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THE HUMAN PREFRONTAL CORTEX AND THE EVOLUTION OF THE SOCIAL BRAIN

A thesis submitted in partial satisfaction of the requirements

for the degree of Master of Arts

in

Anthropology

by

Caroline F. Horton

Committee in Charge:

Professor Katerina Semendeferi, Chair
Professor Margaret Schoeninger
Professor Shirley Strum

2013

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Chair

University of California, San Diego

2013

DEDICATION

I gratefully dedicate this thesis to Dr. Gary Richards, whose devoted and enthusiastic teaching in the undergraduate classroom evoked my passion for Biological Anthropology in the first place, and whose mentorship, support, and friendship provided me with the necessary skills to reach this achievement in my academic career. I further lovingly dedicate this thesis to my parents, Robert and Laurie Horton, for their unconditional love and relentless support of every endeavor I've embarked upon.

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ABSTRACT OF THE THESIS

The Human Prefrontal Cortex and the Evolution of the Social Brain

by

Caroline F. Horton

Master of Arts in Anthropology

University of California, San Diego, 2013

Professor Katerina Semendeferi, Chair

The prefrontal cortex is implicated in the majority of higher order processes of the primate brain. This region is especially important to the study of human evolution, because it is precisely the complex cognitive and socio-emotional processes of the prefrontal cortex that are thought to characterize uniquely human behavior. Here I will explore the role of the prefrontal cortex in the evolution of the social brain in humans. Great Apes, as the extant representatives of the ancestral lineages closely related to our own hominin lineage, are extremely useful to study in a comparative context with humans in order to gain insight into the neuroanatomical changes implicated in the evolution of social cognition, and such findings will be reviewed here. Human

neurological pathologies with distinct social phenotypes are also a powerful area of study in the evolution of the social brain, because comparative studies of pathological individuals with typically developing controls allows for the possibility to associate neural correlates with specific social behaviors that may have been acted upon during the course of human brain evolution. I will focus on three such pathologies: Autism Spectrum Disorder, schizophrenia, and Williams Syndrome. Each of the pathologies have specific social phenotypes that suggest abnormalities of social functions that would have been critical to the evolution of the social brain. Lastly, I will present the findings of an original comparative research investigation of the prefrontal cortex in Williams Syndrome, and discuss the possible evolutionary implications of my findings.

INTRODUCTION

The prefrontal cortex is critically implicated in executive function in the human brain. This region is of special interest to those studying human evolution, because such executive function, especially socio-emotional behavior and elements of complex cognitive processing, collectively referred to as the social brain, is a hallmark of human uniqueness (Sherwood et al. 2008). Many early comparative investigations have emphasized that a dramatic change in the relative size of the prefrontal cortex may have contributed to the evolution of our uniquely human behavior; however, more recent studies have suggested that prefrontal cortex relative size does not deviate dramatically from the great ape trend, and that more subtle differences, including the reorganization of smaller cortical areas and changes in cortical microstructure, may have more significance to the great behavioral divide between humans and other primates.

The goal of this paper is to investigate changes in the human prefrontal cortex that may have been key in the evolution of the social brain, utilizing comparative neuroanatomical methods with nonhuman primates as well as human subjects with neurological pathologies that have a socio-behavioral phenotype. The majority of this is done with an extensive review of the literature, although I also discuss the preliminary findings of my own original research project in chapter IV. This paper will be divided into five chapters. In chapter I, I briefly introduce important anatomical concepts of the prefrontal cortex, including the commonly recognized divisions and sub-regions that will be important for the remainder of the paper. I then discuss what is known or implicated about the function of the prefrontal cortex, most extensively in terms of prefrontal connectivity, but also briefly from implications derived from lesion studies. I lastly

discuss the development of the prefrontal cortex in three sections: prenatal development, development in infancy, childhood and adolescence, and lastly degenerative changes during aging.

In chapter II I discuss the evolution of the prefrontal cortex, focusing on comparative investigations between humans and other primates. I first discuss what is known about differences in size and volume of the entire frontal lobe, as well as differences in grey and white matter. I then discuss microstructural differences between humans and other primates, including the structural reorganization of individual cytoarchitectonic regions, differences in neuronal spacing and organization, including changes in human minicolumns, differences in cortical composition across species, such as glia-neuron and inhibitory interneuron ratios, differences in pyramidal neuron morphology, and differences in innervation by neuromodulators across the species.

In chapter III I investigate abnormalities in the prefrontal cortex in two well-studied neuropathologies, Autism Spectrum Disorder and schizophrenia. While very different in etiology and symptomatic manifestation, both of these disorders are strongly affected by a disruption in normative cognitive function and socio-emotional behavior. Therefore, a comparison of the differences in the prefrontal cortex of the affected subjects and typically developing controls may provide insight into the properties of prefrontal cortex structure that are so critical to typical human behavior. I begin with Autism Spectrum Disorder, in which I investigate the volumetric differences, abnormalities of cortical thickness, differences in neuronal number and density, abnormalities of cortical composition, and minicolumn abnormalities associated with the disorder. I then discuss the MRI imaging and histological studies of schizophrenia, which are somewhat more

difficult to decipher. I discuss the implications of any abnormalities in volume, cortical thickness, neuron numbers and densities, and minicolumns in this pathology. I then relate these findings to possible implications about the critical neural components of the social brain in humans.

In chapter IV I present the results of an original research investigation, consisting of a comparative analysis of cortical layer thickness in area 11 of the prefrontal cortex of a typically developing and a Williams Syndrome post-mortem brain. William's Syndrome is a rare genetic disorder with a unique socio-behavioral phenotype (Galaburta et al. 1994), and thus any quantitative differences identified in these brains may provide insight into the microstructural properties involved in human social cognition. While Area 11 is not extensively studied, it is broadly thought to be implicated in reward-mediated behaviors, and has also been suggested to be involved in the cognitive modulation of empathy, a behavior that is important in the strength and maintenance of complex social relationships (Shamay-Tsoory et al. 2009; Pochon et al. 2002). I first discuss the etiology and cognitive and behavioral phenotype of Williams Syndrome, and give an overview of the few neuroanatomical studies currently available in the literature. I then present the results of a comparative study of neuronal density in prefrontal area 11 of a Williams Syndrome subject and a typically developing control, in which I found that while neuronal density as a whole did not significantly differ from the normal average in the Williams Syndrome subject, the infragranular layers of area 11 in the Williams Syndrome subject displayed a drastic decrease in density. While not statistically significant due to the extremely small sample size, these preliminary findings were significant in their own right and lend some insight into the relationship between neural

abnormalities and the behavioral phenotype in Williams Syndrome, as well as implications into the possible neural changes associated with the evolution of the social brain.

Lastly in chapter V, I discuss how these findings implicate the PFC as a critical structure to the evolution of the sociality in the hominin lineage. I propose that the relationship between cortico-cortical and cortico-subcortical connectivity with the PFC might be critical to the complex socio-emotional and cognitive processes that constitute the social brain, and thus may be an important area of study in the investigation of human uniqueness and the constituent neural changes implicated in the evolution of the social brain. Here I tie together evidence from comparative primate investigations and the neuroanatomical findings in Williams Syndrome, Autism Spectrum Disorder (ASD), and schizophrenia that supports this proposition. Furthermore, based on the findings of abnormal neuronal density in the infragranular layers in Williams Syndrome in my original research investigation, here I also make predictions about how neuronal density might be distributed amongst the individual cortical layers in ASD and schizophrenia, how this might relate to the relationship between cortico-cortical and cortico-subcortical connectivity in these pathologies, and how these possible findings may relate to the evolution of the social brain.

CHAPTER 1: ANATOMY, FUNCTION, AND DEVELOPMENT OF THE PREFRONTAL CORTEX

Introduction

In order to discuss the role of the prefrontal cortex in the evolution of the social brain in humans, an introduction to the fundamentals of this structure is necessary. Here I will briefly introduce the prefrontal anatomy of the PFC, the anatomical subregions, and the cytoarchitectonically defined areas included in this structure. I discuss the extensive cortico-cortical and cortico-subcortical connections of the PFC, which show that this structure receives multimodal sensory information from the external environment as well as maintaining considerable communication with subcortical structures that monitor the internal environment, placing the PFC in an ideal position for mediating behavior in a dynamic social environment. I also summarize the findings of lesion studies in the various subregions, which, while limited, enable some understanding of the functional roles of the PFC, particularly in terms of emotions and social behavior. Lastly, I discuss the development of the PFC, from neural tube formation through neural aging at the end of life. The late maturation of the PFC makes this structure highly plastic and therefore ideal to adaptive development to novel social environments.

ANATOMY AND FUNCTION

The cerebrum of the mammalian brain is traditionally divided into two hemispheres consisting of four lobes, the most anterior of which are the frontal lobes. The neocortex, a layer of cells 2-5 mm thick that contain neural soma, lie on the surface of these lobes (Stiles and Jernigan 2010). The frontal cortex is the portion of cortex that

covers the frontal lobe; the prefrontal cortex is a subregion of the frontal cortex. While the presence of a prefrontal cortex homologue in other mammalian animals is up for debate (see Pruess 1995; Uylings, Groenwegen, and Kolb 2003), it is most confidently referred to in the context of primates. Within primates, the prefrontal cortex consists of the association cortex of the frontal lobe, or the area of the frontal cortex anterior to the motor and premotor cortices (Teffer and Semendeferi 2012). The prefrontal cortex (from here on the PFC) is further anatomically defined by the inclusion of eleven architectonically distinct areas (BA 8,9,10,11,12,13,32,44,45,46, and 47) (Fuster 1997; see Figure 1), each identified and differentiated by morphological properties such as cortical layer thickness and number of layers, and the size, shape and density of cortical neurons (Semendeferi et al. 2001).

The PFC is involved in higher order cognitive processing, or "executive function", a blanket term that can be broadly defined as "the flexible co-ordination of sub-processes to achieve a particular goal in a flexible manner" (Elliot 2003). In terms of executive function, the PFC often is divided into three major functional regions: the orbitofrontal cortex (OFC), the medial PFC (MPFC) and the lateral PFC (LPFC) (see Figure 1). The OFC and MPFC have been linked to emotional processing and social behavior, while the LPFC is mostly involved in the organization of sensory information and behavioral output, as well as reasoning and speech (Fuster 1997). Other anatomical regions of interest commonly recognized in the literature that overlap the major regions are are the ventromedial prefrontal cortex (vmPFC) (includes portions of both the OFC and the MPFC; is attributed to emotions and sociality; Dimitrov et al. 1999) the dorsolateral prefrontal cortex (DLPFC) (includes only the dorsal region of the LPFC;

attributed to organization of behaviors, maintenance of working memory; Elliot 2003), and the frontopolar cortex (the anterior-most portion of the OFC; attributed with the planning of future actions, taking initiative, and the processing of working memory and attention; Semendeferi et al. 2001).

Connectivity

The extensive connectivity of the PFC reflects the general functionality of the structure: subcortical connections tend to reflect the internal environment of the organism, and include those involved in autonomic response, motor movement, and emotion, while the neocortical connections relay information about the external environment from sensory cortices. The PFC integrates these different modes of information, allowing for the complex behavior that is associated with higher order executive processing. Because the connections of the PFC are mostly bidirectional, I will not spend time distinguishing between afferent and efferent connections to the PFC, although it is important to remember that these have distinct roles: afferent connections to the particular area of the PFC provide perceptual and memory input from specific cortical and subcortical regions, while efferent connections allow for a particular area in the PFC to control how this information is processed in other cortical and subcortical areas, thus contributing to behavioral output (Petrides et al. 2012). These bidirectional processes occur both intra-and inter-hemispherically (Jacobsen and Trojanowski, 1977a,b). Below, I review these connections, with an emphasis on those implicated in behavior.

Subcortical Connections

The PFC has connections with the basal ganglia, thalamus, brainstem, and

components of the limbic system (Fuster 2001). Connections between the PFC and the thalamus have the most organized arrangement (Rempel-Clower and Barbas 1998). The connections with the mediodorsal nucleus of the thalamus are most the extensive, and connect to the PFC via the anterior thalamic radiations and the inferior thalamic peduncle. This organization appears to be consistent in all mammalian species documented, including the rat, mouse, cat, dog, and both human and nonhuman primates (Fuster 1997). Within the primates, the mediodorsal nucleus appears to divide into two major cytoarchitectonic areas: the magnocellular component, which has projections to the OFC and MPFC, and the parvocellular component, with projections to the DLPFC (Rempel-Clower and Barbas 1998). While this division of the mediodorsal nucleus, and its afferents to the PFC, are less clear in non-primate species, investigations of primate connections suggest that this organization appears to be conserved throughout the primate lineage, including humans (Fuster 1997).

The majority of the subcortical afferent connections to the PFC are sent from the mediodorsal nucleus of the thalamus, so that this structure serves as an information hub, receiving input from many other structures of the brain to relay to the PFC. These structures are diverse in function, and include the mesencephalic reticular formation (important in sensory and motor systems), the amygdala (many functions, including arousal, autonomic responses to fear, memory, and emotion), the prepiriform cortex, the inferior temporal cortex (important in visual object recognition), the substantia nigra (implicated in eye movement), the cerebellum (important in motor movement), and the globus pallidus (important in voluntary movement) (Tanibuchi and Goldman-Rakic 2005; Barbas et al. 1991; Barbas et al. 2003; Zikopolous and Barbas 2007; Fuster 1997). The

PFC also receives afferent connections from other thalamic nuclei, including the ventralis anterior, the rostral intralaminar nuclei, and the pulvinar, as well as direct connections from the brainstem tegmentum, the pons, the hypothalamus, and the amygdala (Barbas et al. 1991; Fuster 1997). Most of these connections are bidirectional, so that the PFC sends afferents reciprocally and in similar topographical organization to these subcortical structures. However, the PFC also sends efferents to the basal ganglia, from which it does not receive a reciprocal connection (Fuster 1997). Collectively, these subcortical structures relay to the PFC information that integrates the organism's internal milieu to its external environment (such as state of arousal, motivations and desires, visceral complements to emotion) (Barbas et al 2003). Subcortical structures with connections to the PFC that have been found to be of particular importance to behavior include the amygdala, hypothalamus, and hippocampus (Fuster 2001).

The amygdala is suggested to be the center of the emotional memory system, and has connections with other subcortical structures related to autonomic response as well as areas of the neocortex, including the PFC (LeDoux 1993). Numerous primate studies have identified afferents from the amygdala to the posterior OPFC through the mediodorsal nucleus of the thalamus, as well as efferents from the posterior OPFC to the amygdala, and it has been suggested that these connections are involved in the concomitance of emotional behaviors and autonomic responses to external environments (Barbas et al. 2011). One study in the marmoset monkey found direct afferents from three amygdalar nuclei to area 10, the frontal pole of the PFC (Burman et al. 2011).

The same study also found projections to area 10 from the hypothalamus, a structure involved in numerous autonomic responses to emotional stimuli and the

execution of emotional behavior (Burman et al. 2011; Fuster 2001). Another study in the rhesus monkey found both afferent and efferent projections between the posterior hypothalamus to the OPFC and MPFC (Rempel-Clower and Barbas, 1998). It has been suggested that efferent projections from the PFC to the hypothalamus may be part of a descending pathway that allows for an elicitation of autonomic function in response to emotions related to abstract cognitive states of self-awareness (Craig 2004; Rempel-Clower and Barbas1998).

The hippocampus has long been known to be involved in the transformation of short term memories into long term memories, but more recent evidence has suggested that it also has a secondary role in the emotional memory system through its connections to both subcortical structures implicated in emotion, such as the amygdala and hypothalamus, as well as with the association cortices (LeDoux 1993; Barbas 2000). One study supporting this hypothesis found direct connections between the hippocampus and the mPFC in rhesus monkeys (Barbas and Blatt, 1995).

Cortico-cortical Connections

The PFC also has significant connectivity with other areas of the neocortex, including the sensory cortices. Sensory input to the PFC, including afferents from visual, auditory, somatosensory, gustatory, and olfactory cortices varies by region, reflecting the functional roles of that region (Barbas 2000). However, these connections with the PFC are not direct from the primary sensory cortices, but rather are mediated by other areas of association cortex. Sensory input is projected first from a primary sensory area to nearby areas of the cortex, which in turn project to areas in the PFC. The PFC also sends projections back to these other associative areas. The associative areas that are most

clearly involved in sensory projection to the PFC include BA 7 in the parietal cortex, which is associated with bodily perception, BA 22 in the temporal cortex, which is associated with audition, and BA 21 in the inferotemporal cortex, associated with vision. (Fuster 1997).

Sensory input to the PFC is not homogeneous. The lateral PFC receives input mostly from visual, auditory, and somatosensory cortices, with specific areas in the lateral PFC being targeted more robustly than others by certain sensory modalities. For example, visual association cortices have significant afferents to the anterior portion of BA 8, the posterior portion of BA 46, and BA 12 within the LPFC, auditory association cortices target the rostral portion of BA 8, the central and dorsal portion of BA 46, and BA 10, and somatosensory cortices target the central portion of BA 46 and BA 12 (Barbas 2000). In contrast with the LPFC, the OFC receives input from all of the sensory cortices, including gustatory and olfactory, and afferents from each sensory cortex are more diffuse throughout the OFC rather than targeting one region (Barbas 2000). These findings, found most extensively in rhesus monkeys but generally supported by other primate studies, suggest that the PFC is important in sensory processing and integration of external stimuli (Fuster 1997).

Lesion Studies

In the broadest sense, the PFC is believed to be the primary structure involved in executive function, or an organism's active adaptation between self and the environment (Fuster 2000). As a heteromodal association cortex, the PFC is the only structure that receives input from all sensory modalities (Stuss and Benson 1984). Furthermore, as

discussed above, the PFC also has extensive connectivity with subcortical structures so that the PFC is simultaneously receiving information about both the external environment and the organism's own internal milieu. Lesion studies of executive function in the PFC can be broken down into two categories that pertain both to region and functional domain. The first is cognitive function, which is involved in the temporal organization of behavior, and primarily takes place in the dorsolateral PFC (DLPFC). The second is socio-emotional function, which is involved in the relationship between visceral actions, social environment, and emotional behavior, and is thought to primarily engage the orbitofrontal cortex (OFC)/ventromedial PFC (vmPFC) (Fuster 1997; Fuster 2000). In this section, I will briefly discuss what is known about PFC function in each of these categories. I will also discuss some findings of function in the frontopolar cortex.

Dorsolateral Prefrontal Lesions

The dorsolateral regions of the PFC are most critically implicated in cognitive functions, which include all aspects of goal-driven working memory, including attention, temporal integration, and goal-driven behavior and memory-related aspects of self-control (Fuster 2000; Barbas 2000; Hornak et al. 2004; Hare et al. 2009). Lesion studies of the DLPFC on monkeys have shown that lesioned animals have difficulty performing delayed response tasks, and also have attention deficits in learning. For example, lesioned monkeys that were given baited food traps fared well in performance for food retrieval when allowed to respond immediately or a very short time after; however, when required to wait longer than a few seconds after traps were baited, performance drastically declined (Goldman 1971). Another dorsolateral lesion study in monkeys, which tested working memory with objects, found that lesioned animals fared similarly to non-

lesioned animals with relatively few objects to remember, but as the number of objects increased, performance in the lesioned animals declined significantly, suggesting that cognitive load was disrupted in these animals (Petrides et al. 2000).

Similar findings of the failure of working memory in goal-oriented behavior have been found in humans with DLPFC lesions. In a study by Hornak and colleagues (2004), human subjects with DLPFC lesions were given a task in which two patterns were presented on a screen simultaneously, and touching the correct pattern resulted in a reward of imaginary money, while touching the incorrect pattern resulted in a loss. The subjects were required to discover by trial and error which pattern was the correct pattern based on an increase or decrease in their money, as well as keep track of when a reversal occurred, i. e. when the correct pattern became the incorrect pattern and vice versa. The researchers found that the DLPFC lesioned subjects failed to pay attention to the amount of money won or lost after each trial, so their choice of pattern selection was not based on useful external cues. They suggest that the DLPFC is critical in working memory and attention processes that allow an individual to determine what external information is critical to the goal at hand (Hornak et al. 2004).

Qualitative observations of human subjects with DLPFC impairments have found similar results. In general, DLPFC lesioned subjects are associated with a disturbance in attentive behaviors; these individuals are reported to be apathetic to external stimuli and lack drive and awareness (Fuster 1997). Due to this lack of attention to such stimuli, these subjects are unable to act rapidly to a situation, or to take deliberate action to achieve a task (Fuster 1997). They have also been shown to have difficulty in making appropriate contextual decisions in tasks requiring self-control (Hare et al. 2009).

Language difficulties related to the temporal integration of speech are also common to human subjects with DLPFC impairments (Fuster 1997). In order to accomplish a cognitive task, the organism must be able to retain the necessary information temporarily and knowing this information (working memory), be able to modulate and keep track of its own response (Barbas 2000). Subjects with lesions to the DLPFC appear deficient in this process, suggesting that this region is critical to the cognitive processes of executive function. Furthermore, the failure of lesioned subjects to act in an appropriate, timely manner in such tasks, as well as evidence of some speech difficulties related to temporal integration, suggests that the DLPFC is implicated in cognitive operations involved in the mediation of cross-temporal contingencies (Fuster 2000).

Orbitofrontal/Ventromedial Lesions

Lesions in humans and animals in the OFC/vmPFC suggest that these regions are primarily involved in integrating personal drive, decision-making and emotion with social and environmental cues (Fuster 2000). Lesions of these areas in monkeys have shown deficits in decision-making and the ability to predict future outcome. In one such study, monkeys with VMPFC lesions were shown to display abnormal preferences in food choice compared to non-lesioned animals, as well as possessing inconsistent food choices between trials (Baylis and Gaffan 1991). In another study, OFC-lesioned monkeys were shown to respond to a task at erratic or inappropriate times; for instance, they would respond even when responses were no longer rewarded, or would respond to a stimulus that was not associated with reward (Rolls 2004). Monkeys with OFC lesions have also been reported to show changes in emotion, including decreased aggression to humans and stimuli that would normally be perceived as dangerous or threatening (Rolls

2004).

Similar to lesions of these regions in monkeys, humans with OFC/VMPFC lesions also showed abnormalities in decision-making. In one study, subjects with VMPFC lesions were observed in a gambling task, which consisted of a card game in which participants were told to select from one of four decks of cards in order to gain the most amount of money. The choice to select from a given deck of cards is influenced by patterns of reward (gain money) or punishment (lose money); decks A and B, while associated with a somewhat larger immediate gain than C and D when a reward was given, were also associated with a substantially greater loss of money than C and D when a punishment was given. It was found that subjects with vmPFC lesions continued to select cards from decks A and B even after the pattern was established that these cards are associated with much more substantial loss compared to decks C and D. Furthermore, this impairment of profitable decision-making was shown to be consistent even when subjects were re-tested at a later time. These results suggest that in humans, the VMPFC is important in adaptive decision-making, and perhaps the inhibition of impulse (Bechara et al. 2000).

Another study by Young and colleagues (2010) sought to investigate the role of the vmPFC in judgment of the harmful intent of others. Earlier studies have suggested that subjects with vmPFC lesions have difficulty in making action-based decisions on moral situations and also do not experience normal emotional behavior to such situations (Young et al. 2010). In this study, the researchers presented lesioned subjects with scenarios that displayed one individual causing harm to another, and were asked to judge whether the harm caused was socially permissible. These scenarios ranged from more

normal, non-life threatening situations to murder. They found that compared to controls, lesioned subjects consistently judged attempts of harm as morally acceptable, and even judged harm with intent as more acceptable than accidental harm, indicating not only a deviation from normal socio-emotional function but even a reversal. Furthermore, the lesioned subjects failed to display normative emotional reactions to the situations presented. These results suggest that the vmPFC is critical to the processing of socio-emotional stimuli and integration of an adaptive behavioral response (Young et al. 2010).

It has also been suggested that these regions are critical in the integration of self-moderating and interactions involving socio-emotional cognition tasks. In a study by Beer and colleagues (2006), patients with OFC lesions were first asked a series of questions that tested their knowledge of social norms of disclosure of personal information. They were then asked to participate in a task in which a stranger would ask them personal questions, they would answer the question in as much detail as they felt appropriate. They would watch their taped behavior and were asked to judge the appropriateness of their own response. It was found that while the OFC lesioned subjects performed normally on the test of their knowledge of social norms, they had significantly more inappropriate self-disclosure responses in the stranger questioning task. Furthermore, the OFC lesioned subjects underestimated the inappropriateness of their own responses in the final self-judgement task, failing to show any embarrassment at their social error. These findings led the researchers to suggest that the OFC is critical in self-monitoring of behavior during social situations, as well as the ability to produce appropriate socio-emotional behaviors when deviating from social norms (Beer et al. 2006).

Qualitative studies of human patients with lesions in these regions have made similar observations. In general, these individuals are unable to suppress impulsions driven by both internal urges and external stimuli (Fuster 1997). In contrast to lesions of the DLPFC, OFC/vmPFC lesioned patients possess hyperactive motivation to an extent that it often interferes with important physiological processes such as sleep, and can result in manic behaviors and periods of euphoria (Fuster 1997). Appropriate moral judgment and decision-making are also impaired, and there are some cases of criminal sociopathic tendencies in individuals with lesions in these regions (Fuster 1997). Taken together, these findings suggest that the OFC/vmPFC are critically involved in executive functions related to the mediation of inner impulse and the production of socio-emotionally appropriate behavior, as well as the ability to make adaptive long-term decisions based on attentive processing of environmental cues.

Frontopolar Cortex Lesions

The explicit functions of the frontopolar cortex in humans has largely remained a mystery. While functional MRI studies show that this region is engaged in virtually every basic cognitive task, patients with lesions in frontopolar regions still retain normal performance in basic cognitive tasks, including intelligence, memory, language, motor skills and problem-solving (Burgess et al. 2007). The greatest cognitive deficit observed in frontopolar lesion subjects appears to be the ability to multi-task, or specifically, to resist the distraction of the urge to complete one task during the performance of another, and subsequently returning to the postponed task (Koechlin et al. 2007). Given that patients with frontopolar lesions do not show significant deficits in other cognitive functions during which the frontopolar cortex activates, researchers have suggested that

this region in humans has evolved as an adaptation to aid in flexibility in cognitive control and decision-making, acting as a modulatory mechanism to organize multiple cognitive processes simultaneously (Koechlin et al. 2007). In this way, the frontopolar cortex may support the other processes of the PFC that allow humans to attend to simultaneous stimuli, both internal representations of drive and important external cues, and organize this information in such a way that allows for a rapid adaptive behavioral response (Burgess et al. 2007).

DEVELOPMENT

Prenatal Development

Mammalian neural development begins in the second week of gestation with the formation of neuroectodermal stem cells, or neural progenitor cells, from the epidermal layer of the embryo during gastrulation (Stiles and Jernigan 2010). From these neural progenitor cells, the neural tube forms during the third week of gestation (Teffer and Semendeferi, 2012). Shortly before the neural tube closes, the anterior portion of the tube forms into the three primary brain vesicles: the prosencephalon, the precursor to the forebrain, the mesencephalon, the precursor to the midbrain, and the rhombencephalon, which will form the hindbrain (Stiles and Jernigan 2010). The prosencephalon undergoes further division into the telencephalon and the diencephalon, and the rhombencephalon divides into the metencephalon and myelencephalon, so that there are five subdivisions in total that constitute the rudimentary blueprint of the central nervous system (Stiles and Jernigan 2010).

The neocortex later develops from the telencephalon, along with the limbic

lobe/hippocampal formation, the olfactory/piriform cortex, and some areas of the amygdala (Sur and Rubenstein 2005). In humans, the production of neurons begins in the sixth week of gestation, and migration begins on the fortieth day of gestation (Cassanova et al. 2002a), ultimately resulting in the formation of six cortical layers (Stiles and Jernigan 2010). There is an initial exponential increase of neurons in the neocortex between weeks 13 and 20, followed by a slower linear rate of increase between gestational week 22 to birth. At mid-gestation (week 20) neuron numbers are roughly half that of the average adult brain ($19\text{-}23x10^9$ neurons), and one study found that regions of the cortex reach their maximum neuronal density between gestation week 28 and 38. At birth, neuron numbers average $30x10^9$ cells, significantly more than the adult brain (Teffer and Semendeferi 2012). Within the human neocortex, both neurogenesis and cell migration are complete during the prenatal period (Stiles and Jernigan 2010).

Infancy, Childhood and Adolescence

Total brain growth during infancy is relatively rapid, with total brain volume increasing from 25% of adult brain volume at birth to 72% of adult brain volume in the first year of life, and 83% of adult brain volume by age two (Knickmeyer et al. 2008). Total brain volume increases more slowly during childhood, with maximum volume reached in early adolescence between ages 12-15 years (Courchesne et al. 2000). However, this early expansion is not uniform across the different brain regions. Between birth and the first year of life, the volume of the cortical hemispheres increases by 88%, while subcortical structures increase by 130% and the cerebellum increases by 240% (Knickmeyer et al. 2008). Furthermore, a comparison of the structural MRIs of infants

and adult brains showed that the dorsal and medial prefrontal regions, as well as the lateral temporal and lateral parietal regions of the brain are areas of high post-birth developmental expansion, while the medial temporal, occipital, and insular regions are areas of low developmental expansion. It has further been found that regions of high expansion are less mature than other regions at birth functionally, and that in adults, low expansion areas have smaller and more simply branched dendrites than high expansion areas. These low expansion areas also reach maturity in terms of synaptic density, cortical thickness, and grey matter density earlier than the high expansion areas (Hill et al. 2010).

Rates of growth are not uniform in grey matter and white matter either. Total grey matter volume increases by 149% in the first year of life (Knickmeyer et al. 2008), and continues to increase linearly until late childhood, ages 6-9 years, after which grey matter volume decreases by 5% every subsequent ten years (Courchesne et al. 2000). In contrast, white matter increases by only 11% in the first year of life (Knickmeyer et al. 2008), yet increases by 74% from early childhood through adolescence, from which time it continues to increase at a slower rate until reaching a plateau at around 40 years of age (Courchesne et al. 2000).

As stated above, the trend for grey matter volume increase for the entire brain appears to be a rapid expansion in the first few years of life, followed by a slower increase until ages 6-9 years, followed by a slow, steady decrease in subsequent years thereafter. However, grey matter volume within the PFC continues to increase until early adolescence (11-12 years of age), again supporting the previous findings that the PFC is a late-maturing structure (Teffer and Semendeferi 2012). Similarly, white matter appears to

myelinate latest in the PFC, reflecting late maturation of the connections that compose the neural circuits involved in this region (Muftuler et al. 2012).

Other developmental changes that occur throughout childhood and adolescence are apoptosis, synaptogenesis, synaptic pruning, expansion of axonal and dendritic branching within the cortex (Teffer and Semendeferi 2012; Stiles and Jernigan 2010). These processes are thought to be involved in the maturation of the functional neural circuits. Following the trend of grey matter volume, these processes appear to occur latest in the PFC, and earliest in primary cortices that perform lower order processing (Teffer and Semendeferi 2012). The behavioral data also supports the heterochronous maturation of functional circuits: behaviors attributed to the PFC, such as impulse control and evaluation of risk (Blakemore et al. 2012; Vidal et al. 2012), are deficient in children and sub-adults, while children become efficient at lower-order tasks, such as those related to movement, at a young age (Knickmeyer et al. 2008).

Aging

While neurogenesis is complete in the vast majority of brain regions at birth, there is some evidence that neurogenesis and migration continues in the olfactory bulb and the dentate gyrus of the hippocampus until age forty years (Shankle et al. 2009). Furthermore, it is known that myelination of the white matter tracts in the later maturing frontal and parietal regions of the brain is not complete until age 30-40 years (Sowell et al. 1999a). However, studies investigating total brain volume have found that after peak volume is reached in early adolescence, there is a linear decline, followed by an accelerated rate of decline as individuals pass through middle age and beyond. This

decline is so dramatic that total brain volume is less than that of a 2 year old child by age 71-80 years (Courchesne et al. 2011).

Within the PFC, this decline is disproportionately severe, particularly in grey matter volume. Courchesne and colleagues (2000) report a decline of 4. 9% each decade after age 20, whereas other cortical areas such as the primary motor cortex had a much smaller rate of decline. The reason of this decline is unknown, but suggests that the majority of age-related total brain volume decrease occurs within the PFC. Total brain white matter, on the other hand, appears to increase until around age 40, but by ages 71-80 years, was shown to decrease by 13%. However, like the grey matter of the prefrontal cortex, it would appear that the majority of white matter volume decrease occurs within the prefrontal and superior parietal areas, which experience a much more significant decline that any of the primary sensory areas (Courchesne et. al. 2000; Jernigan et al. 2001). Furthermore, it has been found that at age 90 years, the age-related decline of white matter is significantly greater than the age-related decline of grey matter, whereas even in younger old age (70-81 years), this grey to white matter ratio is less significant (Salat et al. 1999). Rapid neural decline in the PFC is also supported by the behavioral evidence of cognitive decline in old age. Deficiencies in working memory have been correlated with age and PFC decline in adults as early as 61, and numerous studies have documented a reduction in inhibitory control in senescence (West 1996). Reduced performance in visuospatial and attention tasks have also been positively correlated with old age (Teffer and Semendeferi 2012).

Together, the developmental trajectory of the brain occurs as follows: there is an acceleration of neurogenesis between gestational weeks 13-20, followed by a slower,

more linear rate of increase until birth, so that the neural numbers at birth exceed adult values significantly. Throughout early childhood and adolescence, however, natural cell death brings neuron numbers down to adult values. Within the first two years of life, total brain volume increases dramatically, from 25% adult volume at birth to 83% adult volume by age two. From this point on, there is a slower increase in volume, reaching adult volume at around age 12 years. Regionally, this volume increase is not uniform, however, with primary cortices maturing earliest and association cortices, including the PFC, occurring last. Grey matter volume changes alone follow a similar trajectory, increasing rapidly in the few first years of life, linearly from ages 6-9, but decreasing steadily in the years after. However, within the PFC, grey matter continues to increase linearly until age 12 years. White matter has little increase in volume within the first year of life, followed by a large increase in volume from childhood through adolescence, then slowing but continuing to increase until age 40 years. As individuals pass through middle age and into senescence, there is an accelerated rate of decline in total brain volume. These changes are most dramatic in the PFC, especially within grey matter, with a 4. 9% decrease in grey matter volume each decade past age 20 years. White matter decreases at a slower rate until very old age, during which volume decline is severe in the PFC.

Conclusions

It is evident from this overview of the PFC that it is a primary structure implicated in executive functions related to socio-emotional behavior. The PFC has extensive connectivity with subcortical structures involved in the emotional memory system, such as the amygdala and the hippocampus, as well as the hypothalamus, which is involved in

autonomic responses to emotional stimuli and the execution of emotional behavior. The PFC also has substantial cortico-cortical connectivity, and is the only structure that receives input from every sensory modality. These connections suggest that the PFC is an ideal intermediary for sensory input from the external environment and the homeostatic regulation of internal environment of the organism, making this structure well equipped for mediating behavioral flexibility in a dynamic social environment. Furthermore, experimental lesion studies in non-human primates and pathological lesion studies in humans have shown that normative social behavior is reliant on the constitution of the PFC. Lesions of the DLPFC result in deficiencies in cognitive functions involving goal-driven working memory, including attention, temporal integration, and goal-driven behavior and memory-related aspects of self-control. Lesions in the vmPFC and OFC have shown significant deficits in a wide variety of socio-emotional behaviors, including awareness of social norms, self-monitoring of behaviors in order to produce appropriate reactions in social situations, and adaptive socio-emotional responses to the intent of others. Lesions in the frontopolar cortex suggest that this region is critical to simultaneously process internal representations of drive and important external cues, and organize this information in such a way that allows for a rapid behavioral response that is adaptive to the social environment. Lastly, development studies have shown that the PFC is the latest-maturing neural structure, with grey matter continuing to mature until age 12 and white matter continuing to myelinate into the fourth decade of life. The late maturation of the PFC allows for neural circuits involved in social behavior to be adaptively shaped by experience in the social environment.

CHAPTER 2: EVOLUTION OF THE PREFRONTAL CORTEX

Introduction

One of the first inquiries in the study of the evolution of the PFC is when, and with what organism, did this structure first appear? For decades, non-primate animal models, such as rats, have been used to study the function of the PFC, and similarities in function and connectivity have led some neuroscientists to suggest that the structure is an ancient region of the mammalian brain (Uylings et al. 2003). However, the original criterion for the PFC is the presence of a granular layer IV, a feature that is unique to primates. Some have argued that this criterion alone is not useful in defining other cortical areas, and so should not be the sole characteristic for defining the PFC. Instead, connectivity and functionality should be considered (Uylings et al. 2003). While there is little debate that rats possess frontal areas that are similar in function and connectivity to the primate medial and orbital PFC, the existence of the DLPFC in rats has been questioned, because some researchers have suggested that the criteria previously used to distinguish the DLPFC is not acceptable because it applies to premotor areas as well (Pruess 1995). However, Uylings and colleagues (2003) performed a side-by-side structural and functional study on the PFC of rats and nonhuman primates, and concluded that rat brains indeed do have anatomical and functional homologues to the primate DLPFC. These researchers do concede, however, that the rat PFC is not as specialized as that of the primate, suggesting that these specializations are more evolutionarily recent. Furthermore, in a study that examined the scaling of the whole frontal cortex between 25 species of primates and 15 species of carnivores, it was found that the frontal cortex of the primate is hyperscaled relative to both the neocortex and the brain in total when

compared to the carnivores (Bush and Allman 2004). This suggests that the frontal cortex of primates and other mammals is significantly different.

It would seem, therefore, that the answer to the question- do rats (and perhaps some other non-primate mammals) have a PFC?- changes depending on the context of the investigation at hand. Rats clearly possess some anatomical and functional similarities to the primate PFC and may be useful animal models for the research of some general PFC functions such as attention or working memory (Uylings et al. 2003). However, for studies interested in the functions of the PFC that appear specialized to the complex social environment of primates, such as socio-emotional behavior, the use of non-primate animal models is suspect. The primary purpose of this paper is to investigate structural properties of the PFC that may be involved in the evolution of human social behavior. Therefore, for the remainder of this section I will be discussing the evolution of the PFC in an exclusively primate context.

The Frontal Lobe

The human brain as a whole is roughly two to three times larger than predicted for a primate of similar body size (Isler and van Shaik 2006). Furthermore, the principal components analysis of brain morphology between humans and the other extant apes has suggested that humans have a common morphological pattern in many regions including the frontal lobe that is unique to the species (Aldridge 2011). These deviations from the primate trend has been of great interest to those studying the evolution of human behavior, and because of the role of the PFC in human behavioral specializations, some have questioned whether differential enlargement of the human frontal lobe plays a role

in human uniqueness. The comparative study of the frontal lobes of other primates, particularly apes, with humans is often limited by the scarcity of ape brains, and so for many years it was thought that the human frontal lobe is larger relative to that of other apes (Deacon 1996). However, the majority of later studies, one of the earliest being that of Semendeferi and colleagues (1997), suggest otherwise. These researchers took MRI scans of four humans, four apes (1 chimpanzee, 1 gibbon, 1 gorilla, and 1 orangutan), and one monkey (macaque), and measured the volumes of not only the total frontal lobe, but also of the three major frontal lobe divisions (dorsal, mesial, and orbital). They found that while the human frontal lobe, like the brain in its entirety, is roughly three times larger than that of other apes, this enlargement does not, in fact, appear to be disproportionate relative to brain size. Furthermore, they found that the relative size of each major divisions is also fairly conserved across the primates, with chimpanzees having almost exactly the same proportions as humans. This study, as well as a subsequent study by the same group of researchers (Semendeferi et al. 2002) provides evidence that a large frontal lobe is not a uniquely human property, but rather a shared characteristic of all primates.

It has also been shown that humans don't stand out from the primates in terms of total grey matter or white matter quantities in the frontal lobe. While some researchers have recently supported the claim that the frontal lobe is disproportionately enlarged in humans due to a robust expansion of the PFC (Elston et al. 2006), these claims are based on very old estimations of volume that have since been revised. One study by Smaers and colleagues (2011) used histological sections of 19 primate species, including 6 apes (which includes humans) and 13 monkeys to compare total brain area, grey matter area, and white matter area in the frontal lobe, analyzing prefrontal and motor areas separately.

These researchers found that across all three parameters, humans do not deviate significantly from the anthropoid primate trend. However, they did find that the smaller-brained primate species in their sample had a significantly smaller left prefrontal area, and that the trajectory of increasing enlargement in the anthropoid primate brain is such that the left prefrontal area enlarges disproportionately more than the right in all three parameters (grey matter area, white matter area, total brain area). These results suggest that lateralization is an important component of the evolution of prefrontal size. To further support this hypothesis, they found that apes are distinguished from monkeys by having evolved a significant increase in the white to grey matter volume ratio in the left prefrontal areas only. Humans have a similar trend to apes in white matter lateralization, but show significant deviation from the allometric scale for this trend in other primates. These researchers suggest that the structural lateralization trends, first in apes and then in humans, implicate possible cognitive shifts in the evolutionary trajectory of the primate brain, with possible behavioral outcomes such as language and handedness, both of which are associated leftward asymmetry in the frontal lobe. Again, it is important to keep in mind that humans, or apes, do not deviate from the primate trend in terms of total prefrontal, prefrontal grey matter, or prefrontal white matter. Instead, these results suggest a smaller, more specific change in regional lateralization (Smaers et al. 2011).

Some species differences have been found when looking at different components of the frontal lobe, however. One study by Schenker and colleagues (2005) investigated the hypothesis of differential enlargement of components of the frontal and temporal lobes in humans compared to other apes, particularly in subdivisions of white matter. Using MRI scans of ten humans, five chimpanzees, three bonobos, two gorillas, four

orangutans, and three gibbons, these researchers parcellated white matter into gyral white matter (the white matter immediately under the cortex) and core white matter (the remaining white matter). They found that humans have more gyral white matter than expected in relation to the entire respective cortical region (frontal and temporal) as to the core white matter. Furthermore, it was found that chimpanzees and bonobos, the closest living relatives of humans, had an increased ratio of gyral white matter to grey matter in the dorsal region of the frontal lobe compared to gorillas and orangutans, with numbers that overlap with human values in that area. These researchers suggest that this difference in gyral white matter may signify a difference in how humans process information relative to other apes- gyral white matter is associated with short adjacent cortico-cortical connections, and given the known functions of the frontal lobe, these connections are most likely important to cognitive function in humans. Furthermore, an increase in gyral white matter to grey matter ratio is associated with faster transmission, which may be critical to processes of cognitive flexibility and complex cognitive function (Schenker et al. 2005).

Another way in which humans are unique from other primates at the anatomical level is in the aging process. A study by Sherwood and colleagues (2011) performed a comparative investigation of the effects of aging in humans and chimpanzees. Using MRI scans of 99 chimpanzees between the ages of 10-51 years and 87 humans between the ages of 22-88 years, they compared total brain volume, grey matter volume, and white matter volume in the brain as a whole as well as in individual structures, including the frontal lobe. These researchers found that in humans, total brain volume, grey matter volume, and white matter volume in the entire brain, and most significantly in the frontal

lobe and hippocampus, show a decrease as a function of age, while chimpanzee brains show no such age decline. Due to these results Sherwood and colleagues suggest that human lifespan longevity, despite the adaptive advantages it is hypothesized to have in indirect fitness, also carries the consequences of progressive neurological decline. The human brain, and particularly the frontal cortex, is large and metabolically costly, and when combined with a longer lifespan, it is logical that degeneration becomes accelerated relative to shorter-lived species.

Cortical Microstructure

The previous section discussed evolutionary trends in the frontal lobe and PFC by proxy on the gross anatomical scale, and as discussed, it has been found that humans do not stand out from apes in most cases on this level. While many of the current experts in the field tend to agree with this position, it is important to note that there remain significant points of contention regarding this stance (for a review see Schoenemann et al. 2005; Sherwood and Holloway 2005). These differences exist in large part due to disagreements in defining the borders of the PFC based on gross anatomical criteria across species, an action that is difficult due to many reasons, including sample sizes, which limit what is known about both intra- and inter-species variation, and to the crude methods employed in gross anatomical observation and MRI scans. However, the smaller differences that were detected, including the differences in gyral white matter, leftward lateralization, and aging processes do seem to suggest that a more precise quantification of species differences may be obtained by looking at the microstructural processes at the root of these differences. In this section, I will discuss the evolutionary changes at the

microstructural level. I will begin by discussing species comparisons in a few cytoarchitectonic areas in the PFC. I will then focus on neuronal spacing and organization, including species differences in neuropil space and minicolumnar organization. I will next discuss differences in cortical composition across species, including glia-neuron ratios and the distribution of inhibitory interneurons. This will be followed by a discussion on the morphological differences of neurons in the PFC. Finally, I will consider the role of neuromodulatory innervation in the PFC.

Cytoarchitectonic Regions

While significant evolutionary changes in size have not been detected at the gross anatomical scale, some differences in size amongst the primate species have been found in a few specific cytoarchitectonic areas of the PFC. Semendeferi and colleagues (2001) performed a histological investigation of BA 10 in all of the extant apes and one monkey (macaque). BA 10 is a prefrontal area associated with planning of future actions, taking initiative, and the processing of working memory and attention, and forms the entire frontal pole in humans, bonobos, chimpanzees, and orangutans, the orbital sector of the frontal pole in gibbons, and has two separate components in the macaque brain. In terms of size, BA 10 is proportionally twice as large in humans as it is in the other great apes. Cytoarchitectonically, this area is fairly consistent across the hominoids, although some species differences, most likely reflecting functional specializations, were observed. Humans, chimpanzees, and bonobos all have larger supragranular layers, which are associated with cortico-cortical connections, and smaller infragranular layers, which are associated with subcortical connections. This is in contrast with the rest of the hominoids, for whom this trend is reversed, and with macaques, whose supragranular-infragranular

layer ratio is nearly equal. All species seem to have the greatest cell density in cortical layer IV, but throughout the entire BA 10, humans stand out as having a decreased density overall, with density increasing with phylogenetic distance across the primates. Furthermore, humans stand out with significantly lower density in the supragranular layers compared to other primates. Lower density indicates greater neuropil space, which implies an increase in space available for connections. The supragranular layers are associated most with cortico-cortical connections, and so having an increased amount of space in these layers may indicate increased connection between BA 10 and other association areas (Semendeferi et al. 2001).

These same researchers used stereological methods to investigate BA 13, an area in the orbitofrontal cortex (OFC) thought to be associated with emotional reactions to the social environment (Semendeferi et al. 1998). This area, which before this study was not clearly known to be present in humans, appears to straddle the limbic lobe and the PFC, although the largest extent of the region is PFC. They found that humans have volumes that are similar in absolute terms to the orangutans, gorillas, and chimpanzees, but that in relative terms, humans (and bonobos) have the smallest BA 13 volumes out of the apes. Humans also were found to have the smallest relative length BA 13 within the OFC, while orangutans had the greatest relative length. Orangutans were also found to have a very short OFC, so BA 13 occupies more relative space in that region. Microstructurally, all the primates examined were fairly consistent in cortical layer ratios: the supragranular layers are thinner and have more neuropil space than the infragranular layers across the species. Humans, chimpanzees, and bonobos had a relatively thinner layer IV than the rest of the primates. Consistent with BA 10, however, humans had the lowest cell density

in BA 13, suggesting that a decrease in cortical cell density is a consequence of an increase in brain size rather than a unique feature of this area. Overall, in size BA 13 appears to be reduced in humans, although many of the cytoarchitectonic features of this area are conserved across the primates studied here (Semendeferi et al. 1998).

Another cytoarchitectonic area that has been compared across the primates is Broca's area (BA 44 and 45). This area has been consistently found in the inferior frontal gyrus of the human, chimpanzee, bonobo, gorilla, and the orangutan (Schenker et al. 2008). This general region of the PFC is thought to contribute to species-specific social behavior, such as the identification and imitation of conspecifics, and in humans, Broca's area is implicated in many important components of language. In this study, Schenker and colleagues (2008) performed a histological comparison of both hemispheres of 11 humans and 9 great apes. They found that the basic cytoarchitecture of Broca's area was fairly consistent across the species, although there were some species-specific differences: bonobos displayed a wider layer I in both BA 44 and 45, as well as a dysgranular (transitional) layer IV; the cortex of BA 45 was thinner than BA 44 in humans, chimpanzees, and bonobos, while both areas had similar cortical thicknesses in gorillas and orangutans; the gorilla had thinner total cortex in Broca's area compared to the other species examined. Consistent with the general trend of the other cytoarchitectonic areas, the human group had the smallest cell density of the primates examined. The human groups also had larger horizontal spacing between minicolumns (vertical conglomerates of cells that have significant interconnections amongst the cortical layers), again supporting the general trend of the human PFC possessing greater space between cell bodies. However, contrary to their hypothesis, they did not find

significant species-specific asymmetry in Broca's area in either the humans or the great apes at the population level, although humans did display the most individual variation in asymmetry in this area.

While there are some overarching trends in the cytoarchitectonic regions discussed, such as decrease in neuronal density and increase in neuropil space in humans, as well as a general conservation of laminar structure in these regions across the primates, most of the differences are only apparent when looking at a specific architectonic area. In humans, BA 10 appears to have undergone significant expansion, while BA 13 appears to have been reduced, and while its size has not changed, Broca's area appears to have undergone significant changes in neuronal structure. More importantly, analysis of these cytoarchitectonic regions together suggests that significant reorganization of functional areas, rather than a homosychronic expansion of the entire PFC, is implicated in the evolution of the hominin PFC and the cultivation of specialized neuronal circuits responsible for human behavior. In the next section, I will look more closely at the microstructural organization of the cortex.

Neuronal Spacing and Organization

One of the most basic ways to investigate neuronal spacing across species is to compare cell densities. It is a commonly observed trend in primates that as brain size increases, cell density in the cortex tends to decrease (Collins 2011). We have already seen that cell densities are lower in humans compared to other primates in several prefrontal areas, including BA 10 (Semendeferi et al. 2001), BA 13 (Semendeferi et al. 1998), and BA 44 and 45 (Schenker et al. 2008). It has also been found that humans have the lowest cell density in cortical areas outside of the PFC, including the planum

temporale (Buxhoevden et al. 2001a), BA 3, BA 4, and BA 17 (Semendeferi et al. 2011). These findings imply that a decrease in cell density is a change that occurred throughout the entire neocortex in hominin brain evolution. However, this decrease in cell density appears to be most significant in prefrontal areas in humans, including BA 10. Furthermore, there does not appear to be a phylogenetic trend in decreasing cell density, either in prefrontal areas or throughout the neocortex- out of the apes, gorillas have the second lowest density in BA 10, while bonobos have the greatest density in BA 3 and BA 4 (Semendeferi et al. 2011).

Some of the discussion above suggested that the decreased density seen throughout the neocortex in humans indicates an increase in neuropil space. Neuropil space is defined as the space between the cell bodies in the cortex (or in other words, any space that is not occupied by a cell body), and is occupied by dendrites, axons, synapses, glial processes, and other components of neuronal transmission (Spocter et al. 2012). The proportion of neuropil space within the cortical grey matter, as measured by binary-color image analysis in Nissl-stained histological sections (Grey Level Ratio, GLR), is therefore taken as an approximate measure of total connectivity. Some researchers have measured neuropil space in layer III in a few cortical areas (these studies are discussed below: Buxhoevden et al. 2001a; Schenker et al. 2008; Semendeferi et al. 2011), but few have looked at the neuropil space throughout the cortical layers. Spocter and colleagues (2012) compared measures of neuropil space in humans and chimpanzees in six architectonically distinct areas spanning the cortex: BA 10 (frontal pole), BA 45 (part of Broca's area), the frontoinsular cortex, the primary motor cortex, the primary auditory cortex, and the planum temporale (part of Wernicke's area). They found that in humans,

BA 10 and the frontoinsular cortex had significantly more neuropil space than the other regions. In contrast, only the primary auditory cortex stood out in chimpanzees, having significantly less neuropil space than the other regions analyzed. The authors suggest that the frontal pole and frontoinsular cortex underwent significant organizational shifts in microstructure in hominin evolutionary history, including an increase in neuropil space. An increase in neuropil space thus implies an increase in intrinsic and extrinsic connectivity in these frontal areas, supporting the hypothesis that unique human behavioral adaptations may be a product of faster, more local, and more extensive connectivity involved in complex cognitive functioning (Spocter et al. 2012).

Minicolumns are another way to gauge neuronal organization and cortical space. As discussed briefly above, minicolumns are vertically oriented conglomerates of cells that extend throughout layers II through VI of the cortex. Minicolumns are considered by most researchers to be the basic anatomical and physiological unit of of the mature mammalian neocortex (Buxhoeveden and Casanova 2002a). A minicolumn is usually comprised of 80 to 100 neurons, and range in size from 40 to 60 microns in most mammals, although minicolumns in the human brain have been found to range from 35 to 60 microns, depending on the region (Buxhoeveden and Casanova 2002b). Functionally, the minicolumn serves as a specialized local network that unites the neuronal differentiation and specialization of the individual horizontal cortical layers with vertical connectivity. A brief description of the relationship between these vertical and horizontal components of the cortex are as follows: afferent signals to the laminae are either thalamic (to layer IV), callosal(from subcortical regions; to layers II, III, and IV), or cortico-cortical (to layers II, III, and IV). Input from the thalamus to layer IV is sent to

inhibitory interneurons as well as up to layers II and III, from which the signal is relayed back down to layers V and VI. (Buxhoeveden and Casanova 2002a). Minicolumns are a vertical unit of specialized cortical layers; therefore, each minicolumn contains a different selection of local inhibitory circuits and afferent and efferent pathways. This selectivity of inhibitory circuits and connectivity pathways are such that the minicolumn allows for local flexibility and plasticity necessary for complex neural networks (Buxhoeveden and Casanova 2002a).

While in recent literature comparative investigations of minicolumns between neurological pathologies have been examined (see next chapter for more detail), little has been done in terms of evolutionarily close cross-species comparison, as most researchers have worked under the assumption that minicolumnar morphology is evolutionarily conserved across species since minicolumn width has not been found to correlate with cortical volume in a comparison of diverse species (Mountcastle 1997). The first in-depth cross-species primate study of minicolumns is attributed to Buxhoeveden and colleagues (2001), who performed a quantitative investigation of minicolumns in humans, chimpanzees, and rhesus macaques in the planum temporale, an area in the temporal lobe that has been associated with language in humans. They found that humans had significantly wider minicolumn width (51 microns) than chimpanzees and macaques (~36 microns for each), as well as significantly more neuropil space in the minicolumn periphery and significantly decreased cell density (although cell density within the minicolumns was the most dense in humans relative to total cell density). These findings led the researchers to conclude that while minicolumnar morphology of the entire neocortex might appear generally conserved amongst a diverse array of species, a closer

investigation of particular cortical regions in evolutionarily close species suggests that there is some correlation to cortical volume and minicolumnar width, which may be related to the morphological changes associated with an increase in extracellular space that accompanies a larger brain. They suggest that minicolumn specializations in a particular region of the cortex are limited by the degree of interconnectivity and complexity of the region, so minicolumns and cortical volumes in a given region are intimately related (Buxhoeveden et al. 2001).

Semendeferi and colleagues (2011) also performed a comparative study of cross-species primate neuronal spatial organization, this time comparing all extant hominoids in BA 10 (frontal pole), BA 4 (primary motor cortex), BA 3 (primary somatosensory cortex), and BA 17 (primary visual cortex). These researchers used measures of horizontal spacing difference (HSD; the spacing between minicolumns horizontally) as well as density estimations using the Grey Level Ratio (GLR; the fraction of area occupied by cell bodies) in order to quantify the species differences in the neuronal organization in various cortical areas. HSD was measured specifically within layer III in all species in each area, because this layer is both the origin of horizontal axonal connections to other minicolumns as well as an important origin and target of axons between the layers. While HSD and GLR do not assume the existence of minicolumns, they are often used to estimate minicolumn width, as increased HSD and decreased GLR indicate wider minicolumns. Semendeferi and colleagues found that only BA 10 appears to differ significantly in humans compared to the other apes; in this area, humans displayed a significantly larger HSD compared to the other apes, and had the smallest GLR (although decreased density in humans is trend that seems to be found in most

cortical areas). In contrast, all species had a similar HSD in BA 17, despite significant differences in brain size. BA 17 also had the smallest HSD in all species, while BA 4 had the largest HSD in all of the apes, and the second-largest HSD in humans. BA 3 had a similar HSD to BA 10 across the apes. These results suggest that BA 10 has undergone a unique reorganization in humans, with wider minicolumns that could allow for locally specialized connectivity necessary for complex cognitive processing (Semendeferi et al. 2011).

Cortical Composition

The previous studies mentioned in this section have investigated questions of neuronal organization in terms spatial distribution of cell bodies, with results suggesting that the observed increase in neuropil space, as well as the discrete organization of cell bodies into minicolumns in the human neocortex and particularly the human PFC, may be proxy evidence for an increase in intrinsic and extrinsic connectivity. A different way of approximately quantifying connectivity is through the observation of glia-neuron ratios. Sherwood and colleagues (2006) compared glia-neuron ratios in three frontal areas thought to be involved in complex cognitive functions attributed to human behavioral uniqueness (BA 9L, BA 32, and BA 44), as well as the motor cortex, BA 4, in humans, chimpanzees, and macaques in order to determine whether there was a disproportionate increase in this ratio in humans. Glia provide neuronal support, so if humans displayed an increase in glia it could suggest that these areas underwent a specialized evolutionary change for increased support due to an increase in energetic demand. However, it was found that while humans do have an increased glia-neuron ratio, this increase is not disproportionate, and instead scales proportionately to the increase in brain size in

humans. Therefore, the authors suggest that this increased ratio is allometrically tied to a larger brain size: the larger the brain, the more intrinsic and extrinsic connections there are, and thusly, more support is needed to the neurons involved in these projections in order to keep up with the metabolic demands. While the human brain does have greater metabolic demand compared to other primates, this does not appear to be a feature that is specialized in humans, but rather an allometric property of increasing brain size (Sherwood et al. 2006). However, it does support the previous findings discussed above that the human frontal areas are characterized by an overall increase in connectivity, and supports the hypothesis that neuronal density is most significantly decreased in the human PFC in order to make room for the connections necessary for complex cognitive function.

Inhibitory GABA-ergic interneurons are another important component of cortical composition in the neocortex. Inhibitory interneurons are critical to thalamic input to the neocortex and influence pyramidal neuron activity. It has been hypothesized that increases in number and type of inhibitory interneurons have played a critical role in human brain evolution. Sherwood and colleagues (2010) sought to examine the distribution of three inhibitory interneuron subtypes in three prefrontal areas (9, 32, and 44) and the motor cortex in 23 anthropoid primates, including humans. These researchers hypothesized that inhibitory interneurons would be increased in the prefrontal areas, given that these areas are associated with cognitive functions that would require complex neural circuitry. This hypothesis was also proximately tested through genetics; researchers analyzed the distribution of 20 genes related to inhibitory interneuron regulation and development. They found, however, that humans did not differ

significantly in the number or subtype distribution of inhibitory interneurons in the prefrontal areas, and that out of all of the species analyzed, only chimpanzees stood out with a species-distinct prefrontal inhibitory interneuron phenotype. Furthermore, most of the genes related to inhibitory interneuron regulation and development appear to be conserved across the primates. Taken together, these results suggest that the distribution of inhibitory interneurons are not critical to the evolution of human cognition (Sherwood et al. 2010).

Neuronal Morphology

Another method that can be used to investigate microstructural differences across species is to look at morphological differences in pyramidal neurons. Pyramidal neurons are by far the most abundant neurons in the cortex, comprising 70-90% of the total neuronal population (Elston et al. 2006). Pyramidal neurons are distinguished by a large apical dendrite and two basal dendrites that form dendritic trees that can become very complex. These neurons make up almost all cortico-cortical connections, as well as the majority of cortico-subcortical connections, and serve as both the main source and target of excitatory synapses. Subtypes of pyramidal neurons are identified by their morphological structure, and particularly the morphological structure of their dendritic trees; differences can be seen across species, brain regions, and even cortical layers. It is hypothesized that morphological specialization of pyramidal cells strongly influences cortical function (Elston et al. 2006; Elston 2007; Elston 2011; Petanjek et al. 2011).

While cross-species comparisons of pyramidal cells and their dendritic tree morphology are extremely valuable to the study of primate brain evolution, methodological considerations necessary for complete dendritic visualization make such

study exceedingly difficult, so few comparative investigations exist in the literature. The few studies that do exist are mostly investigated by the same research group, and are limited by exceedingly small sample sizes (often only 1 individual per species) that do not necessarily meet standard comparative qualifications, such as age matching, sex matching, or even same hemisphere of origin. However, while their findings could be confounded by these sampling issues, they provide some of the best insight currently available to the evolution of neuronal structure. One such study by this research group, Elston and colleagues (2006), compared the pyramidal morphology in layer III of a human, macaque, marmoset, baboon, vervet monkey, owl monkey, and galago in three separate areas: primary V1, primary V2, and the dorsolateral PFC (DLPFC). They found some similar trends amongst the species; for instance, species with larger cortical areas had larger pyramidal neurons, and in all cases, the dendritic branches of the DLPFC were both more spinous and more complex compared to the primary visual areas. Furthermore, the relationship between number of dendritic spines, cortical area, and size of basal dendritic trees was different in the DLPFC than in the primary visual areas in all species. However, they also found that the dendrites of the pyramidal neurons in the human specimen, which had the largest DLPFC of the individuals analyzed, were disproportionately more spinous than the primary visual areas, as well as disproportionately more spinous than the DLPFC of other species (human DLPFC was 70% more spinous than the macaque). These findings suggest that the human DLPFC receives significantly more excitatory inputs than does the corresponding area in other primates, and could be a feature of more complex connectivity in humans that supports the complex cognitive functions of the human PFC (Elston et al. 2006).

A later study by these same researchers (Elston et al. 2011) compared pyramidal neuron structure of layer III in lateral, medial, and orbital PFC of the vervet monkey, macaque, and baboon in order to further investigate morphological trends across species in the PFC. Again, they found that across the species, all three regions of the PFC had significantly more complex pyramidal structures than in other cortical areas (this significant interaction between cortical area and spine density was not found in chimpanzees, however; see Bianchi et al. 2012 for more detail). However, they also found that in all three prefrontal areas of all species examined, there was regional variation in neuronal morphology, and the type of variation amongst areas was not consistent across the species; each species had its own set of regional phenotypes that differed in the parameters of somal size, spine density, and dendritic branching structure. Furthermore, they also found that interindividual variation within the PFC was also significantly greater than reported in other cortical areas. Together, these findings provide evidence that while the pyramidal neurons of the PFC are consistent across species in that they are more complex, each species has its own specialized morphological variation and gradient of complexity that most likely reflect functional differences adapted to meet a given species' environmental demands (Elston et al. 2011).

Another study by a different research group (Bianchi et al. 2012) examined the dendritic morphology of layer III pyramidal neurons in chimpanzees in prefrontal area 10, primary somatosensory area 3b, primary motor area 4, and prestriate visual area 18, and compared these results to the dendritic morphology of pyramidal neurons in the same four areas in humans. They found that the dendritic morphology of the layer III pyramidal neurons in the prefrontal region (area 10) is more complex (ie more branched

and more spinous) compared to the other areas examined, a finding that is consistent with the findings in humans. However, they also found that human pyramidal neurons had more extensive dendritic branching across all regions studied, but that this was not disproportionately increased in the PFC in humans. Therefore, the disproportionately greater neuropil space and minicolumn width found to be unique in humans, as discussed above, is not due to a similarly disproportionate increase in dendritic branching, and must be related to another parameter of cortical microstructure (Bianchi et al. 2012).

While the limitations and confounding variables in these studies require that any statements made on the evolution of the primate brain be approached with apprehension, the findings do point to some interesting interpretations that encourage further investigation. First, it appears that over the course of primate brain evolution, pyramidal neurons in the PFC underwent significant morphological change, in the form of phenotypic complexity and structural gradients, in comparison to the more conserved morphology of the primary sensory cortices. Secondly, the morphological phenotypes of pyramidal neurons do not appear to have a clear phylogenetic trend, but rather vary greatly and regionally from species to species, possibly reflecting functional specializations necessary for the different adaptive cognitive styles of each species. Thirdly, the pyramidal neurons of the human PFC appear to have undergone a drastic morphological change, most pronounced of which is the dramatic increase in dendritic spines. An increase in dendritic spines reflects an increase in excitatory input to the PFC, and suggests that the human PFC is marked by increased, more complex connectivity compared to other primates. This increase in connectivity and connective complexity could have an important functional role in the human-specific cognitive behaviors that

are associated with the PFC (Elston et al. 2006; Elston 2007; Elston et al. 2011).

Innervation

Another important factor in cortical microstructure is the innervation of axonal fibers by neuromodulators. Neuromodulatory systems involve long-term effects in processing, including influencing synaptic transmission and affecting characteristics of pyramidal neurons (Raghanti et al. 2008a). One such critical neuromodulator is acetylcholine (ACh), which is thought to be involved in learning, working memory, and cognitive flexibility. Raghanti and colleagues (2008a) sought to compare cholinergic innervation both quantitatively and qualitatively between humans, chimpanzees, and macaques in two prefrontal areas, 9 (involved in inductive reasoning and working memory), and 32 (involved in theory of mind tasks), as well as area 4 (primary motor cortex). They hypothesized that humans would have denser or more extensive cholinergic innervation in the prefrontal areas compared to other primates, given that cholinergic innervation is critically implicated in complex cognitive processes attributed to human behavior. However, they found that humans do not have greater cholinergic innervation in the prefrontal areas; instead, area 4 had the most dense cholinergic innervation across all of the species. Therefore, quantitative measures of cholinergic innervation cannot be attributed to the maintenance of the complex cognitive functions that influence uniquely human behavior. However, they did find some species-specific patterns of innervation that may be involved in differences in function. For instance, humans and chimpanzees had less cholinergic innervation in layer I compared to layers III and V/VI in all three areas, while macaques had more cholinergic innervation in layer I than in layers V/VI in areas 9 and 32, but not in area 4. Raghanti and colleagues suggest that these differences

could be related to an evolutionary shift in cortical circuitry, such that in chimpanzees and humans greater emphasis in cholinergic innervation was placed in layer III, associated with cortico-cortical connections, and layers V/VI, associated with cortico-subcortical connections. Another important species-specific finding was the presence of clusters of ChAT-ir fibers in humans and chimpanzees, and the absence of these fibers in macaques. Clusters of fibers are thought to be associated with events of cortical plasticity, so the presence of these fibers in human and chimpanzee cortex, and absence in macaque cortex, might contribute to species differences in cognitive flexibility (Raghanti et al. 2008a).

These same researchers also examined differences in dopamine innervation in areas 9,32, and 4 in humans, chimpanzees, and macaques (Raghanti et al. 2008b). Dopamine is a neuromodulator that has been crucially implicated in high cognitive functions such as working memory, language comprehension, and reasoning, as well as some role in general intelligence (Raghanti et al. 2008b). Given that in humans, the prefrontal areas 9 and 32 are involved in such higher cognitive functions, these researchers hypothesized that humans would display differences in dopaminergic innervation in these areas compared to other primates. Similar to the findings of cholinergic innervation, humans did not display a significant increase in dopaminergic innervation in these areas compared to chimpanzees. Some species-wide trends were observed amongst the three areas: in all species, area 4 had the most dense dopaminergic innervation. However, there were some species-specific patterns of innervation observed. While macaques had higher innervation in layers I and II compared to layers III and V/VI in all three cortical areas, chimpanzees had higher innervation in layer I of area 4 only,

and humans had higher innervation in layers V/VI in the prefrontal areas only. In macaques, the density ratio of dopamine-innervated axons to neurons was significantly decreased in layers III and V/VI in areas 9 and 32, but not in area 4. In contrast, these differences were not observed in humans or chimpanzees. Finally, as with the cholinergic innervation study, humans and chimpanzees displayed coils of dopaminergically innervated axon fibers in layer III, while macaques did not. Again, the presence of these coils has been linked to cortical plasticity (Raghanti et al. 2008b).

Finally, these researchers investigated serotonergic innervation in humans, chimpanzees, and macaques, in cortical areas 9, 32, and 4 (Raghanti et al. 2008c). Serotonin is another neuromodulator that is implicated in many functions in the prefrontal cortex; furthermore, it has been demonstrated that patterns of serotonergic innervation is significantly more complex in primates compared to other animals. Like the findings of the previous two studies, humans did not display quantitatively more serotoninergic innervation in areas 9 and 32; instead, in all species, area 4 had the densest innervation, followed by area 32, with least innervation in area 9. However, humans did display a significantly stronger innervation of area 32 compared to the macaques, as did chimpanzees, although to a lesser degree. Furthermore, humans and chimpanzees had greater innervation in layers V/VI of both areas 9 and 32 when compared to the macaques. Humans and chimpanzees also possessed the morphological specialization of serotonin-innervated fiber coils, similar to the coils/clusters observed in the previous two studies (Raghanti et al. 2008c).

All three of these studies show that while primates seem to share general patterns of neuromodulatory innervation in the cortex, the prefrontal areas appear to contain

patterns of innervation that are species-unique. In general, humans appear to have greater innervation of all three neuromodulators to layers III and/or V/VI compared to macaques, followed by chimpanzees to a lesser extent. As discussed previously, these layers are important in complex cognitive circuits, so the presence of greater innervation of these neuromodulators in these layers in the prefrontal cortex in humans and chimpanzees may represent an important evolutionary shift in neurocircuitry, could be related to the cognitive abilities shared by apes that seem to be lacking in monkeys, such as Theory of Mind tasks. Furthermore, in all three studies, the presence of coils or clusters of richly innervated fibers were found in humans and chimpanzees, but not macaques. While the functional significance of such coils is unknown, it is thought to be related to cortical plasticity, and thus may be related to the greater behavioral flexibility seen in the apes compared to other primates (Raghanti et al. 2008a,b,c).

Conclusions

Throughout this chapter, I have discussed the evolution of the human PFC at many levels. First, I looked at evolution of the human frontal lobe at the gross anatomical level. It is evident that while some small differences can be observed (Schenker et al. 2005; Sherwood et al. 2011), the human frontal lobe as a whole does not deviate drastically in terms of relative size, or relative quantities of grey matter or white matter (Semendeferi et al. 1997; Semendeferi et al. 2002; Smaers et al. 2011). However, comparative investigations of cortical microstructure have shown some uniquely human phenotypes in terms of cell density (Semendeferi et al. 1998; Semendeferi et al. 2001; Semendeferi et al. 2011; Schenker et al. 2008; Buxhoevden et al. 2001a), neuropil space

(Buxhoevden et al. 2001; Schenker et al. 2008; Semendeferi et al. 2011; Spocter et al. 2012), minicolumnar organization (Buxhoeveden et al. 2001; Buxhoeveden and Casanova 2002a; Semendeferi et al. 2011), and pyramidal morphology (Elston et al. 2006; Elston 2007; Elston et al. 2011), as well as some aspects of cortical microstructure that are relatively conserved across the primates (Sherwood et al. 2006; Sherwood et al. 2010; Raghanti et al. 2008a,b,c). Many of these observations are tentative; most sample sizes were very small due to methodological considerations, and additional studies are needed before drawing any general conclusions of human brain evolution. This being said, however, one interesting trend to all of this data can be observed- most of this evidence suggests that the human PFC is marked by increases in connectivity, whether this is in terms of a decrease in neuronal density, an increase in space for connections, a shift in minicolumnar organization that allows for more short, local connections to be made, or morphological specializations of pyramidal neurons that allow for greater, more extensive connectivity.

CHAPTER 3: PATHOLOGY

Introduction

It is known that the PFC is a relatively recent structure in mammalian brain evolution, and that it is this region that is associated with much of the higher order cognitive functions, such as reasoning, attention, memory, and social behavior (Teffer and Semendeferi 2012). Similarly, the observed anatomical and microstructural differences between humans and other extant primates suggest that this structure has undergone significant, and relatively rapid, evolution in the hominin lineage. Furthermore, it has been found that genes associated with meeting the high metabolic demands of the PFC have undergone rapid evolutionary changes in humans, and that these genes that evolved most recently and rapidly have been found to be significantly altered in neurological disorders with social phenotypes that implicate dysfunction in the PFC, such as schizophrenia (Khaitovich et al. 2008). These findings suggest that comparisons of neurological pathologies that have abnormal social phenotypes are useful in the study of the evolution of the human PFC, because the features that we are most interested in, those that evolved most recently, and thus make human behavior unique, may be the very features altered in such neurological pathologies, as their recent and rapid evolution has made them most susceptible to dysfunction. Two such neurological pathologies that show significant association with dysfunction in the prefrontal cortex are autism and schizophrenia. In this section, I will review the findings on each of these disorders separately, with a particular focus on cortical thickness and neuronal number and density when possible, given that these are the parameters I will be investigating in Williams Syndrome in the following chapter.

Autism Spectrum Disorder

Autism is neurodevelopmental disorder with a strong genetic component. It is currently known as Autism Spectrum Disorder (ASD), because the disorder is associated with a spectrum of behavioral phenotypes related to deficient social behaviors and language difficulties, restrictive attentive abilities, and highly rigid, repetitive behaviors (Geschwind 2009). ASD is also heterogenous in terms of its genetic phenotype; each genetic locus that has been linked to a case of autism either is only present in a few cases or has an insignificant role in neural dysfunction (Geschwind 2009). However, despite this heterogeneity, many behavioral stereotypes observed in ASD are implicated in prefrontal function. In this section, I will review the anatomical and histological studies of PFC abnormalities in ASD.

Volumetric Studies

Numerous studies have observed that cases of ASD are correlated with abnormally high brain volume within the first 1-2 years of life (Courchesne et al. 2003; Carper et al. 2002; Carper and Courchesne 2000). This observation has been documented both through neuroimaging studies as well as through measurements of head circumference, which is has been found to be an accurate measure of brain volume in the first few years of life (Lemons et al. 1981; Bartholomeusz et al. 2002). One such head circumference study by Courchesne and colleagues (2003) found that compared to normal healthy infants, infants who were later found to have ASD had a significantly smaller head circumference at birth, followed by an extremely rapid increase in head circumference in the first 6-14 months of life. Such a drastic increase in head circumference was seen in 59% of the ASD infants, compared to only 6% in the typically

developing infants. These authors suggest that head circumference may be a useful early sign of risk of ASD in young infants, as behavioral symptoms do not manifest until 2-3 years of life (Courchesne et al. 2003). However, another study found that head circumference of ASD individuals is normal at birth (Lainhart et al. 1997), and in a small number of cases larger than normal at birth (Gilberg and Souza 2005). While these differences reinforce the etiological heterogeneity of ASD, the majority of head circumference studies continue to suggest a developmental trajectory of rapid early brain growth in the first 1-2 years of life, followed by a period of slow growth in later childhood (Courchesne and Pierce 2005).

This pattern of abnormal growth patterns has been consistently observed throughout the neuroimaging literature in ASD patients. Courchesne and colleagues (2011) performed a longitudinal cross-sectional MRI scan analysis on over 500 ASD studies. Compared to typically developing children, they found rapid overgrowth in brain volume in ASD individuals in ages 2-3, followed by a period of extremely reduced growth from ages 4-9 years. Interestingly, it was also found that there is a possible increase in volumetric decline in ASD individuals from adolescence through middle age compared to typically developing individuals. Collectively, these results have led the authors to suggest that the age-specific neuroanatomical changes in autism are representative of underlying dysfunction in the genetic, molecular, and cellular processes that control microstructural development (Courchesne et al. 2011).

More detailed studies in neuroimaging have found that this enlargement seen in early ASD patients is not uniform throughout the brain (Carper et al. 2002; Carper and Courchesne 2000). A neuroimaging study by Carper and colleagues (2002) quantified the

individual lobe volumes and segmented the grey and white matter in typically developing and ASD patients between 2-11 years of age . They found that in ASD subjects aged 2-4 years, the frontal lobes had significantly more white matter volume, as did the parietal lobes, although to a lesser extent. Similarly, the frontal lobe grey matter volume was significantly higher in the ASD patients in the 2-4 year age range, as did the temporal lobes, but to a lesser extent. Furthermore, it was found that the period of early rapid growth in ASD infants is followed by extremely reduced growth, so that between the ages of 4-8 years frontal lobe volume only increased by 1%, while in the typically developing children the frontal lobe volume increased by 20%, and by age 8 brain volumes between ASD and typically developing children were mostly indistinguishable. Interestingly, they also found a slight trend between severity of ASD stereotypic behaviors and enlargement of frontal lobe white matter in early childhood. Taken together, these results suggest that although the genetic and behavioral profile of ASD is highly variable, there appears to be a similar neurodevelopmental trajectory in ASD, of which the frontal lobe is most severely affected. This corresponds well to the behavioral data, which has found that cognitive abilities related to the frontal lobe, such as social function, language and attention are disrupted, while other abilities with functions that are not seated in the frontal lobe are unimpaired (Carper et al. 2002).

Cortical Thickness and Grey-White Matter Boundaries

Abnormalities in cortical thickness in ASD cases have been a source of dispute in the recent literature. As previously discussed, the first few years of life are associated with a dramatic increase in grey matter volume in ASD patients, although there are no studies currently in the literature that examine cortical thickness in infancy and early

childhood. There have also been findings that some ASD individuals also have a greater volume of grey matter in later childhood and adolescence compared to typically developing individuals. Therefore, it has been suggested that a possible source of this grey matter volume increase is an increase in cortical thickness (Hardan et al. 2006). One of the earliest studies of postmortem autistic brains reported an overall increase in cortical thickness in two individuals in their early 20s, although this was not found in the other cases, including a four-year-old (Bailey et al. 1998). An MRI study of 17 ASD children and their age matched controls ranging from 8-12 years of age, found greater cortical thickness overall in the ASD children, but a regional analysis found that thickness was only significantly greater in the temporal and parietal cortices, while the frontal and occipital cortices were not significantly thicker (Hardan et al. 2006). However, others have found a lack of significant differences in absolute cortical thickness between ASD and typically developing individuals (Mak-Fan et al. 2012; Hutsler et al. 2007), while others found a decrease in cortical thickness in individuals with ASD (Misaki et al. 2012).

While absolute differences in cortical thickness are not consistently observed in ASD individuals, there have been some significant differences in change of cortical thickness over time in ASD compared to typically developing individuals. One study by Hardan and colleagues (2009) used MRI scans to analyze the the changes in cortical thickness and grey matter volume over a period of two years in ASD and typically developing male subjects aged 8-12 years. They found that while absolute differences in cortical thickness were not significant between the two groups, changes in cortical thickness over time were significant between the two groups, with ASD individuals

showing a significantly more pronounced decrease in cortical thickness in many cortical areas, including the frontal lobe (Hardan et al. 2009).

Hutsler and colleagues (2007) performed a similar study, but over a larger age range of 14-45 years. Like previous studies, they found that averaged cortical thickness measures were similar between the ASD and typically developing groups. They also found a significant relationship between the magnitude of cortical thickness decrease and age in the ASD group, but not in the typically developing group; however, the difference between these two group correlations was non-significant. They did find that the relationship between age and cortical thickness compared between the two groups has a downward trend with age, which suggests that differences between the changes in cortical thickness with age between the two groups is more pronounced during younger ages (see Figure 2). This pattern of differential developmental progression in cortical thickness is also supported by the findings of another study, which found that this interaction was strongest in the frontal and parietal lobes (Mak-Fan et al. 2012). In this same study, Hutsler and colleagues also performed a histological comparison between post-mortem ASD and typically developing subjects, with which they analyzed the the proportional thicknesses of the individual cortical layers in multiple brain regions. They did not find any significant interaction between individual layer thicknesses and region or diagnosis, leading the researchers to conclude that there are no significant differences in laminar patterning between ASD and typically developing subjects (Hutsler et al. 2007). However, a later study by Avino and Hutsler (2010) found that the grey-white matter subcortical boundary was less distinct in ASD subjects compared to controls, which suggests that cell patterning between layer VI and subcortical white matter may be

disrupted in ASD.

One final study in cortical thickness in ASD individuals is worth noting. Misaki and colleagues (2012) sought to investigate interactions of cortical thickness, age, and intellectual ability (full-scale IQ) in high-functioning ASD and typically developing individuals between 12-24 years of age. As mentioned earlier, these researchers found a significant interaction between diagnosis and cortical thickness, with ASD individuals having thinner cortices overall, as well as an interaction between both groups in terms of cortical thickness and age, with thinning of cortex occurring over time in both groups. However, while they found a positive correlation between IQ and cortical thickness in orbitofrontal, postcentral and superior temporal brain regions in the typically developing group, they did not find such a correlation in the high-functioning ASD group. While definitive conclusions cannot be drawn from these findings because they are only correlational and measures of IQ as a proxy for intellectual ability are controversial, these findings are still interesting because they suggest that the relationship between neural structure and intelligence is different in ASD and typically developing individuals. Furthermore, this suggests that high functioning ASD individuals possess structural alterations in cognitive processing compared to typically developing individuals that may compensate for dysfunctional regions or neural circuits (Misaki et al. 2012).

Neuronal Number

While one possible source of increase in brain volume, and particularly grey matter, in ASD individuals could be a pathological increase in neuronal numbers, few studies have investigated this, as access to post-mortem ASD subjects is limited. This is an interesting line of inquiry, however, because unlike parameters such as cortical

thickness which varies with age, neurogenesis ends during prenatal development, so any differences in neuronal counts in ASD patients should be consistent at all ages prior to neurodegeneration seen in aging. One of the earliest studies of neuronal counts compared the neuronal counts in the primary auditory cortex, Broca's area, and auditory association cortex of a single 21-year-old autistic female, who died of unknown causes,with two age-matched controls (Coleman et al. 1985). These regions were chosen by the researchers due to the effects of autism on language skills and auditory behavior. While counts between regions and diagnosis varied significantly, and the autistic subject consistently had a lower glia/neuron ratio than the controls across all areas, no differences were significant, and variation in neuron counts in any given area was greatest between the two controls than between the autistic subject and either control. While these results failed to provide evidence of abnormality in these brain regions, there were a number of methodological considerations (such as unknown cause of death of the autistic subject, sample size, and counting discrepancies) that could have confounded the results.

A later study by Courchesne and colleagues (2011) performed neuron counts in two prefrontal regions (DLPFC and MPFC) in seven postmortem ASD children (2-16 years of age) and their age-matched controls. In order to test the hypothesis that abnormally large brains in ASD children may be in part accounted for by a pathological increase in neuron number, they also compared the brain weights of their subjects. They found that the ASD group had 79% more neurons than the control group in the DLPFC, and 29% more neurons in the MPFC, for an averaged prefrontal neuron count of 67% greater in the ASD subjects compared to the controls. Furthermore, while they did find that the brain weights of the ASD subjects were greater than those of the control subjects,

the control group showed a strong linear relationship between brain weight and neuron number, while the ASD group had neuron counts that either met or exceeded the predicted number according to their brain weight. These results suggest that the increase in neuron numbers in ASD is indeed pathological, and not only an artifact of a larger brain. The authors hypothesize that molecular and genetic prenatal mechanisms that control neuronal number in the typically developing brain, such as proliferation or apoptosis, may be disrupted in ASD, resulting in pathological increase in neuron numbers. Given that abnormal neuron numbers point to disruption in prenatal development, the authors emphasize that such findings, and further research into their genetic and molecular causes, may contribute to the understanding of the origin of autism (Courchesne et al. 2011).

Two other studies performed counts of spindle neurons (or Von Economo neurons) in the frontoinsular cortex of autistic subjects and typically developing controls (Kennedy et al. 2007; Santos et al. 2011). Spindle neurons, found in the anterior cingulate cortex and frontoinsular cortex in humans, are thought to be involved in functions of social cognition, including empathy, social awareness, and self-control (Allman et al. 2011). Given that aspects of these behaviors have been shown to be abnormal in the ASD phenotype, some have hypothesized that spindle neurons may be affected, with a possible reduction in the number of spindle neurons (Kennedy et al. 2007). However, a study by Kennedy and colleagues (2007), which performed spindle neuron counts in the frontoinsular cortex of four ASD subjects and their age-matched controls, did not find a decrease in spindle neurons in the ASD subjects, but rather a slight increase. Similarly, a later study by Santos and colleagues (2011) investigated ratios of spindle neurons to

pyramidal neurons in four young ASD subjects (4-14 years of age) and their age-matched controls, and found that the ASD subjects had a significantly higher ratio of spindle neurons to pyramidal neurons compared to controls. While the total number of spindle neurons in the cortex is small and therefore cannot account for the excess of brain growth seen in ASD (Courchesne et al. 2011), these findings do provide general support for the hypothesis that ASD is characterized by a period of rapid, excessive neural growth early in postnatal life. Furthermore, the relationship between spindle neurons and social cognitive behaviors that are abnormal in ASD could possibly imply that this excess of spindle neurons in ASD subjects plays a role in the behavioral manifestations of the disorder (Santos et al. 2011).

Cortical Composition

Microglia are important to the organization of neurons in the cortex as they are involved in the support and regulation of neural function, as well as the removal of dysfunctional neurons. Therefore, it has been suggested the neural overgrowth seen in ASD may result in microglia that are activated differentially in response to neuronal excess, dysfunction or altered connectivity (Morgan et al. 2012). Morgan and colleagues (2012) sought to investigate the spatial relationship of microglia and neurons in the DLPFC of 13 postmortem ASD subjects and their age-matched controls, given that this region has been shown to have significant overgrowth in ASD. Recording the coordinates of neurons and immunostained microglia, they analyzed the organization of these elements in terms of microglia-neuron clustering, neuron-neuron clustering, and microglia-microglia clustering. They found that microglia-neuron clustering was increased in the ASD subjects. Furthermore, somal encirclement by the microglial

processes was especially evident in the ASD subjects. They also found that microglia-neuron clustering at short distances was especially increased in ASD subjects ages 6 and under in their sample. Neuron-neuron clustering was not significantly different in ASD subjects overall, although there was a significant increase in neuron-neuron clustering in the adult subgroup of ASD subjects compared to controls. Microglia-microglia clustering was similar to controls in ASD subjects. Given the results, these researchers suggest that these microglial-neurons clustering abnormalities are indicative of increased neuronal recruitment of microglial activation in ASD, perhaps to provide support in the form of remodelling aberrant neurons or to remove neurons that are dysfunctional. The increase of this clustering in short distances in young ASD subjects could also indicate that increased activation of microglia during a time period of rapid neural overgrowth may play a role in the maldevelopment seen in the disorder. Lastly, the increase in neuronal-neuronal clustering in older ASD subjects may reflect an increase in neuronal loss in the DLPFC during the aging process (Morgan et al. 2012).

Minicolumns

As discussed in Chapter 2, minicolumns are vertically oriented aggregates of neurons that lie between layers II through VI of the cortex. The "core area" of a column consists of the neurons, dendrites, synapses, and fibers, while the cell-deficient space on the periphery of the column is usually referred to as the neuropil space (Casanova et al. 2002a). A minicolumn is a functional unit that unites specializations of the horizontal layers of the cortex with vertical connectivity, and is separated from other minicolumns by the lateral inhibition of vertical fiber bundles of GABA-ergic inhibitory interneurons at the edge of the column (Buxhoevden and Casanova 2002a; Casanova et al. 2002a). It

has been suggested that the surface of the neocortex expanded throughout evolution as a result of an increase in the number of cell columns. Furthermore, the increase in columns would have led to new patterns of connectivity, and would only develop if these patterns were adaptive (Casanova et al. 2002a). Given that the minicolumn is the basic functional unit of the cortex, it has been suggested that some aspects of minicolumnar organization may be altered in neuropathologies that have disruptions of neural circuitry or maladaptive patterns of connectivity, such as ASD (Casanova et al. 2002a).

One study by Casanova and colleagues (2002a) compared the layer III minicolumnar morphology of nine ASD and four control subjects in prefrontal area 9 and temporal areas 21 and 22. In this study they analyzed a number of parameters within minicolumns, including column width, neuropil space, and density. They found that in all regions, ASD subjects had narrower minicolumn width, reduced neuropil space, yet similar neuronal density. These findings imply that the ASD brain has a greater number of reduced-sized minicolumns compared to typically developing brains (Casanova et al. 2002a). These same researchers used the subjects and regions from the initial 2002 study in a second study to analyze more closely the Grey-Level Index (GLI) of layer III in each group, in order to use a different method to test the results of their previous findings (Casanova et al. 2002b). As briefly mentioned previously, GLI (more recently known as Grey-Level-Ratio, or GLR) is defined as the ratio of nissl stained elements to unstained elements, and can be used to estimate cell density in cortex, as well as organization and spacing of nissl-stained structures. While the previous study used GLI to measure neuronal density within the minicolumns and found no significant differences between ASD subjects and controls, this second study employed GLI to measure the density of the

vertical structures of cells in layer III (minicolumns). They found that the neocortex of the ASD subjects contained vertical structures that were significantly more tightly packed than those of the controls, supporting the previous findings that minicolumns in ASD are narrower and in greater quantity (Casanova et al. 2002b).

A third study by this same research group (Casanova et al. 2006) sought to compare differences in neuropil space in the cortex of six ASD subjects and their age-matched controls in nine cortical areas, including paralimbic, heteromodal and unimodal association, and primary regions. While their previous study (Casanova et al. 2002a) measured the neuropil space of minicolumns in layer III and found that neuropil space was reduced in ASD subjects, this study sought to expand on these findings by measuring the neuropil space in a greater and more varied number of regions in layers II through VI, or the full extent of the minicolumn. They found that neuropil space was reduced in the ASD subjects in area 17 (visual cortex) and area 9 (prefrontal area), and significantly reduced in area 10 (frontopolar cortex) and area 24 (anterior cingulate gyrus), which suggests that in these areas, minicolumns are more densely packed in the ASD subjects. While three out of four of these areas of reduced neuropil space lie in the prefrontal cortex, other areas in the prefrontal cortex that were examined, including area 11 and area 44 (part of Broca's area), did not show differences in neuropil space. These results suggest that while the PFC does appear to be preferentially affected by more tightly packed minicolumns in ASD, not all regions of the PFC are affected (Casanova et al. 2006a).

A fourth study by this research group (Casanova et al. 2006b) corroborated much of their previous findings in another independent sample of six ASD subjects and their

age-matched controls in areas S1, 4, 9, and 17. In this study, they again examined minicolumn width, as well as neuronal and nucleolus size and two methods of neuronal density. Similar to their initial study (Casanova et al. 2002a), they found evidence of narrower minicolumn spacing in the ASD subjects. They also found that while density within each minicolumn is similar to that of the controls, inter-minicolumnar density was increased in the ASD subjects, suggesting that minicolumns are more tightly packed together. This corroborates with their previous findings (Casanova et al. 2006a), as well as providing more evidence for their previous hypothesis that ASD is marked by an increase in total number of minicolumns. Furthermore, they found that the neuronal soma and nucleolus size was decreased overall in the ASD subjects, which, along with narrower spacing of minicolumns, may also play a role in the reduced size of minicolumns in ASD (Casanova et al. 2006b).

As mentioned previously, some researchers have suggested that the expansion of the neocortex in mammalian brain evolution was due to an increase in the numbers of minicolumns. Given that minicolumns are the smallest functional unit of the mammalian neocortex, an increase in the number of minicolumns would have led to an increase in neural circuitry and complex connectivity that would have been acted upon by natural selection, so that only adaptive patterns of connectivity would be passed down (Casanova et al. 2002a). The finding of less neuropil space, reduced density of minicolumns, and narrower minicolumns in ASD, in addition to the finding of either average-sized or enlarged brain size in ASD, suggests that the ASD neocortex, and particularly areas of the PFC, are marked by a greater number of minicolumns. This increase in minicolumns may result in maladaptive patterns of connectivity in ASD. An increase in connectivity in

ASD is also supported by the previously mentioned findings of an increase in white matter in some areas of the cortex in ASD, including the PFC. Furthermore, it is worth noting that the founder cell divisions that account for the number of minicolumns occur early in gestation, so the origins of ASD may originate very early in embryological development (Casanova et al. 2006b).

The second consistent finding is that ASD is characterized by a decrease in minicolumn width. It has been suggested that wide columns facilitate generalization, while narrow columns facilitate discrimination (Casanova et al. 2002b). This hypothesis is supported by findings of narrower minicolumns in the primate visual cortex compared to other mammals, which has been found to reflect an increase in complexity of primate vision (Casanova et al. 2006b). It is thought that this increase in complexity results in a greater interdependence in neighboring minicolumns. One hypothesis of autism is that a dysfunction in the arousal modulating systems of the brain results in an autistic individual remaining in a constant state of arousal, and is unable to generalize sensory input. Given the finding of narrower minicolumns in larger quantities in ASD subjects, with preferential effect in the PFC, a region that has intricate connectivity with most areas of the brain, it is possible that this excess of narrow minicolumns results in a neurocircuitry system that cannot properly generalize information due to an increase in interdependence in neighboring minicolumns (Casanova et al. 2006b).

Schizophrenia

Schizophrenia is a debilitating mental disorder that is clinically diagnosed by the patient's experience of delusions, hallucinations, and other forms of disordered thought

(Shenton et al. 2001; Harrison 1999). Unlike ASD, the behavioral manifestations of schizophrenia are late-onset, with peak onset at around 30 years of age (Harrison 1999). Similar to ASD however, while schizophrenia has a genetic origin, no particular genetic loci have been pinned to the disorder unequivocally (Harrison 1999). While the behavioral criteria are useful in the diagnosis of schizophrenia, they do not help elucidate a mechanism for the disorder. Therefore, efforts have been made over the past century to identify abnormalities in the brain associated with schizophrenia. As will be discussed below, the PFC in particular seems to be affected in the disorder.

Volumetric studies

Unlike ASD, most studies have not found a significant difference in brain volume in schizophrenia, although those that have found a difference implicate a slight decrease in whole brain volume (Shenton et al. 2001). The most significant whole brain finding in schizophrenia is an increase in ventricle size (Shenton et al. 2001), as well as some findings of volume differences in the temporal lobe. The findings of the frontal lobe, however, are more difficult to interpret. The frontal lobe is of particular interest in schizophrenia because many of the cognitive and behavioral deficits seen in the disorder are similar to those seen in frontal lobe damage (Shenton et al. 2001). One of the earliest MRI studies of 38 schizophrenic subjects found a trend for a smaller frontal lobe, particularly in male subjects (Andreasan et al. 1986). Analysis of individual MRI sections further found that this frontal lobe decrease was due primarily to a decrease in the dorsolateral and orbital regions of the frontal lobe. A second study by Raine and colleagues (1992) compared the brain volumes of 17 schizophrenic subjects and their controls and similarly found a reduction in the frontal lobe in schizophrenia that could not

be explained by changes in the ventricles or temporal lobes. Similarly, another study by Breier and colleagues (1992) analyzing MRI scans of 44 schizophrenic subjects and their controls found a reduction in the prefrontal volumes, as well as reduced amygdala and hippocampal volumes. However, a later study by Wible and colleagues (1995) compared MRI scans of the entire prefrontal cortex in 14 male schizophrenics and their controls, and failed to find any significant structural abnormalities, although there was a slight trend of prefrontal volume reduction that was correlated with reductions in the temporal lobe. Two earlier studies also failed to find any difference in frontal or prefrontal volume (Smith et al. 1987; Kelsoe et al. 1988). Furthermore, a meta-analysis by Shenton and colleagues (2001) found that 60% of the reviewed MRI findings reported some type of abnormality in the frontal lobes in schizophrenia, while 40% did not. Wible and colleagues (1995) suggest that much of these inconsistent results could be due to inconsistent methods of MRI, while Shenton and colleagues (2001) present another possibility that the changes in the PFC in schizophrenia may be too small to detect by MRI. Therefore, in the remaining sections, we will discuss differences observed the in schizophrenic PFC at finer scales.

Cortical Thickness

Unlike total volume, differences in cortical thickness in schizophrenia are somewhat more clear, with the majority of MRI studies showing evidence of cortical thinning in multiple cortical areas and most pronounced thinning in the temporal and prefrontal cortices (Ehrlich et al. 2012). One study by Kuperberg and colleagues (2003) analyzed the MRI scans of the entire brain of 33 schizophrenic subjects and controls, and found that schizophrenic subjects showed a reduced cortical thickness overall, with a

significant degree of cortical thinning bilaterally in the orbitofrontal cortex(OFC). They also found regional differences in thickness unilaterally, with significant left-hemisphere thinning in the left inferior frontal cortex, and significant right-hemisphere thinning in the right medial frontal cortex. Another study that analyzed cortical thickness via MRI scans of 54 schizophrenics and their controls found significantly reduced cortical thickness bilaterally in the ventrolateral prefrontal, dorsolateral prefrontal, superiorfrontal, and frontopolar cortex, as well as right hemisphere cortical thinning in area 44 of Broca's, the orbitofrontal, and the anterior cingulate cortex (Schultz et al. 2010). Other studies in the literature have reported similar findings of cortical thinning in these areas (Cobia et al. 2012; Hulshoff Pol and Kahn 2008).

The observation of significantly reduced cortical thickness in regions involved in cognitive functions that are compromised in schizophrenia led Ehrlich and colleagues (2012) to investigate relationships between cognition and cortical thickness. Using a large sample of 131 schizophrenic subjects and their controls, they analyzed the MRI scans and cognitive tests of executive function of the two subject groups. In addition to the findings of widespread decrease in cortical thickness and overall worse performance on the cognitive tests in the schizophrenic subjects, they found a positive correlation between cortical thickness of the lateral PFC and working memory function in the controls, while the schizophrenic subject group showed a positive correlation between working memory function and middle and superior temporal lobe cortical thickness. These results led the researchers to suggest that cortical abnormalities in the lateral PFC of schizophrenics could result in the reliance of other cortical areas, like the temporal lobe, to compensate, and such a shift in cortical networks may contribute to disrupted cognitive function

(Ehrlich et al. 2012).

A study by Byun and colleagues (2012) sought to investigate the relationship between genetic loading and cortical abnormalities in schizophrenia, which is known to be a highly heritable disorder. In this study, they examined the MRI scans of 31 schizophrenics, 31 individuals with one or more relatives with schizophrenia, and 31 normal controls. They found that the relatives of schizophrenics group displayed significant cortical thinning in multiple areas of the cortex compared to the control group. Like the previous studies in schizophrenic subjects, the majority of this thinning was seen in the fronto-temporal regions. The frontal regions that showed significant thinning in the relatives groups include the ventromedial prefrontal cortex and the frontal pole, as well as the anterior cingulate cortex. Furthermore, in the anterior cingulate cortex, those individuals that had two or more relatives with schizophrenia showed even greater cortical thinning than those with only one schizophrenic relative. Given these results, the researchers suggest that genetic loading has a quantitative effect on cortical abnormalities associated with schizophrenia. Furthermore, given that this pattern of cortical thinning is seen in non-psychotic relatives of schizophrenics, they suggest that cortical thinning is a neurodevelopmental process associated with genetic factors that contribute to a vulnerability to the disorder, rather than the result of degenerative processes associated with the progression of schizophrenia (Byun et al. 2012).

As discussed above, the majority of MRI studies of cortical thickness in schizophrenia show a significant reduction in cortical thickness in a number of regions, with frontal and temporal regions often shown as particularly affected. It is worth noting here that a histological assessment of cortical thickness showed a slight trend of

decreased cortical thickness in the PFC of schizophrenic subjects, but they did not find any significant differences from normal controls (Selemon et al. 1998). However, it has been argued that total cortical thickness is more accurately measured using MRI scans, because only on MRI scans can one verify that a measurement is orthogonal to the pial surface in all three dimensions, and histological measures tend to overestimate thickness due to the inability to measure on such precise axes (Hutsler et al. 2007).

Neuronal Density and Number

Neuronal density abnormalities in the PFC of schizophrenics are extremely unclear, and most likely are not uniform, with the DLPFC being most affected by the disorder (Selemon 2001). One study by Selemon and colleagues (1995), which compared neuronal densities of 16 schizophrenics and controls via stereological counts in prefrontal area 9, found that the schizophrenic subject group had a mean neuron density that was 17% greater than the controls in this region. A second study by this research group again used stereological methods to compare neuronal densities of schizophrenics and controls in prefrontal area 46, and found that this area had a 21% greater neuronal density in the schizophrenic subjects (Selemon et al. 1998). Lastly, a third stereological study by this same group again performed density measurements in prefrontal area 9, as well as area 44 (part of Broca's), again finding that neuronal density of area 9 was significantly increased in schizophrenics compared to controls. However, prefrontal area 44 did not show any significant abnormalities in neuronal density (Selemon et al. 2001). Furthermore, a study by another research group, which performed stereological counts in area 10 in schizophrenic subjects and controls, found a slight trend of decreasing neuronal density in schizophrenic subjects (Benes et al. 1986). A later study by this same

research group again found little difference in neuronal density overall in schizophrenic subjects, although they did find a decrease in density of small neurons in layer II in the anterior cingulate and prefrontal area 10, as well as an increase in pyramidal neuron density in layer V of prefrontal area 10 (Benes et al. 1991). These results indicate that while there do seem to be neuronal abnormalities in the DLPFC(such as area 9 and 46) in schizophrenics, these abnormalities may not be universal throughout the entire PFC (Selemon et al. 2003).

Abnormalities in neuronal density in some regions of the PFC in schizophrenia beg the question of what is causing these changes, the most obvious of which would be a change in the absolute number of neurons. However, the findings of multiple studies suggest that total neuron number in the schizophrenic PFC is similar to that of normal healthy controls (Pakkenberg 1993; Akbarian et al. 1995; Thune et al. 2001) . Given these findings, Selemon and colleagues (1999) suggest that neuronal density is increased in parts of the PFC in schizophrenic subjects as a result of decreasing cortical volume from a loss of neuropil space and possibly an atrophy of neuronal processes, rather than a loss of neurons. Indeed, the previously mentioned findings of cortical thinning and reductions in volume in schizophrenia support this hypothesis. The authors suggest that this decrease of neuropil space and possible atrophy of neuronal processes may result in a deficiency in prefrontal cortical circuitry, causing the deficits in cognitive function associated with schizophrenia (Selemon et al. 1999).

Minicolumns

Given the above observations made on the possibility of neuronal density abnormalities, as well as the possibility of reduced neuropil space contributing to cortical

circuitry dysfunction in the schizophrenic cortex, some researchers have hypothesized that neuronal organization in terms of minicolumn structure may be affected. One study by Casanova and colleagues (2005) compared parameters of minicolumn morphometry, which includes columnarity index (measure of minicolumn orientation), minicolumn width and the dispersion of width, and mean interneuron distance (distance between two neurons), in 13 postmortem schizophrenic subjects and their controls. They found that all parameters were similar in both groups with the exception of mean interneuron distance, which showed a slight trend of decreasing interneuron distance (or increased cell packing) in the prefrontal and motor areas of the schizophrenic subjects. Given that changes did not seem to be present in the minicolumn core or alter minicolumn width, the researchers suggest that this slight decrease in interneuron distance may be related to alterations in the peripheral neuropil space in the vertical dimension of the lamina, rather than the minicolumn itself. This may also be supported by the finding of reduced cortical thickness in some areas of the PFC in schizophrenia. The authors further suggest that the cortical abnormalities seen in schizophrenia occur after the development of the minicolumn, since the minicolumn itself appears unaltered in the disorder. A later study by this same research group re-examined these subjects, confirming that the abnormalities observed correspond to changes in peripheral neuropil space rather than minicolumn structure, which is preserved (Casanova et al. 2008). A reduction in peripheral neuropil space corresponds to abnormalities in intra-columnar circuit features, such as dendritic arborization and inhibitory interneuron connectivity. While minicolumns are responsible for processing information in the cortex, it is thought that intra-columnar circuits are responsible for relating this information to an adaptive

behavioral response. Therefore, Casanova and colleagues (2008) suggest that alterations in the relation of information into a behavioral state may contribute to the behavioral consequences of schizophrenia.

Conclusions

While investigations of PFC abnormalities between these two pathologies, both of which are characterized in part by disturbances in normative social behavior, have profoundly different findings, some general consistencies are observed. As discussed, in ASD the most significant findings have been a decrease in minicolumn width and an increase in minicolumn number, as well as some evidence of increased neuronal density. While findings are somewhat less consistent in schizophrenia, most have found a decrease in cortical thickness in regions of the PFC that is correlated with a developmental, rather than degenerative process. Similarly, while minicolumnar morphology is preserved in schizophrenia, a reduction in peripheral neuropil space amongst minicolumns has been observed. The findings in these two pathologies have a similar trend in that most indicate a reduction and reorganization in the amount of space available for connectivity in the PFC. This suggests that normative socio-emotional and cognitive function in humans is dependent on the presence of such space in order for the necessary neural circuits to be properly developed.

In chapter 2, we saw a similar trend, that the human PFC is marked by increases in space available for connectivity. Humans were shown to have decreased neuronal density, increase in neuropil space, and wider minicolumns compared to other primates. Taken together with the pathological findings in ASD and schizophrenia in the PFC, the

case can be made for increased connectivity as a critical element of human socio-emotional behavior and cognitive function, and that a critical development in the evolution of the human brain in relation to human-specific behavior was the shift towards increased space and decreased cell density. These changes may have allowed for the development of an increase in smaller, more specialized neural circuits that allowed for complex executive functions. While cross-species comparisons allow researchers to form hypotheses about the differences in cortical structure and micro-morphology that might contribute to general types of behavior, it is important to remember that these evolutionary studies are using other extant primates with their own species-specific evolutionary history, including the evolution of their own set of species-specific behaviors. Studies of human pathologies with socially abnormal phenotypes are especially useful to the evolution of human cognition because they provide a means with which to make hypotheses about correlations between cortical differences and human-specific behaviors.

CHAPTER 4: A CASE STUDY FOR THE EVOLUTION OF THE SOCIAL BRAIN: DIFFERENCES IN THE PREFRONTAL CORTEX IN WILLIAMS SYNDROME AND A TYPICALLY DEVELOPING CONTROL

Introduction

As discussed in the previous chapter, the study of human pathologies that have distinct socio-behavioral phenotypes are extremely useful in the study of the evolution of the social brain because abnormal neuroanatomical findings consistently observed in such pathological individuals can possibly be correlated with the specific socio-behavioral abnormalities of such pathologies. These possible neural correlates to social dysfunction in pathological individuals may in turn infer possible neural structures and structural properties that underwent significant changes in hominin evolution, and may be identified as critical components of the social brain. Williams Syndrome is an especially useful pathology for the study of the social brain because it is characterized by a very unique social phenotype, but unlike other disorders with specific socio-behavioral phenotypes, such as ASD, the genotype of Williams Syndrome is relatively universal and identifiable. While research on this pathology is in its infancy, it is important to the study of the evolution of the social brain because it offers a rare insight into the relationship between genes, the brain, and behavior.

The goal of this chapter is to review the previous literature on the socio-behavioral and neurological phenotype of Williams Syndrome, as well as to present the preliminary findings of my original research investigation, a comparative analysis of neuron density distribution in the prefrontal cortex of Williams Syndrome and typically

developing (TD) controls. I first give a brief overview of the etiology and phenotype of William's Syndrome, as well as review the neuroanatomical findings of Williams Syndrome that are currently available in the literature, including those of a previous study conducted in our laboratory examining laminar thickness in the PFC of Williams Syndrome and TD subjects, which was recently presented as a poster at the Society for Neuroscience 2012 symposium (Stefanacci et al. 2012). I then present an original research investigation regarding the distribution of neuronal density in area 11 of a Williams Syndrome subject and a typically developing control. These findings, while not yet statistically significant as they reflect results of a single subject and control, indicate a possible pattern of neuronal density distribution in Williams Syndrome that may be linked to common behavioral traits of the pathology. Lastly, I conclude with a discussion about the implications that abnormal patterning of neuronal density distribution in Williams Syndrome may suggest about the evolution of the social brain.

Cognitive and Social Phenotype of Williams Syndrome

Williams Syndrome is a pathology caused by a single genetic deletion of 26 genes, including the elastin gene (responsible for the functional production of elastin proteins, an important type of connective tissue), on chromosome 7. q11. 23 (Morris 2010a). In addition to the morphological and physiological phenotype of the disorder, which includes dysmorphic facial features, arterial and other connective tissue irregularities, and short stature, most of which are likely related to the deletion of the elastin gene, Williams Syndrome is also characterized by a unique cognitive and socio-behavioral phenotype. Williams Syndrome individuals tend to have a variable degree of intellectual disability. However, they also have specific cognitive abnormalities,

including very strong expressive language and non-verbal reasoning abilities, but poor visuospatial skills. Furthermore, while Williams Syndrome subjects usually have an extensive vocabulary, they struggle with relational language describing temporal or spatial states. Similarly, they have extreme difficulty in spatial construction tasks, such as drawing. These specific cognitive deficits in relational comprehension have been correlated with reduced grey matter in the inferior parietal cortex, a region that is critically involved in the processing of the dorsal stream of information in the visual pathway. It is therefore suggested that this processing is interrupted in Williams Syndrome, and is a source of the characteristic cognitive deficits associated with the disorder (Morris 2010b).

The socio-behavioral phenotype of Williams Syndrome is what truly defines this disorder as an important case study of the social brain. The most prominent characteristic associated with Williams Syndrome is hypersociability, which affects many aspects of social behavior. One of the earliest symptoms of Williams Syndrome in infants is prolonged facial gazing, with preferred attention to the face of others even amidst other sensory stimuli that distract the attention of typically developing infants (Jones et al. 2001). As Williams Syndrome children develop language and communicative skills, they acquire an abnormally increased interest in the desire for social engagement with others, and are abnormally empathetic compared to typically developing children (Morris et al. 2010b). Individuals with Williams Syndrome are also found to be abnormally gregarious and have hyperverbal speech, characterized by excessive use of stereotyped phrases, over-familiar referents, and irrelevant tangents of conversation (Mervis and John 2011). Despite their hypersociability, subjects with Williams Syndrome tend to have difficulty

maintaining social relationships, likely due to their conversational skills and their poor comprehension of social cues (Mervis and John 2011). Furthermore, individuals of all ages with Williams Syndrome have been shown to have a propensity for approaching and interacting with strangers (Doyle et al. 2004), and to have difficulty processing angry facial expressions (Santos et al. 2010), which suggests that they lack an understanding of social dangers. Despite the lack of proper fear or inhibitory response in the face of social dangers, generalized anxiety and specific phobias are also very common in individuals with Williams Syndrome, although many of these lessen in severity with age (Dykens 2011).

Neuroanatomical Phenotype in Williams Syndrome

A number of studies have performed neuroimaging methods on live individuals with Williams Syndrome. In general, these studies have found an overall reduction of the cerebrum, with a preservation of cerebral grey matter volume and a significant reduction of cerebral white matter volume, as well as (Martens et al. 2008). There have also been neuroimaging differences in specific brain regions that seem to parallel the behavioral and cognitive phenotype of Williams Syndrome. Reiss and colleagues (2004) found that the occipital cortex and thalamus had especially reduced volume in Williams Syndrome, and that grey matter density was decreased in the parahippocampal gyri and the parietal and occipital cortices. These regions are critical in visuo-spatial pathways, and so decreases in volume may be related to the poor visuo-spatial skills in Williams Syndrome individuals. These researchers also found that the Williams Syndrome subjects had increases in volumes of structures and regions critically implicated in facial processing and emotion, including the amygdala, anterior cingulate cortex, and the OPFC and

MPFC. These researchers suggest that this volumetric increase may be linked to the behavioral phenotype of hypersociality and empathy that is characteristic of Williams Syndrome. However, an fMRI study by Paul and colleagues (2009), which measured neural activity of Williams Syndrome and typically developing individuals, found that the Williams Syndrome subjects failed to recruit the amygdala in a facial discrimination task. While this result was surprising given that the amygdala is important in facial processing, a skill that has shown to be preserved, and even in some cases enhanced in Williams Syndrome, the amygdala is also involved in the detection of socially relevant information and regulation of social approach behavior. Therefore, these researchers suggest that that common behavioral traits seen in Williams Syndrome, such as an affinity for approaching strangers and the difficulty in perceiving anger in facial processing is linked to a lack of amygdala activation during facial processing in Williams Syndrome (Paul et al. 2009). This hypothesis is supported by similar findings, such as one study which found that individuals with Williams Syndrome showed reduced activation of the amygdala to fearful facial expressions compared to controls, and this abnormal amygdala response was correlated with subjects' affinity for approaching strangers (Haas et al. 2010).

To date, only five postmortem studies have been performed on Williams Syndrome subjects: four by the Galaburda laboratory and one recent study by our laboratory, the Semendeferi Laboratory of Comparative Human Neuroanatomy. The first was a case study of a single 31 year old male with Williams Syndrome, investigated by Galaburda and colleagues (1994). They reported gross anatomical observations, finding that the overall size of the frontal lobe fell in the normal range for typically developing subjects, but the parietal, occipital, and temporal lobes were reduced in size. They also

noted abnormal gyrification in the mesial occipital surface. They performed an architectonic analysis in select regions (inferior frontal gyrus, cingulate and pericingulate gyri, motor and somatosensory cortices, the superior and inferior parietal lobules, the calcarine region, the hippocampus and parahippocampus, the superior temporal gyrus, and the lateral and medial geniculate nuclei and the pulvinar of the thalamus) and found that there was an increase in cell packing density, particularly in small neurons, without a decrease in cortical thickness, which the authors suggest indicate an increase in number of neurons in the Williams brain. They also found abnormal cortical lamination, particularly in occipital area 17, in which horizontal layering appeared rippled. Lastly, they found some abnormalities in cell clustering and orientation throughout the cortical areas examined. The abnormalities in area 17 are interesting in light of the cognitive phenotype of Williams Syndrome; this region is important in the visuospatial stream, and thus structural abnormalities in this area might reflect the visuospatial difficulties that are characteristic of Williams Syndrome (Galaburda et al. 1994).

A second study of the gross anatomical and microstructural abnormalities in Williams Syndrome with a more robust sample size of four subjects (see Table 1 for full subject profiles) was conducted by this same research group (Galaburda and Bellugi 2000). They found that the only consistent gross anatomical abnormality observed in Williams Syndrome was a shortened central sulcus, which resulted in an unusual morphological shape and orientation of the dorsal-central region. This region also consistently demonstrated abnormal gyrification. The authors note that this region is implicated in many of the behaviors that are abnormal in Williams Syndrome. The cytoarchitectonic analysis of this subject group found, in contrast to their previous study,

that in area 17 there was a slightly diminished cell packing density in Williams Syndrome, as well as an increase in neuronal size. The authors suggest that these abnormalities could be indicative of abnormal connectivity which may be implicated in behavioral abnormalities (Galaburda and Bellugi 2000). A later study by the same research group again analyzed only area 17, this time in three middle-aged Williams Syndrome subjects and their age-matched controls (Galaburda et al. 2002). In addition to replicating their previous findings that packing density was actually decreased in area 17, and there was an increase in neuronal size, this study also found that the Williams Syndrome subjects consistently had more small neurons than the controls in the left hemisphere, an effect that was found to be particularly strong in layer IV (Galaburda et al 2002).

The fourth postmortem study on Williams Syndrome by this same research group (Hollinger et al. 2005) examined the primary auditory cortex (area 41) in three Williams Syndrome subjects and their age-matched controls. Given that many Williams Syndrome individuals have superior auditory perception, including musical abilities and heightened sound sensitivity, the researchers hypothesized that area 41 would have more normal cytoarchitecture than area 17 in Williams Syndrome individuals. They found that while area 41 of controls demonstrated a number of asymmetries, including increased cell packing density in the left hemisphere in layer IV, and decreased cell size of layer III in the left hemisphere, Williams Syndrome subjects did not show these asymmetries. Furthermore, there was a bilateral increase in number of large pyramidal neurons in layer II of Williams Syndrome compared to controls. However, there were no significant differences in cell packing density overall in area 41 of Williams Syndrome subjects,

which is in contrast to the decrease in cell packing density seen in area 17. This neuroanatomical finding of few cytoarchitectonic abnormalities in area 41 in contrast to area 17 supports the clinical observations that auditory skills remain intact in Williams Syndrome patients, while visuo-spatial skills are deficient. Given that the auditory cortex, temporopolar regions with significant connectivity to the amygdala tend to project into layer II, the proportional increase of large pyramidal neurons in layer II in Williams Syndrome may suggest an increase in limbic connectivity, and could account for the heightened auditory sensitivity seen in the disorder (Hollinger et al. 2005).

The fifth and final neuroanatomical postmortem study in Williams Syndrome, conducted by the Semendeferi Laboratory for Comparative Human Neuroanatomy, sought to investigate the distribution of individual layer thicknesses in the PFC of three Williams Syndrome subjects and their age-matched controls (Stefanacci et al. 2012). Given that neuroimaging studies in Williams Syndrome have found volumetric increases in regions of the PFC, we hypothesized that these differences may represent changes in cortical thickness in the PFC in Williams Syndrome. Cortical abnormalities in the PFC are of special interest in the evolution of the social brain, as this region is thought to have undergone significant reorganization in hominins and is implicated in many of the social and cognitive behaviors that appear to be unique, or uniquely specialized, in humans. Stefanacci and colleagues (2012) found that while there were no significant differences in layer thickness across the PFC in Williams Syndrome and TD, layer IV was relatively thinner in Williams Syndrome in all prefrontal regions. Furthermore, there was a slight trend for layers II/III and V/VI to be thicker in Williams Syndrome in certain prefrontal regions. These slight differences in laminar distribution might reflect differences at the

microanatomical level.

Original Research Investigation: A Case Study of Neuronal Density Distribution in Area 11 of Williams Syndrome and a Typically Developing Control

The findings of Stefanacci and colleagues (2012) led to the investigation of neuronal density in Williams Syndrome presented here, in which I compare the neuronal distribution of cortical layers II/III and V/VI in prefrontal area 11 in a Williams Syndrome subject and a TD control. Area 11, which lies in the OFC, is involved in neural networks that mediate social behavior and emotion (Purves et al. 2007). Area 11 is also thought to be important to the cognitive modulation of empathy, a behavior that is critical in the maintenance of complex social bonds (Shamay-Tsoory et al. 2009; Pochon et al. 2002). The hypothesis of this present research investigation is that the slight alteration of cortical thicknesses of V/VI demonstrated in the OFC of Williams Syndrome in our previous study might represent a more significant change in neuronal density in layers V/VI (while overall prefrontal cortical thicknesses were altered in both layers II/III and V/VI in the Stefanacci and colleagues (2012) study, only cortical thicknesses in layers V/VI were abnormally greater in the orbital region). One possibility is that neuronal density in these layers might be decreased in Williams Syndrome, suggesting that absolute neuronal numbers remain similar while the thickness of layers V/VI increase. This reduction in density might suggest an increase in neuropil space and thus an increase in neuronal complexity, such as dendritic arborization, in layers V/VI in Williams Syndrome. However, if the densities of layers V/VI do not differ in Williams Syndrome, this may suggest that the increase in laminar thickness of these layers in Williams

Syndrome reflects an increase in total number of neurons in these areas, and thus a decrease in neuropil space and neuronal complexity.

Materials and Methods

(Initials Key for Research Scientists and Research Assistants: KS= Dr. Katerina Semendeferi; LS= Dr. Lisa Stefanacci; CB= Chelsea Brown; LY= Linda Yzurdiaga; LW= Linnea Wilder; CH= Caroline Horton)

Subjects

The Williams Syndrome subject, WS 8, is a 48 year old male, and the age-matched control subject, A0248, is a 51 year old male. Both subjects are part of a larger sample obtained from the Laboratory for Cognitive Neuroscience at the Salk Institute (La Jolla, CA), as part of an ongoing donation-based program aimed at describing the WS phenotype in order to better understand the relationship between genotype, neuroanatomy, and behavior in this syndrome. See Table 1 for subject information.

Tissue Processing

Subjects were fixed in 10% formalin shortly after death and then stored in a sucrose solution until use. KS and LS extracted and blocked area 11 from larger slabs of prefrontal cortex in both subjects. Blocks were placed in a cryoprotectant solution in preparation for freezing, then frozen on dry ice and cut by CB, LY and LW on a Leica SM 2010R sliding microtome at 60 microns, with each section 30 microns apart, and mounted by these same research assistants on subbed glass slides. The slides were then dried in an oven and submerged in 1:1 chloroform-ethanol solution overnight, stained by CH and LW for Nissl substance with . 25% thionin, re-hydrated, submerged in xylenes for 20 minutes, then cover-slipped with permount by LS.

Neuronal Counts and Density Estimations

All stereoanalysis was performed by CH. Neuron numbers in Area 11 of WS8 and the control were estimated by using the optical fractionator probe in combination with fractionator sampling in Stereoinvestigator software (MBF Bioscience, Williston, VT). Stereological analyses were performed on a Dell workstation receiving live video feed from an Optronics MicroFire color video camera (East Muskogee, OK) that is attached to a Nikon Eclipse 80i microscope equipped with a Ludl MAC5000 stage (Hawthorn, NY) and a Heidenhain z-axis encoder (Plymouth, MN). Sections were analyzed using a 100x oil objective. Three regions of interest for each section was drawn at a 1x magnification in a selected portion of cortex that was free of processing damage, with clear top of pial layer and clear layer VI-white matter boundaries, and straight borders. For the first region of interest, a rectangular area was drawn with a lower border on the layer VI-white matter boundary and an upper border on the top of the pial surface, comprising of all six layers (see Figure 4). The second region of interest consisted of a rectangular area delineating only layers II/III, and the third region of interest was a rectangular area delineating only layers V/VI (See Figure 5). A rectangular area is preferable for accurate neuronal density estimation. The grid size was 600 x 600 microns, with a dissector height of 15 microns, a counting frame of 100 x 100 microns and an average of ten counting frame sites per section. Section thicknesses were measured at each site, and thicknesses varied from 15 microns to 30 microns. A range of six to ten sections were analyzed per area per subject. Sections were selected based on the quality of sectioned tissue, in order to ensure accurate neuronal counts. To the best of the researcher's ability, sections were spaced at set intervals that varied depending on the number of sections in the series and number of

good quality sections (usually every fourth section). A neuron in a given counting frame was counted if it met the following criteria: nucleus was visible; nucleolus was visible; the neuron did not touch the line of exclusion; the neuron fell within or touched the line of inclusion. After neuronal counts were completed, volumes of these same regions of interest were estimated using the Stereoinvestigator Cavalieri probe. Densities were then calculated by dividing the neuronal population estimation by the volume estimation.

Results

Total neuronal density of the control in this investigation only differed by 4% from the reported density of area 11 in a control in the literature (Ketzler et al. 1990), therefore confirming inter-rater reliability of neuron counting conducted in this study. Total neuronal density in all layers of Area 11 did not differ significantly between the Williams Syndrome subject and the control, with the Williams Syndrome subject displaying a density only 4. 4% less than the control (WS: 20,746. 47 mm^3; control: 21,700. 68 mm^3). Neuronal density in layers II/III were even more similar, with the Williams Syndrome subject displaying a density only 2.3% less dense than the control (WS: 25,116. 17 mm^3; control: 25,752. 45 mm^3). Neuronal density in layers V/VI was significantly different between the two individuals, however, with the Williams Syndrome subject displaying a neuronal density 38.5% less dense than the control (WS: 20,630.94 mm^3; control: 33,541.67 mm^3). See Table 2 and Table 3 for a summary of the results.

Conclusions

While this study consists of only one Williams Syndrome individual and a TD control, the extremely reduced density (38.5% less dense than the control) in layers V/VI in Williams Syndrome does support the hypothesis that the slight increase in cortical thickness of layers V/VI in the PFC of Williams Syndrome is indicative of a greater difference in neuronal density in this region. While layers II/III did not show a significant difference in density between the two subjects in area 11, this actually corresponds to the findings of Stefanacci and colleagues (2012), because while layer thickness was greater in the Williams Syndrome subjects in the PFC in layers II/III and V/VI overall, only layers V/VI were thicker in the OFC (see Figure 3). Area 11 is part of the orbitofrontal cortex (OFC), and the present finding suggests a significant decrease in density only in layers V/VI of area 11.

A decrease in neuronal density along with an increase in laminar thickness in layers V/VI implies that there is an increase in neuropil space in layers V/VI in Williams Syndrome. Neuropil space, defined as the space in the cortex not occupied by cell bodies, is generally occupied by components of neuronal transmission, including dendrites, axons, and synapses (Spocter et al. 2012). Therefore an increase in neuropil space in layers V/VI in Williams Syndrome suggests that there is an increase in connectivity and neuronal complexity in these layers. Furthermore, projections from layers V/VI are mostly subcortical; neurons in layer V give rise to subcortical projections and receive input from other cortical areas and the brainstem, while neurons in layer VI send projections to the thalamus and receive input from the brainstem (Purves et al. 2007). An increase in neuronal complexity in these layers therefore suggests an increase in

subcortical projections. Given that OFC has extensive connectivity with subcortical structures involved in the regulation of emotional behavior and the social environment, such as the amygdala and the hypothalamus, the current finding that there is decreased neuronal density and therefore possibly increased neuronal connectivity in layers V/VI in area 11 in Williams Syndrome may indicate that there is abnormal subcortical connectivity associated with this pathology. An increase in subcortical connectivity in prefrontal regions involved in socio-emotional behavior such as area 11, which is thought to play a functional role in empathy, may be linked to the hypersocial behavioral phenotype observed in Williams Syndrome. Abnormalities in cortico-subcortical connectivity may also reduce inhibitory responses involved in the appropriate behavioral responses to important social cues, such as angry facial expressions or the recognition of social danger, and could be linked to the deficits of such inhibited behavior in Williams Syndrome.

Area 10, or the frontopolar cortex of humans, chimpanzees, and bonobos is marked by thicker supragranular layers and thinner infragranular layers, while this trend is reversed in other primates (Semendeferi et al. 2001). The cortical layer thickness study performed by our research group (Stefanacci et al. 2012) also demonstrated the same trend as seen in area 10 in humans in both the controls and the Williams Syndrome subjects. These findings may imply that an increased reliance on cortico-cortical connectivity (supragranular layers) and a decreased reliance on cortico-subcortical connectivity (infragranular layers) were important in the evolution of the hominin PFC (although prefrontal area 13, considered part of the limbic cortex, was demonstrated to have thicker infragranular layers in humans; see Semendeferi et al. 1998). While the

Williams Syndrome subjects retained the typically developing human trend of thicker supragranular and thinner infragranular layers throughout the PFC, perhaps an abnormal emphasis on subcortical connectivity, as evidenced by both the slight increase in infragranular thickness and the significant decrease of neuronal density in these layers in area 11, may play a role in the abnormal socio-behavioral phenotype in Williams Syndrome. If this is the case, it may imply that ratios of cortico-cortical and cortico-subcortical connectivity in the PFC played an essential role in the evolution of hominin sociality. Emotional stimuli in the mammalian brain are processed within two different, simultaneous pathways: the direct thalamo-amygdala pathway, which processes stimuli quickly and produces a rapid, instinctual response, and the more indirect thalamo-cortico-amygdala pathway, which produces a more delayed, yet more sophisticated response based on more refined processing of information from the given stimulus (Vermeire et al. 2011). The PFC in particular is important in the thalamo-cortico-amygdala pathway, as it has been shown to exhibit significant inhibitory control on the amygdala (Vermeire et al. 2011; Quirk et al. 2003). Functional neuroimaging studies in individuals with anxiety disorders have shown that there is a negative coupling between activity in the amygdala and the PFC, which suggests that anxiety is linked to a reduction of inhibitory control by the PFC on the amygdala (Monk et al. 2008). While hypersociality is the most significant behavioral characteristic of Williams Syndrome, anxiety is also very common in the disorder. Perhaps abnormalities of cortico-subcortical projections in layers V/VI of regions of the PFC in Williams Syndrome result in a decreased inhibitory effect of the cortex on subcortical structures, which in turn causes the individual to have a more impulsive response to socio-emotional stimuli, as well as the failure to process external

social cues and flexibly modify behavior to that which is more socially advantageous.

The findings in this study of decreased neuronal density in area 11 of Williams Syndrome are not statistically supported, given that only one Williams Syndrome individual and one age-matched control have so far been analyzed, so any hypotheses made from this finding is suggested with caution. These instead represent the preliminary results of a future study which will include a more robust sample size in both number of individuals and the number of prefrontal areas studied. However, if a future, larger study demonstrates similar findings to that of this single individual, this may lend support to two functional possibilities. The first is that Williams Syndrome may be marked by abnormal cortico-subcortical projections, which may result in a disruption of these connections. These disruptions might entail failing to properly identify important social cues necessary to modulate behavior to a response that is socially appropriate, and/or a failure of inhibitory processes by the PFC onto subcortical structures, resulting in behavior that is more impulsive and less environmentally-advantageous. The second hypothesis is that changes in the complexity and distribution of cortico-subcortical projections in certain functional regions of the PFC might have been a crucial specialization in the evolution of the social brain, involved in the fine tuning of cortical inhibitory control for refined informational processing, as well as the flexible modulation of behavioral response to socio-emotional stimuli. The present study does not lend strong enough support to either of these possible hypotheses; however, the preliminary results at hand do provide some insight for future directions of research for both the neural correlates of the abnormal social phenotype in Williams Syndrome as well as possible neural changes implicated in the evolution of the social brain.

CHAPTER 5: CONCLUSIONS

As the past four chapters have demonstrated, there are many lines of evidence to suggest that the PFC is critically implicated in human behaviors that can be attributed to the social brain. In this final chapter, I briefly summarize the findings from each chapter that most implicate the importance of the PFC in human social behavior, as well as discuss how these findings may contribute to a better understanding of the evolution of the social brain in humans.

In the first chapter, I discussed how studies in humans and nonhuman primates have demonstrated an extensive connectivity of the PFC with subcortical structures that are known to be involved in emotion, including the amygdala, suggested to be the center of the emotional memory system, the hypothalamus, involved in autonomic responses to emotional stimuli and the execution of emotional behavior, and the hippocampus, suggested to be another important structure in the emotional memory system (LeDoux 1993; Burman et al. 2011). In addition, the PFC has extensive connectivity to other cortices of the neocortex, and is the only structure that receives input from all sensory modalities (Barbas 2000). Because the PFC in primates receives sensory input from the environment as well as playing a critical role in modulating response to emotional stimuli, this structure is well equipped for the behavioral flexibility that is needed to navigate complex social environments. Similarly, lesion studies in both humans and non-human primates have shown that individuals with prefrontal lesions, and particularly lesions in the vmPFC and OFC, have shown significant deficits in a wide variety of socio-emotional behaviors, including awareness of social norms, self-monitoring of behaviors in order to produce appropriate reactions in social situations, and adaptive

socio-emotional responses to the intent of others (Rolls 2004; Bechara et al. 2000; Young et al. 2010; Beer et al. 2006). This suggests that these areas of the PFC are critically involved in executive functions related to the mediation of inner impulse and the flexible production of appropriate social behavior, as well as the ability to make adaptive decisions based on the processing of social cues. It is reasonable, therefore, to suspect that extensive connectivity with other regions of the brain, both subcortical and cortical, was an adaptive advantage for an environment that was becoming increasingly social, and so was a critical change in the evolution of the social brain in hominins.

Chapter I also discussed the development of the PFC in humans. The human PFC is less functionally mature at birth than other areas, and has high post-developmental expansion. In adults, high expansion areas are larger and have larger and more complex dendritic branching compared to the low expansion areas, which reach maturity in terms of synaptic density, cortical thickness, and grey matter density much earlier in development (Hill et al. 2010). The PFC is also latest to mature in terms of grey matter volume, not reaching maximum volume until age 11-12 years, in contrast the rest of the brain, which on average reaches maximum grey matter volume around age 6-9 years (Courchesne et al. 2000). Similarly, white matter continues to increase in volume until the fourth decade of life, and and has been shown to myelinate latest in the PFC, reflecting the late maturation of neural circuits in this region (Muftuler et al. 2012). Furthermore, other neural processes that are critical to brain development, including apoptosis, synaptogenesis, synaptic pruning, and expansion of axonal and dendritic branching appear to occur latest in the PFC, and earliest in low-expansion areas, such as the primary cortices (Stiles and Jernigan 2010). Perhaps the relatively late development

of the PFC was an adaptive advantage in the early hominin brain, allowing for neural circuits important in social behavior to be influenced by experience in the social environment. It is of particular interest that the majority of PFC development continues through adolescence, during which time humans start to become social beings within the larger community. This late maturation of the PFC may allow for a neural plasticity that allows the individual to develop adaptive behavior not only for complex social interaction but also for adaptive integration into the particular social environment that the individual belongs to.

In chapter II I investigated the evolution of the PFC through a comparative analysis of differences between human and extant great ape neuroanatomy. Although it is important to remember that extant great apes do not serve as replicas of a hominin ancestor and have continued to evolve along their own trajectory after the ancestral divergence, differences that are unique to humans in comparison with the rest of the great apes may be useful in identifying the neural structures in humans that underwent the greatest evolutionary change, and thus may be linked to our unique social behavior. While principle components analysis has revealed that humans display a pattern of brain morphometry that is unique to the species in comparison to the other great apes (Aldridge 2011), numerous studies have demonstrated that the human PFC as a whole does not significantly deviate from that of the great apes in terms of overall relative size or relative quantities of grey matter or white matter (Semendeferi et al. 1997; Semendeferi et al. 2002; Smaers et al. 2011). However, there is evidence that there are differences between humans and great apes in terms of size of certain functional areas. In humans, the frontopolar cortex BA 10, which is associated with planning, taking initiative, and

working memory and attention processing, has undergone significant expansion compared to other great apes (Semendeferi et al. 2001). In contrast, BA 13, an area that straddles the limbic lobe and OFC and is thought to be involved in emotional reactions to the social environment, appears reduced in size in humans compared to the great apes (Semendeferi et al. 1998). Findings such as these suggest that evolutionary changes in the hominin PFC occurred through a reorganization and a partially mosaic evolution of distinct functional areas, rather than a general expansion or change of the PFC as a whole.

Given the above findings, it is likely that neural differences associated with uniquely human social behavior occur at the microstructural or molecular level. As I discussed in chapter II, within the microstructural differences observed in humans compared to other great apes, there appears to be a trend of an increase in connectivity and complexity of such connections in humans. Humans have lower neuronal densities compared to the great apes, the magnitude of which are particularly pronounced in prefrontal regions (Semendeferi et al. 1998; Semendeferi et al. 2001; Semendeferi et al. 2011; Schenker et al. 2008; Buxhoevden et al. 2001a). Humans also have an increase in neuropil space in prefrontal regions compared to other regions of the cortex, and this trend was not seen in chimpanzees (Spocter et al. 2012). Both a decrease in neuronal density and an increase in neuropil space suggest that the human PFC is marked by an increase in space for greater connectivity. This is further supported by the findings that humans have a significantly greater minicolumn width compared to other primate species, a feature that would support an increase in local and specialized connectivity between minicolumns, which would be necessary for complex cognitive processing

(Buxhoeveden et al. 2001b; Semendeferi et al. 2011). Humans have also been found to have greater complexity in pyramidal neuron morphology compared to other primates, such as an increase in dendritic spine density in the DLPFC (Elston et al. 2006; Elston et al. 2011). An increase in dendritic spines reflects an increase in excitatory input to the PFC, and suggests that the human PFC has a more extensive and complex connectivity compared to other primates. Taken together, these unique findings in the human PFC imply an increase in intrinsic and extrinsic connectivity in this structure, supporting the hypothesis that distinctly human behaviors may be a product of faster, more local, and more extensive connectivity within the PFC necessitated by complex cognitive processing.

Chapters III and IV investigated abnormalities in the PFC of individuals with neuropathologies of Autism Spectrum Disorder (ASD), schizophrenia, and Williams Syndrome, all of which are in part defined by distinct socio-behavioral phenotypes. As discussed in Chapter III, the PFC is a relatively recent structure in mammalian brain evolution, and the many anatomical and microstructural differences between humans and the great apes suggests that this structure has undergone recent significant and rapid change over the course of the evolution of the hominin lineage. Furthermore, many of the genes associated with brain metabolism in the PFC in humans have similarly undergone recent and rapid evolutionary change in order to meet the increasing demands of a larger and more complex structure, and many of these PFC metabolism genes have been found to be significantly altered in neurological disorders with social phenotypes, which suggests that these relatively recent neural changes are more susceptible to dysfunction (Khaitovich et al. 2008). Therefore the observed abnormalities in the PFC in such

pathologies may be useful in the study of the evolution of social behavior, because the very features that are altered in these pathologies may be features that are critical in behaviors that are uniquely human.

While each of the three pathologies examined all vary in the manifestation of abnormalities, each characteristic abnormality changes the amount of space available for connectivity between the PFC and other structures. In ASD, there were consistent findings of an increase in neuronal number, an increase in number of minicolumns, and a decrease in minicolumn width (Courchesne et al. 2011; Casanova et al. 2002a,b; Casanova et al. 2006a,b). All of these imply that ASD is marked by an overcrowding of neural networks; too many neurons, an overabundance of minicolumns and a decrease in minicolumn width all reduce the amount of space available for minicolumns to communicate with each other, and may result in dysfunctional neural circuitry that could possibly account for the behavioral phenotype characteristic of ASD. While the minicolumn morphology itself appears to be preserved in schizophrenia, there is an observed reduction in the neuropil space surrounding the minicolumns (Casanova et al. 2005; Casanova et al. 2008), which similarly would reduce the available space for networks of communication between the minicolumns. Schizophrenia was furthermore marked by significant cortical thinning in the PFC (Ehrlich et al. 2012; Kuperberg et al. 2003; Schultz et al. 2010; Cobia et al. 2012; Hulshoff Pol and Kahn 2008) and increased cortical density (Selemon et al. 1995; Selemon et al. 1998; Selemon et al. 2001), which would again account for a reduction in space available for connectivity. Although minicolumn analysis in the PFC has not yet been investigated in Williams Syndrome, given that density in layer III (the layer in which the minicolumns in the previous studies

were analyzed) is within the normal range in Williams Syndrome and the thickness of layer III is not drastically different, I would not predict that the same decrease in space between minicolumns in ASD and schizophrenia would be seen in Williams Syndrome, because Williams Syndrome has so far been shown to have typical overall neuronal density. This does not mean that minicolumns are unaltered in Williams Syndrome, but rather that at this time there is no evidence to suggest abnormal spacing of minicolumns in layer III in this pathology.

However, in layers V/VI, Williams Syndrome appears to have an abnormal relative increase in space for connectivity, given the slight increase in thickness and drastic decrease in neuronal density observed in these layers compared to the typically developing individual. Furthermore, the thickness and neuronal density of layers II/III and total cortical density are not significantly different from the normal range, which suggests that this increase in space for connectivity lies only in the infragranular layers. In the previous chapter, I suggested that this indicates a possible over-emphasis on subcortical connectivity associated with this pathology. Supragranular projections are primarily subcortical, and BA 11, the area that I investigated in my study, lies in the OFC, which is known to have extensive connectivity with subcortical structures involved in the regulation of emotional behavior and the social environment (Purves et al. 2007; Shamay-Tsoory et al. 2009; Pochon et al. 2002). Therefore, I suggested that an increase in subcortical connectivity in prefrontal regions involved in socio-emotional behavior such as BA 11, which is thought to play a functional role in empathy, might be associated with hypersocial behaviors observed in Williams Syndrome. Given these findings, I further hypothesized that ratios of cortico-cortical and cortico-subcortical connectivity in

the PFC may have played an essential role in the evolution of hominin sociality.

While abnormalities in neuronal density and thickness of the individual cortical layers has not yet been reported in the literature for ASD, this does not necessarily mean that these abnormalities are not present. ASD is characterized by a significant increase in neuron number in the PFC compared to typically developing controls, but not a significant increase in overall cortical thickness, which implies an abnormally high neuronal density overall in this pathology. However, a breakdown of neuronal distribution and layer thickness within the individual layers may reveal consistent trends in supragranular versus infragranular cortical layers in ASD that are masked by reports of overall neuronal density and cortical thickness. Given that ASD is characterized by asocial behavior and difficulties in applying the appropriate socio-emotional response to social stimuli (Geschwind 2009), perhaps the infragranular layers are marked by a greater increase in neuronal density compared to the supragranular layers, so that there is less space for connectivity with subcortical structures and thus cortico-subcortical neural circuitry critical to socio-emotional behavior is de-emphasized and/or detrimentally altered. Furthermore, as discussed in chapter III, some studies have shown that there is a slight increase in spindle neurons in ASD individuals (Kennedy et al. 2007; Santos et al. 2011). Spindle neurons, which are thought to be involved in functions of social cognition, including empathy, social awareness, and self-control, are found in layer Vb in the anterior cingulate cortex and frontoinsular cortex in humans (Allman et al. 2011). Perhaps in these areas, an increase in spindle neurons may result in an increase in neuronal density in layer V, which could lead to a functional disruption of social cognition, resulting in the characteristic ASD phenotype. In contrast, if the supragranular

layers have an abnormally greater neuronal density in ASD, this would suggest that cortico-cortical connectivity is disrupted, which could perhaps be linked to deficiencies in the generalized processing of sensory stimuli characteristic of ASD that are often attributed the restrictive attentive abilities and rigid, repetitive behaviors common to the disorder (Geschwind 2009). Lastly, it is possible that the abnormally high neuronal density observed in ASD is consistent throughout the cortical layers, and thus could lead to disrupted circuitry in both cortico-cortical and cortico-subcortical connectivity that could be functionally related to the characteristic behaviors of ASD just described.

It is also possible that patterns of thickness and neuronal distribution may be altered in schizophrenia. The most consistent neuroanatomical finding in schizophrenia is an abnormal thinning of the cortex, which is most pronounced in prefrontal regions (Ehrlich et al. 2012). However, total neuron number in the PFC of schizophrenic individuals is similar to that of normal healthy controls (Pakkenberg 1993; Akbarian et al. 1995; Thune et al. 2001). While a reduced cortical thickness coupled with the maintenance of normal neuron number implies that total neuron density should be increased in schizophrenia relative to controls, the findings in the literature are inconsistent, so that while some show an increase in neuronal density, a few studies have shown no difference or even a slight decrease in neuronal density (Benes et al. 1986; Benes et al. 1991). Many of these inconsistencies may be related to the different prefrontal regions sampled in each of the studies, which suggests that in schizophrenia, some functional areas are affected while others remain intact. Unlike ASD and Williams Syndrome, schizophrenia is usually late-onset, and the behavioral manifestations of the pathology are primarily abnormalities in mental states. One of the hypothesized

mechanisms for much of the abnormal behaviors associated with schizophrenia (hallucinations, paranoia, lack of drive) is a defect in working memory due to reduced prefrontal neuropil space and alterations in neural circuits between the dlPFC and other cortical and subcortical regions (Andreasan 1997; Selemon and Goldman-Rakic 1999). As with the other pathologies previously described, further investigation into neuronal density distribution and thickness of the individual cortical layers in functional areas related to working memory circuits in schizophrenia may provide useful insight into the relationship between cortico-cortical and cortico-subcortical connectivity in this pathology, the role of this relationship in normative social behavior, and possibly the importance of this relationship in the evolution of the social brain in the human species.

In summary, given the developmental evidence and the comparative evidence in both non-human primates and the neural pathologies reviewed above, it is apparent that the unique connectivity between the human PFC and other neural structures is critically implicated in the collective neural circuits and systems involved in the rapid detection and processing of important social stimuli, and the flexible and adaptive modification of behaviors in a dynamic social environment. These neural circuits and systems involved in such complex socio-emotional and cognitive processes are what constitute the social brain, and are attributed to many of the behaviors that are uniquely human. Further investigation into the relationship between the cortico-cortical and cortico-subcortical connectivity in the PFC through comparative work with other primates and neural pathologies that affect the social brain may further inform an understanding of the properties and changes in neural structure that were essential to the evolution of the social brain in the hominin lineage.

APPENDIX

FIGURES

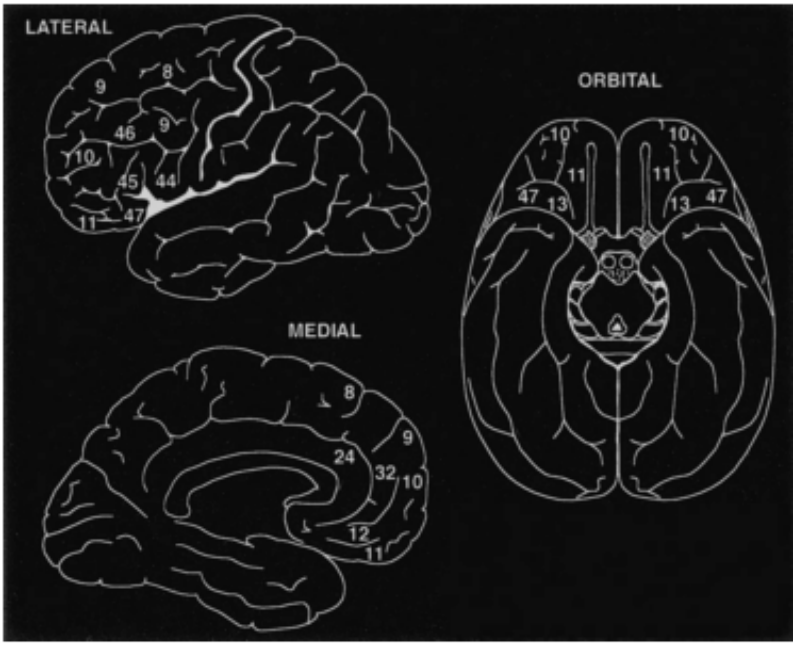


Figure 1: Subdivisions of the human prefrontal cortex. Prefrontal areas are numbered according to Brodmann's cytoarchitectonic map. (Adapted from Fuster 2001)

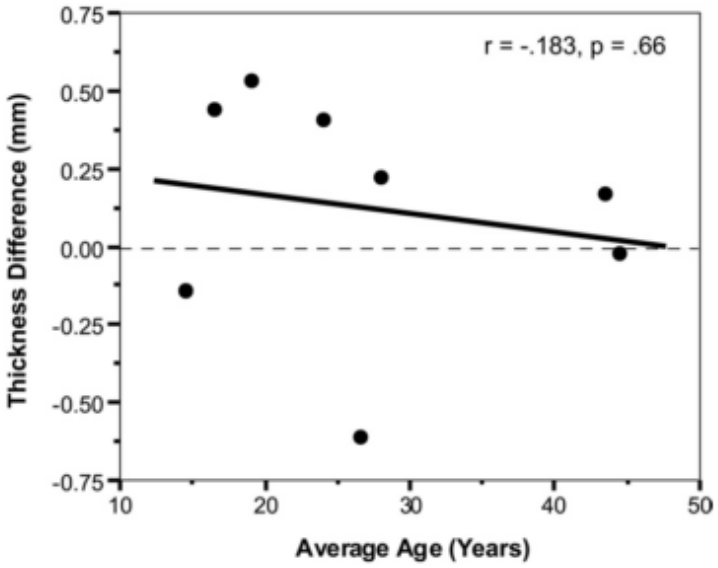


Figure 2: Cortical thickness differences between age-matched subject pairs as a function of age. Differences are much greater in the younger ages, and much smaller in middle age. Adapted from Hutsler et al. 2007.

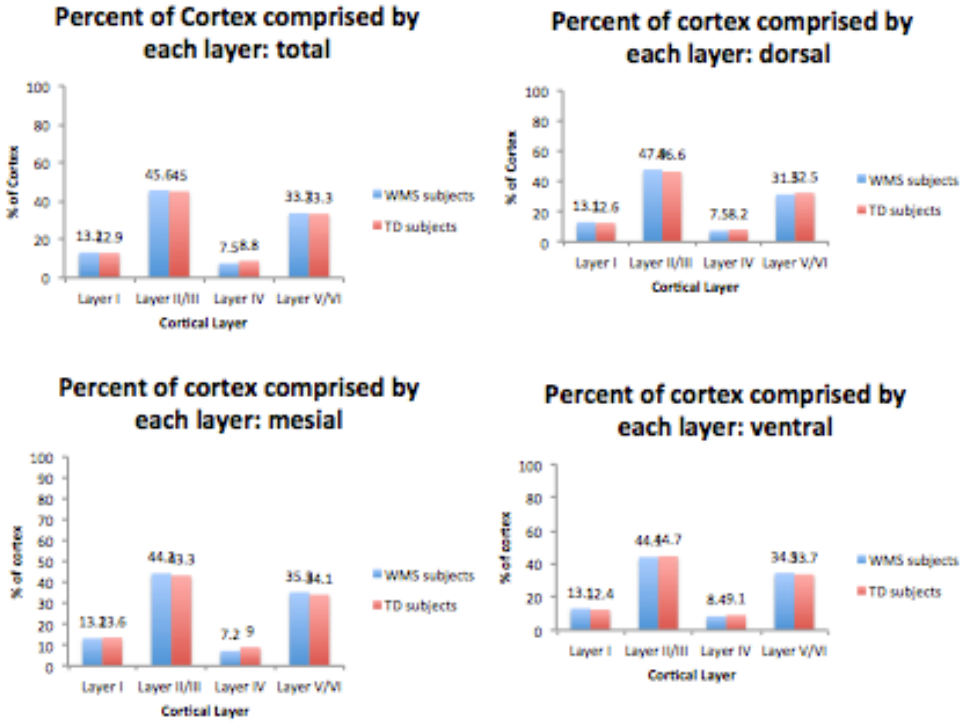


Figure 3: Percent of cortex comprised by each layer in Williams Syndrome subjects (WS) and typically developing controls (TD). (Adapted from Stefanacci et al. 2012)



Figure 4: Photograph of WS 8 cortex boundary delineation for neuron counting and density estimation in all six cortical layers.

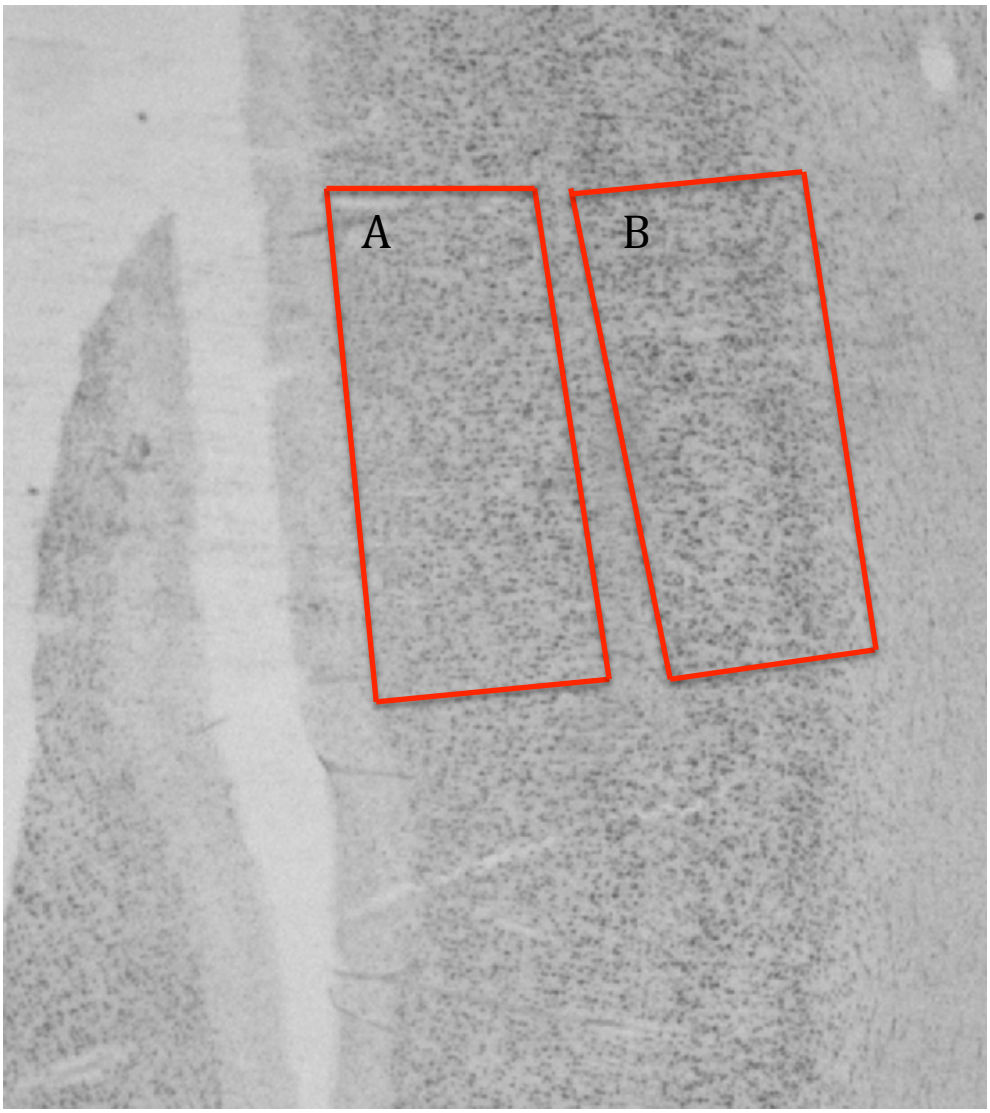


Figure 5: Photograph of WS 8 cortex boundary delineation for neuron counting and density estimation in layers II/III (A) and layers V/VI (B).

TABLES

Subject	Age	Sex	Hemisphere
WS 1	31 years	M	L,R
WS 2	248 days	M	L,R
WS 3	53 years	F	L,R
WS 4	46 years	F	L,R

Table 1: Subject background information of Williams Syndrome subjects analyzed in Galaburda and Bellugi (2000).

Subject	Age	Sex	Hemisphere	Cause of Death
Control A0248	51 years	M	L	Unknown
WS 8	48 years	M	L	Cardiac Complications

Table 2: Subject background information for control subject (A0248) and Williams Syndrome subject (WS 8)

Subject	Density: total (mm^3)	Density: Layers II/III (mm^3)	Density: Layers V/VI (mm^3)
Control A0248	21,700	25,752	33,541
WS 8	20,746	25,116	20,630

Table 3: Neuronal density (mm^3) in total cortical layers, cortical layers II/III, and cortical layers V/VI in a typically developing control (A0248) and a Williams Syndrome subject (WS 8).

WS % Less Density than Control: Total	WS % Less Density than Control: Layers II/III	WS % Less Density than Control Layers V/VI
4. 4%	2. 3%	38. 5%

Table 3: Percentage of decrease in neuronal density in Williams Syndrome subject (WS) compared to the typically developing control, for total density, density of layers II/III, and density of layers V/VI.

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