UNIVERSITY OF CALIFORNIA SAN DIEGO

Cellular Disturbances in the Williams Syndrome Cortex: The Impact of Glia on Social Behavior

A dissertation submitted in partial satisfaction of the requirements
for the degree Doctor of Philosophy

in

Anthropology with a
Specialization in Anthropogeny

by

Linnea Lorene Wilder

Committee in charge:

Professor Katerina Semendeferi, Chair
Professor Gedeon Deák
Professor Terry Jernigan
Professor Amy Non
Professor Shirley Strum

2020

The Dissertation of Linnea Lorene Wilder is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

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Chair

University of California San Diego

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CURRICULUM VITAE

Education

Ph.D. Anthropology, Specialization in Anthropogeny — 2020
University of California San Diego — San Diego, CA
Dissertation Title: Cellular Disturbances in the Williams Syndrome Cortex: The Impact of Glia on Social Behavior

M.A. Anthropology
University of California San Diego — June 2014
Thesis Title: The Human OFC, vmPFC, and ACC: Development and Evolution

B.A. Anthropology — May 2010
Washington State University, Pullman, WA

Teaching Experience

Instructor

University of California San Diego
Human Developmental Sciences

Evolution in Human Development	Fall 2020
	Fall 2019
	Fall 2018
	Spring 2018

Department of Anthropology

How Monkeys See The World	Summer 2020
Human Origins	Spring 2020
Human Skeleton	Winter 2020
Human Origins	Summer 2019
Humans are Cultural Animals	Winter 2019
	Summer 2018
Human Origins	Summer 2017

MiraCosta College, Oceanside, CA
Department of Anthropology

Introduction to Biological Anthropology	Fall 2018

Teaching Assistant

University of California San Diego
Department of Anthropology

Evolution of Human Disease	Spring 2019
The Evolution of the Human Diet	Fall 2017
Debating Multiculturalism	Winter 2017
Human Origins	Fall 2016

Debating Multiculturalism	Spring 2016
	Winter 2016
	Fall 2015
Human Origins	Fall 2015
Debating Multiculturalism	Spring 2015
	Winter 2015
	Fall 2014

Human Development Program

Brain and Behavioral Development	Winter 2019
	Winter 2018

Research Experience

University of California San Diego
Graduate Student Researcher 2012-present
Laboratory for Human Comparative Neuroanatomy (Dr. Katerina Semendeferi, PI)

Funding

CARTA Fellowship	2017-2020
F.G. Bailey Fellowship Department of Anthropology, University of California San Diego	2018-2019
Research Stipend Department of Anthropology, University of California San Diego	2014-2016
M.E. Spiro Fellowship Department of Anthropology, University of California San Diego	2013-1014
Research Stipend Department of Anthropology, University of California San Diego	2012-2013

Publications/Presentations

Wilder, L., Lew, C., & Semendeferi, K., Glia density in the Williams Syndrome cortex (Brodmann Areas 10, 4, 3, and 18). *Manuscript Under Review, Autism Research*.

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Wilder, L., Hanson, K., Lew, C., Bellugi, U., & Semendeferi, K. (2018). Decreased Neuron Density and Increased Glia Density in the Ventromedial Prefrontal Cortex (Brodmann Area 25) in Williams Syndrome. *Brain sciences*, 8(12), 209.

Wilder, L., Hanson, K., Lew, C.H., Brown, C., Cuevas, D., Greiner, D., Groeniger, K., Bellugi, U., & Semendeferi, K. (2018). A stereological study of glia density in the cortex in Williams syndrome. Poster Presentation: Society for Neuroscience Annual Meetings, November 2018.

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ABSTRACT OF THE DISSERTATION

Cellular Disturbances in the Williams Syndrome Cortex:
The Impact of Glia on Social Behavior

by

Linnea Lorene Wilder

Doctor of Philosophy in Anthropology with a Specialization in Anthropogeny

University of California San Diego, 2020

Professor Katerina Semendeferi, Chair

The evolution of the human brain has involved a substantial increase in size as well as modifications to the internal, cellular organization and developmental trajectory. These changes have resulted in enhanced cognition, highly complex social behavior, and an increased susceptibility to neurological dysfunction. Comparative neuroanatomical studies of human neurodevelopmental disorders that present with altered cognition or social behavior can increase our understanding of how microstructural changes in the brain may impact uniquely human traits

and behaviors. Williams Syndrome (WS) is a rare neurodevelopmental disorder, caused by a hemi-deletion of approximately 25-28 genes on chromosome band 7q11.2, characterized by altered social and emotional behavior, specifically hypersociability, lack of social inhibition, and increased anxiety. We identified microstructural changes in the ventromedial prefrontal cortex (vmPFC) and in glia density across the cortex that may contribute to the social and emotional features of WS. We found decreased neuron density and increased glia density in the vmPFC, increased astrocyte/microglia density in unimodal cortical areas, and increased oligodendrocyte density in prefrontal and unimodal cortical areas. These results suggest that alterations in glia may be a common feature across the WS cortex, potentially affecting neuron-glia interaction and neuronal function. These findings provide evidence for the role of glia in social behavior, and aid in identifying the mechanisms underlying alterations in social function. Additionally, this work contributes to our understanding of the link between microstructural variation and brain function, and the mechanisms that link genetic variation to neuroanatomical variation.

Chapter 1

Introduction

Humans occupy a unique adaptive niche, they are highly intelligent, social, technological, and cultural. This required the evolution of a large, complex brain. The human brain is over three times larger than that of our closest relatives, the great apes. It contains approximately 86 billion neurons and roughly the same number of glial cells (Herculano-Houzel, 2012). The increase in brain size was accompanied by microstructural alterations in neuron organization (Barger et al., 2012; Semendeferi et al. 1998, 2001, 2011) and glia distribution (Sherwood et al., 2006). The demands of a complex social environment may have contributed to the evolution of the human brain, particularly in regions of the brain implicated in social cognition, including portions of the prefrontal cortex (PFC) (Amodio & Frith, 2006). These changes were achieved through shifts in brain development in humans relative to non-human primates, which likely increased the cognitive flexibility of humans, but may have also increased the susceptibility to neurological dysfunction.

Neurodevelopmental disorders and brain evolution

Multiple lines of evidence can be used to understand the evolution of the human brain. One of those is examination of neurodevelopmental disorders or neurological diseases that disrupt neural systems underlying uniquely human behavior, to link cognitive and behavioral differences to differences in neural phenotype. Williams Syndrome (WS) is a rare neurodevelopmental disorder caused by a hemizygous deletion of approximately 25-28 genes on chromosome band 7q11.23 (Strømme et al., 2002). It is characterized by cardiac abnormalities, a distinct facial morphology, and a unique cognitive and behavioral phenotype. Cognitively, individuals with WS present with low global IQ, spatial processing deficits, relatively preserved

although abnormal language and face processing, and frequent anxiety. Perhaps of greatest interest to an evolutionary approach to the study of the brain, WS is characterized by unique social behaviors including hypersociability and decreased inhibition in social approach (Bellugi et al., 2000). Interestingly, chromosome 7 has undergone purifying selection in the hominid lineage, and may be particularly susceptible to change in humans. Many neurodevelopmental disorders are associated with duplications of genes on chromosome 7, including duplication of genes at 7q11.23 which is implicated in a subset of Autism Spectrum Disorders (ASD), marked by speech and language deficits and social avoidance (Hanson et al., 2014; Velleman & Mervis, 2011).

The behavioral features of WS are hypothesized to be a deficit in inhibitory control. This may be the result of abnormal functional connectivity between the prefrontal cortex and subcortical structures involved in emotional processing and reward, such as the amygdala and striatum (Meyer-Lindenberg et al., 2005; Mobbs et al., 2007). There is evidence of altered fronto-striatal circuitry in WS, with slower response time and reduced PFC activity during a task of inhibition (Mobbs et al., 2007), and evidence of altered fronto-amygdala activity while viewing threatening images (Meyer-Lindenberg et al., 2005). Abnormalities in neurons have been found in orbital regions of the PFC. WS subjects have lower neuron density and less dendritic branching in BA 10 and BA 11 than controls, and these areas were more affected than unimodal cortical areas (Hrvoj-Mihic et al. 2017; Lew et al., 2017). Although little is known about glial abnormalities in WS, there are deficits in myelination, suggesting oligodendrocytes may play a role in the disorder. There is a disproportionate decrease in white matter relative to total brain volume, and a lower fractional anisotropy in prefrontal-amygdala pathways in WS (Avery et al., 2012; Faria et al., 2012). Williams Syndrome provides an excellent model for

studying the link between genetic changes, neuroanatomy, and social and emotional behaviors that show evidence of specialization in human evolution.

Region of interest: the ventromedial prefrontal cortex

The prefrontal cortex (PFC), which comprises the anterior portion of the neocortex on the frontal lobe of the brain, is implicated in cognitive and behavioral traits that are considered specialized in humans, including executive functions and understanding social cues (Fuster, 1997; Bechara et al. 2000). The ventromedial prefrontal cortex (vmPFC) in particular is implicated in social function, emotional regulation, and decision making (Amodio & Frith, 2006). The vmPFC is located on the ventral surface of the medial portion of the PFC, and it made up of parts of Brodmann areas (BA) 10, 11, 14, 24, 25, and 32 (Öngür et al., 2003). Most of the PFC is organized into six cortical layers that are functionally and architectonically distinct. Within the vmPFC, there is a rostral to caudal gradient in the presence or absence of granular layer IV. More caudal areas of BA 14, 24, 25, and 32 are agranular, entirely lacking layer IV in the adult cortex. Granular layer IV begins to emerge in the intermediate areas of the vmPFC, becomes thicker in more rostral areas 10 and 11. The most well-developed lamination is found in the frontal pole, BA10 (Öngür et al., 2003). The vmPFC has projections to and from subcortical structures, including hypothalamus, periaqueductal gray region, striatum, and amygdala. These projections are particularly strong within BA 25 (Öngür et al., 1998; Ferry et al., 2000).

Glia in human evolution and neurodevelopmental disorders

Historically, most research on the brain focused on neurons, with glia largely seen as simple support cells. More recently, the importance of glia to neural functioning has increasingly been acknowledged, although there are still questions regarding how changes in glia can affect human social behavior and cognition. Glia, including astrocytes, oligodendrocytes, and

microglia, are non-neuronal cells of the central nervous system that do not produce electrical impulses. They do however, play crucial roles in neural communication.

Astrocytes regulate synaptic development, both inhibiting and inducing synapse formation through secreted factors. They also play a role in activity-dependent synaptic pruning, engulfing live synapses both early in development and throughout adulthood in rodents, suggesting a role in adult brain plasticity and learning. In addition to influencing the development of neural circuits, they actively impact excitatory communication of neurons, by altering glutamate receptors at the synapse (Chung et al., 2015). Astrocytes of great apes are larger than those of rodents, and they are the largest and most complex in humans relative to the other great apes (Herculano-Houzel, 2014; Oberheim et al., 2006). Human astrocytes propagate calcium waves more quickly than those of rodents, and when grafted to mouse brains, they enhance learning and memory (Han et al., 2013). The size, complexity, and functioning of human astrocytes may contribute to human-specific higher order cognitive processing.

Oligodendrocytes produce myelin in the central nervous system and play a role in maintaining the health of neurons (Berto et al., 2019). Myelin is necessary for neural networks to function optimally, and is important for human cognitive functions and behaviors. In adults, mature oligodendrocytes continue to produce myelin. This is particularly prolonged in the neocortex of the human brain relative to non-human primates, which may increase neural plasticity in adulthood (Miller et al., 2012; Yeung et al., 2014). Relative to chimpanzees, there is evidence of upregulated gene expression in human oligodendrocytes, suggesting an enhancement of oligodendrocyte networks that may contribute to both enhanced cognitive capacity and susceptibility to certain disorders (Berto et al., 2019). Microglia impact neural circuits in typical brain development through their role in synapse formation, maturation, and elimination in typical

brain development (Chung et al., 2015). In addition to their more classically recognized roles in apoptosis and synaptic pruning, microglia promote the formation of new excitatory synaptic spines. Microglia also help regulate inhibitory neural communication by modulating activity and wiring of Parvalbumin expressing interneurons (Thion & Garel, 2020).

Alterations in glia are found in many neurodevelopmental disorders. Neurodevelopmental disorders frequently involve deficits in synapse formation and function. This can be impacted by astrocyte or microglia development and function. In typical development, neural stem cells switch from producing neurons to producing glia, starting with astrocytes then oligodendrocytes. If the timing of this switch is disrupted, it can result in over- or under-production of astrocytes, which can impact neural network formation and function (Krencik et al., 2017). In rodent models of Down Syndrome and Rett syndrome abnormalities in astrocytes leads to fewer synapses and synaptic spines than controls (Allen, 2013). Alterations in microglia are commonly found in both Autism Spectrum Disorders (ASD) and schizophrenia, both neurodevelopmental disorders with a social component. Due to their role in regulating both excitatory and inhibitory neural circuits, perturbation in microglia may impact the excitatory/inhibitory balance, which may underlie many neurodevelopmental disorders (Thion & Garel, 2020). Excessive microglia activation can also result in neuroinflammation, which is a common feature of many neurodevelopmental disorders. This inflammation can impact neuron-glia signaling and neuron survival, resulting in cognitive and behavioral abnormalities (Chung et al., 2015).

Oligodendrocytes and deficits in myelin are also commonly found in neurodevelopmental disorders, and particularly those involving abnormalities in social functioning and mood regulation (Berto et al., 2019). Myelin deficits can alter the function of neural networks by impacting the timing and synchronization of neural signals. Genes with human-specific

expression in oligodendrocytes are associated with schizophrenia, Bipolar Disorder, and Major Depressive Disorder and white matter deficits are found in both schizophrenia and Williams Syndrome (Berto et al., 2019; Haroutunian et al., 2014; Nir & Barak 2020). Abnormal social interaction may itself be enough to produce myelin deficits, as is seen in the medial PFC of mice following social defeat or isolation (Lehmann et al., 2017; Liu et al., 2012). Humans may be more vulnerable to developmental disruption of myelin than non-human primates due to prolonged neocortical myelination (Miller et al., 2012).

Outline of the dissertation

This dissertation examines the postmortem neuroanatomy of WS in BA 25 of the vmPFC and in glia across the cortex, in BA 10, 4, 3, and 18 to investigate neuroanatomical abnormalities that may underlie the unique cognitive and behavioral phenotype in this disorder.

Chapter 2 provides theoretical background to the dissertation, reviewing the evolution of brain development and how evolutionary modifications to brain development in the human lineage affects brain plasticity and susceptibility to developmental disturbances. In chapter 3, we used Nissl-stained tissue to examine neuron and glia density in BA 25 in WS adults and infants. Previous research in our lab found decreased neuron density in the orbitofrontal cortex (BA 10 and BA 11) in adult WS (Lew et al., 2017). We extended this work to the vmPFC, and examined whether the abnormalities present in adult WS can be seen in the first year of life. In chapter 4, we focus on glia density across the WS cortex, separately quantifying oligodendrocytes and non-oligodendrocyte glia (astrocytes and microglia). Increased glia density has been found in WS the caudate nucleus, where it was demonstrated to be driven by an increase in oligodendrocyte density (Hanson et al., 2018). We chose to examine glia density across the cortex to determine if increased glia density was restricted to regions of the brain with known functional abnormalities,

or if there is a systemic perturbation of glia in WS. Chapter 6 serves as a general discussion and conclusion for the dissertation, summarizing our findings on cortical abnormalities in WS. This chapter also includes future directions for investigating WS, including future work on glia subtypes to better define the neuroanatomical phenotype of WS and to shed light on the mechanisms underlying these changes and their link to social behavior.

References

Allen, N. J. (2013). Role of glia in developmental synapse formation. *Current opinion in neurobiology*, *23*(6), 1027-1033.

Amodio, D. M., & Frith, C. D. (2006). Meeting of minds: the medial frontal cortex and social cognition. *Nature reviews neuroscience*, *7*(4), 268-277.

Avery, S. N., Thornton-Wells, T. A., Anderson, A. W., & Blackford, J. U. (2012). White matter integrity deficits in prefrontal–amygdala pathways in Williams syndrome. *Neuroimage*, *59*(2), 887-894.

Barger, N., Stefanacci, L., Schumann, C.M., Sherwood, C.C., Annese, J., Allman, J.M., Buckwalter, J.A., Hof, P.R. and Semendeferi, K. (2012). Neuronal populations in the basolateral nuclei of the amygdala are differentially increased in humans compared with apes: a stereological study. *Journal of Comparative Neurology*, *520*(13), 3035-3054.

Bechara, A., Damasio, H., and Damasio, A. R. (2000). Emotion, decision making and the orbitofrontal cortex. *Cerebral cortex*, 10(3), 295-307.

Bellugi, U., Lichtenberger, L., Jones, W., Lai, Z., and St. George, M. (2000). I. The neurocognitive profile of Williams Syndrome: a complex pattern of strengths and weaknesses. *Journal of cognitive neuroscience*, 12(Supplement 1), 7-29.

Berto, S., Mendizabal, I., Usui, N., Toriumi, K., Chatterjee, P., Douglas, C., Tamminga, C.A., Preuss, T.M., Soojin, V.Y. and Konopka, G. (2019). Accelerated evolution of oligodendrocytes in the human brain. *Proceedings of the National Academy of Sciences*, *116*(48), 24334-24342.

Chung, W. S., Welsh, C. A., Barres, B. A., & Stevens, B. (2015). Do glia drive synaptic and cognitive impairment in disease?. *Nature neuroscience*, *18*(11), 1539-1545.

Faria, A.V., Landau, B., O'Hearn, K.M., Li, X., Jiang, H., Oishi, K., Zhang, J. and Mori, S. (2012). Quantitative analysis of gray and white matter in Williams syndrome. *Neuroreport*, *23*(5), 283.

Ferry, A. T., Öngür, D., An, X., & Price, J. L. (2000). Prefrontal cortical projections to the striatum in macaque monkeys: evidence for an organization related to prefrontal networks. *Journal of Comparative Neurology*, *425*(3), 447-470.

Fuster, J. M. (1997). *The prefrontal cortex: anatomy, physiology, and neuropsychology of the frontal lobe* (3rd edition). Philadelphia and New York:Lippincott-Raven Publishers.

Han, X., Chen, M., Wang, F., Windrem, M., Wang, S., Shanz, S., Xu, Q., Oberheim, N.A., Bekar, L., Betstadt, S. and Silva, A.J. (2013). Forebrain engraftment by human glial progenitor cells enhances synaptic plasticity and learning in adult mice. *Cell stem cell*, *12*(3), 342-353.

Hanson, K. L., Hrvoj-Mihic, B., and Semendeferi, K. (2014). A dual comparative approach: integrating lines of evidence from human evolutionary neuroanatomy and neurodevelopmental disorders. *Brain, behavior and evolution*, 84(2), 135-155.

Hanson, K. L., Lew, C. H., Hrvoj-Mihic, B., Groeniger, K. M., Halgren, E., Bellugi, U., and Semendeferi, K. (2018). Increased glia density in the caudate nucleus in Williams syndrome: Implications for frontostriatal dysfunction in autism. *Developmental neurobiology*, 78, 531-545.

Haroutunian, V., Katsel, P., Roussos, P., Davis, K. L., Altshuler, L. L., & Bartzokis, G. (2014). Myelination, oligodendrocytes, and serious mental illness. *Glia*, *62*(11), 1856-1877.

Herculano-Houzel, S. (2012). The remarkable, yet not extraordinary, human brain as a scaled-up primate brain and its associated cost. *Proceedings of the National Academy of Sciences*, *109*(Supplement 1), 10661-10668.

Herculano-Houzel, S. (2014). The glia/neuron ratio: how it varies uniformly across brain structures and species and what that means for brain physiology and evolution. *Glia*, *62*(9), 1377-1391.

Hrvoj-Mihic, B., Hanson, K. L., Lew, C. H., Stefanacci, L., Jacobs, B., Bellugi, U., & Semendeferi, K. (2017). Basal dendritic morphology of cortical pyramidal neurons in Williams syndrome: prefrontal cortex and beyond. *Frontiers in neuroscience*, *11*, 419.

Krencik, R., van Asperen, J. V., & Ullian, E. M. (2017). Human astrocytes are distinct contributors to the complexity of synaptic function. *Brain research bulletin*, *129*, 66-73.

Lehmann, M. L., Weigel, T. K., Elkahloun, A. G., & Herkenham, M. (2017). Chronic social defeat reduces myelination in the mouse medial prefrontal cortex. *Scientific reports*, *7*, 46548.

Lew, C. H., Brown, C., Bellugi, U., and Semendeferi, K. (2017). Neuron density is decreased in the prefrontal cortex in Williams syndrome. *Autism Research*, 10, 99-112.

Liu, J., Dietz, K., DeLoyht, J.M., Pedre, X., Kelkar, D., Kaur, J., Vialou, V., Lobo, M.K., Dietz, D.M., Nestler, E.J. and Dupree, J. (2012). Impaired adult myelination in the prefrontal cortex of socially isolated mice. *Nature neuroscience*, *15*(12), 621-1623.

Meyer-Lindenberg, A., Hariri, A. R., Munoz, K. E., Mervis, C. B., Mattay, V. S., Morris, C. A., & Berman, K. F. (2005). Neural correlates of genetically abnormal social cognition in Williams syndrome. *Nature neuroscience*, *8*(8), 991-993.

Miller, D.J., Duka, T., Stimpson, C.D., Schapiro, S.J., Baze, W.B., McArthur, M.J., Fobbs, A.J., Sousa, A.M., Šestan, N., Wildman, D.E. and Lipovich, L. (2012). Prolonged myelination in human neocortical evolution. *Proceedings of the National Academy of Sciences*, *109*(41), 6480-16485.

Mobbs, D., Eckert, M. A., Mills, D., Korenberg, J., Bellugi, U., Galaburda, A. M., and Reiss, A. L. (2007). Frontostriatal dysfunction during response inhibition in Williams syndrome. *Biological psychiatry*, 62(3), 256-261.

Nir, A., & Barak, B. (2020). White matter alterations in Williams syndrome related to behavioral and motor impairments. *Glia*.

Oberheim, N. A., Wang, X., Goldman, S., & Nedergaard, M. (2006). Astrocytic complexity distinguishes the human brain. *Trends in neurosciences*, *29*(10), 547-553.

Öngür, D., Drevets, W. C., & Price, J. L. (1998). Glial reduction in the subgenual prefrontal cortex in mood disorders. *Proceedings of the National Academy of Sciences*, 95(22), 13290-13295.

Öngür, D., Ferry, A. T., & Price, J. L. (2003). Architectonic subdivision of the human orbital and medial prefrontal cortex. *Journal of Comparative Neurology*, 460(3), 425-449.

Semendeferi, K., Armstrong, E., Schleicher, A., Zilles, K., & Van Hoesen, G. W. (1998). Limbic frontal cortex in hominoids: a comparative study of area 13. *American Journal of Physical Anthropology: The Official Publication of the American Association of Physical Anthropologists*, *106*(2), 129-155.

Semendeferi, K., Armstrong, E., Schleicher, A., Zilles, K., & Van Hoesen, G. W. (2001). Prefrontal cortex in humans and apes: a comparative study of area 10. *American Journal of Physical Anthropology: The Official Publication of the American Association of Physical Anthropologists*, *114*(3), 224-241.

Semendeferi, K., Teffer, K., Buxhoeveden, D.P., Park, M.S., Bludau, S., Amunts, K., Travis, K. and Buckwalter, J. (2011). Spatial organization of neurons in the frontal pole sets humans apart from great apes. *Cerebral cortex*, *21*(7), 1485-1497.

Sherwood, C.C., Stimpson, C.D., Raghanti, M.A., Wildman, D.E., Uddin, M., Grossman, L.I., Goodman, M., Redmond, J.C., Bonar, C.J., Erwin, J.M. and Hof, P.R., 2006. Evolution of increased glia–neuron ratios in the human frontal cortex. *Proceedings of the National Academy of Sciences*, *103*(37), pp.13606-13611.

Strømme, P., Bjømstad, P. G., & Ramstad, K. (2002). Prevalence estimation of Williams syndrome. *Journal of child neurology*, *17*(4), 269-271.

Thion, M. S., & Garel, S. (2020). Microglial ontogeny, diversity and neurodevelopmental functions. *Current Opinion in Genetics & Development*, *65*, 186-194.

Velleman, S. L., & Mervis, C. B. (2011). Children with 7q11. 23 duplication syndrome: speech, language, cognitive, and behavioral characteristics and their implications for intervention. *Perspectives on language learning and education*, *18*(3), 108-116.

Yeung, M.S., Zdunek, S., Bergmann, O., Bernard, S., Salehpour, M., Alkass, K., Perl, S., Tisdale, J., Possnert, G., Brundin, L. and Druid, H. (2014). Dynamics of oligodendrocyte generation and myelination in the human brain. *Cell*, *159*(4), pp.766-774.

Chapter 2

Infant Brain Development and Plasticity from an Evolutionary Perspective

Abstract

The evolution of the human brain following the split from the last common ancestor of hominins and *Pan* has involved a substantial increase in size as well as modifications to the internal, cellular organization. These changes were likely achieved through modifications in the timing and rate of development during hominin evolution. The result of those changes is a uniquely derived developmental trajectory of the brain in humans compared to non-human primates, which includes an accelerated rate of growth prenatally and in infancy, prolonged development, and substantial postnatal plasticity. The outcome of these evolutionary modifications is significant brain growth and development occurring postnatally. This allows the brain to be shaped by the physical and social environment outside of the uterus to a greater degree than is seen in non-human primates, contributing to the cognitive flexibility, intelligence, and brain plasticity of humans.

Introduction

The evolution of the human brain following the split from the last common ancestor of hominins and *Pan* (*Pan troglodytes* and *Pan paniscus*) has involved a substantial increase in size as well as modifications to the internal, cellular organization. These changes were likely achieved through modifications in the timing and rate of development during hominin evolution. The result of those changes is a uniquely derived developmental trajectory of the brain in humans compared to non-human primates, which includes an accelerated rate of growth prenatally and in infancy, prolonged development, and substantial postnatal plasticity.

The outcome of these evolutionary modifications is significant brain growth and development occurring postnatally. This allows the brain to be shaped by the physical and social environment outside of the uterus to a greater degree than is seen in non-human primates, contributing to the cognitive flexibility, intelligence, and brain plasticity of humans (Leigh & Park, 1998; Neubauer & Hublin, 2012; Piantadosi & Kidd, 2016).

Brain Development in Infancy

General Primate Development

Brain development in primates begins with the formation of the neural tube, the first structure of the central nervous system to arise in primate embryos, which develops to form the brain and spinal cord. In humans, neural tube formation is complete by the end of the third week of gestation. A region of the interior of the neural tube known as the ventricular zone is lined with neural progenitor cells. Initially, these cells divide symmetrically, with each cell producing two progenitor cells. This increases the pool of progenitor cells and thus the number of neurons that can be produced. They later gradually shift to asymmetric division, producing one neural progenitor and one neuron. The progenitor cells remain in the ventricular zone producing more neurons, which then migrate out of the ventricular zone (Stiles & Jernigan, 2010).

Newly born cortical neurons migrate to the emerging cortical plate, where they will form the laminar structure of the cortex in an inside-out manner, with the earliest born neurons populating the deeper cortical layers (V/VI), and later born neurons populating more superficial layers (II/III) (Cooper, 2008). Neural proliferation occurs almost exclusively prenatally, with a peak in neuron density in the second half of gestation. This is followed by a period of *apoptosis*, where approximately half of all neurons produced undergo programmed cell death, resulting in neuron numbers at birth that are near adult values (Rabinowicz et al., 1996; Stiles & Jernigan,

2010). Proliferation and migration of glial cells begin prenatally after the onset of neurogenesis, first with astrocytes, then oligodendrocytes (Lee et al., 2000). Neurogenesis continues throughout life in primates in only two regions, the dentate gyrus of the hippocampus and the olfactory bulb (Taupin & Gage, 2002; Kornack & Rakic, 2001). There is no reliable evidence of post-natal neurogenesis in the primate neocortex, but production of glial cells continues throughout life (Gage, 2019; Lee et al., 2000).

As neurons reach their target destinations, they begin to extend axons, and dendrites form synapses with other cells. This begins prenatally and continues after birth. This initial cell growth and *synaptogenesis* is followed by a period of pruning unnecessary or unused connections. This activity-dependent fine-tuning of connections allows the brain to be modified in response to the environment (Oppenheim, 1989; Buss et al., 2006). Following *gliogenesis*, oligodendrocytes begin to form myelin sheaths around axons, aiding in the transmission of neural signals.

The microstructural changes described above are reflected in changes to the gross anatomy of the brain. Dendritic growth causes an increase in gray matter (mostly neurons and glia cells) volume early in life, followed by a later decline. This is paralleled by changes in cortical thickness, which initially increases, then thins as synapses and dendrites are pruned (Brown et al., 2012; Lyall et al., 2015). Myelination causes growth of white matter (mostly myelinated axons), which begins slowly prenatally and continues steadily until reaching maximum volume (Sakai et al., 2011). In some primates these changes occur at different times (i.e., are heterochronous) across different regions of the brain (Bianchi et al., 2013). Generally, primary processing regions develop first, and association regions such as the prefrontal cortex (PFC), which serves higher-order cognitive and emotional functions, develop later (Petanjek et al., 2011; Bianchi et al., 2013).

Less is known about the development of subcortical structures in primates, but there are data available for amygdala development in humans and non-human primates (Payne et al., 2010; Ulfig et al., 2003). The amygdala is known to play an important role in primate behavior and typical development in the production and processing of social and emotional behavior (Gabard-Durnama et al., 2018). The amygdala is of particular interest to human evolution as it shows evidence for reorganization in humans relative to non-human apes. The human amygdala has a larger lateral nucleus, with a greater number of neurons than expected for an ape brain of its size. This expansion of the lateral nucleus may contribute to enhanced social cognition in humans (Barger et al., 2007; 2012). The primate amygdala emerges early in prenatal development but is immature at birth, allowing post-natal experience to shape its development (Payne et al., 2010; Ulfig et al., 2003).

Although all primates follow the same general pattern of brain development, there are differences in the timing of these events, which have been best studied in macaques (*Macaca mulatta)*, common chimpanzees (*Pan troglodytes*), and humans.

Macaque

The majority of growth in total brain volume in macaques, approximately 60%, occurs prenatally, with slower growth postnatally (Leigh, 2004; Malkova et al., 2006). Total brain volume, as well as both gray and white matter volume, increase for the first five postnatal months. This is followed by a slight decline in gray matter volume by the end of infancy after which it stabilizes. White matter volume continues to increase through infancy and into the juvenile period, with both gray matter and white matter volumes reaching adult values by the onset of puberty (Kim et al., 2020; Knickmeyer et al., 2010). Postnatal growth of the amygdala is

greatest in the first two weeks, with growth slowing and finally stabilizing around 8 months of age, near the end of infancy, when adult volume is reached (Payne et al., 2010).

At the cellular level, neurogenesis in macaques begins on embryonic day 40, with synaptogenesis starting by embryonic day 65 (average gestation length = 167 days) (Bourgeois & Rakic, 1993). Across the macaque cortex there is a rapid increase in density of synapses in the last two prenatal months that takes place concurrently in all cortical layers. This increase continues throughout the first six postnatal months, the majority of the infancy period, after which the density of synapses begins to decline as unused connections are pruned (Rakic et al., 1986; Bourgeois et al., 1994; Rakic et al., 1994).

Chimpanzee

Combining longitudinal magnetic resonance imaging (MRI) data with cross-sectional measures of brain volume at death allows the rate of chimpanzee brain growth to be described throughout the prenatal and infant period. As shown in MRI studies, the rate of brain growth in chimpanzees accelerates in utero until it reaches its peak growth of approximately 12cc/week at gestation week (GW) 22, after which the rate of brain growth gradually slows, reaching 4.1cc/week at the end of gestation (see Figure 2.1). At birth, the neonatal chimpanzee brain is approximately 150cc, 40% of its adult volume (Sakai et al., 2012). Shown through measures of brain size at death, postnatally, the rate of brain growth continues to slow until around 5 years of age, when adult brain volume, 380cc, is achieved (Herdon et al., 1999; Leigh, 2004).

Gray matter volume in chimpanzees increases throughout infancy. In non-prefrontal regions gray matter volume reaches its peak at the end of infancy, 3 years of age. After 3 years of age gray matter volume in non-prefrontal regions declines until reaching adult values. Gray matter growth is prolonged in prefrontal regions, with gray matter volume continuing to increase

past infancy before eventually declining. White matter growth occurs throughout infancy, growing 173% and 62% of the volume at birth in the prefrontal and non-prefrontal regions of the cerebrum, respectively. Cerebral white matter reaches 50% (prefrontal) and 64% (non-prefrontal) of adult volume by 3 years of age. This growth continues past infancy, only reaching adult volume after puberty (Sakai et al., 2011). The growth of white matter is largely the result of changes in myelin. Myelination begins prenatally, with 20% of neocortical myelination complete at birth, and continues throughout infancy and the juvenile period before reaching adult levels around the time of sexual maturity, with myelin growth in prefrontal regions slightly prolonged relative to other brain regions (Miller et al., 2012).

At the cellular level, brain development is described through post-mortem histological studies of neural anatomy at different ages throughout life. Synaptogenesis in chimpanzees occurs concurrently across cortical areas, similar to macaques, with peak density of synapses reached at 3-5 years of age, just after infancy ends (Bianchi et al., 2013). There is growth of dendrites across the cortex in infancy, which appears to be prolonged in the prefrontal cortex. In adult primates, including macaques, chimpanzees, and humans, dendritic trees of pyramidal neurons in PFC are longer, and have more branches and spines than those of primary sensory areas (Jacobs et al., 2001; Hrvoj-Mihic et al., 2013). In infant chimpanzees, the dendritic trees and spine density in the PFC are not as elaborate as in other cortical areas, indicating continued development of pyramid neurons in the PFC past infancy (Bianchi et al., 2013).

Human

Prenatally, the rate of growth of the human brain accelerates from the period of GW 16 to GW 32, reaching a growth rate of 26.1cc/week at GW 32, more than six times the rate of growth in a chimpanzee fetus of the same gestational age (Figure 2.1). This rate of growth is maintained

throughout the remainder of the fetal period, with brain size at birth being approximately 400cc, about 30% of adult brain volume (Holland et al., 2014). The growth rate begins to decrease after birth.

Total brain volume increases dramatically in humans during infancy, doubling in size in the first year of life, to approximately 72% of adult volume (Figure 2.2). This growth is largely due to increases in gray matter, which grows by 149%, while white matter increases by 11% at the same time. In the second year of life there is a 15% increase in total brain volume, to approximately 83% of adult volume. In this period gray matter increases by 14%, while white matter increases by 19% (Knickmeyer et al., 2008). The dramatic increase in gray matter in the first year of life is in part due to growth of the cerebellum, which doubles in volume in the first three postnatal months, and increasing another 70% from three months to one year (Knickmeyer et al., 2008; Holland et al., 2014). In the cerebral hemispheres both gray and white matter volume increase in infancy, with gray matter possibly peaking at age 4, while white matter continues to slowly increase (Figure 2.2) (Matsuzawa et al., 2001; Pfefferbaum et al., 1994;). In the first two years of life, cerebral gray matter growth is slowest in primary processing areas, with frontal association cortices (including portions of the PFC) growing more rapidly (Gilmore et al., 2012).

Throughout infancy cerebral white matter grows at a faster rate than cerebral gray matter, growing 185% in prefrontal regions from 1 to 6 years of age, reaching 76.2% of adult volume, and 80% in non-prefrontal regions in the same period, reaching 77.2% of adult volume (Matsuzawa et al., 2001). The growth in white matter continues past infancy, particularly in prefrontal regions, due to prolonged myelin growth. Myelination begins prenatally. In the

neocortex this is restricted to postnatal development, and continues beyond adolescence (Miller et al., 2012).

The amygdala in humans can be found as early as GW 8, and structural connectivity is present by GW 13 (Gabard-Durnama et al., 2018). Postnatally, there is a large increase in amygdala volume in the first three months of life, with continued growth until around 4 years of age (Holland et al., 2014; Tottenham, 2012). Development continues past this point, with mature amygdala-PFC connectivity emerging after 10 years of age in typical development (Gee et al., 2013a).

Neurogenesis begins at GW 6 in humans, with the number of neurons reaching a peak at GW 28 then declining due to cell death to adult values at the time of birth (Rabinowcz et al., 1996). Postnatal growth in gray matter is due to *neuropil* growth, which reflects cellular development, specifically the growth of dendrites and axons (Knickmeyer et al., 2008; Rabinowcz et al., 1996). Neuronal development in the PFC is prolonged relative to other cortical regions. At birth, the earlier generated layer V pyramidal neurons of the PFC have larger and more complex dendritic trees than those of the later generated layer III pyramidal neurons. In the first year of life the dendritic trees of layer III pyramidal neurons grow more rapidly than those of layer V, with neurons in both layers reaching similar growth. This is followed by a period of minor dendritic growth, a "plateau" period, until two years of age. Between the ages of two and three years these neurons undergo a period of substantial dendritic elaboration, resulting in dendritic trees that are more extensive that those of layer V pyramidal neurons (Petanjek et al., 2008; Hrvoj-Mihic et al., 2013).

Synaptogenesis begins by GW 27 in humans and peaks around five years of age, with synaptic refinement and pruning continuing into adulthood (Huttenlocher & Dabholkar, 1997;

Petanjek et al., 2011). Development of synapses is heterochronous in different cortical areas, with primary processing areas developing more rapidly, while development of the PFC is prolonged (Huttenlocher & Dabholkar, 1997; Petanjek et al., 2011).

Uniquely Human Features of Infant Brain Development

Beginning prenatally, and continuing through early postnatal life, the rate of brain growth in humans is much greater than in chimpanzee (Figure 2.1). In the prenatal period this rate of growth is greater even relative to the greater volume of the human fetal brain. At birth, the human brain is approximately 2.5 times larger than a neonatal chimpanzee brain, and slightly larger than the average adult chimpanzee brain. However, humans achieve a smaller percentage of brain growth prenatally than chimpanzees, ≈30% compared to ≈40% of adult volume at birth, respectively. Postnatally, humans and chimpanzees appear to share a derived pattern of a rapid rate of postnatal brain growth compared to Old World monkeys.

After 18 months of age, the rate of brain growth in humans and chimpanzees slows significantly, with both species reaching a similar growth rate at this time (Figure 2.2). Brain growth duration does not vary substantially between species, suggesting that the change in the rate of growth during prenatal and early postnatal life is what allows the human brain to grow so large. This rapid growth is energetically costly. Approximately 87% of the resting metabolic rate of a newborn human is devoted to brain growth and function, while a chimpanzee of the same age only requires 45% of the resting metabolic rate to maintain brain growth and function (Bogin, 2007). In addition to a greater rate of overall brain growth, humans have more rapid growth of white matter in the brain than chimpanzees during infancy, particularly in prefrontal regions, and myelination is prolonged past sexual maturity well into adulthood.

At the cellular level, brain maturation follows the same overall pattern in humans as in non-human primates. There is a period of exuberant neuron production and synaptogenesis, with neurons and synapses later pruned in an activity-dependent manner, allowing the brain to be modified in response the environment. Nevertheless, there are differences in the timing of these events in humans relative to non-human primates. Maturation of pyramidal neurons in humans appears prolonged compared to non-human primates. Human neurons display delayed growth in dendritic length and dendritic spine number, with substantial developmental remodeling of synaptic spine density in the prefrontal cortex continuing until approximately 30 years of age (Petanjek et al., 2011). Novel experimental studies using induced pluripotent stem cells to directly compare human and chimpanzee neuronal development showed that human pyramidal neurons develop and migrate more slowly than chimpanzee pyramidal neurons. Even though initial growth of dendrites and dendritic spines occurs earlier in chimpanzees, growth is prolonged in human neurons. This allows humans to have a greater total dendritic length and greater number of dendritic spines (Marchetto et al., 2019).

Synaptogenesis in both humans and chimpanzees is prolonged compared to macaques. This is additionally prolonged in humans at least in the prefrontal cortex, and is reflected in changes in gene expression, with synaptic genes reaching peak expression in the prefrontal cortex before 1 year of age in chimpanzee and macaques, and 5 years of age in humans (Liu et al., 2012). Functionally important aspects of synaptic remodeling may occur during “plateau” periods, described for humans above. These occur in both humans and non-human primates later in development (Levitt, 2003). However, the early phase of limited growth in layer III of the PFC has only been observed in humans and may represent a specialization for enhanced cognition and cortico-cortical connectivity (Petanjek et al., 2008).

Brain development in human infancy is characterized by both rapid early growth and prolonged cellular maturation. Particularly interesting is the fact that regions with prolonged cellular maturation, such as the PFC, also exhibit the most rapid growth in the early postnatal period in humans (Gilmore et al., 2012). These features contribute to neuroplasticity in infancy, allowing the brain to be modified by the environment while the infant is in a critical period of social and cultural learning.

Neuroplasticity and Atypical development

Typical neural development in primates relies on both experience-expectant and experience-dependent plasticity. *Experience-dependent plasticity* is idiosyncratic, knowledge that is unique to the individual, shaping them for their particular environment. *Experience-expectant plasticity* is associated with critical periods in development, when expected experiences shape the developing brain in a species-typical way (Greenough & Black, 1999). These expected experiences are required for typical neural development, and may be experienced atypically in individuals with some neurodevelopmental disorders.

In social primates, there is a critical period during infancy for the development of social behaviors. Both common marmosets and macaques deprived of social contact as infants display aberrant social behaviors, including increased fear and a lack of social play, which persist into adulthood (Harlow & Harlow, 1962; Dettling et al., 2002). This atypical development does not require complete social deprivation. Macaques raised without their mother, but with age-matched peers until 6 months of age, also displayed aberrant social behaviors (Spinelli et al., 2009). In common marmosets, typically in constant contact with their parents early after birth, separation from both parents for just two hours per day in the first month of life is enough to induce stress

and decreased social play (Dettling et al., 2002). This suggests that social contact with the parent(s) during infancy is required for species-typical development of social behavior to occur.

Early life stress in infancy has similar results in humans. Children who experience early life neglect have higher levels of anxiety and depression that may be tied to altered connectivity between the amygdala and PFC (Tottenham, 2012). Early life stress in humans results in reduced integrity of white matter between frontal and limbic regions of the brain, as well as accelerated development of functional connectivity between the amygdala and medial PFC (Bick et al., 2015; Gee et al., 2013b). This accelerated development may be adaptive, as it does appear to provide greater resilience to stress. However, it also decreases early life plasticity and may prevent optimal neural development (Gee et al., 2013b). In the extreme case of institutionalization devoid of typical parental care, children show significant decreases in both cortical gray matter and white matter volumes. These negative effects can be partially ameliorated by providing a replacement parental figure, as long as this is done prior to two years of age (Nelson et al., 2009). Even less extreme stress can negatively impact brain development in infancy, as is the case with socioeconomic status, now known to positively correlate with cortical surface area (Noble et al., 2015).

Studies of brain development in neurodevelopmental disorders, in conjunction with studies of behavior and cognition in these disorders, shed light on the impact of *neuroplasticity*, which can be seen in Autism Spectrum Disorder (ASD) and Williams Syndrome (WS). ASD is complex and heterogenous, genetically and phenotypically, while WS has a known deletion of approximately 25-28 genes that underlies all typical WS cases, and a more consistent behavioral and cognitive profile (Bellugi et al., 2000; Strømme et al., 2002). Despite the differences in

etiology, both ASD and WS involve altered social behavior, and both provide examples of alterations to typical development.

ASD is characterized by early overgrowth in the brain, likely the result of exuberant neurogenesis or reduced apoptosis prenatally, and impaired white matter tracts in infancy (Shen & Piven, 2017). The growth of axons and dendrites from an excessive number of neurons may accelerate the rate of brain growth in infancy. This is seen in the PFC, where children with ASD have 67% more neurons than typically developing controls (Courchesne et al., 2011). Young children with ASD experience early overgrowth of the amygdala so that in childhood they have enlarged amygdalas relative to controls. They do not show typical growth of the amygdala during childhood that occurs in controls, and by adolescence amygdala volume in ASD subjects is equal to that of controls (Schumann et al., 2004). Overall neuron number is decreased in the ASD amygdala, and serotonergic innervation is increased relative to controls (Schumann & Amaral, 2006; Lew et al., 2020). Alterations to brain growth and neural development during infancy are not limited to the area directly affected. During this critical period of development, while the brain is highly sensitive to external input, disruptions to development can alter functional connectivity across the brain, resulting in substantial cognitive and behavioral consequences. In ASD it is hypothesized that early life neural overgrowth may alter the course of typical development, resulting in overconnectivity of local circuits and underconnectivity of long-range networks, impacting neural function throughout life (Courchesne & Pierce, 2005).

In WS, alterations to the brain appear to be more consistent and genetically guided than in ASD. WS is characterized by hypersociability, a lack of social inhibition, along with high levels of anxiety (Bellugi et al., 2000). In adulthood, WS subjects have alterations in parts of the brain known to be involved in social behavior. They have reduced neuron density and higher glia

density, along with relatively decreased dendritic trees in some regions of the PFC (Lew et al., 2017; Wilder et al., 2018; Hrvoj-Mihic et al., 2017). They also have a higher number of neurons, and decreased serotonergic innervation in the amygdala (Lew et al., 2018; 2020). There are very few studies on morphology of the infant and developing WS brain, but it appears that the alterations present in WS adults begin prenatally or in infancy. Increased neuron number in the lateral nucleus of the amygdala is present even in the youngest WS subject examined (<1 month of age), and increased glia density in the PFC is present at 8 months of age (Lew et al., 2018; Wilder et al., 2018). These early life alterations in WS may impact functional connectivity and future development within these brain regions, limiting neuroplasticity in some respects. This could be reflected in the behavioral profile of WS, which is consistent even cross-culturally (Zitzer-Comfort et al., 2007).

Brain Development in Human Evolution

As the brain does not fossilize, and development itself cannot be directly observed in extinct species, it is difficult to determine exactly when the *Homo sapiens* pattern of prolonged brain development, with rapid early growth, emerged during hominin evolution. However, by examining the fossil record and the timing of genetic changes, some inferences can be made.

Extinct Hominins

In two early species of hominins, *Sahelanthropus tchadensis* and *Ardipithecus ramidus*, adult endocranial volume was under 400cc, within the range of present-day chimpanzees. Brain growth in these species was likely relatively conserved and similar to the pattern that would have been present in last common ancestor of the *Pan* genus and hominins (Zollikofer & de León, 2013).

The earliest evidence of possible changes to the brain in hominin evolution has been demonstrated in Australopithecines. Adult Australopithecine endocranial volume (410-550cc) is slightly larger than that of chimpanzees (approximately 380cc) (Falk et al., 2000). Estimated neonatal brain size in Australopithecines is also slightly larger than average neonatal brain size in chimpanzees, 179.8cc and 145.7cc, respectively. Based on these estimates, Australopithecines would have completed a slightly lower percentage of brain growth prenatally than chimpanzees, 38% and 40% respectively (DeSilva & Lesnik, 2008). More direct evidence of brain ontogeny in Australopithecines comes from the endocasts of the Dikika child (*Austalopithecus afarensis*) and the Taung child (*Austalopithecus africanus*). The Dikika child, which dates to 3.3 million years ago, was approximately 3 years old at the time of death and had an estimated endocranial volume of 275-330cc (Alemseged et al., 2006). The Taung child, dated to 2.5 million years ago, was approximately 3-4 years old at the time of death, with an estimated endocranial volume of 382-405cc (Falk et al., 2000; Falk & Clarke, 2007). Relative to endocranial volume in adults of the same species, the values of both of these specimens fit within what would be expected for chimpanzees (Zollikofer & de León, 2013). Further evidence of brain ontogeny in the Taung child is found in its metopic suture, which had not completely fused at the time of death. In great apes, this occurs shortly after birth, while in *Homo sapiens* this suture fuses much later, possibly to accommodate a greater degree of brain growth. This delayed fusion of the metopic suture in the Taung child may indicate a higher rate of growth in the very early postnatal period in Australopithecines (Falk et al., 2012). Overall, brain growth in Australopithecines appears to be similar to that of chimpanzees, while possibly shifting very gradually to more prolonged postnatal growth, in the direction of the *Homo sapiens* growth pattern. Due to the limited number

of juvenile fossils found from the various Australopithecine species, there is not enough evidence to conclusively identify a change from the ancestral pattern of brain growth.

Brain volume increased significantly in the *Homo* genus relative to earlier hominins, with endocranial volumes of nearly all *Homo habilis* specimens being greater than 580cc. Estimates based on adult endocranial volume and pelvis size suggest neonatal brain size in early *Homo* species (*Homo habilis* and *Homo rudolfensis*) may have been approximately 225cc, only 35% of average adult values (Falk et al., 2000). The lack of any infant or young juvenile specimens from either of these species makes these estimates somewhat speculative, however.

The earliest species in which brain growth trajectory has been thoroughly examined is *Homo erectus*, where there is evidence of a change in the rate of brain growth compared to chimpanzees and relative to earlier hominins. The skull cap of an infant *Homo* erectus was found in Mojokerto, Indonesia and dated to 1.43-1.49 million years ago. The endocranial volume of the Mojokerto specimen is between 630 and 660cc, approximately 70% of the adult cranial capacity in *Homo erectus* from this time period (Balzeau et al., 2005). The individual was most likely younger than 4 years of age at death and may have been as young as 0.5-1.5 years of age, although estimates of age range widely due to the lack of information on *Homo erectus* somatic growth (Coqueugniot et al., 2004). By 0.5-1.5 years of age, humans achieve an average of approximately 62% of their adult endocranial volume, while chimpanzees reach approximately 80% by the same age (Figure 2.2). These values suggest an intermediate pattern of postnatal brain growth in *Homo erectus*, falling somewhere between *Homo sapiens*-like growth and non-human ape-like growth (O'Connell & DeSilva, 2013). This is supported by simulated annual growth rates of endocranial volume in *Homo erectus*, based on the Mojokerto specimen. These results suggest a rate of brain growth in *Homo erectus* that is on the lower end of the range of

Homo sapiens growth from 0.5-1.5 years of age, falling below the human range after 2 years of age, and elevated above that of chimpanzees or gorillas for the entire period from 0.5-2 years of age (Zollikofer & de León, 2010; Cofran & DeSilva, 2015).

Rapid brain growth in the early postnatal period appears to have emerged at least 1.8 million years ago with *Homo erectus*. The rapid growth period is even more prolonged in later hominins, *Homo neanderthalensis* and *Homo sapiens*. In *Homo neanderthalensis* this rate of growth was likely even greater than in *Homo sapiens*. This higher rate of growth resulted in a larger adult brain volume, despite a similar percentage of brain growth completed prenatally, and similar duration of brain growth (de León et al., 2008).

One feature of brain ontogeny that appears to be completely unique to *Homo sapiens* is *globularization* – developmental changes that result in a more rounded, globular braincase – that occurs in the perinatal, or immediate postnatal period. This postnatal change in brain shape is not seen in chimpanzees or in *Homo neanderthalensis*, likely only having evolved after *Homo sapiens* and *Homo neanderthalensis* diverged. It is achieved through parietal lobe expansion, expansion of posterior cranial fossa and basicranial flexion, which together result in a more rounded shape to the braincase (Neubauer et al., 2010). As this globularization takes place prior to complete ossification of the skull, while cranial sutures remain unfused, this change in the shape of the braincase is more likely the result of brain growth, suggesting possible subtle changes to the internal brain organization in early infancy that is unique to *Homo sapiens* (Neubauer et al., 2018)

Genetic Changes in the Human Lineage

There are many genes expressed in the brain that have undergone evolutionary modifications in the human lineage. Several of these genes, discussed here, have known

functions that impact brain development and may contribute to the greater size and plasticity of the human brain.

The difference in brain size between humans and closely related non-human primates has been attributed to differences in the proliferative capacity of neural progenitors during prenatal development. A larger pool of progenitor cells can produce greater neuron numbers, resulting in a substantially larger brain (Rakic, 2000). *ARHGAP11B*, which arose through incomplete duplication of the ancestral *ARHGAP11A* approximately 5 million years ago, promotes the production of basal neural progenitors, which are responsible for the production of most cortical neurons (Florio et al., 2016). Similarly, *TBC1D3* has been duplicated in the human lineage, and there are multiple copies of this gene in humans, while there is only one copy in chimpanzees. Overexpression of this gene in mice increases the number of basal neural progenitor cells (Ju et al., 2016). Partial duplication of *NOTCH2* resulted in the human-specific *NOTCH2NLA*, *NOTCH2NLB*, and *NOTCH2NLC* genes, which are all highly expressed in human radial glial cells. Overexpression of human *NOTCH2NL* in mice results in downregulation of genes involved in neuronal differentiation, suggesting a possible delay in the differentiation of neural progenitor cells (Fiddes et al., 2018). Such delay would allow for more rounds of symmetric cell division and thus a larger pool of neural progenitors.

Not every alteration to the genome contributing to changes in brain size has been in protein coding genes. The non-coding human accelerated *HARE5* gene acts as a regulatory enhancer of *FZD8*. In comparison to chimpanzee *HARE5*, human *HARE5* enhanced expression of *FZD8* accelerates neural progenitor cell cycle (Boyd et al., 2015). These genetic changes work together, promoting the production of neural progenitors, allowing them to divide more rapidly

and for a longer period of time, producing a substantially larger pool of neural progenitors and therefore greater neuron numbers and a larger brain.

Genes contributing to neuronal and synaptic maturation have been altered over the course of human evolution as well. Of particular interest is the *SRGAP2* gene, which has undergone three human-specific duplications to form *SRGAP2B* (3.4 million years ago), *SRGAP2C* (2.4 million years ago), and *SRGAP2D* (1 million years ago) (Dennis et al., 2012). The ancestral form of *SRGAP2*, *SRGAP2A* is upregulated at the end of cortical migration and promotes synapse maturation. *SRGAP2C* inhibits the functions of *SRGAP2A*, delaying synapse maturation and extending spine production in excitatory and inhibitory cortical neurons (Charrier et al., 2012; Fossati et al., 2016). This prolongs the period of cortical synaptogenesis in humans, and may underlie the extreme plasticity of the human brain during infancy and beyond.

Causes and Consequences of Brain Plasticity in Infancy

In the last 6-7 million years, since the split from the last common ancestor of humans and the *Pan* genus, brain size has more than tripled over the course of human evolution. This large brain has allowed humans to exploit a unique adaptive niche, becoming highly cultural and intelligent. The evolution of this large brain required developmental shifts, including a smaller relative brain at birth and more postnatal growth and development compared to non-human primates and earlier hominins. While energetically costly, these shifts to accommodate a larger brain resulted in increased neuroplasticity, along with increased potential for perturbations of development.

Human brain development is characterized by rapid growth prenatally and postnatally, as well as prolonged neuronal maturation and synaptic refinement. This is most extreme in regions of the brain devoted to higher level cognitive processes, such as areas of the prefrontal cortex.

These features underlie the extreme plasticity of the human brain in infancy. The brains of human infants have the capacity to be highly responsive to the environment during the development of critical cognitive and social skills, such as joint attention and language, ultimately allowing the emergence of uniquely human cognition and behavior.

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Figures

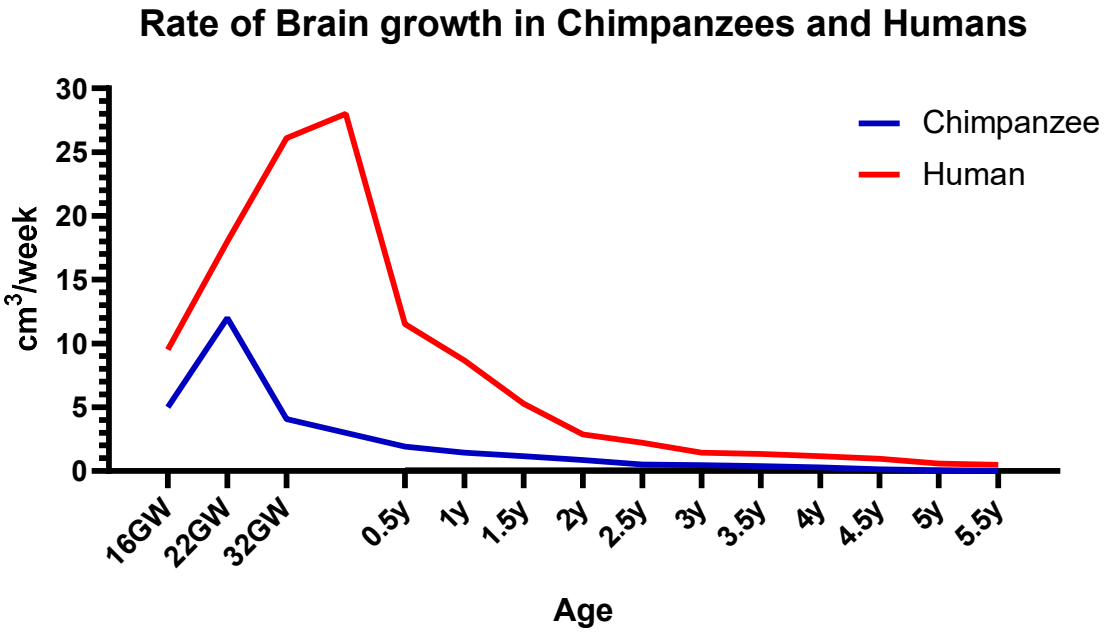

Figure 2.1. Approximate rate of total brain volume growth by week in chimpanzees and humans. Adapted from Leigh, 2004 and Sakai et al., 2012.

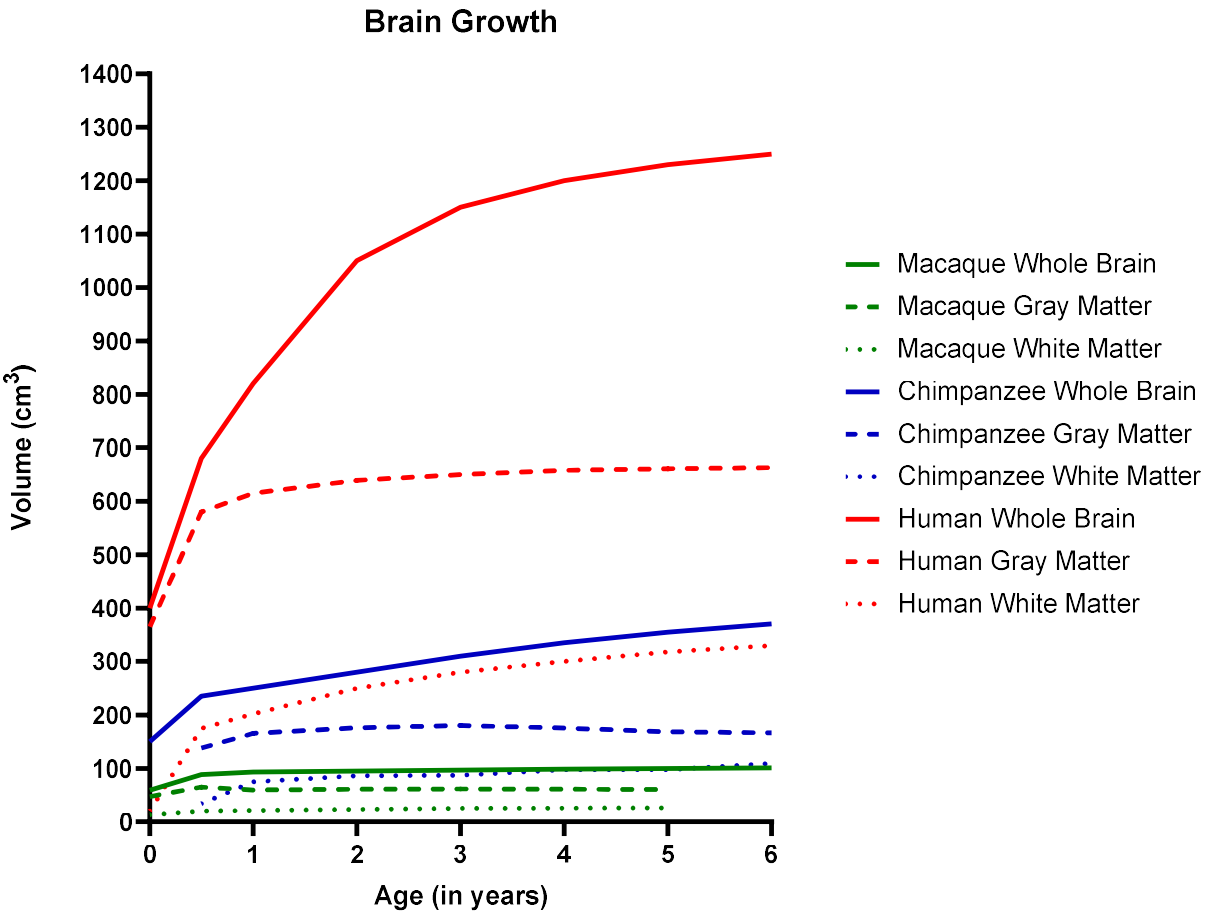

Figure 2.2. Growth in total brain volume, cerebral gray matter and white matter in macaques, chimpanzees, and humans. Adapted from Herdon et al., 1999, Kim et al., 2020, Leigh , 2004, Matsuzawa et al., 2001, and Sakai et al., 2011.

References

Alemseged, Z., Spoor, F., Kimbel, W. H., Bobe, R., Geraads, D., Reed, D., & Wynn, J. G. (2006). A juvenile early hominin skeleton from Dikika, Ethiopia. *Nature*, *443*(7109), 296–301.

Balzeau, A., Grimaud-Hervé, D., & Jacob, T. (2005). Internal cranial features of the Mojokerto child fossil (East Java, Indonesia). *Journal of Human Evolution*, *48*(6), 535–553.

Barger, N., Stefanacci, L., Schumann, C. M., Sherwood, C. C., Annese, J., Allman, J. M., Buckwalter, J. A., Hof, P. R., & Semendeferi, K. (2012). Neuronal populations in the basolateral nuclei of the amygdala are differentially increased in humans compared with apes: A stereological study. *The Journal of Comparative Neurology*, *520*(13), 3035–3054.

Barger, N., Stefanacci, L., & Semendeferi, K. (2007). A comparative volumetric analysis of the amygdaloid complex and basolateral division in the human and ape brain. *American Journal of Physical Anthropology*, *134*(3), 392–403.

Bellugi, U., Lichtenberger, L., Jones, W., Lai, Z., & St. George, M. (2000). I. The neurocognitive profile of Williams Syndrome: A complex pattern of strengths and weaknesses. *Journal of Cognitive Neuroscience*, *12*(supplement 1), 7–29.

Bianchi, S., Stimpson, C. D., Duka, T., Larsen, M. D., Janssen, W. G. M., Collins, Z., Bauernfeind, A. L., Schapiro, S. J., Baze, W. B., McArthur, M. J., Hopkins, W. D., Wildman, D. E., Lipovich, L., Kuzawa, C. W., Jacobs, B., Hof, P. R., & Sherwood, C. C. (2013). Synaptogenesis and development of pyramidal neuron dendritic morphology in the chimpanzee neocortex resembles humans. *Proceedings of the National Academy of Sciences*, *110*(Supplement_2), 10395–10401.

Bick, J., Zhu, T., Stamoulis, C., Fox, N. A., Zeanah, C., & Nelson, C. A. (2015). Effect of early institutionalization and foster care on long-term white matter development: A randomized clinical trial. *JAMA Pediatrics*, *169*(3), 211.

Bogin, B. (2007). The evolution of human brain and body growth patterns. In J. H. Kaas & T. M. Preuss (Eds), *Evolution of nervous systems* (pp. 337–345). London: Elsevier.

Bourgeois, J., & Rakic, P. (1993). Changes of synaptic density in the primary visual cortex of the macaque monkey from fetal to adult stage. *The Journal of Neuroscience*, *13*(7), 2801–2820.

Bourgeois, J.-P., Goldman-Rakic, P. S., & Rakic, P. (1994). Synaptogenesis in the prefrontal cortex of rhesus monkeys. *Cerebral Cortex*, *4*(1), 78–96.

Boyd, J. L., Skove, S. L., Rouanet, J. P., Pilaz, L.-J., Bepler, T., Gordân, R., Wray, G. A., & Silver, D. L. (2015). Human-chimpanzee differences in a FZD8 enhancer alter cell-cycle dynamics in the developing neocortex. *Current Biology*, *25*(6), 772–779.

Brown, T. T., Kuperman, J. M., Chung, Y., Erhart, M., McCabe, C., Hagler, D. J., Venkatraman, V. K., Akshoomoff, N., Amaral, D. G., Bloss, C. S., Casey, B. J., Chang, L., Ernst, T. M., Frazier, J. A., Gruen, J. R., Kaufmann, W. E., Kenet, T., Kennedy, D. N., Murray, S. S., Sowell, E. R., Jernigan, T. L., & Dale, A. M. (2012). Neuroanatomical assessment of biological maturity. *Current Biology*, *22*(18), 1693–1698.

Buss, R. R., Sun, W., & Oppenheim, R. W. (2006). Adaptive roles of programmed cell death during nervous system development. *Annual Review of Neuroscience*, *29*(1), 1–35.

Charrier, C., Joshi, K., Coutinho-Budd, J., Kim, J.-E., Lambert, N., de Marchena, J., Jin, W.-L., Vanderhaeghen, P., Ghosh, A., Sassa, T., & Polleux, F. (2012). Inhibition of SRGAP2 function by its human-specific paralogs induces neoteny during spine maturation. *Cell*, *149*(4), 923–935.

Cofran, Z., & DeSilva, J. M. (2015). A neonatal perspective on *Homo erectus* brain growth. *Journal of Human Evolution*, *81*, 41–47.

Cooper, J. A. (2008). A mechanism for inside-out lamination in the neocortex. *Trends in Neurosciences*, *31*(3), 113–119.

Coqueugniot, H., Hublin, J.-J., Veillon, F., Houët, F., & Jacob, T. (2004). Early brain growth in *Homo erectus* and implications for cognitive ability. *Nature*, *431*(7006), 299–302.

Courchesne, E., Mouton, P. R., Calhoun, M. E., Semendeferi, K., Ahrens-Barbeau, C., Hallet, M. J., Barnes, C. C., & Pierce, K. (2011). Neuron number and size in prefrontal cortex of children with autism. *JAMA, 306*(18), 2001-2010.

Courchesne, E., & Pierce, K. (2005). Why the frontal cortex in autism might be talking only to itself: Local over-connectivity but long-distance disconnection. *Current Opinion in Neurobiology*, *15*(2), 225–230.

De Leon, M. S., Golovanova, L., Doronichev, V., Romanova, G., Akazawa, T., Kondo, O., Ishida, H., & Zollikofer, C. P. E. (2008). Neanderthal brain size at birth provides insights into the evolution of human life history. *Proceedings of the National Academy of Sciences*, *105*(37), 13764–13768.

Dennis, M. Y., Nuttle, X., Sudmant, P. H., Antonacci, F., Graves, T. A., Nefedov, M., Rosenfeld, J. A., Sajjadian, S., Malig, M., Kotkiewicz, H., Curry, C. J., Shafer, S., Shaffer, L. G., de Jong, P. J., Wilson, R. K., & Eichler, E. E. (2012). Evolution of human-Specific specific neural SRGAP2 genes by incomplete segmental duplication. *Cell*, *149*(4), 912–922.

DeSilva, J. M., & Lesnik, J. J. (2008). Brain size at birth throughout human evolution: A new method for estimating neonatal brain size in hominins. *Journal of Human Evolution*, *55*(6), 1064–1074.

Dettling, A. C., Feldon, J., & Pryce, C. R. (2002). Repeated parental deprivation in the infant common marmoset (callithrix jacchus, primates) and analysis of its effects on early development. *Biological Psychiatry*, *52*(11), 1037–1046.

Falk, D., Redmond, J. C., Guyer, J., Conroy, C., Recheis, W., Weber, G. W., & Seidler, H. (2000). Early hominid brain evolution: A new look at old endocasts. *Journal of Human Evolution*, *38*(5), 695–717.

Falk, D., Zollikofer, C. P. E., Morimoto, N., & Ponce de Leon, M. S. (2012). Metopic suture of Taung (*Australopithecus africanus*) and its implications for hominin brain evolution. *Proceedings of the National Academy of Sciences*, *109*(22), 8467–8470.

Falk, D., & Clarke, R. (2007). Brief communication: New reconstruction of the Taung endocast. *American Journal of Physical Anthropology*, *134*(4), 529–534.

Fiddes, I. T., Lodewijk, G. A., Mooring, M., Bosworth, C. M., Ewing, A. D., Mantalas, G. L., Novak, A. M., van den Bout, A., Bishara, A., Rosenkrantz, J. L., Lorig-Roach, R., Field, A. R., Haeussler, M., Russo, L., Bhaduri, A., Nowakowski, T. J., Pollen, A. A., Dougherty, M. L., Nuttle, X., Addor, M., Zwolinkski, S., Katzman, S., Kriegstein, A., Eichler, E. E., Salama, S. R., Jacobs, F. M., & Haussler, D. (2018). Human-specific NOTCH2NL genes affect notch signaling and cortical neurogenesis. *Cell*, *173*(6), 1356-1369.e22.

Florio, M., Namba, T., Pääbo, S., Hiller, M., & Huttner, W. B. (2016). A single splice site mutation in human-specific *ARHGAP11B* causes basal progenitor amplification. *Science Advances*, *2*(12), e1601941.

Fossati, M., Pizzarelli, R., Schmidt, E. R., Kupferman, J. V., Stroebel, D., Polleux, F., & Charrier, C. (2016). SRGAP2 and its human-Specific paralog co-regulate the development of excitatory and inhibitory synapses. *Neuron*, *91*(2), 356–369.

Gabard-Durnam, L. J., O'Muircheartaigh, J., Dirks, H., Dean, D. C., Tottenham, N., & Deoni, S. (2018). Human amygdala functional network development: A cross-sectional study from 3 months to 5 years of age. *Developmental Cognitive Neuroscience*, *34*, 63–74.

Gage, F. H. (2019). Adult neurogenesis in mammals. *Science*, *364*(6443), 827–828.

Gee, D. G., Humphreys, K. L., Flannery, J., Goff, B., Telzer, E. H., Shapiro, M., Hare, T. A., Bookheimer, S. Y., & Tottenham, N. (2013a). A Developmental shift from positive to negative connectivity in human amygdala-prefrontal circuitry. *Journal of Neuroscience*, *33*(10), 4584–4593.

Gee, D. G., Gabard-Durnam, L. J., Flannery, J., Goff, B., Humphreys, K. L., Telzer, E. H., Hare, T. A., Bookheimer, S. Y., & Tottenham, N. (2013b). Early developmental emergence of human amygdala-prefrontal connectivity after maternal deprivation. *Proceedings of the National Academy of Sciences*, *110*(39), 15638–15643.

Gilmore, J. H., Shi, F., Woolson, S. L., Knickmeyer, R. C., Short, S. J., Lin, W., Zhu, H., Hamer, R. M., Styner, M., & Shen, D. (2012). Longitudinal development of cortical and subcortical gray matter from birth to 2 years. *Cerebral Cortex*, *22*(11), 2478–2485.

Greenough, W. T., & Black, J. E. (1999). Experience, neural plasticity, and psychological development. The role of early experience in infant development, In N. A. Fox , L. A. Leavitt , & J. G. Warhol (Eds.), *The role of early experience in infant development* (pp. 29 – 40). New Brunswick , NJ : Johnson & Johnson Consumer.

Harlow, H. F., & Harlow, M. K. (1962). Social deprivation in monkeys. *Scientific American*, *207*(5), 136–147.

Herndon, J. G., Tigges, J., Anderson, D. C., Klumpp, S. A., & Mcclure, H. M. (1999.). Brain weight throughout the life span of the chimpanzee. *Journal of Comparative Neurology*, 409(4), 567-572.

Holland, D., Chang, L., Ernst, T. M., Curran, M., Buchthal, S. D., Alicata, D., Skranes, J., Johansen, H., Hernandez, A., Yamakawa, R., Kuperman, J. M., & Dale, A. M. (2014). Structural growth trajectories and rates of change in the first 3 months of infant brain development. *JAMA Neurology*, *71*(10), 1266.

Hrvoj-Mihic, B., Bienvenu, T., Stefanacci, L., Muotri, A. R., & Semendeferi, K. (2013). Evolution, development, and plasticity of the human brain: From molecules to bones. *Frontiers in Human Neuroscience*, *7,* 707.

Hrvoj-Mihic, B., Hanson, K. L., Lew, C. H., Stefanacci, L., Jacobs, B., Bellugi, U., & Semendeferi, K. (2017). Basal dendritic morphology of cortical pyramidal neurons in Williams Syndrome: Prefrontal cortex and beyond. *Frontiers in Neuroscience*, *11*, 419.

Huttenlocher, P. R., & Dabholkar, A. S. (1997). Regional differences in synaptogenesis in human cerebral cortex. *Journal of comparative Neurology, 387*(2), 167-178.

Jacobs, B. (2001). Regional dendritic and spine variation in human cerebral cortex: A quantitative golgi study. *Cerebral Cortex*, *11*(6), 558–571.

Ju, X.-C., Hou, Q.-Q., Sheng, A.-L., Wu, K.-Y., Zhou, Y., Jin, Y., Wen, T., Yang, Z., Wang, X., & Luo, Z.-G. (2016). The hominoid-specific gene TBC1D3 promotes generation of basal neural progenitors and induces cortical folding in mice. *ELife*, *5*, e18197.

Kim, J., Jung, Y., Barcus, R., Bachevalier, J. H., Sanchez, M. M., Nader, M. A., & Whitlow, C. T. (2020). Rhesus macaque brain developmental trajectory: A longitudinal analysis using tensor-based structural morphometry and diffusion tensor imaging. *Cerebral Cortex*, *30*(8), 4325–4335.

Knickmeyer, R. C., Gouttard, S., Kang, C., Evans, D., Wilber, K., Smith, J. K., Hamer, R. M., Lin, W., Gerig, G., & Gilmore, J. H. (2008). A structural MRI study of human brain development from birth to 2 years. *Journal of Neuroscience*, *28*(47), 12176–12182.

Knickmeyer, Rebecca C., Styner, M., Short, S. J., Lubach, G. R., Kang, C., Hamer, R., Coe, C. L., & Gilmore, J. H. (2010). Maturational trajectories of cortical brain development through the pubertal transition: Unique species and sex differences in the monkey revealed through structural magnetic resonance imaging. *Cerebral Cortex*, *20*(5), 1053–1063.

Kornack, D. R., & Rakic, P. (2001). The generation, migration, and differentiation of olfactory neurons in the adult primate brain. *Proceedings of the National Academy of Sciences*, *98*(8), 4752–4757.

Lee, J. C., Mayer-Proschel, M., & Rao, M. S. (2000). Gliogenesis in the central nervous system. *Glia*, 30(2), 105-121.

Leigh, S. R. (2004). Brain growth, life history, and cognition in primate and human evolution. *American Journal of Primatology*, *62*(3), 139–164.

Leigh, S. R, & Park, P. B. (1998). Evolution of human growth prolongation. *American Journal of Physical Anthropology*: 107(3), 331-350.

Levitt, P. (2003). Structural and functional maturation of the developing primate brain. *The Journal of Pediatrics*, *143*(4), 35–45.

Lew, C. H, Brown, C., Bellugi, U., & Semendeferi, K. (2017). Neuron density is decreased in the prefrontal cortex in Williams syndrome. *Autism Research*, *10*(1), 99–112.

Lew, C. H., Groeniger, K. M., Bellugi, U., Stefanacci, L., Schumann, C. M., & Semendeferi, K. (2018). A postmortem stereological study of the amygdala in Williams syndrome. *Brain Structure and Function*, *223*(4), 1897–1907.

Lew, C. H., Groeniger, K. M., Hanson, K. L., Cuevas, D., Greiner, D. M. Z., Hrvoj-Mihic, B., Bellugi, U., Schumann, C. M., & Semendeferi, K. (2020). Serotonergic innervation of the amygdala is increased in autism spectrum disorder and decreased in Williams syndrome. *Molecular Autism*, *11*(1), 1-10.

Liu, X., Somel, M., Tang, L., Yan, Z., Jiang, X., Guo, S., Yuan, Y., He, L., Oleksiak, A., Zhang, Y., Li, N., Hu, Y., Chen, W., Qiu, Z., Paabo, S., & Khaitovich, P. (2012). Extension of cortical synaptic development distinguishes humans from chimpanzees and macaques. *Genome Research*, *22*(4), 611–622.

Lyall, A. E., Shi, F., Geng, X., Woolson, S., Li, G., Wang, L., Hamer, R. M., Shen, D., & Gilmore, J. H. (2015). Dynamic development of regional cortical thickness and surface area in early childhood. *Cerebral Cortex*, *25*(8), 2204–2212.

Malkova, L., Heuer, E., & Saunders, R. C. (2006). Longitudinal magnetic resonance imaging study of rhesus monkey brain development. *European Journal of Neuroscience*, *24*(11), 3204–3212.

Marchetto, M. C., Hrvoj-Mihic, B., Kerman, B. E., Yu, D. X., Vadodaria, K. C., Linker, S. B., Narvaiza, I., Santos, R., Denli, A. M., Mendes, A. P., Oefner, R., Cook, J., McHenry, L., Grasmick, J. M., Heard, K., Fredlender, C., Randolph-Moore, L., Kshirsagar, R., Xenitopoulos, R., Chou, G., Hah, N., Moutri, A. R., Padmanabhan, K., Semendeferi, K., & Gage, F. H. (2019). Species-specific maturation profiles of human, chimpanzee and bonobo neural cells. *ELife*, *8*, e37527.

Matsuzawa, J. (2001). Age-related volumetric changes of brain gray and white matter in healthy infants and children. *Cerebral Cortex*, *11*(4), 335–342.

Miller, D. J., Duka, T., Stimpson, C. D., Schapiro, S. J., Baze, W. B., McArthur, M. J., Fobbs, A. J., Sousa, A. M. M., Sestan, N., Wildman, D. E., Lipovich, L., Kuzawa, C. W., Hof, P. R., & Sherwood, C. C. (2012). Prolonged myelination in human neocortical evolution. *Proceedings of the National Academy of Sciences*, *109*(41), 16480–16485.

Nelson, C., Furtado, E., Fox, N., & Zeanah, C. (2009). The deprived human brain. *American Scientist*, *97*(3), 222-229.

Neubauer, S., Gunz, P., & Hublin, J.-J. (2010). Endocranial shape changes during growth in chimpanzees and humans: A morphometric analysis of unique and shared aspects. *Journal of Human Evolution*, *59*(5), 555–566.

Neubauer, S., Hublin, J.-J., & Gunz, P. (2018). The evolution of modern human brain shape. *SCIENCE ADVANCES*, *4*(1).

Neubauer, S., & Hublin, J.-J. (2012). The Evolution of Human Brain Development. *Evolutionary Biology*, *39*(4), 568–586.

Noble, K. G., Houston, S. M., Brito, N. H., Bartsch, H., Kan, E., Kuperman, J. M., Akshoomoff, N., Amaral, D. G., Bloss, C. S., Libiger, O., Schork, N. J., Murray, S. S., Casey, B. J., Chang, L., Ernst, T. M., Frazier, J. A., Gruen, J. R., Kennedy, D. N., Van Zijl, P., Mostofsky, S., Kaufmann, W. E., Kenet, T., Dale, A. M., Jernigan, T. L., & Sowell, E. R. (2015). Family income, parental education and brain structure in children and adolescents. *Nature Neuroscience*, *18*(5), 773–778.

O'Connell, C. A., & DeSilva, J. M. (2013). Mojokerto revisited: Evidence for an intermediate pattern of brain growth in Homo erectus. *Journal of Human Evolution*, *65*(2), 156–161.

Oppenheim, R. W. (1989). The neurotrophic theory and naturally occurring motoneuron death. *Trends in Neurosciences*, *12*(7), 252–255.

Payne, C., Machado, C. J., Bliwise, N. G., & Bachevalier, J. (2010). Maturation of the hippocampal formation and amygdala in Macaca mulatta: A volumetric magnetic resonance imaging study. *Hippocampus*, *20*(8), 922–935.

Petanjek, Z., Judas, M., Kostovic, I., & Uylings, H. B. M. (2008). Lifespan alterations of basal dendritic trees of pyramidal neurons in the human prefrontal cortex: A layer-specific pattern. *Cerebral Cortex*, *18*(4), 915–929.

Petanjek, Zdravko, Judaš, M., Šimić, G., Rašin, M. R., Uylings, H. B. M., Rakic, P., & Kostović, I. (2011). Extraordinary neoteny of synaptic spines in the human prefrontal cortex. *Proceedings of the National Academy of Sciences*, *108*(32), 13281–13286.

Pfefferbaum, A., Mathalon, D. H., Sullivan, E. V., Rawles, J. M., Zipursky, R. B., & Lim, K. O. (1994). A quantitative magnetic resonance imaging study of changes in brain morphology from infancy to late adulthood. *Archives of Neurology*, *51*(9), 874–887.

Piantadosi, S. T., & Kidd, C. (2016). Extraordinary intelligence and the care of infants. *Proceedings of the National Academy of Sciences*, *113*(25), 6874–6879.

Rabinowicz, T., de Courten-Myers, G. M., Petetot, J. M. C., Guohua, X. I., & de los Reyes, E. (1996). Human cortex development: estimates of neuronal numbers indicate major loss late during gestation. *Journal of Neuropathology & Experimental Neurology, 55*(3), 320-328.

Rakic, P. (2000, May). Radial unit hypothesis of neocortical expansion. In Novartis Foundation Symposium (pp. 30-52). Chichester; New York, NY; John Wiley.

Rakic, P, Bourgeois, J., Eckenhoff, M., Zecevic, N., & Goldman-Rakic, P. (1986). Concurrent overproduction of synapses in diverse regions of the primate cerebral cortex. *Science*, *232*(4747), 232–235.

Rakic, Pasko, Bourgeois, J.-P., & Goldman-Rakic, P. S. (1994). Synaptic development of the cerebral cortex: Implications for learning, memory, and mental illness. In *Progress in Brain Research* (Vol. 102, pp. 227–243). London: Elsevier.

Sakai, T., Mikami, A., Tomonaga, M., Matsui, M., Suzuki, J., Hamada, Y., Tanaka, M., Miyabe-Nishiwaki, T., Makishima, H., Nakatsukasa, M., & Matsuzawa, T. (2011). Differential prefrontal white matter development in chimpanzees and humans. *Current Biology*, *21*(16), 1397–1402

Sakai, T., Hirata, S., Fuwa, K., Sugama, K., Kusunoki, K., Makishima, H., Eguchi, T., Yamada, S., Ogihara, N., & Takeshita, H. (2012). Fetal brain development in chimpanzees versus humans. *Current Biology*, *22*(18), R791–R792.

Schumann, C. M. (2004). The amygdala is enlarged in children but not adolescents with autism; The hippocampus is enlarged at all ages. *Journal of Neuroscience*, *24*(28), 6392–6401.

Schumann, C. M., & Amaral, D. G. (2006). Stereological analysis of amygdala neuron number in autism. *Journal of Neuroscience*, *26*(29), 7674–7679.

Sedmak, D., Hrvoj-Mihić, B., Džaja, D., Habek, N., Uylings, H. B. M., & Petanjek, Z. (2018). Biphasic dendritic growth of dorsolateral prefrontal cortex associative neurons and early cognitive development. *Croatian Medical Journal*, *59*(5), 189–202.

Shen, M. D., & Piven, J. (2017). Brain and behavior development in autism from birth through infancy. *Dialogues in Clinical Neuroscience, 19*(4), 325.

Spinelli, S., Chefer, S., Suomi, S. J., Higley, J. D., Barr, C. S., & Stein, E. (2009). Early-life stress induces long-term morphologic changes in primate brain. *Archives of general psychiatry, 66*(6), 658-665.

Stiles, J., & Jernigan, T. L. (2010). The basics of brain development. *Neuropsychology review, 20*(4), 327-348.

Strømme, P., Bjømstad, P. G., & Ramstad, K. (2002.). Prevalence estimation of Williams Syndrome. 3. *Journal of child neurology, 17*(4), 269-271.

Taupin, P., & Gage, F. H. (2002). Adult neurogenesis and neural stem cells of the central nervous system in mammals. *Journal of Neuroscience Research, 69*(6), 745-749.

Tottenham, N. (2012). Human amygdala development in the absence of species-expected caregiving. *Developmental Psychobiology*, *54*(6), 598–611.

Ulfig, N., Setzer, M., & Bohl, J. (2006). Ontogeny of the human amygdala. *Annals of the New York Academy of Sciences*, *985*(1), 22–33.

Wilder, L., Hanson, K. L., Lew, C. H., Bellugi, U., & Semendeferi, K. (2018). Decreased neuron density and increased glia density in the ventromedial prefrontal cortex (Brodmann area 25) in Williams syndrome. *Brain Sciences, 8*(12), 209.

Zitzer-Comfort, C., Doyle, T., Masataka, N., Korenberg, J., & Bellugi, U. (2007). Nature and nurture: Williams syndrome across cultures. *Developmental Science*, *10*(6), 755–762.

Zollikofer, Christoph P.E., & Ponce de León, M. S. (2010). The evolution of hominin ontogenies. *Seminars in Cell & Developmental Biology*, *21*(4), 441–452. Academic Press.

Zollikofer, Christoph Peter Eduard, & De León, M. S. P. (2013). Pandora's growing box: Inferring the evolution and development of hominin brains from endocasts: Pandora's Growing Box. *Evolutionary Anthropology: Issues, News, and Reviews*, *22*(1), 20–33.

Chapter 3

Decreased Neuron Density and Increased Glia Density in the Ventromedial Prefrontal Cortex (Brodmann Area 25) in Williams Syndrome

Abstract

Williams Syndrome (WS) is a neurodevelopmental disorder caused by a deletion of 25–28 genes on chromosome 7 and characterized by a specific behavioral phenotype, which includes hypersociability and anxiety. Here, we examined the density of neurons and glia in fourteen human brains in Brodmann area 25 (BA 25), in the ventromedial prefrontal cortex (vmPFC), using a postmortem sample of five adult and two infant WS brains and seven age-, sex- and hemisphere-matched typically developing control (TD) brains. We found decreased neuron density, which reached statistical significance in the supragranular layers, and increased glia density and glia to neuron ratio, which reached statistical significance in both supra- and infragranular layers. Combined with our previous findings in the amygdala, caudate nucleus and frontal pole (BA 10), these results in the vmPFC suggest that abnormalities in frontostriatal and frontoamygdala circuitry may contribute to the anxiety and atypical social behavior observed in WS.

Introduction

Williams Syndrome (WS) is a rare (<1 in 7500) neurodevelopmental disorder resulting from a deletion of approximately 25–28 genes on chromosome band 7q11.23 (Strømme et al., 2002). Individuals with WS have a specific and well defined cognitive and behavioral phenotype. The cognitive profile of WS is characterized by deficits in global IQ and spatial processing, and relatively preserved language and face processing. However, even in these relatively spared skills, WS individuals demonstrate delayed and abnormal development, along

with atypical cognitive processing during some language and face tasks (Bellugi et al., 2000; Karmiloff-Smith et al., 2004). WS behavior is marked by high levels of sociability and anxiety. WS individuals have a high drive to engage in social interactions with others, and a tendency to approach even unfamiliar individuals to engage them in conversation (Doyle et al., 2004; Klein-Tasman & Mervis, 2003). In striking contrast to this, Autism Spectrum Disorders (ASD) are characterized often by social avoidance (Geschwind, 2011; Miles, 2011). Unlike WS, ASD are genetically complex and heterogenous (Geschwind, 2011; Miles, 2011). Interestingly, however, duplication of the WS gene deletion appears to cause ASD in a small subset of cases, demonstrating the range of behavioral effects that alterations at this locus can cause (Merla et al., 2010).

Abnormalities in the structure and function of the prefrontal cortex (PFC) have been demonstrated in imaging studies of WS. Overall cortical surface area, including surface area in two regions linked to emotion processing and social behavior, the orbital and medial prefrontal cortices, is decreased in WS. Cortical thickness, however, appears increased in these regions, and relative to brain size, total gray matter volume may also be increased in the orbital and medial prefrontal cortices in WS (Fan et al., 2017; Meda et al., 2012; Reiss et al., 2004). Functional imaging studies (fMRI) provide evidence of deficits in behavioral inhibition in WS. WS subjects had slower response times on an inhibition task than TD controls and displayed lower levels of activation in the striatum and frontal cortex (Mobbs et al., 2007). These abnormalities in fronto-striatal circuitry, and deficits in behavioral inhibition may relate to WS hypersociability, which has been described as an inability to inhibit the desire to approach and engage with others (Jones et al., 2000). In a functional imaging study examining response to threat, WS individuals displayed lower levels of activation in the amygdala and ventromedial prefrontal cortex vmPFC

while viewing threatening faces, but higher levels of activation in these regions while viewing threatening scenes, compared to TD controls (Meyer-Lindenberg et al., 2005). Atypical communication between frontal and limbic regions has been suggested as a possible factor in the high anxiety seen in WS (Ng et al., 2016). At the cellular level, microstructural analyses of WS subjects demonstrated lower neuronal density in the infragranular layers of the rostral orbitofrontal cortex (Lew et al. 2017). An increase in the ratio of glia to neurons, and in the density of oligodendrocytes in WS, has been found in the in the medial caudate nucleus, a region that receives projections from the vmPFC (Ferry et al., 2000; Hanson et al., 2018). In the amygdala of WS subjects, neuron number was higher in the lateral nucleus (Lew et al., 2018). Taken together, these findings suggest that abnormalities in PFC cytoarchitecture, and altered prefrontal inhibitory control of the amygdala and striatum, may be linked to the atypical anxiety and social behavior characteristic of WS.

Here, we examined one area of the vmPFC, Brodmann area 25 (BA 25), that is critically involved in social behaviors and related functions of inhibition and decision making (Ruff & Fehr, 2014). This area is heavily connected to several subcortical structures, including the amygdala and striatum, both of which are altered in WS, and in other disorders including autism (Barbas et al., 2006; Hanson et al., 2018; Lew et al., 2018; Morgan et al., 2014; Schumann et al., 2006). Using postmortem tissue from ten adult and four infant subjects, seven WS and seven age, sex, and hemisphere matched typically developing (TD) controls, we measured the density of neurons and glia in the supragranular (II/III) and infragranular (V/VI) layers of BA 25 in the vmPFC to test whether the previously observed decreases in neuron density in WS are restricted to rostral orbitofrontal cortical areas, or if there are widespread alterations to the frontal cortex in WS.

Materials and Methods

Brain Tissue

We examined cortical tissue from BA 25 in the vmPFC in a total of fourteen postmortem human subjects, including five adult WS and five adult TD subjects, as well as two WS infant subjects and two TD infant subjects (Table 3.1). TD subjects were matched with WS subjects for age (110/114 and 234/245 days for infants, 18–43 years for adults), sex, and hemisphere (right), to control for possible cytoarchitectonic asymmetries and age and sex-related differences (Rentería, 2012; Zilles et al., 1997).

All subjects in the Bellugi Williams Syndrome Brain Collection are part of an ongoing donation-based program now run by the Laboratory for Human Comparative Neuroanatomy at UCSD (La Jolla, CA, USA).

Regions of Interest

The region of interest (ROI) was identified using anatomical landmarks and by the absence of any visible border between cortical layers II and III and between layers V and VI. BA 25 occupies a portion of the brain immediately caudal and ventral to genu of the corpus callosum. It is agranular, lacking a visible layer IV, and poorly laminated compared to surrounding cortical areas (Dombrowski et al., 2001; Öngür et al., 2003). Cortical layers II/III and V/VI were analyzed as two distinct ROIs (Figure 3.1).

Processing of Tissue

Blocks of tissue containing BA 25 were extracted and cryoprotected using a series of 10%, 20%, and 30% sucrose solutions with 0.1 M phosphate buffer until saturated. Frozen tissue was cut on a Leica SM 2010R (Leica Biosystems, Wetzlar, Germany) sliding microtome into ten series of 40 micrometer (μm) thick sections in adult subjects. Due to the fragility of infant tissue,

infant subjects were cut into five series of 80 µm thick sections. One series was rehydrated for 48 h in a neutral phosphate buffer, then mounted on gelatin-coated slides. Mounted sections were dried for 48 h at room temperature, then dehydrated in a 1:1 chloroform ethanol solution overnight. These sections were stained with a 0.25% thionine stain for Nissl substance to visualize cell bodies, rehydrated, submerged in xylenes or citrisolv for 15 min after staining, and then cover-slipped with permount. The remaining series were stored for use in later processing, including a variety of immunohistological staining experiments.

Unbiased, Design-Based Stereology

Data collection was performed using StereoInvestigator software (MBF Bioscience, Williston, VT, USA) on a Dell workstation receiving live video feed from an Optronics MicroFire color video camera (East Muskogee, OK, USA) attached to a Nikon Eclipse 80i microscope (Nikon Instruments, Melville, NY, USA) equipped with a Ludl MAC5000 stage (Ludl, Hawthorn, NY, USA) and a Heidenhain z-axis encoder (Heidenhain, Plymouth, MN, USA). To increase the accuracy and consistency of measurements across all subjects, we report neuron and glia density rather than number, a standard practice for data collection in the cortex (Benes et al., 1986; Lew et al., 2017; Oblak et al., 2011; Smiley et al., 2012).

All data were collected by a single rater (LW). Inter-rater reliability was ensured through repeated neuron density estimations on a sample previously reported in the literature to 95% concordance (Lew et al., 2017). Sections were coded before data collection to blind the rater to diagnosis. Six sections per subject, spaced as equidistantly as allowed by individual section quality, were analyzed, representing the maximum extent of the area in the coronal plane. Neuron and glia densities in layers II/III and V/VI were estimated using the Optical Fractionator probe in StereoInvestigator. Two regions of interest per section, one bounding layers II/III and

the other bounding layers V/VI, were drawn at a 1× magnification, consistent with previous work on WS cortex (Lew et al., 2017). BA 25 in adults has no visible layer IV. Neurons and glia were counted using a 1.4 numerical aperture, 100× oil objective lens, with a grid size of 300 × 300 microns, a dissector height of 9 microns, and a counting frame of 85 × 85 microns. For infant subjects, a 50 × 50 micron counting frame was used. Within this frame, neurons and glia not touching the line of exclusion were counted using different markers. Cells were distinguished based on their morphology. Neurons were identified by the presence of a distinct nucleolus, and a lightly stained nucleus surrounded by cytoplasm. Glia were identified by their smaller size and lightly or darkly stained nucleus, with very little or no staining of the surrounding cytoplasm (Figure 3.2) (García-Cabezas et al., 2016). For each ROI (layers II/III and layers V/VI, respectively), neuron and glia densities were calculated by dividing population estimate of each cell type by the planimetric volume estimate from the Optical Fractionator probe.

Statistical Analysis

Standard two-tailed t-tests ($p < 0.05$) were used to compare neuron density, glia density, and glia to neuron ratio in WS and TD. Supragranular and infragranular layers were compared separately, as well as the average density of these layers combined. Percent difference in WS compared to TD was calculated as the difference in mean value of WS from TD, in relation to the mean TD value, for neuron density, glia density, and glia to neuron ratio, in each ROI.

Results

Adult Neuron Density

Results are summarized in Table 3.2 and Figure 3.3. In supragranular layers, neuron density was significantly decreased in WS compared to TD ($p = 0.046$, 17% decrease). Neuron

density infragranular layers were decreased in WS, but this was not statistically significant ($p = 0.186$, 9% decrease)

Adult Glia Density and Glia to Neuron Ratio

Results are summarized in Tables 3.3 and 3.4, and Figure 3.4. Mean glia density was significantly increased in WS compared to TD, in both supragranular (83% increase, $p = 0.00007$) and infragranular (116% increase, $p = 0.000001$) layers. Glia to neuron ratio was also increased in WS compared to TD in supragranular (125% increase, $p = 0.003$) and infragranular (140%, $p = 0.0003$) layers.

Infant Neuron Density, Glia Density, and Glia to Neuron Ratio

Results are summarized in Figure 3.5. In the 114 (WS) and 110 (TD) day-old subject pair, neuron density, glia density, and glia to neuron ratio were quite similar between the TD and WS subject in the supragranular layers (within 1%). In the infragranular layers, neuron density, glia density, and glia to neuron ratio were lower in the WS subject (33% lower, 55% lower, and 16% lower respectively). In the 234- and 245-day pair, across all layers, neuron density was lower (35% lower supragranular, 16% lower infragranular), and glia density (5% higher supragranular, 16% higher infragranular) and glia to neuron ratio (63% higher supragranular, 61% higher infragranular) were both higher in the WS subject. Table 3.5 summarizes results for all ages.

Discussion

Very few histological studies of BA 25 in TD adults have been conducted (Mackey & Petrides, 2010; Mackey & Petrides, 2014; Öngür et al., 2003) and none on infants. All data from these few adult studies are qualitative rather than quantitative. The present study provides the first quantitative data for neuron and glia density in TD adult and infant BA 25. Our findings

demonstrate variation in cell density between cortical layers consistent with expected patterns based on adult TD brains from the limited reports available. Qualitative description of human BA 25, along with quantitative findings in macaques, show that caudal, agranular regions of the vmPFC, such as BA 25, are characterized by higher neuron density in infragranular layers compared to supragranular layers, and lower glia density than neuron density in all layers (Dombrowski et al., 2011; Öngür et al., 2003). As expected, neuron density is much higher in infants than adults (Huttenlocher, 1990). This study builds on previous research on the adult PFC in both TD and WS, a region implicated in the behavioral phenotype of the disorder, and is the first to examine the PFC in WS infants (Fan et al., 2017; Huttenlocher et al., 1990; Lew et al., 2017).

Adults: A previous postmortem histological study of WS, which included three of the same adult WS subjects utilized here, along with an additional three adult WS subjects and six adult TD controls, found decreased neuron density in BA 10 and 11 of the prefrontal cortex (PFC), with the greatest difference observed in the infragranular layers (Lew et al., 2017). A study on the morphology of basal dendrites in adult WS subjects found that dendritic length and branching were compromised in the supragranular layers in BA 10 and 11, relative to more posterior areas of the cortex, BA 4, 3 and 18 (Hrvoj-Mihic et al., 2017). Based on the above study, we expected to find decreased neuron density in BA 25 in WS adults compared to TD adults, which is consistent with our results. This difference was greater in supragranular layers than in infragranular layers. In the present study, we additionally found significant increases in glia density and glia to neuron ratio, in both supragranular and infragranular layers of BA 25 in WS adults. An increase in glia was also observed in the caudate nucleus in WS, which seems to be driven by an increase in oligodendrocytes (Hanson et al., 2018).

Infants: As expected, in both the TD and WS infants, overall neuron density was lower in the eight-month-old subjects than in the four-month-old subjects. In the TD infants, this difference was greatest in infragranular layers, while in WS infants there was a greater decrease in supragranular layers. Additionally, glia density increased with age in both the TD and WS infants, although this increase was far greater in WS. In the older TD infant, both neuron density and glia density were elevated compared to adult TD subjects, but glia to neuron ratio was very close to the adult mean. In the older WS infant, neuron density was elevated compared to adult WS. However, overall glia density in this subject was similar to the WS adult mean, and glia to neuron ratio was much lower.

In the youngest infant pair examined here (about four months old), differences in both neuron and glia density between TD and WS appear almost exclusively in the infragranular layers. Additionally, this was the only pair examined in which the WS subject had lower glia density than the TD subject. In the older infant pair (about eight months old) examined here, differences in neuron and glia density occurred in a similar pattern as was seen in adults. There was a greater difference in neuron density in supragranular layers, and a greater difference in glia density in infragranular layers, suggesting that this pattern is not present at birth, but is established in infancy. At both time points in the infants, overall neuron density was lower in WS than TD, as it is in adults, but the difference in neuron density in the supragranular layers may develop postnatally, in infancy. In the four-month-old infant pair, glia density was lower in WS than in TD, in contrast to all other pairs examined. Although these results represent only two WS infants, one at each age point, this suggests there may be disruptions in prenatal gliogenesis or glial migration in WS, followed by a significant increase in glial cells which begins in infancy, and continues beyond the ages of the infants included in this study.

Here, we demonstrated decreased neuron density in WS compared to TD subjects starting in early infancy; and increased in glia density in WS older infants and adults compared to TDs, but not in the youngest infant pair examined. Although the exact mechanisms for the decrease in neuron density, and the differences in glia development observed here in BA 25 in WS are unknown, they may be due in part to the deletion of *GTF2I*, *GTF2IRD1*, and *FZD9* genes crucially involved in neural development, cell division, and cell fate and neuroinflammatory processes increasing glia and decreasing neuronal survival (Chailangkarn et al., 2016; Mitew et al., 2014; Sakurai et al., 2011). Given that the decrease in neuron density in BA 25 is present even early in the first year of life, it likely results from a combination of deficient neurogenesis prenatally and increased apoptosis prenatally, possibly extending slightly into the postnatal period. WS neural progenitor cells (NPCs), differentiated from WS induced pluripotent stem cells (iPSCs), were found to have increased doubling time, resulting in a smaller population of NPCs, and increased levels of apoptosis compared to TD NPCs. *FZD9* appears to be critically involved in these processes in WS. By restoring *FZD9*, both apoptosis and the doubling time of WS NPCs and apoptosis were reduced to a similar level as in TD controls, creating the same number of NPCs as TD controls (Chailangkarn et al., 2016).

The increase in glia density in BA 25 in WS could be due to excess production of glial cells, deficits in apoptosis, or disrupted migration. Glia have critical roles in neural development and neurological functions, affecting neuronal survival, and synapse formation, elimination, and functioning (Bayraktar et al., 2015; Molofsky et al., 2012; Petrelli et al., 2016). Changes in glia cells, or in the ratio of glia to neurons, can alter the typical course of neurodevelopment and the formation and functioning of neural circuits (Sloan & Barres, 2014). Abnormalities in glia cells have been linked to many neurological or neurodevelopmental disorders, including major

depressive disorder, ASD, and schizophrenia (Petrelli et al., 2016; Sloan & Barres, 2014; Zhan et al., 2014). Decreased glia density has been found in the orbitofrontal cortex of subjects with major depressive disorder, and increased microglia density has been found in the prefrontal cortex in both ASD and schizophrenia (Garey, 2010; Morgan et al., 2010; Rajkowska et al., 1999).

Deletion of *FZD9* gene has been shown to affect neural progenitor cells through the canonical Wnt pathway, a pathway necessary to inhibit the differentiation of oligodendrocyte progenitor cells (OPCs) (Chailangkarn et al., 2016; Mitew et al., 2014). The proliferation of oligodendrocytes occurs in a series of successive waves, beginning prenatally, with later generated cells replacing earlier derived populations (Kessaris et al., 2006; Lee et al., 2000; Mitew et al., 2014). OPCs continue to proliferate while migrating to white matter until an appropriate density of OPCs has been reached (Orentas & Miller, 1998). OPCs remain proliferative in the subventricular zone throughout postnatal life, although cell turnover is low in typically developing adults (Mitew et al., 2014; Orentas & Miller, 1998).

In typical development, neural stem cells switch from neurogenic to gliogenic, producing astrocytes and then oligodendrocytes, in the prenatal period. The timing of this switch is critical. If the switch happens too early, it can result in overproduction of astrocytes and deficits in some neuronal populations. If it occurs too late, this can reduce the number of astrocytes produced, limiting the signals they provide for axonal guidance, neuronal survival, and synaptogenesis (Homem et al., 2015; Kohwi & Doe, 2013; Sloan & Barres, 2014; Ren et al., 2017). Chronic neuroinflammation, which is found in many neurological disorders, could cause increased density and activation of microglia, and potentially atrophy in astrocytes, leading to the excessive pruning of synapse and neuronal death. This may result in underconnectivity in the

brain and contribute to the phenotypes of neurodevelopmental disorders (Petrelli et al., 2016; Rodriguez & Kern, 2011; Zhan et al., 2014) Inflammation may also alter synaptic transmission through changes to astrocyte function, further affecting cognition and behavior (Petrelli et al., 2016).

In contrast to the findings in WS, increases in neuron number have been found in the PFC of young subjects with ASD, age range 2–16 years, with no significant difference in glia number (Courchesne et al., 2011). Impaired connectivity between regions critical to social cognition and emotional regulation may be a common factor underlying some of the social and emotional abnormalities seen in both ASD and WS. In ASD, reduced fractional anisotropy was found in white matter adjacent to the vmPFC, and in the temporal lobe approaching the amygdala, suggesting disrupted connections between frontal and limbic brain regions (Barnea-Goraly et al., 2004). Additionally, there is evidence of atypical activation of the vmPFC while evaluating emotional faces, as well as altered functional connectivity in fronto-striatal and fronto-amygdala circuits (Di Martino et al., 2016; Monk et al., 2010). Although no differences were found in total glia number in the PFC, increased density, along with increased activation, of microglia has been demonstrated in the PFC in ASD (Morgan et al., 2010). The increased activation of microglia may reflect ongoing neuroinflammatory processes, which may contribute to loss of synaptic connections and under-connectivity in ASD (Morgan et al., 2010).

Neuroinflammatory mechanisms could account for the increased glia density in WS. Although no studies have been conducted to examine microglia in WS specifically, chronic neuroinflammation is common in many neurodevelopmental disorders, including ASD and schizophrenia, and is often the cause of increased glia density (Morgan et al., 2010; Petrelli et al., 2016; Rodriguez & Kern, 2011; Zhan et al., 2014). The results from infant subjects suggest that

the increased glia density in WS may develop over the first year of life, but it does not appear to be present before 4 months of age. It is not currently known if this increase is restricted to certain types of glia and to frontal and striatal regions, or if it represents a systemic perturbation of glia. Further investigation, utilizing immunohistochemical staining to determine what type of glia cells are affected, and examination of more brain regions in WS could help elucidate this matter.

The results presented here, combined with prior findings of decreased neuron density in BA 10 and 11 in WS, suggest that neuronal abnormalities in WS may be a common feature across the PFC (Lew et al., 2017). Additionally, abnormalities have been reported in the striatum and the amygdala in WS, two subcortical structures heavily connected to BA 25 (Hanson et al., 2018; Lew et al., 2018). Together, these findings suggest that abnormalities in PFC cytoarchitecture, and altered prefrontal inhibitory control of the amygdala and striatum, may be linked to the atypical anxiety and social behaviors characteristic of WS.

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Chapter 3, in full, is an adaptation of a primary data paper published in *Brain Sciences*. Citation: Wilder, L., Hanson, K. L., Lew, C. H., Bellugi, U., & Semendeferi, K. (2018). Decreased neuron density and increased glia density in the ventromedial prefrontal cortex (Brodmann area 25) in Williams syndrome. *Brain sciences*, *8*(12), 209. The dissertation author was the primary investigator and author of this paper.

Tables and Figures

Table 3.1. Subject Information.

Subject	Age at Death	Sex	Hemisphere	PMI (hours)	Cause of death
WS 7	114d	M	R	<30	Multiorgan failure
TD 5883	110d	M	R	34	Sudden unexplained death in infancy
WS 2	245d	F	R	N/A	Sudden infant death syndrome
TD 4392	234d	F	R	13	Intussuseption of Meckel's diverticulum
WS 10	18y	M	R	24	Cardiac complications
TD 4916	19y	M	R	5	Drowning
WS 15	24y	F	R	20	Pneumonia, Sepsis
TD 5350	25y	F	R	26	Sepsis
WS 1	31y	M	R	26	Cardiac complications
TD 5539	31y	M	R	24	Acute drug intoxication
WS 14	42y	F	R	18	Cardiac complications
TD 5445	42y	F	R	10	Pulmonary thromboembolism
WS 9	43y	F	R	12	Cardiac complications
TD 4636	43y	F	R	19	Pulmonary thromboembolism

Table 3.2. Mean Neuron Density (neurons/mm3) and Standard Deviation in BA 25.

Cortical Layers	II/III	V/VI
TD	30882±2537	36506±2567
WS	25594±4157	33094±4417
% Difference	-17%	-9%

Table 3.3. Mean Glia Density (glia/mm^3) and Standard Deviation in BA 25.

Cortical Layers	II/III	V/VI
TD	18756±426	19721±465
WS	34355±2038	42510±1844
% Difference	+83%	+116%

Table 3.4. Glia to Neuron Ratio in BA 25.

Cortical Layers	II/III	V/VI
TD	0.61	0.54
WS	1.38	1.30
% Difference	+125%	+140%

Table 3.5. Summary Table of Results.

Age	Layer	Neuron Density	Glia Density
4 months	II/III	No difference	No difference
	V/VI	33% Lower	55% Lower
8 months	II/III	35% Lower	5% Higher
	V/VI	16% Lower	16% Higher
Adult	II/III	17% Lower *	83% Higher *
	V/VI	9% Lower	116% Higher *

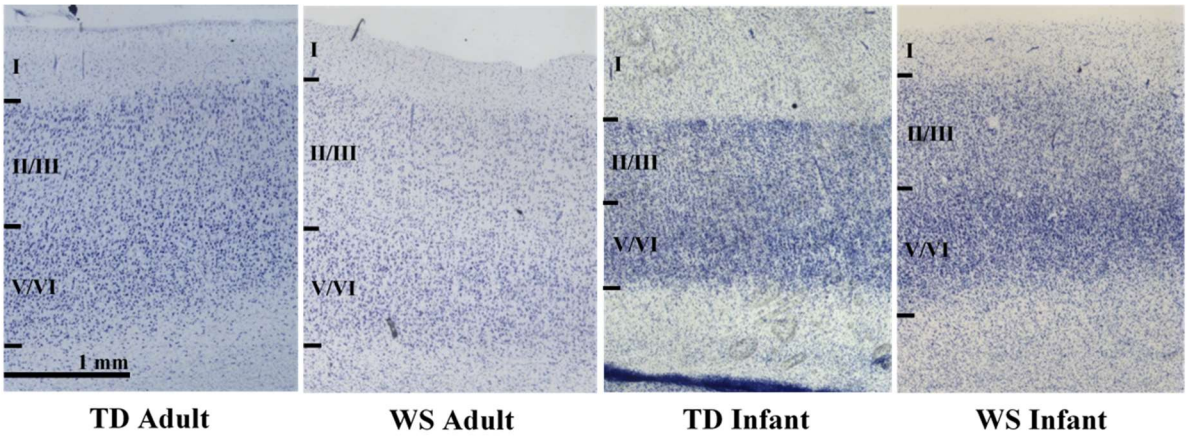

Figure 3.1. Microphotographs of BA 25 in adult and infant WS and TD. Images taken at 2X.

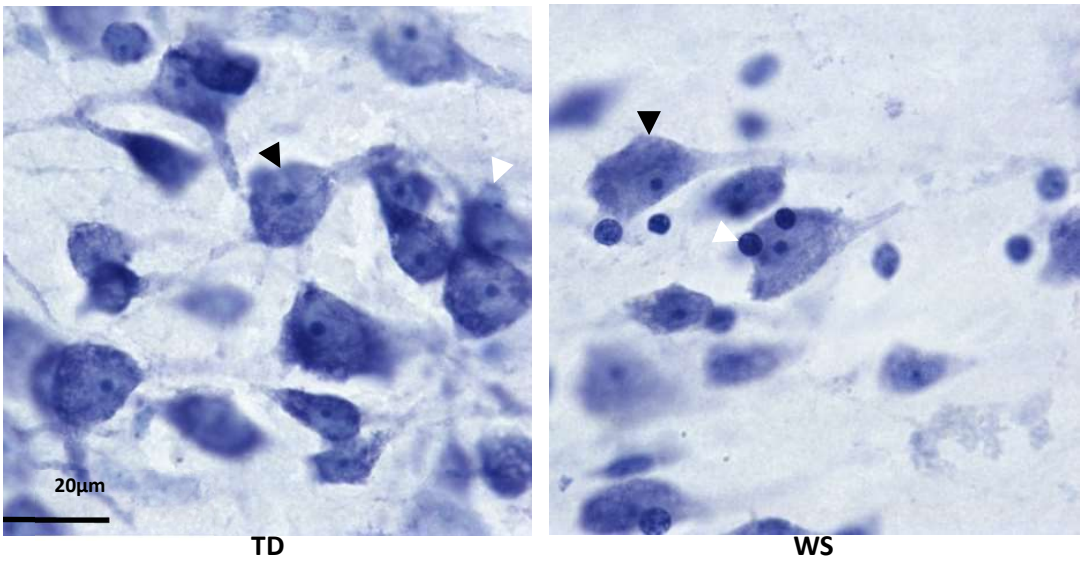

Figure 3.2. Microphotograph of BA 25 adult WS and TD. Neurons (black arrows) were distinguished from glia (white arrows) by their large size and distinctly stained nucleolus. Images taken at 100X.

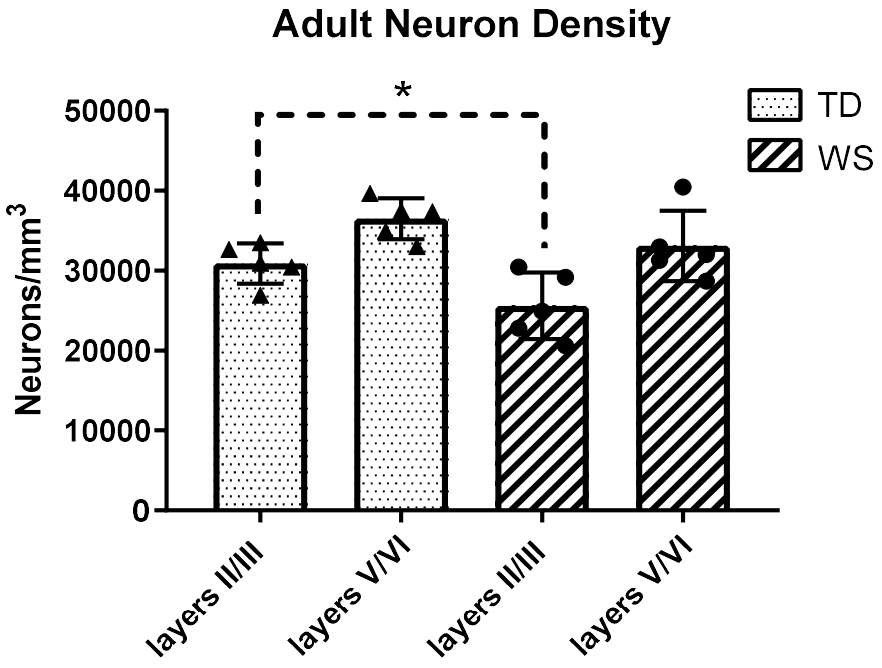

Figure 3.3. Mean neuron density in adult BA 25.

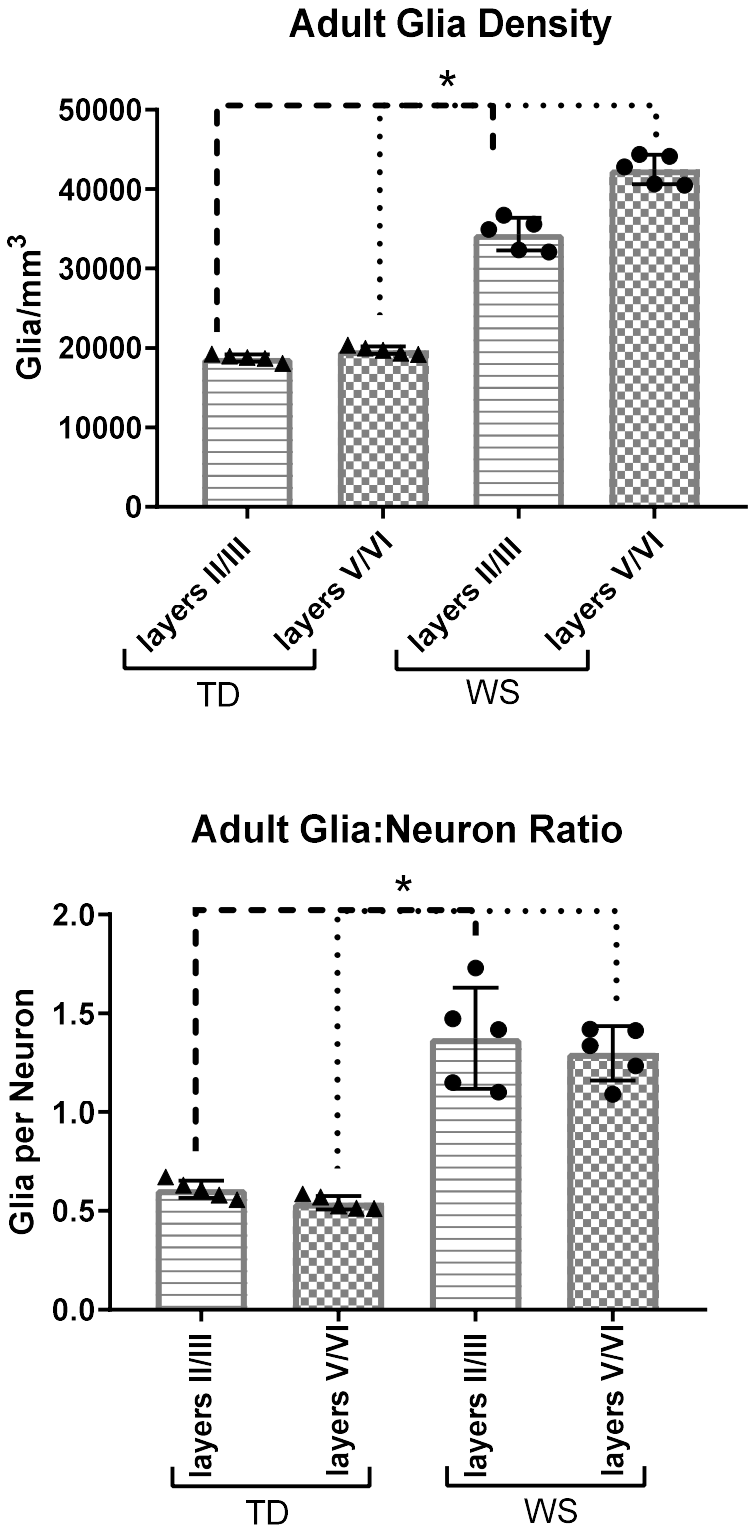

Figure 3.4. Mean glia density and glia to neuron ratio in adult BA 25.

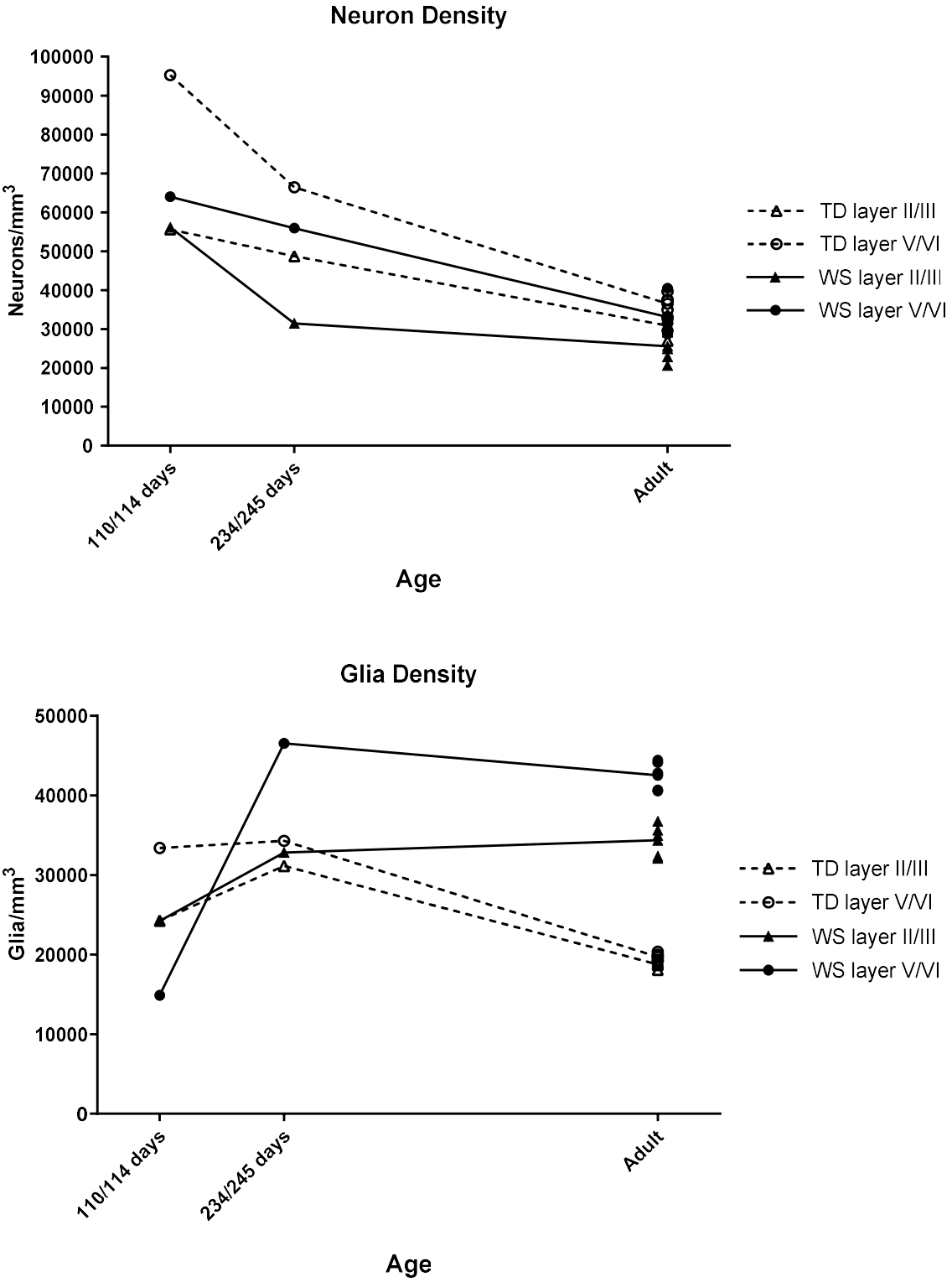

Figure 3.5. Neuron density, glia density, and glia to neuron ratio in infant BA 25.

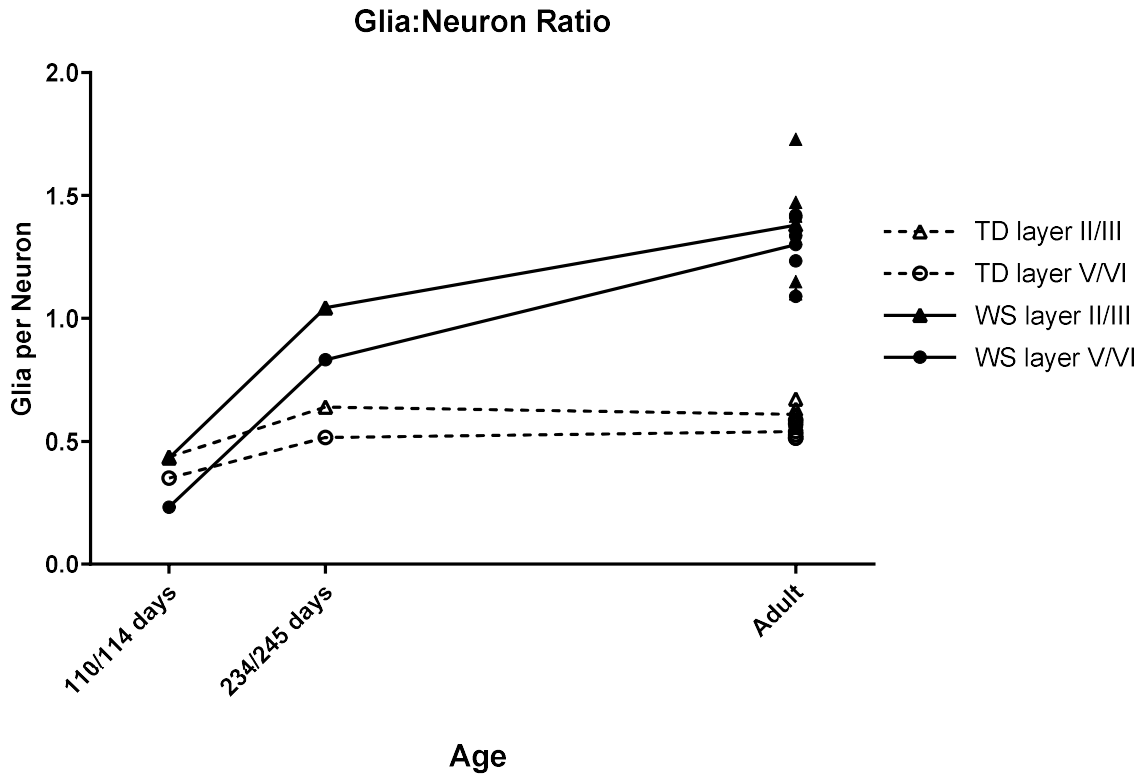

Figure 3.5. Neuron density, glia density, and glia to neuron ratio in infants, Continued.

References

Barbas, H.; Zikopoulos, B. (2006). Sequential and parallel circuits for emotional processing in primate orbitofrontal cortex. *Orbitofrontal Cortex*. 1, 57–93.

Barnea-Goraly, N., Kwon, H., Menon, V., Eliez, S., Lotspeich, L., & Reiss, A. L. (2004). White matter structure in autism: preliminary evidence from diffusion tensor imaging. *Biological psychiatry*, *55*(3), 323-326.

Bayraktar, O. A., Fuentealba, L. C., Alvarez-Buylla, A., & Rowitch, D. H. (2015). Astrocyte development and heterogeneity. Cold Spring Harbor perspectives in biology, 7(1), a020362.

Bellugi, U., Lichtenberger, L., Jones, W., Lai, Z., & St. George, M. (2000). I. The neurocognitive profile of Williams Syndrome: a complex pattern of strengths and weaknesses. *Journal of cognitive neuroscience*, *12*(Supplement 1), 7-29.

Benes, F. M., Davidson, J., & Bird, E. D. (1986). Quantitative cytoarchitectural studies of the cerebral cortex of schizophrenics. *Archives of general psychiatry*, *43*(1), 31-35.

Chailangkarn, T., Trujillo, C.A., Freitas, B.C., Hrvoj-Mihic, B., Herai, R.H., Diana, X.Y., Brown, T.T., Marchetto, M.C., Bardy, C., McHenry, L. and Stefanacci, L. (2016). A human neurodevelopmental model for Williams syndrome. *Nature*, *536*(7616), 338-343.

Courchesne, E., Mouton, P.R., Calhoun, M.E., Semendeferi, K., Ahrens-Barbeau, C., Hallet, M.J., Barnes, C.C. and Pierce, K. (2011). Neuron number and size in prefrontal cortex of children with autism. *Jama*, *306*(18), 2001-2010.

Di Martino, A., Choi, E. Y., Jones, R. M., Castellanos, F. X., & Mukerji, A. (2016). *Imaging the striatum in autism spectrum disorder* (pp. 189-218). Boca Raton, FL: CRC Press.

Dombrowski, S. M., Hilgetag, C. C., & Barbas, H. (2001). Quantitative architecture distinguishes prefrontal cortical systems in the rhesus monkey. *Cerebral Cortex*, *11*(10), 975-988.

Doyle, T. F., Bellugi, U., Korenberg, J. R., & Graham, J. (2004). "Everybody in the world is my friend" hypersociability in young children with Williams syndrome. *American Journal of Medical Genetics Part A*, *124*(3), 263-273.

Fan, C.C., Brown, T.T., Bartsch, H., Kuperman, J.M., Hagler Jr, D.J., Schork, A., Searcy, Y., Bellugi, U., Halgren, E. and Dale, A.M. (2017). Williams syndrome-specific neuroanatomical profile and its associations with behavioral features. *NeuroImage: Clinical*, *15*, 343-347.

Ferry, A. T., Öngür, D., An, X., & Price, J. L. (2000). Prefrontal cortical projections to the striatum in macaque monkeys: evidence for an organization related to prefrontal networks. *Journal of Comparative Neurology*, *425*(3), 447-470.

García-Cabezas, M. Á., John, Y. J., Barbas, H., & Zikopoulos, B. (2016). Distinction of neurons, glia and endothelial cells in the cerebral cortex: an algorithm based on cytological features. *Frontiers in neuroanatomy*, *10*, 107.

Garey, L. (2010). When cortical development goes wrong: schizophrenia as a neurodevelopmental disease of microcircuits. *Journal of anatomy*, *217*(4), 324-333.

Geschwind, D. H. (2011). Genetics of autism spectrum disorders. *Trends in cognitive sciences*, *15*(9), 409-416.

Hanson, K. L., Lew, C. H., Hrvoj-Mihic, B., Groeniger, K. M., Halgren, E., Bellugi, U., & Semendeferi, K. (2018). Increased glia density in the caudate nucleus in williams syndrome: Implications for frontostriatal dysfunction in autism. *Developmental neurobiology*, *78*(5), 531-545.

Homem, C. C., Repic, M., & Knoblich, J. A. (2015). Proliferation control in neural stem and progenitor cells. *Nature Reviews Neuroscience*, *16*(11), 647-659.

Hrvoj-Mihic, B., Hanson, K. L., Lew, C. H., Stefanacci, L., Jacobs, B., Bellugi, U., & Semendeferi, K. (2017). Basal dendritic morphology of cortical pyramidal neurons in Williams syndrome: prefrontal cortex and beyond. *Frontiers in neuroscience*, *11*, 419.

Huttenlocher, P. R. (1990). Morphometric study of human cerebral cortex development. *Neuropsychologia*, *28*(6), 517-527.

Jones, W., Bellugi, U., Lai, Z., Chiles, M., Reilly, J., Lincoln, A., & Adolphs, R. (2000). II. Hypersociability in Williams syndrome. *Journal of cognitive neuroscience*, *12*(Supplement 1), 30-46.

Karmiloff-Smith, A., Thomas, M., Annaz, D., Humphreys, K., Ewing, S., Brace, N., Van Duuren, M., Pike, G., Grice, S. and Campbell, R. (2004). Exploring the Williams syndrome face-processing debate: the importance of building developmental trajectories. *Journal of Child Psychology and Psychiatry*, *45*(7), 1258-1274.

Kessaris, N., Fogarty, M., Iannarelli, P., Grist, M., Wegner, M., & Richardson, W. D. (2006). Competing waves of oligodendrocytes in the forebrain and postnatal elimination of an embryonic lineage. *Nature neuroscience*, *9*(2), 173-179.

Klein-Tasman, B. P., & Mervis, C. B. (2003). Distinctive personality characteristics of 8-, 9-, and 10-year-olds with Williams syndrome. *Developmental neuropsychology*, *23*(1-2), 269-290.

Kohwi, M., & Doe, C. Q. (2013). Temporal fate specification and neural progenitor competence during development. *Nature Reviews Neuroscience*, *14*(12), 823-838.

Lee, J. C., Mayer-Proschel, M., & Rao, M. S. (2000). Gliogenesis in the central nervous system. *Glia*, *30*(2), 105-121.

Lew, C. H., Brown, C., Bellugi, U., & Semendeferi, K. (2017). Neuron density is decreased in the prefrontal cortex in Williams syndrome. *Autism Research*, *10*(1), 99-112.

Lew, C. H., Groeniger, K. M., Bellugi, U., Stefanacci, L., Schumann, C. M., & Semendeferi, K. (2018). A postmortem stereological study of the amygdala in Williams syndrome. *Brain Structure and Function*, *223*(4), 1897-1907.

Mackey, S., & Petrides, M. (2014). Architecture and morphology of the human ventromedial prefrontal cortex. *European Journal of Neuroscience*, *40*(5), 2777-2796.

Mackey, S., & Petrides, M. (2010). Quantitative demonstration of comparable architectonic areas within the ventromedial and lateral orbital frontal cortex in the human and the macaque monkey brains. *European Journal of Neuroscience*, *32*(11), 1940-1950.

Meda, S. A., Pryweller, J. R., & Thornton-Wells, T. A. (2012). Regional brain differences in cortical thickness, surface area and subcortical volume in individuals with Williams syndrome. *PloS one*, *7*(2), e31913.

Merla, G., Brunetti-Pierri, N., Micale, L., & Fusco, C. (2010). Copy number variants at Williams–Beuren syndrome 7q11. 23 region. *Human genetics*, *128*(1), 3-26.

Meyer-Lindenberg, A., Hariri, A. R., Munoz, K. E., Mervis, C. B., Mattay, V. S., Morris, C. A., & Berman, K. F. (2005). Neural correlates of genetically abnormal social cognition in Williams syndrome. *Nature neuroscience*, *8*(8), 991-993.

Miles, J. H. (2011). Autism spectrum disorders—a genetics review. *Genetics in Medicine*, *13*(4), 278-294.

Mitew, S., Hay, C. M., Peckham, H., Xiao, J., Koenning, M., & Emery, B. (2014). Mechanisms regulating the development of oligodendrocytes and central nervous system myelin. *Neuroscience*, *276*, 29-47.

Mobbs, D., Eckert, M. A., Mills, D., Korenberg, J., Bellugi, U., Galaburda, A. M., & Reiss, A. L. (2007). Frontostriatal dysfunction during response inhibition in Williams syndrome. *Biological psychiatry*, *62*(3), 256-261.

Molofsky, A.V., Krenick, R., Ullian, E., Tsai, H.H., Deneen, B., Richardson, W.D., Barres, B.A. and Rowitch, D.H. (2012). Astrocytes and disease: a neurodevelopmental perspective. *Genes & development*, *26*(9), 891-907.

Monk, C.S., Weng, S.J., Wiggins, J.L., Kurapati, N., Louro, H.M., Carrasco, M., Maslowsky, J., Risi, S. and Lord, C. (2010). Neural circuitry of emotional face processing in autism spectrum disorders. *Journal of psychiatry & neuroscience: JPN*, *35*(2), 105.

Morgan, J. T., Barger, N., Amaral, D. G., & Schumann, C. M. (2014). Stereological study of amygdala glial populations in adolescents and adults with autism spectrum disorder. *PloS one*, *9*(10), e110356.

Morgan, J.T., Chana, G., Pardo, C.A., Achim, C., Semendeferi, K., Buckwalter, J., Courchesne, E. and Everall, I.P. (2010). Microglial activation and increased microglial density observed in the dorsolateral prefrontal cortex in autism. *Biological psychiatry*, *68*(4), 368-376.

Ng, R., Brown, T. T., Järvinen, A. M., Erhart, M., Korenberg, J. R., Bellugi, U., & Halgren, E. (2016). Structural integrity of the limbic–prefrontal connection: Neuropathological correlates of anxiety in Williams syndrome. *Social neuroscience*, *11*(2), 187-192.

Oblak, A. L., Rosene, D. L., Kemper, T. L., Bauman, M. L., & Blatt, G. J. (2011). Altered posterior cingulate cortical cyctoarchitecture, but normal density of neurons and interneurons in the posterior cingulate cortex and fusiform gyrus in autism. *Autism Research*, *4*(3), 200-211.

Öngür, D., Ferry, A. T., & Price, J. L. (2003). Architectonic subdivision of the human orbital and medial prefrontal cortex. *Journal of Comparative Neurology*, *460*(3), 425-449.

Orentas, D. M., & Miller, R. H. (1998). Regulation of oligodendrocyte development. *Molecular neurobiology*, *18*(3), 247-259.

Petrelli, F., Pucci, L., & Bezzi, P. (2016). Astrocytes and microglia and their potential link with autism spectrum disorders. *Frontiers in cellular neuroscience*, *10*, 21.

Porter, M. A., Coltheart, M., & Langdon, R. (2007). The neuropsychological basis of hypersociability in Williams and Down syndrome. *Neuropsychologia*, *45*(12), 2839-2849.

Rajkowska, G., Miguel-Hidalgo, J.J., Wei, J., Dilley, G., Pittman, S.D., Meltzer, H.Y., Overholser, J.C., Roth, B.L. and Stockmeier, C.A. (1999). Morphometric evidence for neuronal and glial prefrontal cell pathology in major depression. *Biological psychiatry*, *45*(9), 1085-1098.

Reiss, A.L., Eckert, M.A., Rose, F.E., Karchemskiy, A., Kesler, S., Chang, M., Reynolds, M.F., Kwon, H. and Galaburda, A. (2004). An experiment of nature: brain anatomy parallels cognition and behavior in Williams syndrome. *Journal of Neuroscience*, *24*(21), 5009-5015.

Ren, Q., Yang, C.P., Liu, Z., Sugino, K., Mok, K., He, Y., Ito, M., Nern, A., Otsuna, H. and Lee, T. (2017). Stem cell-intrinsic, seven-up-triggered temporal factor gradients diversify intermediate neural progenitors. *Current Biology*, *27*(9), 1303-1313.

Rentería, M. E. (2012). Cerebral asymmetry: a quantitative, multifactorial, and plastic brain phenotype. *Twin Research and Human Genetics*, *15*(3), 401-413.

Rodriguez, J. I., & Kern, J. K. (2011). Evidence of microglial activation in autism and its possible role in brain underconnectivity. *Neuron glia biology*, *7*(2-4), 205-213.

Ruff, C. C., & Fehr, E. (2014). The neurobiology of rewards and values in social decision making. *Nature Reviews Neuroscience*, *15*(8), 549-562.

Sakurai, T., Dorr, N. P., Takahashi, N., McInnes, L. A., Elder, G. A., & Buxbaum, J. D. (2011). Haploinsufficiency of Gtf2i, a gene deleted in Williams Syndrome, leads to increases in social interactions. *Autism Research*, *4*(1), 28-39.

Schumann, C. M., & Amaral, D. G. (2006). Stereological analysis of amygdala neuron number in autism. *Journal of Neuroscience*, *26*(29), 7674-7679.

Sloan, S. A., & Barres, B. A. (2014). Mechanisms of astrocyte development and their contributions to neurodevelopmental disorders. *Current opinion in neurobiology*, *27*, 75-81.

Smiley, J. F., Konnova, K., & Bleiwas, C. (2012). Cortical thickness, neuron density and size in the inferior parietal lobe in schizophrenia. *Schizophrenia research*, *136*(1-3), 43-50.

Strømme, P., Bjømstad, P. G., & Ramstad, K. (2002). Prevalence estimation of Williams syndrome. *Journal of child neurology*, *17*(4), 269-271.

Zhan, Y., Paolicelli, R.C., Sforazzini, F., Weinhard, L., Bolasco, G., Pagani, F., Vyssotski, A.L., Bifone, A., Gozzi, A., Ragozzino, D. and Gross, C.T. (2014). Deficient neuron-microglia signaling results in impaired functional brain connectivity and social behavior. *Nature neuroscience*, *17*(3), 400-406.

Zilles, K., Schleicher, A., Langemann, C., Amunts, K., Morosan, P., Palomero-Gallagher, N., Schormann, T., Mohlberg, H., Bürgel, U., Steinmetz, H. and Schlaug, G., 1997. Quantitative analysis of sulci in the human cerebral cortex: development, regional heterogeneity, gender difference, asymmetry, intersubject variability and cortical architecture. *Human brain mapping*, *5*(4), pp.218-221.

Chapter 4

Glia density in the Williams Syndrome cortex (Brodmann Areas 10, 4, 3, and 18)

Abstract

Williams Syndrome (WS) is a rare neurodevelopmental disorder caused by a hemideletion of approximately 25-28 genes on chromosome 7, characterized by hypersociability and anxiety. It is hypothesized that these behaviors may reflect a deficit in inhibitory control of behavior due to abnormalities in frontostriatal and fronto-amygdala connectivity. Previous studies of WS have shown abnormalities in neuron density in the prefrontal cortex and amygdala, and in glia density in the striatum. Abnormalities in glia density are a common finding in Autism Spectrum Disorder (ASD), another neurodevelopmental disorder characterized by altered social behavior, but relatively little is known about glia density across the WS cortex. Here we examine total glia density, oligodendrocyte density, and combined density of astrocytes and microglia in four areas of the cortex (BA 10, 4, 3, and 18) using a sample of five matched pairs of WS and typically developing (TD) controls. We found increased total glia density in BA 4, 3, and 18, reaching statistical significance in BA 4 and 3. Astrocyte/microglia density was decreased in BA 10 and 18, reaching statistical significance in BA 10, and was increased in BA 4 and 3, reaching statistical significance in BA 3. Oligodendrocyte density was significantly increased in WS in all cortical areas we examined. The current study demonstrates that differences in glia density are not restricted to frontostriatal regions. Glia abnormalities are also present in areas without known functional abnormalities in WS, and may represent a systemic perturbation of glia across the WS cortex.

Introduction

Williams Syndrome (WS) is a rare neurodevelopmental disorder, resulting from a hemideletion of approximately 25-28 genes on chromosome band 7q11.23 (Strømme et al., 2002). It is characterized by a specific and well defined cognitive and behavioral phenotype, including anxiety and altered social behavior, specifically hypersociability and lack of social inhibition (Klein-Tasman & Mervis, 2003). Previous postmortem histological studies of WS revealed decreased neuron density in the prefrontal cortex (PFC), layer V/VI in the orbitofrontal cortex in particular (Lew et al., 2017) and layers II/III of BA 25 in the ventromedial PFC (Wilder et al., 2018), and increased neuron number in the lateral nucleus of the amygdala (Lew et al., 2018). These findings suggest abnormalities in frontostriatal and frontoamygdala circuits, which may underlie the anxiety and atypical social behavior observed in WS.

Abnormalities in glia cells have been linked to many neurological or neurodevelopmental disorders, including major depressive disorder, ASD, and schizophrenia (Petrelli et al., 2016; Sloan & Barres, 2014; Zhan et al., 2014). Glia have critical roles in neural development and neurological functions, affecting neuronal survival, and synapse formation, elimination, and communication (Bayraktar et al., 2015; Molofsky et al., 2012; Petrelli et al., 2016). Changes in glia cells, or in the ratio of glia to neurons, can alter the typical course of neurodevelopment and the formation and functioning of neural circuits, and may contribute to WS social and behavioral phenotype (Sloan & Barres, 2014).

Despite the importance of glia, we have only a couple of reports on glia density in WS, that have demonstrated increased glia density in BA 25 (Wilder et al., 2018), and increased glia density in the caudate nucleus of the striatum, mostly driven by increased oligodendrocyte density (Hanson et al., 2018). These findings pointed to the possibility that glia abnormalities in

WS are found specifically in frontostriatal systems. In the present study we test this hypothesis by targeting glia cells in four Brodmann areas throughout the cortex (BA 10, 4, 3, and 18). Neuron density in these areas in WS varies compared to controls, with a significant difference found in the neuron density of BA 10, while other areas were unaffected (Lew et al. 2017).

Materials and Methods

Brain tissue

We examined histological sections from human tissue from a total of ten adult subjects, five WS and five typically developing (TD) controls (Table 4.1). The four areas targeted include prefrontal BA 10, primary motor BA 4, primary somatosensory BA 3, and visual area BA 18 (Table 4.1). All five WS subjects, and four of the five TD controls were used in the Lew et al. 2017 study. One control (TD 16339) was added to replace a control that was unavailable. WS and TD subjects were matched for age, sex, and hemisphere to control for possible cytoarchitectonic asymmetries and age and sex-related differences (Rentería 2012; Zilles et al., 1997). All subjects in the Bellugi Williams Syndrome Brain Collection are part of an ongoing donation-based program now run by the Laboratory for Human Comparative Neuroanatomy at UCSD (La Jolla, CA, USA).

Regions of interest

The regions of interest (ROIs) were identified using anatomical landmarks and at the microscopic level using cytoarchitectonic characteristics of each area as described in Lew et al. 2017. BA 10 was sampled from the tip of the frontal pole and all six cortical layers are present and well defined (Semendeferi et al., 2001). BA 4 was sampled from the motor hand region and lacks layer IV (Amunts et al., 1995). BA 3 was sampled from the post-central gyrus in the parietal cortex, adjacent to the motor hand region and is identified by a wide layer IV, and large

Betz-like cells in layer III (Geyer et al., 1997). BA 18 occupies regions of the cuneus, lingual gyrus, and middle occipital gyrus in the occipital cortex, and is identified by high cell density, with a dense layer IV, and large pyramidal cells in deep layer III and layer V (Amunts et al., 2000).

Tissue processing

Blocks of tissue containing each cortical area of interest were extracted and cryoprotected using a series of 10%, 20%, and 30% sucrose solutions with 0.1 M phosphate buffer until saturated. Frozen tissue was cut on a Leica SM 2010R (Leica Biosystems, Wetzlar, Germany) sliding microtome into an alternating series of 60 micrometer and 30 micrometer thick sections. The 60 micrometer thick series was mounted on gelatin coated slides and stained with a 0.25% thionine stain for Nissl substance to visual cell bodies. The remaining, 30 micrometer thick, series was stored for use in later processing (see also Lew et al, 2017 for more details).

Data collection

Data collection was performed using StereoInvestigator software (MBF Bioscience, Williston, VT, USA) on a Dell workstation receiving live video feed from an Optronics MicroFire color video camera (East Muskogee, OK, USA) attached to a Nikon Eclipse 80i microscope (Nikon Instruments, Melville, NY, USA) equipped with a Ludl MAC5000 stage (Ludl, Hawthorn, NY, USA) and a Heidenhain z-axis encoder (Heidenhain, Plymouth, MN, USA). To increase the accuracy and consistency of measurements across all subjects, we report glia density rather than number, a standard practice for data collection in the cortex (Benes et al., 1986; Lew et al., 2017; Oblak et al., 2011; Smiley et al., 2012).

Ten sections per subject were analyzed in each cortical area examined. Glia cell density was estimated using the using the Optical Fractionator probe in StereoInvestigator software.

Consistent with a prior study on neuron density in the WS cortex, two regions of interest in each section, one bounding infragranular layers (V/VI), the other bounding supragranular layers (V/VI), were drawn at a 1x magnification and cells were counted at 100x magnification (Lew et al., 2017). Glia density data were collected by a single rater (LW) and inter-rater reliability was ensured through repeated neuron density estimations on a sample previously reported in the literature to 95% concordance (Lew et al., 2017). Cells were distinguished based on their morphology. Glia cells were distinguished from neurons by their smaller size and lightly or darkly stained nucleus and a lack of stained cytoplasm surrounding the nucleus. Due to the difficulty in reliably distinguishing microglia from astrocytes in Nissl-stained tissue, these cells were counted together, consistent with a previous study on glia density in WS (Hanson et al., 2018). Oligodendrocytes were identified by their small, round shape, darkly stained euchromatin, and distribution of nuclear heterochromatin (García-Cabezas et al., 2016) (Figure 4.1). Density was calculated as the estimated number of each cell type in the region sampled, divided by the planimetric volume of the ROI sampled.

Statistical Analysis

Standard two-tailed t-tests ($p < 0.05$) were used to compare glia density in WS and TD. Supragranular and infragranular layers were compared separately. Percent difference in WS compared to TD was calculated as the difference in mean value of WS from TD, in relation to the mean TD value, for neuron density, glia density, and glia to neuron ratio, in each ROI.

Results

General findings: In TD subjects, total mean glia density (combined astrocyte, microglia, and oligodendrocyte density) was lowest in BA 10, followed by BA 3, then BA 4, and was highest in BA 18. In every cortical area examined here mean glia density was higher in infragranular layers

than in supragranular layers. Total mean glia density for individual subjects ranged from 29,000-51,000 cells/mm^3 in BA 10, 30,000-57,000 cells/mm^3 in BA 4, 27,000-50,000 cells/mm^3 in BA 3, and 37,000-64,000 cells/mm^3 in BA 18.

In WS subjects, mean glia density was also lowest in BA 10, this was followed by BA 18, then BA 4, with the highest density in BA 3. As in TD controls, glia density was higher in infragranular layers than in supragranular layers for all cortical areas examined. There is a wider range of glia density between individuals in WS than in TD, with values ranging from 28,000-60,000 cells/mm^3 in BA 10, 49,000-96,000 cells/mm^3 in BA 4, 51,000-84,000 cells/mm^3 in BA 3, and 40,000-76,000 cells/mm^3 in BA 18.

Total mean glial cell density was higher in WS than in TD controls in three of the four cortical areas examined, BA 18, 3, and 4. This difference was greatest in BA 3 and BA 4, where it reached statistical significance. There was a smaller, non-statistically significant increase in BA 18. Total mean glia density was similar between WS and TD controls in BA 10. The density of astrocytes/microglia was lower in WS than in TD controls in BA 10 and BA 18, and was higher in WS in BA 3 and BA 4. Oligodendrocyte density was higher in WS than in TD controls in all cortical areas examined.

BA 10: Total mean glia cell density was similar in WS and TD controls. There was a slight decrease in supragranular layers (p=0.942), and a slight increase in infragranular layers (p=0.778), but neither reached statistical significance (Table 4.2). In BA 10 there was considerable overlap in total mean glia density between TD and WS subjects, with four of the five WS subjects falling within the range of the TD controls. Of the five subjects with the lowest glia density, three were WS (WS 10, WS 8, WS 9) and two were TD (TD 4916, TD 16339). In

the five subjects with the highest glia density, three were TD (TD A02048, TD 5552) and two were WS (WS 1, WS 12) (Figure 4.2).

The combined density of astrocytes and microglia was decreased in WS. This was statistically significant in supragranular layers (p=0.03), but not in infragranular layers (p=0.096) (Table 4.2 and Figure 4.2). Density of oligodendrocytes was higher in WS. This was statistically significant in supragranular layers (p=0.042) and approached but did not reach significance in infragranular layers (p=0.056) (Table 4.2 and Figure 4.2).

BA 4: Total mean glia cell density was significantly higher in WS compared to TD controls, in both supragranular (p=0.002) and infragranular layers (p=0.013) (Table 4.3). There was no overlap of total glia density between TD and WS subjects when comparing the same cortical layers. All WS subjects had glia densities that were higher than all TD subjects (Figure 4.3).

The combined density of astrocytes and microglia was increased in WS, but this was not statistically significant in either supragranular (p=0.067) or infragranular layers (p=0.134) (Table 4.3 and Figure 4.3). Density of oligodendrocytes was also higher in WS, but this was not statistically significant in either supragranular (p=0.052) or infragranular layers (p=0.065) (Table 4.3 and Figure 4.3).

BA 3: Total mean glia cell density was significantly increased in WS compared to TD controls, in both supragranular layers (p=0.005) and infragranular layers (p=0.0007) (Table 4.4). There was no overlap between TD and WS subjects in total glia density. All WS subjects had glia densities that were higher than all TD subjects (Figure 4.4).

The combined density of astrocytes and microglia was increased in WS. This statistically significant in both supragranular (p=0.031) and infragranular layers (p=0.0008) (Table 4.4 and Figure 4.4). Density of oligodendrocytes was also higher in WS. This was statistically significant

in infragranular (p=0.031), but not supragranular layers (p=0.056) and (Table 4.4 and Figure 4.4).

BA 18: Total mean glia cell density was increased in WS compared to TD controls, but there was no significant difference in either supragranular (p=0.121) or infragranular (p=0.132) layers (Table 4.5). As in BA 10, there was overlap in the individual values for glia density between TD and WS subjects, although only two fell within the range of TD controls (WS 8, WS 10), while the three subjects with the highest glia density were all WS (WS 12, WS 1, WS 9) (Figure 4.5).

The combined density of astrocytes and microglia was decreased in WS. This was not statistically significant in either supragranular layers (p=0.587) or in infragranular layers (p=0.292) (Table 4.5 and Figure 4.5). Density of oligodendrocytes was higher in WS. This was statistically significant in both supragranular layers (p=0.03) and in infragranular layers (p=0.036) (Table 4.5 and Figure 4.5).

Individual results: There were no noticeable differences between subjects on the basis of sex or age, but two particular individuals, TD 5552 and WS 8, do stand out with respect to their oligodendrocyte density. In four of the five control subjects, oligodendrocyte density was very low (<150 cells/mm^3) in all cortical areas examined here. TD 5552 had much higher oligodendrocyte density (≈3,300-11,400 cells/mm^3). In BA 4 the oligodendrocyte density of TD 5552 was within the range of typical WS values. In contrast, WS 8 had much lower oligodendrocyte density than other WS subjects. In the remaining four WS subjects oligodendrocyte density ranged from ≈5,900-33,600 cells/mm^3, while in WS 8 oligodendrocyte density was <380 cells/mm^3 in all cortical areas examined here, very similar to TD controls.

Discussion

Summary

The present study demonstrates that glia abnormalities are found across the cortex in WS. Total mean glia density was higher in WS than in TD controls in supragranular and infragranular layers of BA 18, 3, and 4, and in infragranular layers of BA 10. This difference was significant in supragranular and infragranular layers in BA 3 and BA 4. Not only was the difference significant in these areas, but even with the high degree of interindividual variability in glia density, there was no overlap in the range of glia density in these areas between TD and WS subjects. That is, mean glia density in every WS subject was higher than mean glia density in any TD subject in BA 4 and BA 3, suggesting a robust increase in glia in in these areas is a common feature of WS. In both TD controls and WS, glia density shows considerable variation. This variation does not appear to relate to sex or age in the current sample, although a larger sample with a greater age range may reveal differences not observed here.

These abnormalities in glia density found here appear to be independent of alterations found in neuron density in the cortex. Prior work on these cortical areas in WS revealed decreased neuron density that was specific to the PFC, without significant differences in other cortical areas (Lew et al. 2017, Wilder et al., 2018). The current study expands on that work, demonstrating that alterations to glia density as well occur in the PFC, but are also present in areas without known functional abnormalities in WS. While total mean glia density is significantly higher in WS compared to TD in BA 4 and BA 3, as well as in BA 25, and was very similar to TD in BA 10, mean neuron density is lower in WS in BA 10 and BA 25. Neuron density in WS in BA 4 is very similar to that of controls, and is slightly elevated in BA 3, although this difference was not statistically significant (Lew et al., 2017; Wilder et al., 2018). This demonstrates that increased glia density can be found in WS in areas with increased (BA 3), decreased (BA 25), or no difference (BA 4) in neuron density between WS and TD, and that

areas significant differences in neuron density (BA 10) can be found without differences in total mean glia density.

While total mean glia density in WS is generally higher than or similar to TD in the areas of the cortex examined here, that obscures some of the differences that may exist in subtypes of glia. The combined astrocyte/microglia density is elevated in BA 4 and 3, but it is decreased in BA 10 and 18. This difference was significant in all layers of BA 3 and supragranular layers of BA 10. Mean oligodendrocyte density however, was higher is all layers of all cortical areas examined, and these differences reached significance in infragranular layers of BA 3, all layers of BA 18, and supragranular layers of BA 10. The increase in oligodendrocytes specifically appears to be a widespread feature in the cortex in WS, and qualitative observations suggest it may be present in BA 25 as well. However, it may not be a universal feature of WS. Oligodendrocyte density in TD controls was very low in the cortical areas examined here. In three of the five TD subjects, no oligodendrocytes were seen despite extensive sampling. One TD control (TD 5552, 42 years old, female) had much higher oligodendrocyte density than the other four TD controls, the reasons for this are unclear. The subject's age is the median age of the sample, so age is unlikely to play a role. This subject is the only female control however, observations of a different, similarly aged female control in another cortical area (the cortical areas examined here were not available for this subject) suggest that this difference is not due to sex, and may simply be an abnormal feature of this subject. Among the WS subjects, four had elevated oligodendrocyte density, while one (WS 8, 48 years old, male) did not. In this subject, no oligodendrocytes were found in BA 4 or BA 3, and density in BA 10 and 18 was less than 380 oligodendrocytes/mm^3, far closer to the TD average than the WS average. Qualitatively, this subject more closely visually resembled the TD subjects in all cortical areas examined as the

subject almost completely lacked the darkly stained oligodendrocytes that characterized the other four WS subjects. Despite the lower oligodendrocyte density, astrocyte/microglia density and total glia density in this subject was similar to other WS subjects. This subject is the oldest WS subject (48 years old) included in the present study however, it is unlikely that this contributes to the lower oligodendrocyte density as two other WS subjects are close in age (45 and 43 years old) and oligodendrocyte density did not appear to decrease with age in the TD or other WS subjects.

TD studies on glia

There is considerable heterogeneity present in glia density in TD controls however, there are a few patterns present across different studies, including lower density in supragranular layers than in infragranular layers, and greater variability than neuron density reflected in greater standard deviations (see Table 4.6) (Benes et al., 1986; Cotter et al., 2002; Gittens & Harrison, 2004; Leuba & Garey, 1989; Ongur et al., 1998; Rajkowska et al., 1999; Sherwood et al., 2006). All of these common glia findings were found in the current study in both TD controls and WS subjects. In the present study, glia density is lower in BA 10, 4, and 3, and higher in BA 18 than has been previously reported. This could be due to the high variability that appears to be common across studies of glia density. The reported numbers could also be impacted by methodological differences. When looking at glia to neuron ratio, our values are quite similar to those previously published in BA 10 and 18. Additionally, glia density, along with glia to neuron ratio are not stable throughout adult life. Both measures have been shown to increase with age in some cortical areas, including BA 10, but not BA 18, where they may in fact decrease with age (Terry et al., 1987; Leuba & Garey, 1989; Tetreault et al., 2012). The average age of our subjects (37 years old, with the oldest subject being 51 years old) is relatively young compared to other

reports where average age varied from 49 (Ongur et al., 1998), 66 (Benes et al., 1986), and 68 years of age (Leuba & Garey, 1989). This age difference may account for the lower glia density we found in BA 10, 4, and 3, and higher density in BA 18.

ASD studies on glia

Alterations in glia appear to be a common feature of many neurological disorders, including ASD, schizophrenia, and depression (Almeida et al., 2020; Uranova et al., 2003; Vostrikov et al., 2007; Zeidan-Chulia et al., 2014). Only a few studies have looked at glia density of all glia subtypes combined in ASD, and of those that have, the results have not always been consistent (see Table 4.7). In the frontal cortex, studies have found no significant difference in glia numbers in the dorsolateral or medial PFC (Courchesne et al., 2011). In contrast an increase in glia density has also been shown in the frontal cortex (Mukaetova-Ladinska et al., 2004; Edmonson et al., 2014), although the former study included only two ASD subjects, one of whom suffered from epilepsy, and the latter study only including individuals under the age of 20, both of which may have impacted the results. In language areas of the cortex, BA 22, BA 39, and BA 44, increased glia density has been found in ASD (Lopez-Hurtado & Prieto 2008). In one study of the amygdala, there was no difference in average overall non-neuronal cells numbers (Morgan et al., 2014). Most studies of ASD have focused on specific subtypes of glia, most often microglia or astrocytes, although there is one report of decreased oligodendrocytes in the amygdala of adults with ASD (Morgan et al., 2014). Excessive microglia activation has been found in the frontal cortex in ASD (Courchesne & Pierce, 2005), and specifically in the dorsolateral PFC (Morgan et al., 2010), although these results include samples that were processed at two different facilities and the microglia measures may correlate to seizure activity, rather than just ASD (Casanova 2014). One study found no difference in astrocyte density in

dorsolateral PFC (BA 9) (Lee et al., 2017), while another found evidence of an increase of astrocytes in BA 9 (Laurence & Fatemi, 2005). This difference could have been due to differences in age, the former study includes subjects from 4-51 years of age, while the latter only included subjects from 19-30 years of age. In the visual cortex and fronto-insular cortex an increase in microglia density has been found in ASD (Tetreault et al., 2012). In the amygdala, although there was not a difference in overall microglia density between ASD and TD controls, two of the eight ASD subjects showed strong microglia activation, while the other six did not (Morgan et al., 2014), suggesting that there is considerable heterogeneity among ASD subjects, which may account for differing findings presented here. The most common finding in ASD is increased microglia, either density or activation (Courchesne & Pierce, 2005; Vargas et al., 2005; Morgan et al., 2010; Morgan et al., 2012; Rodriguez & Kern, 2012; Tetreault et al., 2012). These microglia changes may additionally be driving changes in astrocytes and oligodendrocytes, due to the role microglia plays in cell survival (Courchesne & Pierce 2005; Zeidan-Chulia et al., 2014).

ASD and WS are both neurodevelopmental disorders characterized by alterations in social behavior, and both demonstrate some abnormalities in glia density. Unlike WS, which has a clear genetic cause underlying all cases, and a consistent cognitive and behavioral phenotype, ASD is far more heterogenous in both cause and presentation. Likewise, the differences in the glia density in ASD appear to be more heterogenous than in WS. These consistent differences in glia density in WS may aid in the identification of genetic mechanisms underlying glia abnormalities and their link to social behavior in neurodevelopmental disorders.

Mechanisms

The exact mechanisms for the changes in glia density in the WS cortex are unknown, but we hypothesize that it may be due in part to deletions of *GTF2i* and *GTF2iRD1*, genes that play critical roles in neuron and glia development and cell division. The increase in oligodendrocytes could be due to excess production, deficits in migration, or disrupted migration of oligodendrocytes. Mouse models of the deletion of *GTF2i* have shown a decrease in oligodendrocyte number and reduced myelination (Barak et al., 2019). Deletion of both *GTF2i* and *GTF2iRD1* in mice can disrupt vasculature and endothelial cell development, which may impact oligodendrocyte migration as oligodendrocyte progenitors migrate along vasculature guided by endothelial cells (Enkhmandakh et al., 2009). Together these gene deletions in WS may disrupt oligodendrocyte differentiation and disrupt migration of oligodendrocytes into white matter, resulting in excess oligodendrocytes in gray matter of the cortex, while potentially reducing the overall number of oligodendrocytes in the brain. The lack of oligodendrocytes in the cortex of WS 8 may be due to an atypical gene deletion that resulted in fewer oligodendrocyte abnormalities, or oligodendrocyte differentiation may have be so impacted that there are fewer oligodendrocytes in this subject than is typical in WS.

These disruptions to oligodendrocyte likely have critical consequences for myelination in WS. Structural imaging studies of WS have shown reduction in white matter volume relative to gray matter volume, suggesting deficits in myelination (Faria et al., 2012). Typical social behavior is dependent upon connectivity between the PFC and subcortical regions such as the amygdala and striatum, which is impacted by the development of myelin. In mice, reduced myelination due to the deletion of *GTF2i* is enough to cause increased sociability and anxiety in mice, both behavioral features of WS (Barak et al., 2019; Nir & Barak, 2020). Further work

investigating oligodendrocytes changes and myelination would help identify the exact abnormalities that are present in WS.

The changes to the combined density of astrocytes and microglia have a number of possible causes, and changes in density in one cell type may mask changes in the other. The increase in astrocyte/microglia density in BA 4 and BA 3 could be due to increases in either the density of astrocytes, microglia, or both. If there is a great enough increase in one cell type, there could also be a decrease in the other cell type that is hidden here. The decrease in astrocyte/microglia density in BA 10 and BA 18 could be due to decreases in density of one or both cell types. Similar to BA 4 and BA 3, if the decrease in one cell type is great enough, there could be an increase in the other cell type. Chronic neuroinflammation, common to many neurodevelopmental disorders, could cause all of those changes. The deletion of *GTF2i* may influence microglia density as well as oligodendrocyte density, as it has been shown to increase the number of microglia in the mouse cortex (Bar & Barak 2019).

Microglia play a crucial role in typical neurological development, and can affect the survival and activity of astrocytes, oligodendrocytes, and neurons (Czeh & Nagy, 2018). Microglia appear to be particularly important in establishing neuron numbers, and in the formation and elimination of synapses. Inflammation caused by microglia can inhibit neurogenesis prenatally, potentially leading to decreased neuron numbers. Microglia additionally regulate axonal extension, and mediate dendritic spine formation and elimination. Mice with decreased microglia numbers show higher neural progenitor and mature neuron numbers in the cortex, greater axonal extension, decreased dendritic spine formation and elimination, along with delayed synaptic pruning (Hammond et al. 2018; Jiang et al., 2018). Increased microglia activity can result in increased levels of pro-inflammatory cytokines, leading to increased neuronal death

(Almeida et al., 2020). Chronic inflammation and increased microglia numbers may also affect astrocyte survival and function, further altering synaptic transmission, cognitive functioning, and behavior (Petrelli et al., 2016). Elevated levels of microglia in WS, particularly early in development, could have a significant impact on future development, potentially contributing to the abnormalities in neurons and all types of glia observed in the disorder (Hanson et al., 2018; Lew et al., 2017; Wilder et al., 2018).

Future work with larger samples would be useful to identify if the low oligodendrocyte density seen in this one WS subject (WS 8) is unique to that individual, or if there is a subset of WS cases that do not present with increased oligodendrocyte density in the cortex. Due to the limitations of reliably distinguishing astrocytes versus microglia morphology based on Nissl stain, future work utilizing immunohistochemical staining is needed to separately target microglia and astrocyte density. This would elucidate the precise glia abnormalities present in WS and may provide a greater understanding of the mechanisms behind these changes.

Conclusions

Glia play critical roles in neural development and functioning. Chronic neuroinflammation, together with deficits in myelination due to oligodendrocyte abnormalities, can impact cell survival and connectivity between brain regions, and may contribute to the cognitive and behavioral phenotype seen in WS. The clear genetic etiology and consistent behavior phenotype of WS provide an excellent model for linking genes, glia abnormalities, and social behavior. The present study expands our knowledge of glia in the TD and WS cortex. The results presented here, combined with prior work on WS, demonstrate that glia abnormalities are common to many regions of the WS brain. They are not restricted to frontostriatal or fronto-amygdala systems. Glia abnormalities in the WS cortex are present in areas with and without

known functional abnormalities in WS and may represent a systemic perturbation of glia development (Hanson et al., 2018; Wilder et al., 2018).

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Chapter 4, in full, is an adaptation of a primary data paper currently under review in the journal *Autism Research.* Wilder, Linnea, Lew, Caroline, and Semendeferi, Katerina. The dissertation author was the primary investigator and author of this paper.

Tables and Figures

Table 4.1. Subject Information. WS: Williams Syndrome; TD: typically developing control; PMI: postmortem interval in hours.

Subject	Age at Death (y)	Sex	Hemisphere	PMI	Cause of Death
WS 10	18	M	R	24	Cardiac complications
TD 4916	19	M	R	5	Drowning
WS 1	31	M	R	26	Cardiac complications
TD 16339	31	M	R	NA	NA
WS 9	43	F	R	12	Cardiac complications
TD 5552	42	F	R	18	Heart Disease
WS 12	45	M	R	24	Cardiac complications
TD 4598	45	M	R	6	Dilated myocardiopathy
WS 8	48	M	L	<30	Respiratory illness
TD A04028	51	M	L	18	Accident

Table 4.2. Glia density in BA 10.

Cell Type	All Glia		Astrocytes/Microglia		Oligodendrocytes	
Cortical Layers	II/III	V/VI	II/III	V/VI	II/III	V/VI
TD	35081±5539	43722±6848	34119±4764	42496±6128	962±2082	1226±2742
WS	34836±4892	45308±10005	25678±5312	31780±10632	9158±6324	13529±10426
% Difference	-1%	+4%	-25%*	-25%	+852%*	+1004%

Table 4.3. Glia Density in BA 4.

Cell Type	All Glia		Astrocytes/Microglia		Oligodendrocytes	
Cortical Layers	II/III	V/VI	II/III	V/VI	II/III	V/VI
TD	37687±4389	48522±8420	35909±4634	46242±7434	1778±3977	2281±5100
WS	59683±8550	72421±13439	50498±13173	62381±18917	9185±5860	10040±6267
% Difference	+58%*	+49%*	+41%	+35%	+417%	+340%

Table 4.4. Glia Density in BA 3.

Cell Type	All Glia		Astrocytes/Microglia		Oligodendrocytes	
Cortical Layers	II/III	V/VI	II/III	V/VI	II/III	V/VI
TD	34520±7348	46213±5555	33408±6022	45546±5313	1112±2487	667±1492
WS	61299±12229	72753±3866	50914±12424	61982±4504	10385±7867	10771±7059
% Difference	+76%*	+57%*	+52%*	+32%*	+834%	+1515%*

Table 4.5. Glia Density in BA 18.

Cell Type	All Glia		Astrocytes/Microglia		Oligodendrocytes	
Cortical Layers	II/III	V/VI	II/III	V/VI	II/III	V/VI
TD	41925±4298	54000±8046	40469±3966	51774±6037	1455±3254	2226±4977
WS	52124±11497	64766±11653	37649±10142	46470±8507	14475±9134	18295±11937
% Difference	+24%	+20%	-7%	-10%	+895%*	+722%*

Table 4.6. TD studies on glia density.

BA	Cortical Layers	Glia density (cells/mm^3)	Glia to neuron ratio	Sample size	Sex	Age in years (mean±SD)	Hemisphere	Reference
BA 9	2	71,000	0.66	12	6F, 6M	43.3±16.8	Left	Rajkowska et a., 1999
	3	91,000	1.63					
	2/3	NA	0.74	15	6F, 9M	48.1±10.7	8L, 7R	Cotter et al., 2002
		155,064	1.65	6	3F, 3M	45.5±7.5	Left	Sherwood et al., 2006
BA 24b	2-6	41,700	1.67	5	1F, 4M	72.4±8.0	Left	Ongur et al., 1998
		45,700	1.81	11	4F, 7M	49.1±3.4	6L, 5R	
		72,000	0.8	5	3F, 2M	42.6±22.2	5L, 5R	Gittens & Harrison, 2004
BA 10	2	52,800	0.85	9	NA	66.0±13.1	NA	Benes et al., 1986
	3	52,100	1.11					
	5	55,700	1.22					
	6	50,800	1.14					
BA 4	2	63,800	1.49					
	3	66,300	2.35					
	5	68,900	2.89					
	6	60,100	2.63					
BA 3	2-6	55,900	1.63	11	4F, 7M	49.1±3.4	6L, 5R	Ongur et al., 1998
BA 18	1-6	32,200	1.02	11	7F, 4M	68.4±23.6	NA	Leuba & Garey, 1989
BA 10	2/3	35,081	0.91	5	1F, 4M	37.6±12.7	1L, 4R	Present study
	5/6	43,722	1.26					
BA 4	2/3	37,687	1.24					
	5/6	48,522	1.90					
BA 3	2/3	34,520	0.96					
	5/6	46,213	1.63					
BA 18	2/3	41,925	0.84					
	5/6	54,000	1.41					

Table 4.7. Glia findings in ASD and WS relative to TD.

Disorder	Glia findings in ASD and WS	Sample size	Sex	Age Range (mean±SD)	Hemi	Reference
ASD	Increased glia density in frontal cortex	5	M	4-20 years (10.9±7.2)	NA	Edmonson et al., 2014
ASD	Increased astrocyte activation in middle frontal gyrus and anterior cingulate gyrus Increased microglia activity at junction of cortex and white matter in middle frontal gyrus and anterior cingulate gyrus	11	M	7-44 years (18.4±11.8)	NA	Vargas et al., 2005
ASD	No significant difference in glia density in dlPFC and mPFC	7	M	2-16 years (6.1±4.8)	NA	Courchesne et al., 2011
ASD	No difference in astrocyte density or total glia density in dlPFC	8	M	4-51 years (24.1±17.7)	NA	Lee et al., 2017
ASD	Increased glia density in dlPFC	2	M	29-31 years (30.0±1.4)	NA	Mukaetova-Ladinska et al., 2004
ASD	Increased astrocyte density in dlPFC	5	M	19-28 years (23.2±4.1)	NA	Laurence & Fatemi, 2005
ASD	Increased microglia activation and microglia cell density in dlPFC	13	M	3-41 years (15.8±12.7)	5L, 8R	Morgan et al., 2010
ASD	Increased close associate between microglia and neurons in dlPFC	13	M	3-41 years (15.8±12.7)	5L, 8R	Morgan et al., 2012
ASD	Increased glia density in BA 22, 39, 44	8	M	7-44 years (20.8±13.0)	Left	Lopez-Hurtado & Prieto 2008
ASD	Increase in microglia density in insular cortex	10	2F, 8M	3-22 years (10.6±6.1)	Right	Tetreault et al., 2012
ASD	Increase in microglia density in visual cortex	9	2F, 7M	3-22 years (11.7±7.2)	Right	Tetreault et al., 2012
ASD	No difference in overall non-neuronal cell numbers in amygdala No overall difference in microglia density in amygdala Decreased oligodendrocyte density in amygdala	8	M	10-44 years (23.0±11.9)	3L, 5R	Morgan et al., 2014
WS	Increased total glia density, oligodendrocyte density, and glia to neuron ratio in caudate nucleus of striatum	5	2F, 3M	18-45 years (35.8±11.3)	Right	Hanson et al., 2018
WS	Increased glia density in BA 25	5	3F, 2M	18-43 years (31.6±11.0)	Right	Wilder et al., 2018
WS	Increased total glia density in BA 4, 3, and 18 Decreased astrocyte/microglia density in BA 10 and BA 18 Increased astrocyte/microglia density in BA 3 and BA 4 Increased oligodendrocyte density in BA 10, 4, 3, and 18	5	1F, 4M	18-48 years (37.0±12.43)	1L, 4R	Present study

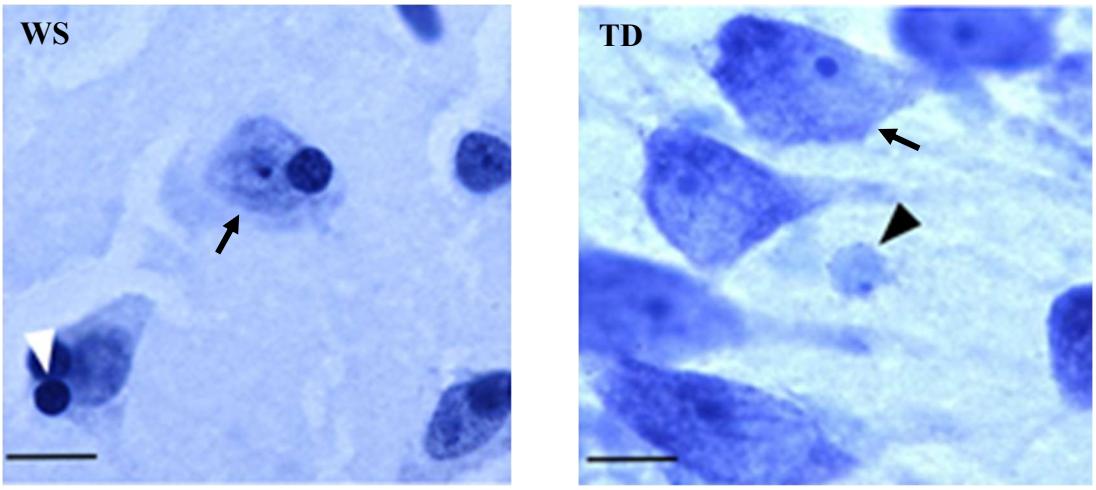

Figure 4.1. Microphotograph of glia subtypes in the cortex. Black arrow: neuron. White arrowhead: oligodendrocyte. Black arrowhead: astrocyte/microglia. Images taken at 100x.

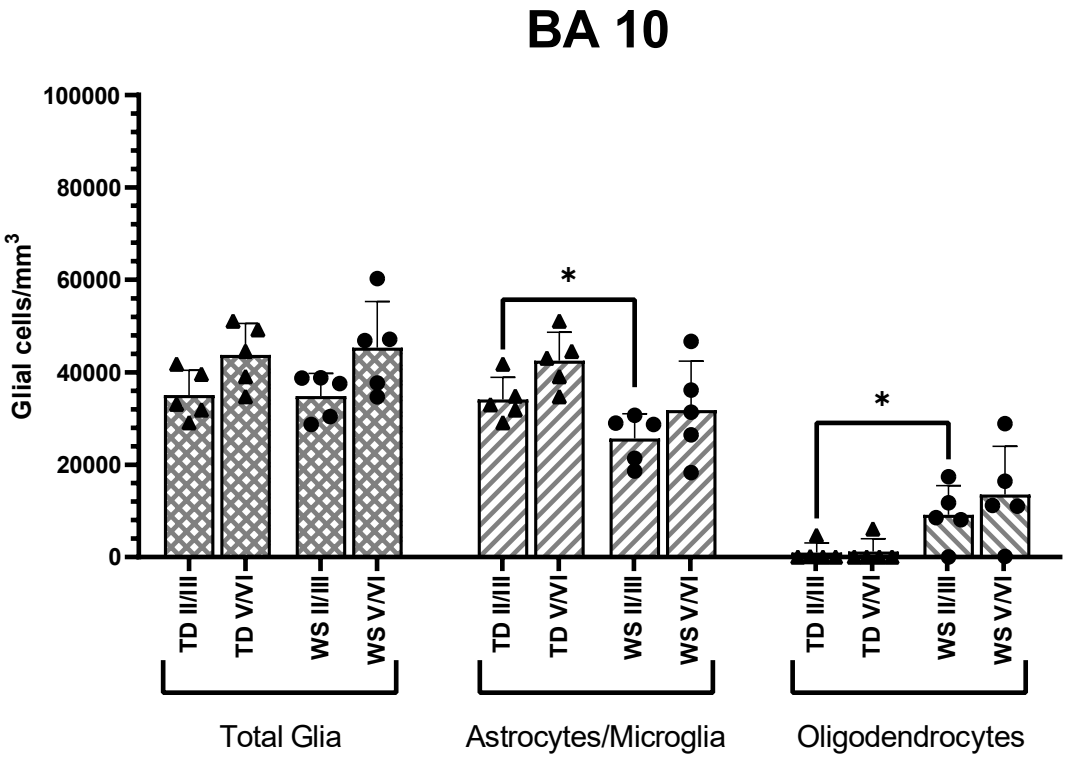

Figure 4.2. Mean glia density in BA 10. *Statistically significant results.

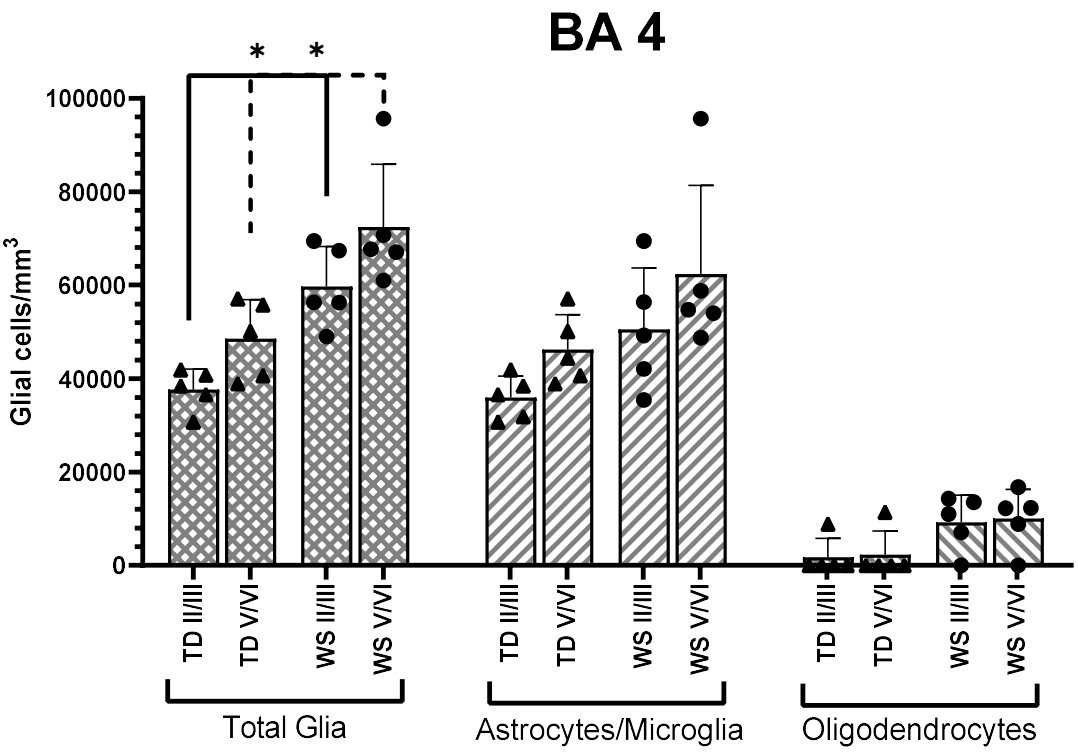

Figure 4.3. Mean glia density in BA 4. *Statistically significant results.

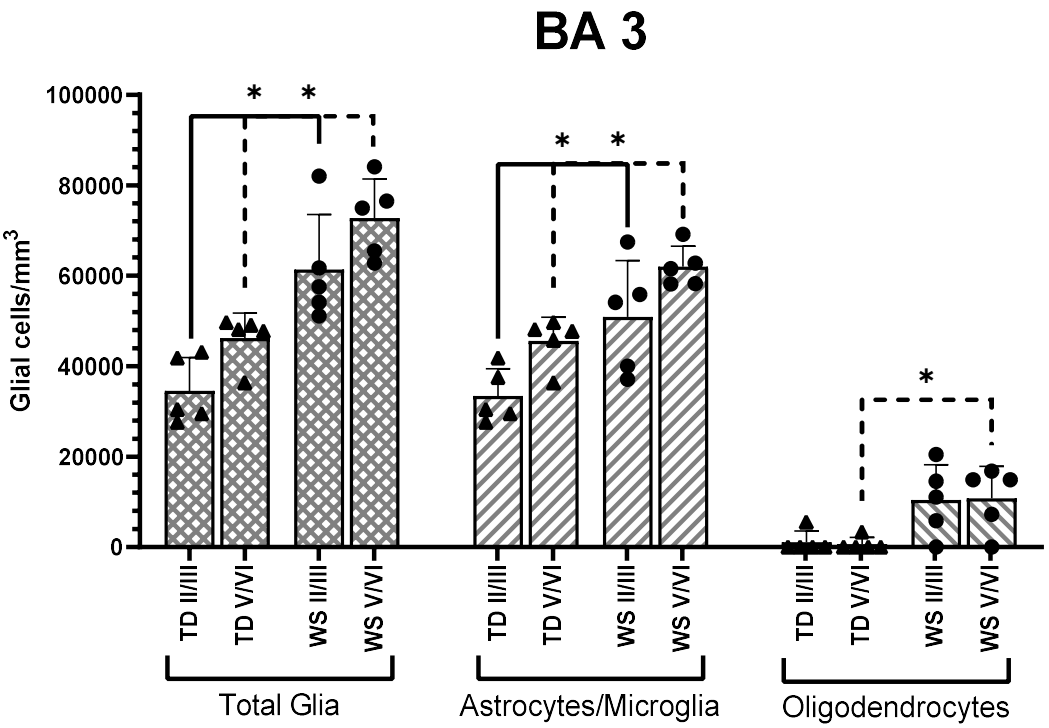

Figure 4.4. Mean glia density in BA 3. *Statistically significant results.

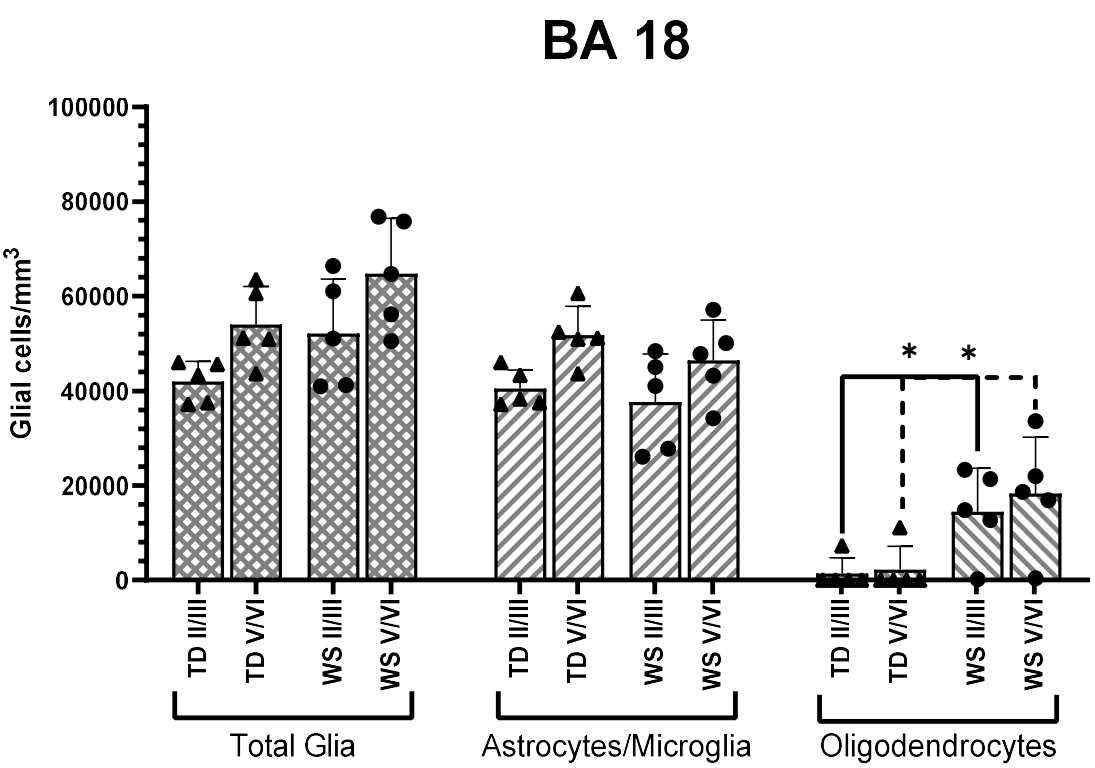

Figure 4.5. Mean glia density in BA 18. *Statistically significant results.

References

Almeida, P. G. C., Nani, J. V., Oses, J. P., Brietzke, E., & Hayashi, M. A. F. (2020). Neuroinflammation and glial cell activation in mental disorders. *Brain, Behavior, & Immunity - Health*, *2*, 100034.

Amunts, K., Istomin, V., Schleicher, A., & Zilles, K. (1995). Postnatal development of the human primary motor cortex: A quantitative cytoarchitectonic analysis. *Anatomy and Embryology*, *192*(6).

Amunts, K., Malikovic, A., Mohlberg, H., Schormann, T., & Zilles, K. (2000). Brodmann's Areas 17 and 18 Brought into Stereotaxic Space—Where and How Variable? *NeuroImage*, *11*(1), 66–84.

Bar, E., & Barak, B. (2019). Microglia roles in synaptic plasticity and myelination in homeostatic conditions and neurodevelopmental disorders. *Glia*, *67*(11), 2125–2141.

Barak, B., Zhang, Z., Liu, Y., Nir, A., Trangle, S. S., Ennis, M., Levandowski, K. M., Wang, D., Quast, K., Boulting, G. L., Li, Y., Bayarsaihan, D., He, Z., & Feng, G. (2019). Neuronal deletion of Gtf2i, associated with Williams syndrome, causes behavioral and myelin alterations rescuable by a remyelinating drug. *Nature Neuroscience*, *22*(5), 700–708.

Bayraktar, O. A., Fuentealba, L. C., Alvarez-Buylla, A., & Rowitch, D. H. (2015). Astrocyte Development and Heterogeneity. *Cold Spring Harbor Perspectives in Biology*, *7*(1), a020362.

Benes, F. M. (1986). Quantitative Cytoarchitectural Studies of the Cerebral Cortex of Schizophrenics. *Archives of General Psychiatry*, *43*(1), 31.

Bernstein, H.-G., Steiner, J., Guest, P. C., Dobrowolny, H., & Bogerts, B. (2015). Glial cells as key players in schizophrenia pathology: Recent insights and concepts of therapy. *Schizophrenia Research*, *161*(1), 4–18.

Casanova, M. F. (2014). The neuropathology of autism. *Handbook of Autism and Pervasive Developmental Disorders, Fourth Edition.*

Cotter, D. (2002). Reduced Neuronal Size and Glial Cell Density in Area 9 of the Dorsolateral Prefrontal Cortex in Subjects with Major Depressive Disorder. *Cerebral Cortex*, *12*(4), 386–394.

Courchesne, E., Mouton, P. R., Calhoun, M. E., Semendeferi, K., Ahrens-Barbeau, C., Hallet, M. J., Barnes, C. C., & Pierce, K. (2011). Neuron number and size in prefrontal cortex of children with autism. *Jama*, *306*(18), 2001-2010.

Courchesne, E., & Pierce, K. (2005). Why the frontal cortex in autism might be talking only to itself: Local over-connectivity but long-distance disconnection. *Current Opinion in Neurobiology*, *15*(2), 225–230.

Edgar, N., & Sibille, E. (2012). A putative functional role for oligodendrocytes in mood regulation. *Translational Psychiatry*, *2*(5), e109–e109.

Edmonson, C., Ziats, M. N., & Rennert, O. M. (2014). Altered glial marker expression in autistic post-mortem prefrontal cortex and cerebellum. *Molecular Autism*, *5*(1), 3.

Enkhmandakh, B., Makeyev, A. V., Erdenechimeg, L., Ruddle, F. H., Chimge, N.-O., Tussie-Luna, M. I., Roy, A. L., & Bayarsaihan, D. (2009). Essential functions of the Williams-Beuren syndrome-associated TFII-I genes in embryonic development. *Proceedings of the National Academy of Sciences*, *106*(1), 181–186.

Faria, A. V., Landau, B., O'Hearn, K. M., Li, X., Jiang, H., Oishi, K., Zhang, J., & Mori, S. (2012). Quantitative analysis of gray and white matter in Williams syndrome: *NeuroReport*, *23*(5), 283–289.

García-Cabezas, M. Á., John, Y. J., Barbas, H., & Zikopoulos, B. (2016). Distinction of Neurons, Glia and Endothelial Cells in the Cerebral Cortex: An Algorithm Based on Cytological Features. *Frontiers in Neuroanatomy*, *10*.

Geyer, S., Schleicher, A., & Zilles, K. (1997). The Somatosensory Cortex of Human: Cytoarchitecture and Regional Distributions of Receptor-Binding Sites. *NeuroImage*, *6*(1), 27–45.

Gittins, R., & Harrison, P. J. (2004). A quantitative morphometric study of the human anterior cingulate cortex. *Brain Research*, *1013*(2), 212–222.

Hammond, T. R., Robinton, D., & Stevens, B. (2018). Microglia and the Brain: Complementary Partners in Development and Disease. *Annual Review of Cell and Developmental Biology*, *34*(1), 523–544.

Hanson, K. L., Lew, C. H., Hrvoj-Mihic, B., Groeniger, K. M., Halgren, E., Bellugi, U., and Semendeferi, K. (2018). Increased glia density in the caudate nucleus in Williams syndrome: Implications for frontostriatal dysfunction in autism. *Developmental neurobiology*, 78, 531-545.

Jiang, N. M., Cowan, M., Moonah, S. N., & Petri, W. A. (2018). The Impact of Systemic Inflammation on Neurodevelopment. *Trends in Molecular Medicine*, *24*(9), 794–804.

Klein-Tasman, B. P., & Mervis, C. B. (2003). Distinctive Personality Characteristics of 8-, 9-, and 10-Year-Olds With Williams Syndrome. *Developmental Neuropsychology*, *23*(1–2), 269–290.

Laurence, J. A., & Fatemi, S. H. (2005). Glial fibrillary acidic protein is elevated in superior frontal, parietal and cerebellar cortices of autistic subjects. *The Cerebellum*, *4*(3), 206–210.

Leuba, G., & Garey, L. J. (1989). Comparison of neuronal and glial numerical density in primary and secondary visual cortex of man. *Experimental Brain Research*, *77*(1).

Lew, Caroline H., Groeniger, K. M., Bellugi, U., Stefanacci, L., Schumann, C. M., & Semendeferi, K. (2018). A postmortem stereological study of the amygdala in Williams syndrome. *Brain Structure and Function*, *223*(4), 1897–1907.

Lew, C. H., Brown, C., Bellugi, U., & Semendeferi, K. (2017). Neuron density is decreased in the prefrontal cortex in Williams syndrome. *Autism Research*, *10*(1), 99–112.

Lopez-Hurtado, E., & Prieto, J. J. (2008). A microscopic study of language-related cortex in autism. *American Journal of Biochemistry and Biotechnology*, 4(2), 130-45.

Molofsky, A. V., Krenick, R., Ullian, E., Tsai, H. -h., Deneen, B., Richardson, W. D., Barres, B. A., & Rowitch, D. H. (2012). Astrocytes and disease: A neurodevelopmental perspective. *Genes & Development*, *26*(9), 891–907.

Morgan, J. T., Barger, N., Amaral, D. G., & Schumann, C. M. (2014). Stereological Study of Amygdala Glial Populations in Adolescents and Adults with Autism Spectrum Disorder. *PLoS ONE*, *9*(10), e110356.

Morgan, J. T., Chana, G., Abramson, I., Semendeferi, K., Courchesne, E., & Everall, I. P. (2012). Abnormal microglial–neuronal spatial organization in the dorsolateral prefrontal cortex in autism. *Brain Research*, *1456*, 72–81.

Morgan, J. T., Chana, G., Pardo, C. A., Achim, C., Semendeferi, K., Buckwalter, J., Courchesne, E., & Everall, I. P. (2010). Microglial Activation and Increased Microglial Density Observed in the Dorsolateral Prefrontal Cortex in Autism. *Biological Psychiatry*, *68*(4), 368–376.

Mukaetova-Ladinska, E. B., Arnold, H., Jaros, E., Perry, R., & Perry, E. (2004). Depletion of MAP2 expression and laminar cytoarchitectonic changes in dorsolateral prefrontal cortex in

adult autistic individuals: MAP2 and laminar cytoarchitectonic changes in autism. *Neuropathology and Applied Neurobiology*, *30*(6), 615–623.

Nir, A., & Barak, B. (2020). White matter alterations in Williams syndrome related to behavioral and motor impairments. *Glia*.

Oblak, A. L., Rosene, D. L., Kemper, T. L., Bauman, M. L., & Blatt, G. J. (2011). Altered posterior cingulate cortical cytoarchitecture, but normal density of neurons and interneurons in the posterior cingulate cortex and fusiform gyrus in autism. *Autism Research*, *4*(3), 200-211.

Ongur, D., Drevets, W. C., & Price, J. L. (1998). Glial reduction in the subgenual prefrontal cortex in mood disorders. *Proceedings of the National Academy of Sciences*, *95*(22), 13290–13295.

Petrelli, F., Pucci, L., & Bezzi, P. (2016). Astrocytes and Microglia and Their Potential Link with Autism Spectrum Disorders. *Frontiers in Cellular Neuroscience*, *10*.

Rajkowska, G., Miguel-Hidalgo, J. J., Wei, J., Dilley, G., Pittman, S. D., Meltzer, H. Y., Overholser, J. C., Roth, B. L., & Stockmeier, C. A. (1999). Morphometric evidence for neuronal and glial prefrontal cell pathology in major depression. *Biological psychiatry*, *45*(9), 1085-1098.

Rentería, M. E. (2012). Cerebral Asymmetry: A Quantitative, Multifactorial, and Plastic Brain Phenotype. *Twin Research and Human Genetics*, *15*(3), 401–413.

Rodriguez, J. I., & Kern, J. K. (2011). Evidence of microglial activation in autism and its possible role in brain underconnectivity. *Neuron Glia Biology*, *7*(2–4), 205–213.

Sherwood, C. C., Stimpson, C. D., Raghanti, M. A., Wildman, D. E., Uddin, M., Grossman, L. I., Goodman, M., Redmond, J. C., Bonar, C. J., Erwin, J. M., & Hof, P. R. (2006). Evolution of increased glia-neuron ratios in the human frontal cortex. *Proceedings of the National Academy of Sciences*, *103*(37), 13606–13611.

Sloan, S. A., & Barres, B. A. (2014). Mechanisms of astrocyte development and their contributions to neurodevelopmental disorders. *Current Opinion in Neurobiology*, *27*, 75–81.

Smiley, J. F., Konnova, K., & Bleiwas, C. (2012). Cortical thickness, neuron density and size in the inferior parietal lobe in schizophrenia. *Schizophrenia research*, *136*(1-3), 43-50.

Strømme, P., Bjømstad, P. G., & Ramstad, K. (2002). Prevalence estimation of Williams syndrome. *Journal of child neurology*, *17*(4), 269-271.

Terry, R. D., DeTeresa, R., & Hansen, L. A. (1987). Neocortical cell counts in normal human adult aging. *Annals of Neurology*, *21*(6), 530–539.

Tetreault, N. A., Hakeem, A. Y., Jiang, S., Williams, B. A., Allman, E., Wold, B. J., & Allman, J. M. (2012). Microglia in the Cerebral Cortex in Autism. *Journal of Autism and Developmental Disorders*, *42*(12), 2569–2584.

Uranova, N. A., Vostrikov, V. M., Orlovskaya, D. D., & Rachmanova, V. I. (2004). Oligodendroglial density in the prefrontal cortex in schizophrenia and mood disorders: A study from the Stanley Neuropathology Consortium. *Schizophrenia Research*, *67*(2–3), 269–275.

Vargas, D. L., Nascimbene, C., Krishnan, C., Zimmerman, A. W., & Pardo, C. A. (2005). Neuroglial activation and neuroinflammation in the brain of patients with autism. *Annals of Neurology*, *57*(1), 67–81.

Vostrikov, V. M., Uranova, N. A., & Orlovskaya, D. D. (2007). Deficit of perineuronal oligodendrocytes in the prefrontal cortex in schizophrenia and mood disorders. *Schizophrenia Research*, *94*(1–3), 273–280.

Wilder, L., Hanson, K., Lew, C., Bellugi, U., & Semendeferi, K. (2018). Decreased Neuron Density and Increased Glia Density in the Ventromedial Prefrontal Cortex (Brodmann Area 25) in Williams Syndrome. *Brain Sciences*, *8*(12), 209.

Zeidán-Chuliá, F., Salmina, A. B., Malinovskaya, N. A., Noda, M., Verkhratsky, A., & Moreira, J. C. F. (2014). The glial perspective of autism spectrum disorders. *Neuroscience & Biobehavioral Reviews*, *38*, 160–172.

Zhan, Y., Paolicelli, R. C., Sforazzini, F., Weinhard, L., Bolasco, G., Pagani, F., Vyssotski, A. L., Bifone, A., Gozzi, A., Ragozzino, D., & Gross, C. T. (2014). Deficient neuron-microglia signaling results in impaired functional brain connectivity and social behavior. *Nature Neuroscience*, *17*(3), 400–406.

Zilles, K., Schleicher, A., Langemann, C., Amunts, K., Morosan, P., Palomero-Gallagher, N., Schormann, T., Mohlberg, H., & Roland, P. E. (1997). Quantitative analysis of sulci in the human cerebral cortex: development, regional heterogeneity, gender difference, asymmetry, intersubject variability and cortical architecture. *Human brain mapping*, *5*(4), 218-221.

Chapter 5

Conclusions

This dissertation sought to identify microstructural changes in the vmPFC and in glia density across the cortex that may contribute to the social and emotional features of Williams Syndrome (WS), a disorder with a well-known genetic etiology and distinct cognitive and behavioral phenotype. We targeted the ventromedial cortex (BA 25), a region of the brain with critical roles in social behavior and emotion regulation, along with one orbitofrontal area (BA 10), and three unimodal cortical areas (BA 4, 3, and 18) in a postmortem sample of brains from individuals diagnosed with WS and typically developing (TD) controls, to perform stereological analysis of neuron and glia density in adults and infants in BA 25, and densities of astrocytes/microglia and oligodendrocytes in BA 10, 4, 3, and 18. Variations in cell density of neurons and glia in BA 25, and of specific glia subtypes in BA 10, 4, 3, and 18 were observed that may contribute to the unique behavioral phenotype of WS.

Summary of key findings

Neuron and glia density in adult BA 25

In the adult samples, neuron density was decreased in WS relative to TD in both infragranular and supragranular layers. This difference reached statistical significance in supragranular layers. Glia density and glia to neuron ratio was significantly increased in both infragranular and supragranular layers in adult WS relative to adult TD. Two other studies examining neurons in the postmortem WS cortex found decreased neuron density in BA 10 and BA 11 (Lew et al., 2017), as well less dendritic branching in BA 10 and BA 11 than expected relative to BA 4, 3, and 18 (Hrvoj-Mihic et al., 2017). Together these findings demonstrate that neuronal abnormalities may be a common feature across the PFC in WS.

Neuron and glia density in infant BA 25

In a 110 day old/114 day old infant pair, neuron density, glia density, and glia to neuron ratio were similar in supragranular layers. In infragranular layers neuron density, glia density, and glia to neuron ration were all lower in the WS subject. In a 234 day old/245 day old infant pair, neuron density was lower in all layers examined in the WS subject. Glia density and glia to neuron ratio were higher in all layers examined in the WS subject compared to the TD subject, but still lower than the adult mean. These finding suggest that the neuronal and glia abnormalities in the WS cortex are not fully present at birth, but they do emerge within the first year of life. The decrease in neuron density could be due in part to the deletion of *FZD9*, which is involved in neural development and cell division (Chailangkarn et al., 2016). A study examining neural progenitors using induced pluripotent stem cells found that WS neural progenitors had increased doubling time, reducing the number of neural progenitors, and increased apoptosis, which could decrease eventual neuron number. These effects were ameliorated by restoring *FZD9* in WS neural progenitors (Chailangkarn et al., 2016).

Glia density in BA 10, 4, 3, and 18

Total glia density was increased in WS in all layers examined in BA 4, 3, and 18, this reached statistical significance in supragranular and infragranular layers of BA 4 and BA 3. In BA 10 total mean glia density was similar in WS and TD. Combined density of astrocytes and microglia was decreased in WS in supragranular and infragranular layers of BA 10 and BA 18, this reached statistical significance only in supragranular layers of BA 10. Combined density of astrocytes and microglia was increased WS in supragranular and infragranular layers of BA 4 and BA 3, this reached statistical significance only in BA 3. Oligodendrocyte density was increased in WS in all layers examined in BA 10, 4, 3, and 18. This reached statistical

significance in supragranular layers of BA 10, and in supragranular and infragranular layers of BA 3 and BA 18.

These findings demonstrate that neuroanatomical abnormalities in the WS cortex are not restricted to regions with known functional abnormalities in WS, suggesting that there may be a systemic perturbation of glia in WS. In mice, deletions of *GTF2i* and *GTF2iRD1* have been shown to decrease oligodendrocyte number, reduce myelination, and disrupt vasculature and endothelial cell development, which could impact oligodendrocyte migration (Barak et al., 2019; Enkhmandakh et al., 2009). Together these gene deletions in WS may disrupt oligodendrocyte differentiation and migration into white matter, resulting deficits in myelination. These deletions may contribute to the atypical social behavior and anxiety in WS, as myelin tracts can influence social behavior, and copy number variation in *GTF2i* in correlates to measures of anxiety and reaction to aversive social stimuli in typically developing humans (Jabbi et al., 2015; Nir & Barak, 2020).

Future Directions

Glia play critical roles in neural development and functioning, and are crucial to typical human cognition, emotion, and social behavior. The number of glia does not appear to be differentially increased in the human brain, but there is evidence of changes to the size, complexity, and functioning of human glia relative to those in non-human apes (Oberheim et al., 2006; Berto et al., 2019). These evolutionary changes in human glia may contribute to human-specific cognition and social behavior, while also increasing the susceptibility of glia to pathological change during development (Berto et al., 2019).

Alterations in glia are a common feature in many neurodevelopmental disorders, but they have only recently been studied in WS. This dissertation represents the first quantitative study of

glia density across the cortex in WS, and of infant neuron and glia density in the WS cortex. The findings presented here demonstrate that abnormalities in glia in WS are found in regions of the brain associated with higher-order cognition and behavior (BA 25 and BA 10), as well as unimodal cortical areas (BA 4, BA 3, and BA 18), and that these glia abnormalities can develop within the first year of life. Future work utilizing immunohistochemical staining to separately target all glia subtypes would elucidate the precise glia abnormalities present in WS. Larger samples, particularly with a greater number of young subjects would aid in identifying what exactly what neuroanatomical abnormalities are present in WS, what mechanisms underlie these changes, and when these alterations emerge in development.

The findings from this dissertation provide fundamental information for understanding how alterations in glia can affect social behavior, and the developmental mechanisms that link genes to neuroanatomical changes.

References

Berto, S., Mendizabal, I., Usui, N., Toriumi, K., Chatterjee, P., Douglas, C., Tamminga, C.A., Preuss, T.M., Soojin, V.Y. and Konopka, G. (2019). Accelerated evolution of oligodendrocytes in the human brain. *Proceedings of the National Academy of Sciences*, *116*(48), 24334-24342.

Barak, B., Zhang, Z., Liu, Y., Nir, A., Trangle, S. S., Ennis, M., Levandowski, K. M., Wang, D., Quast, K., Boulting, G. L., Li, Y., Bayarsaihan, D., He, Z., & Feng, G. (2019). Neuronal deletion of Gtf2i, associated with Williams syndrome, causes behavioral and myelin alterations rescuable by a remyelinating drug. *Nature Neuroscience*, *22*(5), 700–708.

Chailangkarn, T., Trujillo, C.A., Freitas, B.C., Hrvoj-Mihic, B., Herai, R.H., Diana, X.Y., Brown, T.T., Marchetto, M.C., Bardy, C., McHenry, L. and Stefanacci, L. (2016). A human neurodevelopmental model for Williams syndrome. *Nature*, 536(7616), 338-343.

Enkhmandakh, B., Makeyev, A. V., Erdenechimeg, L., Ruddle, F. H., Chimge, N.-O., Tussie-Luna, M. I., Roy, A. L., & Bayarsaihan, D. (2009). Essential functions of the Williams-Beuren syndrome-associated TFII-I genes in embryonic development. *Proceedings of the National Academy of Sciences*, *106*(1), 181–186.

Hrvoj-Mihic, B., Hanson, K. L., Lew, C. H., Stefanacci, L., Jacobs, B., Bellugi, U., & Semendeferi, K. (2017). Basal dendritic morphology of cortical pyramidal neurons in Williams syndrome: prefrontal cortex and beyond. Frontiers in neuroscience, 11, 419.

Jabbi, M., Chen, Q., Turner, N., Kohn, P., White, M., Kippenhan, J.S., Dickinson, D., Kolachana, B., Mattay, V., Weinberger, D.R. and Berman, K.F. (2015). Variation in the Williams syndrome GTF2I gene and anxiety proneness interactively affect prefrontal cortical response to aversive stimuli. Translational psychiatry, 5(8), e622-e622.

Lew, C. H., Brown, C., Bellugi, U., & Semendeferi, K. (2017). Neuron density is decreased in the prefrontal cortex in Williams syndrome. *Autism Research*, *10*(1), 99–112.

Nir, A., & Barak, B. (2020). White matter alterations in Williams syndrome related to behavioral and motor impairments. *Glia*.

Oberheim, N. A., Wang, X., Goldman, S., & Nedergaard, M. (2006). Astrocytic complexity distinguishes the human brain. *Trends in neurosciences*, *29*(10), 547-553.