971-983.

Jakob H, Beckmann H (1986) Prenatal development disturbances in the limbic allocortex in schizophrenics. J of Neural Transmission 65:303-26.

Johnstone EC, Crow TJ, Frith CD, Stevens M, Kreel L, Husband J (1976) Cerebral ventricular size and cognitive impairment in chronic schizophrenia. Lancet ii:924-926.

Jones GE (1997) Cortical development and thalamic pathology in schizophrenia. 23(3):483-501.

Jones L, Fischer I, Levitt P (1996) Region-specific alteration of dendritic development in the cerebral cortex following prenatal cocaine exposure. Cereb Cortex 6:431-5.

Jones L, Johnson N, Byne W (2002) Alterations in MAP2 immunocytochemistry in area 9 and 32 of schizophrenic prefrontal cortex. Psychiatry Res.Neuroimag. 9:3621-3625.

Kalus, P., Muller, T.J., Zuschratter, W., Senitz, D., (2000) The dendritic architecture of prefrontal pyramidal neurons in schizophrenic patients. Neuroreport. 9,3621-3625.

Karson CN, Griffin WS, Mrak RE, Sturner WQ, Shillutt S, Guggenheim FG (1996) Reduced levels of synaptic proteins in the prefrontal cortex in schizophrenia. Soc Neurosci Abstr 22:1677.

Keshavan, MS, Bagwell WW, Haas GL, Sweeney JA, Schooler NR, Pettegrew JW (1994) Changes in caudate with neuroleptic treatment. Lancet 344:1434.

Kindermann SS, Brown G, Zorrilla LE, Olsen RK, Jeste DV (2004) Spatial working memory among middle aged and older patients with schizophrenia and volunteers using fMRI. Schizophrenia Res 68:203-216.

Klinzova AJ, Haselhorst U, Uranova NA, Schenk H, Isomin VV (1989) The effect of haloperidol on synaptic plasticity in rat medial prefrontal cortex. J Hirnforsch 30:51-57.

Lawrie SM, Abukmeil SS (1998) Brain abnormality in schizophrenia. A systematic and quantitative review of volumetric magnetic resonance imaging studies. Br J Psychiatry 172:110-120.

Leonard C M (1972) The connections of the dorsomedial nuclei. Brain Behav Evol 6:524-541.

Levin S (1984) Frontal lobe dysfunction in schizophrenia-II. Impairments of psychological and brain functions. J Psychiatry Res 18:57-72.

Levitt J, Lewis DA, Yoshioka T, Lund JS (1993) Topography of pyramidal neuron intrinsic connections in macaque monkeys prefrontal cortex (areas 9 & 46). J Comp Neurol 338:360-76.

Levitt P, Reinoso B, Jones L (1998) The critical impact of early cellular environment on neuronal development. Brain Research 27:180-83.

Lewis DA, Harris HW (1991) Differential laminar distribution of tyrosine hydroxylase-immunoreactive axons in infant and adult monkey prefrontal cortex. Neurosci Lett 125:151-54.

Lewis DA, Anderson SA (1995) The functional architecture of the prefrontal cortex and schizophrenia. Psychol Med 25: 887-894.

Lewis DA (1997) Development of the prefrontal cortex during Adolescence: Insights into vulnerable neural circuits in schizophrenia. Neuropsychopharmacology 16:385-89.

Lewis DA, Cruz DA, Melchitzky DS, Pierri JN (2001) Lamina-specific decifits in parvalbumin-immunoreactive varicosities in the prefrontal cortex of subjects with schizophrenia: Evidence for fewer projections from the thalamus. The American J of Psychiatry 158:1411-22.

Liddle P, Friston KJ, Frith CD (1992) Cerebral blood flow and mental processes in schizophrenia. Journal of the Royal Society Medicine 85:224-226.

Lidow MS, Songa Z, Castnerb SA, Allenc PB, Greengarde P, Goldman-Rakic PS (2001) Antipsychotic treatment induces alterations in dendrite and spine-associated proteins in dopamine-rich areas of the primate cerebral cortex. Biol Psychiatry 49:1-12.

Lund JS, Lund RD, Hendrickson AE, et al. (1975) The origin of efferent pathways from the primary visual cortex, area 17, of the macaque monkey as shown by retrograde transport of horseradish peroxidase. J comp Neurol 164:287-303.

Maccioni R and Cambiazo V (1995) Role of microtubule-associated proteins in the control of microtubule assembly. Physiol Rev 75:835-864.

Madsen AL, Keiding N, Karle A, Esbjerg S, Hemmingsen AL (1998) Neuroleptics in progressive structural brain abnormalities in psychiatric illness. Lancet 352:784-785.

Mates SL, Lund JS (1983) Spine formation and maturation of type I synapses on spiny stelate neurons in primary visual cortex. Journal of Comparative Neurology 221:91-97.

Matus A, (1988) Microtubule-associated proteins: their potential role in determining neuronal cytoarchitecture. Brain Research Reviews 13:179-212.

Matus A, Bernhardt R, Bodmer R, and Alaimos D (1986) Microtubule-associated protein 2 and tubulin are differentially distributed in the dendrites of developing neurons. Neuroscience 17:371-389.

Meshul CK, Janowsky A, Casey DE, Stallbaumer RK, Taylor B (1992) Effects of haloperidol and clozapine on the density of “perforated” synapses in caudate, nucleus accumbens, and medial prefrontal cortex. Psychopharmacology 106:45-52.

Melchitcky DS, Sesack SR, Pucak ML, Lewis DA (1995) Synaptic targets of pyramidal neuron axon collaterals providing horizontal connections in monkey prefrontal cortex. Soc Neurosci Abstr 21: 409.

Milner B, Petrides M (1984) Behavioral effects of frontal-lobe lesion in man. Trends Neurocsi 7:403-7.

Molnar Z, Adams R, Blakemore C (1998) Mechanisms underlying the early establishment of thalamocortical connections in the rat. J of Neurosci 18:5723-45.

Murphy, DB, Borist BB (1975) Association of high molecular weight proteins with microtubules and their role in microtubule assembly in vitro. Proc. Natl. Acad. Sci. USA 72:2696-2700.

Murray RM, O'Callaghan E, Castle DJ, Lewis SW (1992) A neurodevelopmental approach to the classification of schizophrenia. Schicophr Bull 18:319-32.

Myers R, Vonsattel J, Stevens T, Cupples L, Richardson E, Martin J, Bird E (1988) Clinical and neuropathologic assessment of severity in Huntington Disease. Neurology 38:341-7.

Neuner-Jehle M, Denizot J P, and Mallet (1996) Neurogranin is locally concentrated in rat cortical and hippocampal neurons. Brain Res. 733:149-154.

Noga JT, Bartley AJ, Jones DW, Torrey EF, Weinberger DR. (1996) Cortical gyral anatomy and gross brain dimensions in monozygotic twins discordant for schizophrenia. Schizophr Res 22:27-40.

Nopoulos P, Torres I, Flaum M, Andreasen NC, Ehrhardt JC, Yuh WTC (1995) Brain morphology in first episode schizophrenia. Am J Psychiatry 152(12):1721-24.

Norman DA (1970) Models of human memory (New York: Academic Press).

Pakkenberg B (1987) Post-mortem study of chronic schizophrenic brains. Br J Psychiatry 151: 744-52.

Pakkenberg B. (1990) Pronounced reduction of total neuron number in mediodorsal thalamic nucleus and nucleus accumbens in schizophrenics. Arch Gen Psychiatry 47:1023-8.

Pakkenberg B (1992) The volume of the MD thalamic nucleus in treated and untreated schizophrenics. Schicophr Res 7:95-100.

Pakkenberg B (1993) Total nerve cell number in neocortex in chronic schizophrenics and controls estimated using optical dissectors. Biol Psychiatr 34:768-772.

Park S, Holzman PS (1992) Schizophrenics show spatial working memory deficits. Arch Gen Psychiatry 49:975-82.

Perrone-Bizzozero NI, Sower AC, Bird ED, Benowitz LI, Ivins KJ, Neve RL (1996) Levels of the growth-associated protein GAP-43 are selectively increased in association cortices in schizophrenia. Proc Natl Acad Sci USA 93:14182-87.

Peters A, Kaiserman-Abramof IR (1970) The small pyramidal neuron of the rat cerebral cortex. The perikaryon , dendrites and spines. Am. J. Anat. 127:321-55.

Petersen A, Mani K, Brundin P (1999) Recent advances on the pathogenesis of Huntington's disease. Exp Neurol May;157 (1):1-18.

Pierri, J.N., Volk, C.l., Auh, S., Sampson, A., Lewis, D.A., (2001) Decreased somal size of deep layer 3 pyramidal neurons in the prefrontal cortex of subjects with schizophrenia. Arch Gen Psychiatry. 58,466-473.

Popken JG, Bunney EW, Potkin GS, Jones GE (2000) Subnucleus-specific loss of neurons in medial thalamus of schizophrenics. PNAS 97(15):9276-80.

Powell TPS (1981) Certain aspects of the intrinsic organization of the cerebral cortex. Pp1-19. Raven press. New York.

Prichard, L., Deloulmes, J. C., Storm, D.R. 1999. Interactions between neurogranin and calmodulin *in vivo*. J. Bio. Chem. 274:7689-7694.

Rajakumar N, Williamson PC, Stoessl JA, Flumerfelt BA. (1996) Neurodevelopmental pathogenesis of schizophrenia. Neurosci Abs 467.2.

Rajkowska G, Selemon LD, Goldman-Rakic PS (1998) Neuronal and somal size in the prefrontal cortex. Arch Gen Psychiatry 55:215-224.

Rakic P (1977) Prenatal development of the visual system in rhesus monkey. Philos Trans R Soc London (Biol) 278:245-60.

Reveley AM, Riveley MA, Clifford CA, Murray RM (1982) Cerebral ventricular size in twins discordant for schizophrenia. Lancet 1:540-1.

Roberts GW, Colter N, Lofthouse R, Johnstone EC, Crow TJ (1987) Is there gliosis in schizophrenia? Investigation of the temporal lobe. Biol Psychiatry 22:1459-68.

Roberts GW (1991) A neuropathological perspective. Br J Psychiatry 158:8-17.

Schapira AHV (1997) Mitochondrial function in Huntington's disease: clues for the pathogenesis and prospects for treatment. Ann Neurol 41:141-2.

Scheibel AB, Kovelman JA (1981) Disorientation of the hippocampal pyramidal cell and it processes in the schizophrenic patient. Biological Psychiatry 16:101-2.

Schroeder J, Buchsbaum MS, Siegel BV, Geider FJ, Niethammer R (1995) Structural and functional correlates of subsyndromes in chronic schizophrenia. Psychopathology 28:38-45.

Seeman P (1992) Dopamine receptors sequences: Therapeutic levels of neuroleptics occupy D2 receptors, clozapine occupies D4. Neuropsychopharmacology 7:261-284.

Selemon LD, Rajkowska G, Goldman-Rakic PS (1995) Abnormally high neuronal density in the schizophrenic cortex: A morphometric analysis of prefrontal area 9 and occipital area17. Arch Gen Psychiatr 52:805-818.

Selemon LD, Rajkowska G, Goldman-Rakic PS (1998) Elevated neuronal density in prefrontal area 46 in brains from schizophrenic patients. Journal of Comparative Neurology 392:402-12.

Selemon LD, Goldman-Rakic (1999) The reduced neuropil hypothesis: A circuit based model of schizophrenia. Biol Psychiatry 45:17-25.

Shallice T (1982) Specific impairments in planning. Transactions of the Royal Society of London Series 298:199-209.

Shapiro RM (1993) Regional neuropathology in schizophrenia: Where are we? Where are we going? Schizophr Res 10:187-239.

Sharma T, Lancaster E, Lee D, Lewis S, Sigmundsson T, Takei N (1998) Brain changes in schizophrenia. Br J Psychiatry 173:132-8.

Shatz CJ (1990) Impulse activity and patterning of connections during CNS development. Neuron 5:745-56.

Shelton RC, Weinberger DR (1986) X-ray computerized tomography studies in schizophrenia: A review and synthesis. Hand book of schizophrenia. Elsevier Science, Amsterdam, 207-250.

Silbersweig DA, Stern F, Frith C, and others (1995) A functional neuronatomy of hallucinations in schizophrenia. Nature 378:176-9.

Silverman JM, Smith CJ, Guo SL, Mohs RC, Siever LJ, Davis KL (1998) Lateral ventricular enlargement in schizophrenic probands and their siblings with schizophrenia-related disorders. Biol Psychiatry 43:97-106.

Smiley JF, Williams SM, Szigeti K (1992) Light electron microscopic characterization of dopamine-immunoreactive axons in human cerebral cortex. Comp. Neurol. 321:325-35.

Staal WG, Hulshoff Pol HE, Schnack H, van der Schot AC, Kahn RS (1998) Partial volume decrease of the thalamus in relatives of patients with schizophrenia. Am J Psychiatry 155:1784-86.

Stroop interference and facilitation: Comparing normal individuals and individuals with schizophrenia. Journal of Experimental Psychology 126:19-36.

Suddath RL, Christison GW, Torrey EF, Casanova MF, Weinberger DR (1990) Anatomical abnormalities in the brains of monozygotic twins discordant for schizophrenia. N Engl J Med 322:789-94.

Tamminga CA, Thaker GK, Buchanan RW et al., (1992) Limbic-system abnormalities identified in schizophrenia using positron emission tomography with florodeoxyglucose and neocortical alternations with deficit syndrome. Archives of General Psychiatry. 49:522-530.

Thune JJ, Uylings HBM, Pakkenberg B (2001) No deficit in total number of neurons in the prefrontal cortex in schizophrenics. J of Psychiatric Res 35:15-21.

Turner EE, Fedtsova N, Jeste DV (1997) Cellular and molecular neuropathology of schizophrenia: New directions from developmental neurobiology. Schizophrenia Res 27:169-80.

Uylings H B M, Van Eden C G, Parnavelas J G, Kalsbeek A (1990) The prenatal and postnatal development of rat cerebral cortex. The Cerebral Cortex of the Rat MIT Press, Cabbridge, MA, pp. 35-76.

Van Horn JD, McManus IC (1992) Ventricular enlargement in schizophrenia. Am J Psychiatry 160:687-697.

Van Ooyen A, van Pelt J, Corner MA. (1995) Implication of activity dependent neurite outgrowth for neuronal morphology and network development. J. Theor. Bio. 172:63-82.

Van Pelt J, van Ooyen A, Corner MA. (1996) Growth cone dynamics and activity-dependent processes in neuronal network development. Progress Brain Res. 108:333-46.

Volkow ND, Wolf AP, Van Gelder P et al., (1987) Phenomenological correlates of metabolic activity in 18 patients with chronic schizophrenia. American Journal of Psychiatry 144:151-158.

Vonsattel, JP, Myers RH, Stevens TJ, Ferrante RJ, Bird ED, Richardson EP (1985) Neuropathological classification of Huntington's disease. Journal of Neuropathology and Experimental Neurology 44:559-77.

Watson J B, Battenberg E F, Wong K K, Bloom F and Sutcliffe J G (1990) Subtractive cDNA cloning of RC3, a rodent cortex-enriched mRNA encoding a novel 78 residue protein. J. Neurosci. Res. 26:397-408.

Watson J B, Sutcliffe J G, Fisher R S (1992) Localization of the protein kinase C phosphorylation/calmodulin-binding substrate RC3 in dendritic spines of neostriatal neurons. Proc. Natl. Acad. Sci. USA 89:8581-8585.

Weickert CS, Kleinman JE (1998) The neuroanatomy and neurochemistry of schizophrenia. Schizophrenia 21:57-75.

Weinberger DR, Berman KF, Zec RF (1986) Physiologic dysfunction of dorsolateral prefrontal cortex in schizophrenia: I. Regional cerebral blood flow (rCBF) evidence. Archives of General Psychiatry 43:114-25.

Weinberger DR (1987) Implications of normal brain development for the pathogenesis of schizophrenia. Arch Gen Psychiatry 44:660-9.

Weinberger DR (1988) Schizophrenia and the frontal lobe. Trends in Neurosci 11:367-70.

Weinberger DR, Berman KF, Suddath RS (1992) Evidence of disfunction of a prefrontal-limbic network in schizophrenia: A MRI and regional cerebral blood flow study of discordant monozygotic twins. Am J of Psychiatry. 149:890-97.

Weinberger DR, Aloia MS, Goldberg TE, Berman KF (1994) The frontal lobe of schizophrenia. J Neuropsychiatry Clin. Neurosci 6:419-27.

Wise SP, Jones EG (1978) Developmental studies of thalamocortical and commissural connections in the rat somatic sensory cortex. J comp Neurol 178:187-208.

Wolkin A, Sanfilipo M, Wolf AP (1992) Negative symptoms and hypofrontality in chronic schizophrenics. Archives of Feneral Psychiatry 49:959-965.

Young K, Manaye KF, Liang C, Hicks PB, German DC (2000) Reduced number of MD and anterior thalamic neurons in schizophrenia. Biol Psychiatry 47:944-53.