THE ROLE OF ASTROCYTES IN THE PATHOPHYSIOLOGY OF SCHIZOPHRENIA

by

Amy E. Steffek

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Doctoral Committee:

Professor James H. Meador-Woodruff, Co-Chair
Professor Stephen K. Fisher, Co-Chair
Professor Kirk A. Frey
Associate Professor Gregory W. Dalack
Associate Professor Juan F. Lopez

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LIST OF ABBREVIATIONS

ACC	Anterior cingulate cortex
AMPA	α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid
ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
ATP	Adenosine triphosphate
BDNF	Brain derived neural growth factor
CA	Cornu Ammonis
Ca^{2+}	Calcium
CNP	2',3'-cyclic nucleotide 3'-phosphodiesterase
CNS	Central nervous system
COMT	Catechol-O-methyl transferase
CSF	Cerebrospinal fluid
DAAO	D-amino acid oxidase
DAOA	D-amino acid oxidase activator
DLPFC	Dorsolateral prefrontal cortex
DMSO	Dimethyl sulfoxide
DTT	Dithiothreitol
EAAT	Excitatory amino acid transporters
ECL	Enhanced chemiluminescence
GABA	Gamma-aminobutyric acid
GFAP	Glial fibrillary acidic protein
GLUT	Glucose transporter protein
GRIP	Glutamate receptor interacting protein
IF	Intermediate filaments
IP_3	Inositol 1,4,5-trisphosphate
LTD	Long term depression
LTP	Long term potentiation
MAG	Myelin-associated glycoprotein
MAL	Myelin and lymphocyte protein
mGluR	Metabotropic glutamate receptors
MK-801	(+)-5-methyl-10, 11-dihydro-5H-dibenzo [a, d] cyclohepten-5, 10-imine
NGF	Neurotrophic growth factor
NMDA	N-methyl-D-aspartate
NO	Nitric oxide
NRG1	Neuregulin 1
OD	Optical density
OLIG2	Oligodendrocyte lineage transcription factor 2
ORA	Outward rectifying astrocyte

PA	Passive rectifying astrocyte
PCP	Phencyclidine
PCR	Polymerase chain reaction
PFC	Prefrontal cortex
PICK1	Protein interacting with C-kinase
pnd	Postnatal day
SNARE	Soluble N-ethylmaleimide-sensitive factor attachment protein receptor
SVZ	Subventricular zone
TGF	Transforming growth factor
VGLUT	Vesicular glutamate transporter
VRA	Variably rectifying
wm	White matter

CHAPTER 1

THE EMERGING ROLE OF ASTROCYTES IN THE PATHOPHYSIOLOGY OF SCHIZOPHRENIA

1.1 Schizophrenia is a severe psychiatric disorder

Schizophrenia is a chronic, debilitating psychiatric illness afflicting over three million people in the United States and 1% of the population worldwide and is associated with a high incidence of drug abuse, depression, homelessness, and suicide (Tamminga and Holcomb, 2005). There is no test or biological marker that unambiguously identifies this illness, which manifests with a heterogeneous pattern of symptoms that can obscure personality and intellect and rob individuals of their ability to pursue an independent life.

The symptoms of schizophrenia, which affect thought, perception, affect, and behavior, fall into three broad categories: positive, negative, and cognitive symptoms. Positive symptoms are usually rapid in onset and are characterized by the existence of behaviors not normally present, such as hallucinations, delusions, and disorders of thought, speech, and movement. Conversely, negative symptoms are characterized by the absence of normal mood and behaviors, including flattening of affect, poverty of speech, diminished pleasure and volition, social withdrawal, and, in extreme cases, catatonia. Cognitive symptoms, the third broad category, are a central feature of this disease and are characterized by deficits in attention, working memory, and executive

functioning. While there is no cure for schizophrenia, there are pharmacological and nonpharmacological methods of treatment. The drugs currently available are only moderately effective for relieving many of these symptoms and, unfortunately, medication-induced side effects often make treatment a potentially undesirable alternative to living with the untreated illness. On average, symptoms of schizophrenia emerge beginning in adolescence to about 30 years of age, though males tend to present at a younger age than females.

1.2 A developmental hypothesis of schizophrenia

It is hypothesized that schizophrenia is the result of genetic and environmental factors. Monozygotic twins have a 40-65% concordance rate and about 10% of people with schizophrenia in first degree relatives will go on to develop the illness. Several genes associated with developmental functions including neuronal migration, synaptogenesis, neuronal and glial differentiation, neurotransmission, synaptic plasticity, and receptor trafficking and anchoring have been identified as increasing susceptibility to schizophrenia (Harrison and Weinberger, 2005). Clearly, a genetic predisposition is not solely culpable. When paired with *in utero* complications (especially during the second trimester) such as maternal exposure to viruses, or perinatal complications such as fetal hypoxia, or even those that occur early in life following birth, neurodevelopment may be disrupted and thus set the stage for later emergence of the illness (Cannon et al., 2003; Rapoport et al., 2005). However, neither one specific genetic profile nor a critical threshold for environmental insult is known. Even with these purported factors in place, there is a period of relative quiescence from birth to late adolescence when symptoms are

absent or significantly muted. As adolescence gives way to adulthood, biological events such as neurological restructuring may perturb developmental abnormalities and unmask previously dormant vulnerabilities (Cotter and Pariante, 2002). Psychosocial stressors and environmental factors such as urban vs. rural upbringing and substance abuse are also correlated with the emergence of schizophrenia (Hambrecht and Hafner, 1996; Henquet et al., 2005; Allardyce and Boydell, 2006; Kristensen and Cadenhead, 2007).

Despite the fact that overt symptoms are initially not present, there is evidence that disease-related abnormalities are in place before diagnosable symptoms of schizophrenia materialize. For example, early in childhood the offspring of affected individuals have been shown to demonstrate schizophrenia-like symptoms and structural brain anomalies prior to illness manifestation (Cannon et al., 2003; Rajarethinam et al., 2004). Additionally, the unaffected siblings of individuals with schizophrenia often show hallmark characteristics of the illness such as structural brain changes, decreased performance on cognitive tasks, and behavioral symptoms that resemble schizophrenia (Kendler and Diehl, 1993; Cannon et al., 2000), although it is not understood why certain individuals with these phenotypic characteristics do not become ill.

The advent of brain imaging tools has allowed researchers to identify underlying brain pathology in schizophrenia, which include enlarged ventricles, an overall decrease in cerebral gray and white matter volume, loss of cortical and hippocampal volume, and altered metabolic activity in the brain (Cotter and Pariante, 2002; Tamminga and Holcomb, 2005). Many of these changes are detectable at the first onset of schizophrenia. Work with postmortem brain tissue has revealed that the macroscopic neuropathology identified by *in vivo* imaging is associated with cytoarchitectural

abnormalities, including changes in cellular size, morphology, location, and orientation (Harrison, 1999). Until recently, a majority of these abnormalities were thought to be static, and thus, a neuroprogressive model of illness development was not strongly embraced. There are now reports, however, which suggest that the initial onset of schizophrenia may be characterized by rapid and progressive degeneration that eventually tapers off to form a more stable set of illness characteristics (Ho et al., 2003; Pantelis et al., 2005; Rapoport et al., 2005). One longitudinal neuroimaging study showed that adolescent patients with schizophrenia had a significant and rapid loss of gray matter compared to typical age-related losses in a non-psychiatrically ill comparison group (Thompson et al., 2001). These losses progressed across a span of several cortical regions in a temporal manner. The parietal and motor cortices showed rapid loss of gray matter that appeared in early adolescence, whereas frontal and temporal regions did not exhibit deficits until late adolescence after the appearance of symptoms (Cannon et al., 2000).

A conventional sign of an active degenerative state in the brain is "reactive gliosis" of a specialized type of glial cell—the astrocyte, which includes hypertrophy of astrocytic processes and upregulation of intermediate filament proteins. This has been reported in progressive neurodegenerative disorders such as Parkinson's, Huntington's, and Alzheimer's diseases, and amyotrophic lateral sclerosis (Maragakis and Rothstein, 2006; Wilhelmsson et al., 2006). Gliosis is a sensitive indicator of neurodegeneration and injury in the brain and was hypothesized to exist in schizophrenia. Although reported in early studies of schizophrenia (Stevens, 1982), these findings have not been replicated, and instead may have been due to brain abnormalities secondary to the illness

(Roberts et al., 1986; Roberts et al., 1987; Stevens et al., 1988; Casanova et al., 1990; Falkai et al., 1999; Damadzic et al., 2001).

1.3 Neurotransmitter system abnormalities in schizophrenia

Alterations in several neurotransmitter systems have been identified in schizophrenia. A model of dopamine hyperfunction arose amid findings that dopaminergic agonists, such as the psychostimulant amphetamine, were found to induce a schizophrenia-like psychosis in some nonpsychiatrically ill individuals and exacerbate existing symptoms in individuals with schizophrenia (Guillin et al., 2007). Additional pharmacological support for this model arose when antipsychotic medications that act by blocking D2-like dopamine receptors were shown to successfully reduce some symptoms of schizophrenia (Laruelle et al., 2003). Today, there are ample data that reflect dysfunction in the dopaminergic system in schizophrenia (Carlsson et al., 2001). One major caveat to the dopamine hypothesis of schizophrenia, however, is that D2 receptor antagonists only effectively treat positive symptoms and have far more modest effects on cognitive and negative symptoms. Other dopamine receptor subtypes have since been identified and implicated in this illness, and consequently, a refined theoretical model has been developed to link the idea of both hyper- and hypo-function of the dopamine system, which better accounts for cognitive and negative symptoms (Davis et al., 1991; Guillin et al., 2007).

Although other transmitter systems have emerged as potential contributors to this illness, including, but not limited to, serotonin, gamma-aminobutyric acid (GABA), and acetylcholine, dysfunctional glutamatergic transmission, in particular, has emerged as a

predominant hypothesis for the pathophysiology of schizophrenia (Roth et al., 2003; Wassef, 2003; Sarter et al., 2005; Coyle, 2006; Abi-Dargham and Guillin, 2007; Guillin et al., 2007). An extensive amount of research has produced data to support the idea of glutamate dysfunction in schizophrenia, including findings of altered expression of numerous molecules involved in glutamate synthesis, transport, binding, reuptake, and recycling (Ohnuma et al., 1998; Ibrahim et al., 2000; Ohnuma et al., 2000; Meador-Woodruff et al., 2001; Smith et al., 2001b; Smith et al., 2001a; Gluck et al., 2002; McCullumsmith and Meador-Woodruff, 2002; Burbaeva et al., 2003; Bruneau et al., 2005 and Harrison et al., 2003 for review). Furthermore, unlike the dopamine hypothesis, glutamatergic dysfunction accounts for the spectrum of symptoms seen in schizophrenia. Some have suggested that dopamine dysfunction may in fact be secondary to glutamatergic abnormalities (Laruelle et al., 2003).

In glutamatergic transmission, glutamine is supplied to neurons by transport from astrocytes. Within neurons, glutamine is converted to glutamate and packaged into synaptic vesicles. The release of this presynaptic glutamate from neurons activates receptors located on pre- and postsynaptic neurons, as well as those located perisynaptically on astrocytes. These receptors include metabotropic glutamate receptors (mGluRs), which are G-protein coupled, and three types of ionotropic glutamatergic receptors: the N-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA), and kainate receptors (Figure 1.1). The NMDA receptor is unique in several ways. First, it is both a ligand and ion gated receptor, meaning that it requires binding of an agonist (in this case glutamate) as well as membrane depolarization to become active. Second, it requires the binding of a co-agonist for

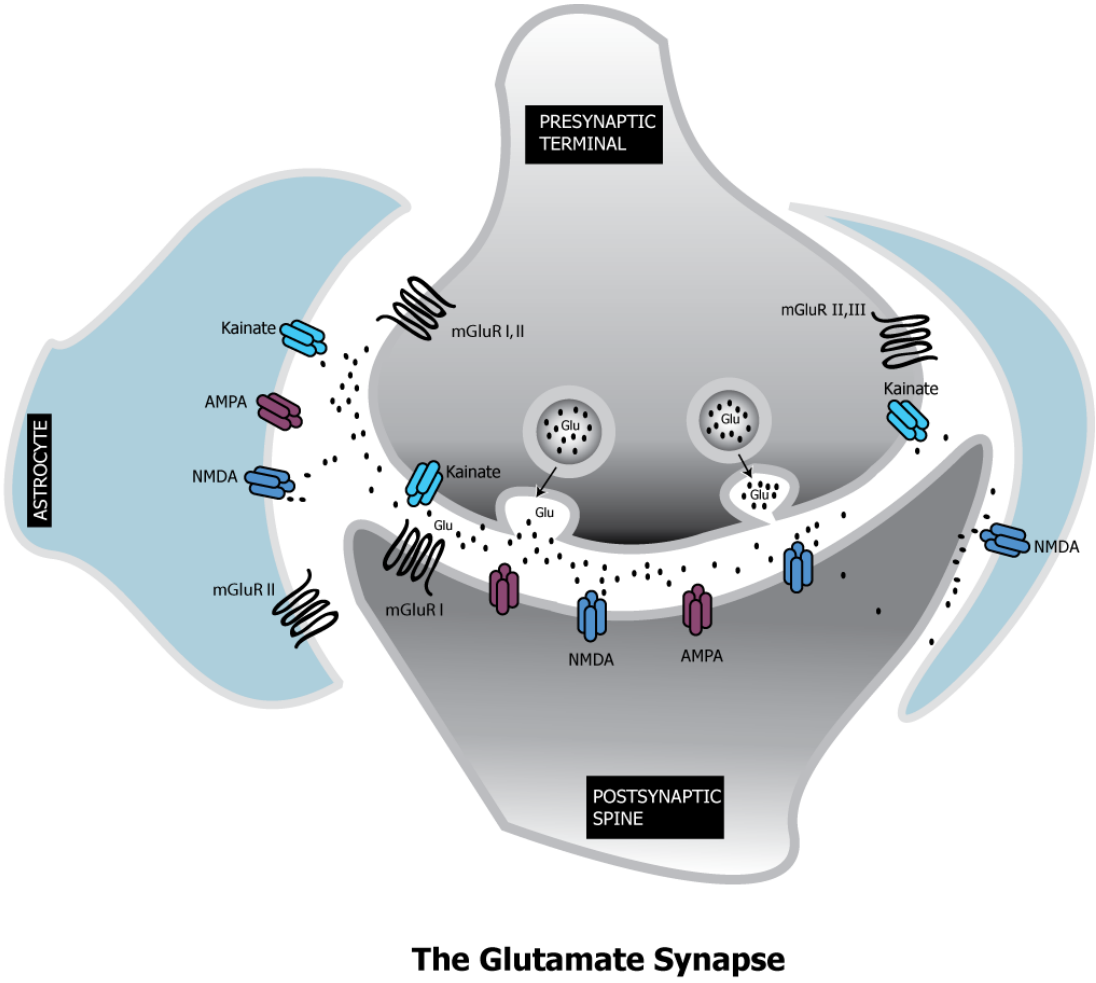

Figure 1.1 The glutamate synapse. The release of glutamate from neuronal presynaptic terminals activates receptors located on pre- and postsynaptic neurons and astrocytes. These receptors include mGluRs, which are G-protein coupled, and three types of ionotropic glutamatergic receptors: the NMDA, AMPA, and kainate receptors.

activation. Several agents act on the co-agonist binding site of the NMDA receptor, including glycine and D-serine (Matsui et al., 1995). Upon receptor activation, calcium (Ca^{2+}) flows into the cell and modulates gene expression through a cascade of signaling mechanisms. Extended activation of NMDA, along with AMPA receptors, can cause morphological changes to postsynaptic dendritic spines and facilitate long term potentiation (LTP), a momentary or long term enhanced synaptic signaling that occurs in learning and memory (Mothet et al., 2006).

A glutamate hypothesis of schizophrenia arose, like the dopamine hypothesis, from a pharmacological observation. Phencyclidine (PCP), which had been used as an anesthetic, was shown to induce symptoms resembling the positive, negative and cognitive symptoms of schizophrenia in non-psychiatrically ill patients, and magnify those symptoms in patients with schizophrenia (Javitt, 1987). PCP was shown to act as a noncompetitive antagonist of the NMDA receptor. This finding, together with the fact that other NMDA receptor antagonists, including ketamine, also elicit a "schizophrenia-like" syndrome, helped establish a model of NMDA receptor hypofunction in schizophrenia (Lahti et al., 1995; Coyle, 1996). Many studies have since reported altered expression of the NMDA receptor, including receptor subunits and binding sites, as well as associated postsynaptic density proteins which facilitate intracellular signaling (Clinton et al., 2003; Laruelle et al., 2003; Meador-Woodruff et al., 2003; and for review see Kristiansen et al., 2007). Furthermore, NMDA receptor hypofunction is supported by data demonstrating that co-agonists of the receptor, which facilitate receptor function, can attenuate some symptoms of schizophrenia (Heresco-Levy et al., 2005).

1.4 Structural and functional brain abnormalities in schizophrenia

The positive, negative, and cognitive symptoms of schizophrenia are most certainly the result of impairment in multiple brain regions. The prefrontal cortex (PFC) emerged early on as a major site of this dysfunction (Weinberger et al., 1986). Normal functioning of this area depends on reciprocal connections with other structures including, but not limited to, the superior temporal gyrus, cingulate cortex, hippocampus, and primary visual cortex. All of these corticolimbic structures have been implicated in the pathophysiology of schizophrenia by volumetric changes, abnormal activation, and aberrant functional connectivity (Assaf et al., 2006; Garrity et al., 2007).

1.4.1 Prefrontal cortex.

Perhaps the most prevalent subset of symptoms, as well as the most devastating and treatment-resistant, is cognitive impairment. This often manifests as the inability to sustain attention, loss of executive functioning, and loss of the ability to initiate and execute goal-directed behaviors. Working memory, the capacity to actively hold information in short term memory, is key to this type of cognition. The PFC plays a critical role in high-level executive functioning. Years of research have clearly shown that this area is compromised in schizophrenia (Weinberger and Berman, 1996; Manoach, 2003). Imaging studies demonstrate that working memory deficits are associated with reduced activation in the dorsolateral PFC during cognitive tasks (Weinberger et al., 1986; Perlstein et al., 2001; Assaf et al., 2006). While some postmortem studies demonstrate layer-specific reductions in neuronal size and density in the PFC (Rajkowska et al., 1998), others report increased neuronal density and reduced interneuronal space

between pyramidal and nonpyramidal neurons, suggesting an overall loss of neuropil (Selemon et al., 1995; Selemon and Goldman-Rakic, 1999). The PFC is one of the last brain areas to reach maturation. This maturation culminates with processes such as synaptic pruning of prefrontal cortical regions in late adolescence, a time that coincides with the emergence of symptoms of schizophrenia, adding further evidence to support the notion of prefrontal cortical dysfunction playing a role in this illness (Woo and Crowell, 2005).

1.4.2 Superior temporal gyrus.

The superior temporal gyrus has been associated with abnormalities in speech, language processing, and the performance of working memory tasks. This brain region is comprised of several specialized areas, including Wernicke's area, which is vital for processing and understanding speech. The superior temporal gyrus also contains the primary auditory cortex, which integrates auditory information into perception, and has also been implicated in the manifestation of auditory hallucinations (Stephane et al., 2001). *In vivo* imaging studies that have focused on the superior temporal gyrus show that this area does not exhibit typical levels of deactivation during working memory tasks in patients with schizophrenia compared to normal controls (Walter et al., 2007). Furthermore, treatment with antipsychotic drugs relieves symptoms, improves working memory, and returns activation patterns during working memory tasks to levels comparable to nonpsychiatrically ill individuals (Wolf et al., 2007). Other *in vivo* imaging studies show a volumetric loss in the left anterior superior temporal gyrus in schizophrenia, a change that correlates with the presence of severe thought disorder

(Rajarethinam et al., 2000). Consistent with loss of volume and aberrant connectivity, postmortem studies show evidence of synaptic abnormalities in this region that become more pronounced with age (Sokolov et al., 2000)

1.4.3 Cingulate cortex.

The cingulate cortex plays an important role in cognition, the ability to sustain attention and motivation, as well as emotion and affect. Studies have uncovered multiple abnormalities of the cingulate in schizophrenia, including structural, functional, and gene expression changes in this region (Tamminga et al., 2000). A meta-analysis of multiple *in vivo* imaging studies examining the cingulate cortex reported a volume reduction in schizophrenia (Baiano et al., 2007). Other studies report that diminished performance on semantic memory tasks correlates with increased activation of the cingulate cortex (Assaf et al., 2006). Postmortem evidence of pathology in the cingulate, which includes decreased neuronal density in this region, is consistent with volume decreases reported in *in vivo* imaging studies (Benes et al., 1986; Benes et al., 1991; Benes et al., 2001). A large scale proteomics study found that 36 different proteins, including metabolic, cytoskeletal, synaptic, signaling, trafficking, and glial proteins, were abnormally expressed in the cingulate cortex in schizophrenia, suggesting wide-spread dysfunction (Clark et al., 2006).

1.4.4 Hippocampus.

The hippocampus plays a pivotal role in learning as well as verbal and spatial memory via its connections with virtually all association cortices. Diminished cognitive

processing and evidence of structural and functional pathology have also implicated the hippocampus in schizophrenia (Heckers, 2001; Harrison, 2004). *In* vivo imaging studies of the hippocampus show volumetric losses in schizophrenia, especially on the left side, even in first episode patients (Heckers, 2001; Steen et al., 2006). Consistent with this evidence of hippocampal volume loss, postmortem studies have revealed evidence of cytoarchitectural abnormalities in the hippocampus of patients with schizophrenia, including decreased neuronal density and decreased expression of synaptic proteins such as NMDA and AMPA receptors (Eastwood and Harrison, 2000; Gothelf et al., 2000; Harrison et al., 2003; Coyle, 2004). Additionally, positive symptoms involving auditory hallucinations have been attributed to hippocampal dysfunction (Boyer et al., 2007). All of these data have been hypothesized to reflect abnormal circuitry between the hippocampal region and the PFC (Winterer et al., 2003; Meyer-Lindenberg et al., 2005).

1.4.5 Primary visual cortex.

The primary visual cortex has not always been thought to be involved in the pathophysiology of schizophrenia, and in fact, has been used as a "control" cortical region which is compared to other areas in some studies. Evidence is emerging, however, that may suggest otherwise (Dracheva et al., 2001; Dracheva et al., 2005). The primary visual cortex is involved in both form recognition and object location, the latter of which involves saccadic eye movements, a mechanism governed by the frontal cortex and a common deficit in schizophrenia (Tamminga and Holcomb, 2005). *In vivo* imaging studies have revealed decreased regional activation in the primary visual cortex of patients with schizophrenia who have received minimal antipsychotic treatment, as well

as in those who have had long term treatment (Desco et al., 2003). Several postmortem studies show that cortical volume, neuronal density, and neuropil volume are altered in the visual cortex in schizophrenia, although there is disagreement regarding the direction of changes (Selemon et al., 1995; Selemon and Goldman-Rakic, 1999; Dorph-Petersen et al., 2007).

This is by no means a complete picture of the pathology present or the multiple regions affected in this illness; rather it is a simplified snapshot of broad cortical and limbic dysfunction inherent in schizophrenia. The pathological aspects of schizophrenia, including evidence of degeneration (despite a lack of gliosis), macroscopic structural and functional abnormalities, neurotransmitter-related dysfunction, and cell level pathology, can all be traced back to disruption during neurodevelopment.

1.5 Nervous system development

Neurodevelopment consists of distinct segments, all of which are highly conserved phylogenetically. From neurogenesis, the initial birth of cells, through differentiation into specific cell types, migration to destined regions, synapse formation, and survival beyond pruning, multiple signaling molecules, both secreted and local, induce, guide, and organize cells into a structurally and functionally complex units (Sun et al., 2002). Early in the development of the nervous system, cells from the ectoderm layer begin to acquire neuronal characteristics. These progenitor cells located in the ventricular zone, an area of extensive neurogenesis, will differentiate to give rise to various types of neural tissue. This differentiation process produces two major cell lineages in the brain, neurons and glia, and is driven by a series of inducing factors, such

as sonic hedgehog and glial growth factor, from neighboring cells (Gotz and Huttner, 2005). Differentiation is followed by a period of mass cellular migration that coincides with the second trimester of pregnancy in humans. During this time, cells leave the ventricular zone and form the cortical plate, which eventually gives rise to cortical gray matter.

Radial glial cells play several critical roles during these early developmental stages of neurogenesis, differentiation, and migration (Ever and Gaiano, 2005). Most, if not all, neuronal cell types can arise from radial glia (Malatesta et al., 2000; Anthony et al., 2004; Gotz and Huttner, 2005). These glial progenitor cells reside in the ventricular zone and extend processes that terminate at the upper pial surface, thus providing a physical guide to which newly differentiated cells may cling as they migrate from the ventricular zone to their destined location in the developing brain. With the radial glia assisting, these differentiated cells arrange in a predictable manner to form cortical layers via an "inside out" pattern. Radial glial cells also provide metabolic support for neighboring cells and release signaling factors to guide axonal and dendritic differentiation (Song et al., 2002). Concurrent with neurogenesis, but not before, radial glia can themselves differentiate into many types of glial cells.

During the final phase of early neurodevelopment, newly migrated cells differentiate to express a specific neurotransmitter phenotype. Intracellular factors, as well as environmental signals from cells targeted for synaptic connection, help determine this fate. Whether synaptic connections or the cells themselves survive, and a majority do not, depends on a class of trophic factors from the target cells, such as neurotrophic growth factor (NGF), brain derived neural growth factor (BDNF), and transforming

growth factor (TGFβ) (Alvarez-Buylla and Garcia-Verdugo, 2002). These neurotrophins incite a cascade of intracellular signaling which results in either cell death or survival. In this manner, cells organize into functional tissue with sensing and signaling capabilities.

1.5.1 Developmental gene abnormalities in schizophrenia.

Schizophrenia is a complex illness that involves the dysfunction of multiple components, all of which are consistent with a developmental origin. A heritable predisposition for abnormal gene function that disrupts cellular migration and/or alters the mechanisms of growth factors could establish a prenatal vulnerability to this illness. This vulnerability, when paired with pre- or postnatal epigenetic factors, can result in structural brain abnormalities that lead to schizophrenia.

Several genes regulating developmental processes described above have been implicated in schizophrenia (Harrison and Weinberger, 2005). For example, neuregulin 1 (NRG1), which plays a role in cellular differentiation in association with glial growth factor, has been linked to genetic susceptibility for schizophrenia. Recent reports indicate that abnormal cellular migration during development, which is thought to account for many of the cytoarchitectural abnormalities seen in schizophrenia, may be attributed to NRG1 dysfunction, and that catechol-O-methyl transferase (COMT), another molecule thought to impart susceptibility to schizophrenia, may compound this dysfunction (Jakob and Beckmann, 1985; Sei et al., 2007). Additional evidence derives from animal studies, which show that modifying NRG1 gene expression can perturb early neurodevelopment and produce animals that exhibit abnormal behavior analogous to that induced by PCP, a drug that, as noted earlier, causes a spectrum of schizophrenia-like symptoms in humans

(Falls, 2003). Consistent with these data, human postmortem studies have shown increased NRG1 mRNA in the dorsolateral prefrontal cortex (DLPFC) and hippocampus in schizophrenia (Harrison and Law, 2006).

In addition to abnormal differentiation and migration, studies suggest that alterations in genes that affect synapse formation and neuronal survival, such as BDNF, may contribute to the development of structural abnormalities seen in schizophrenia (Weickert et al., 2003; Sugai et al., 2005). What is particularly notable is that these structural changes, even when localized, can have a global impact. Studies find that prenatal hippocampal lesions in the rat result in predominant abnormalities in the cortex (Bertolino et al., 2002; Lipska, 2004). These structural abnormalities coincide with marked behavioral changes and sensitivity to NMDA receptor antagonists such as PCP, suggesting a relationship between structural abnormalities, behavior, and glutamatergic dysfunction. Likewise, structural abnormalities in patients with schizophrenia, such as enlarged ventricles and loss of cortical gray and white matter, correlate with symptom severity (Ho et al., 2003; Bearden et al., 2007).

Together, this body of research shows that when neurodevelopmental processes are disturbed, even at the level of individual genes or signaling factors, they can produce significant structural, functional and behavioral abnormalities. Schizophrenia research abounds with evidence of such abnormalities, which have until recently been largely attributed to neurons. However, a novel component of brain dysfunction in schizophrenia is emerging: the glial cell.

1.6 The role of glial cells in brain function

The name glia has had several meanings attributed to it, some rather unflattering (e.g. slime) though the most likely is that of "glue", which is based on the perceived function of these cells as structural cohesive elements within the brain. This task of providing support to neurons, the "functional units" of the brain, was thought to be the raison d'etre of glia. Thus, not much interest was shown in them beyond their initial characterization. Research efforts have since uncovered diverse and important functions for these cells which far exceed their role as mere scaffolding.

Brain matter is composed of cells that derive from two major cell lineages: neuronal cells, which are comprised of a number of morphologically and functionally distinct members, and glial cells, which may be further defined as either macro- or microglia. Glial cells outnumber neurons in the mammalian central nervous system (CNS) by about ten to one (Coyle and Schwarcz, 2000). Compared to other primates, humans have an even higher glia to neuron ratio in the frontal cortex, the area of the brain most associated with higher cognition. It seems likely that through evolution the successful emergence of more complex neural connections was paralleled by a system that could accommodate increased metabolic demands and facilitate more rapid transmission (Sherwood et al., 2006). Vertebrates are thought to have developed cells specialized to meet those demands, a role fulfilled today by macroglial cell types, including astrocytes and oligodendrocytes.

1.6.1 Biology and function of oligodendrocytes.

Oligodendrocytes are found exclusively in the CNS, where they produce a lipid-

rich myelin sheath to extend and wrap in layers around neuronal axons. The myelination process promotes neuronal development and survival and facilitates rapid and accurate transmission of nerve impulses (Griffiths et al., 1998). The propagation of nerve impulses is further mediated by oligodendritic influence on the formation and maintenance of sodium channels at the nodes of Ranvier (Dupree et al., 2005). Reciprocal communication between oligodendrocytes and neurons during brain development is crucial. Later, in the mature CNS, communication between oligodendrocytes, neurons, and astrocytes ensures that the integrity of the myelin sheath, and, therefore, neuronal signaling, is preserved (Itoh et al., 1995; Barres and Raff, 1999; Ishibashi et al., 2006).

1.6.2 Identity of astrocytes.

During the later stages of cortical development that follow neurogenesis, radial glial cells lose their stem cell qualities except in specific locations such as the subventricular zone (SVZ) and the subgranular layer of the dentate gyrus in the hippocampus (Alvarez-Buylla and Garcia-Verdugo, 2002) and differentiate into macro- and microglia (Figure 1.2). Within the CNS, macroglia further differentiate into several different types of cells, including astrocytes that can be further classified as protoplasmic astrocytes of the gray matter and the more ramified fibrous astrocytes of the white matter. Oligodendrocytes are also located in CNS white matter and are responsible for myelination there. Other CNS macroglia include the radial shaped Bergmann glia of the cerebellum and Müller cells of the retina (Alvarez-Buylla et al., 2002). Recently, a novel astrocyte subtype has been proposed, the synantocyte (Butt et al., 2005). These cells,

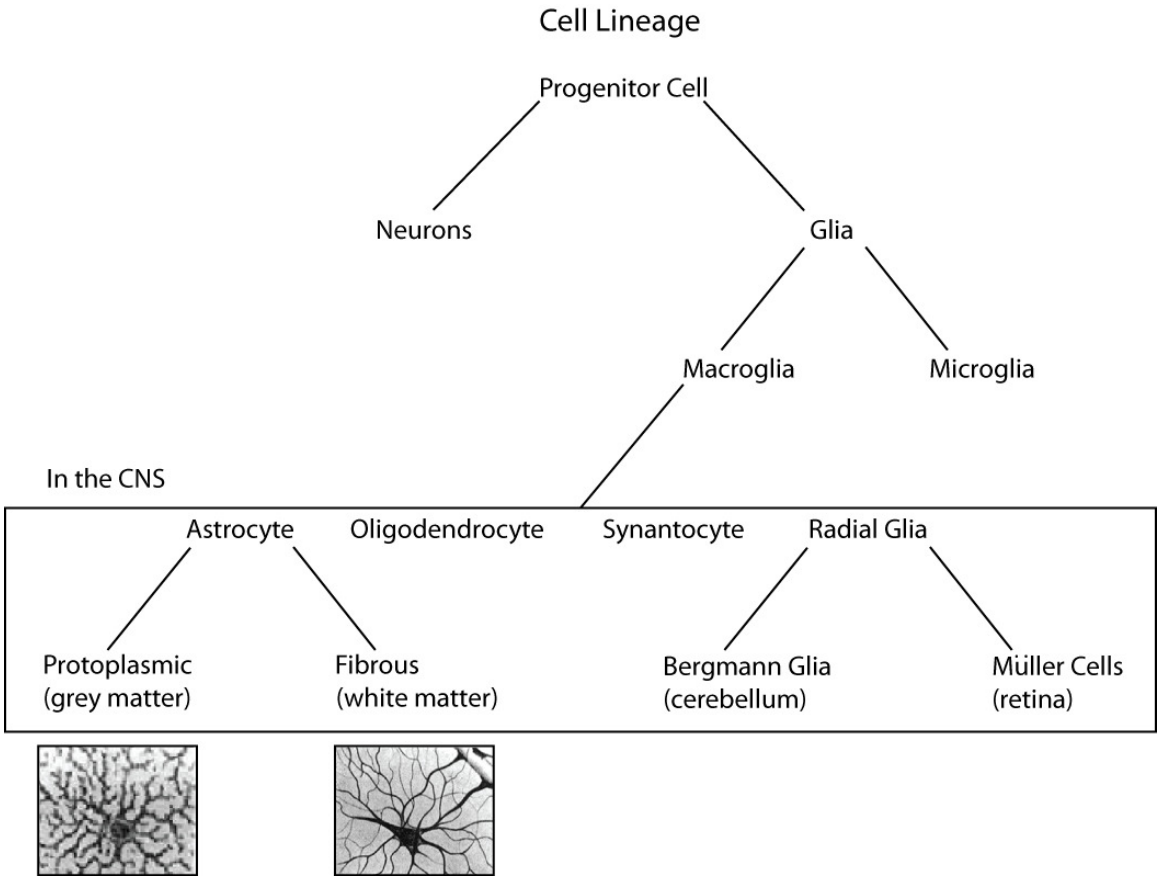

Figure 1.2 Cell lineage. Neurons and glia arise from progenitor cells and differentiate into specific cell types. Within the CNS, astrocytes may differential into either macro- or microglial cells. Microglial cells are small mobile cells that phagocytize debris in the CNS, act as immune cells, and mount an inflammatory response to brain trauma. Macroglia further differentiate into: astrocytes, oligodendrocytes, synantocytes, and radial glial cells.

named for the Greek “to contact” because of their characteristic interactions in synapses and at nodes of Ranvier, comprise a group of cells that morphologically resemble the protoplasmic variety of astrocytes (Butt et al., 2002). However, synantocytes can be identified by their thin, pale processes, irregular shaped nuclei, thinner mitochondria, and a greater number of ribosomes associated with the endoplasmic reticulum (Peters, 2004). Some of these cell types are relatively easy to discriminate from one another because of distinctive localization, morphology, and function. Within the cerebrum, however, defining what is and is not an astrocyte is the subject of some debate (Walz, 2000).

Astrocytic cells exhibit a high degree of heterogeneity, which has created controversy as to whether astrocytes should be thought of as one cell type that displays multiple characteristics or if there are actually several subtypes, each with distinguishing physiological and morphological features that persist throughout development to eventually give rise to distinct astrocyte populations in the mature brain. Often, several of these characteristics must be used to differentially identify this diverse cell type (Walz, 2000; Kimelberg, 2004).

Since there is evidence which demonstrates that multiple astrocyte subtypes exhibit unique K^+ currents, one method of distinguishing astrocyte subtypes is to compare the electrophysiological characteristics of the cells (Steinhauser et al., 1994). Astrocytes can be categorized as either “complex” or “passive” depending upon whether they demonstrated a voltage-dependent outward or an inward rectifying K^+ current, respectively. Using this classification, a pattern of traits supports the existence of at least two subtypes of astrocytes in the mouse hippocampus. These two groups appear to differ in morphology, glutamate uptake currents and the expression of molecules that bind or

transport glutamate (Matthias et al., 2003).

Further research by Zhou *et al.* has revealed that astrocytes exhibit distinct developmental phases where they display different electrophysiological phenotypes (Zhou et al., 2006). Within the developing rat Cornu Ammonis (CA) 1 region of the hippocampus, three astrocytic phenotypes were described: astrocytes that are (a) outward rectifying (ORA), (b) variably rectifying (VRA), and (c) passive rectifying (PA). These phenotypes appear in development in a linear manner beginning with the ORA type that is present and predominates at birth. The VRA phenotype is also present at the time of birth, but to a lesser degree; it shows peak expression around postnatal day (pnd) 5, and then gradually tapers off to very low levels in the adult rat. The ORA phenotype also declines with age, but unlike VRA astrocytes, it disappears around pnd 21. The third astrocyte subgroup, the PA, appears at low levels around pnd 4, doubles its expression levels at pnd 14, and doubles again around pnd 21, making it the predominant astrocyte phenotype in the adult rat CA1 region. The developmental progression of these three cell types include periods of overlap, especially during pnd 21, which suggests that this is a dynamic period of astrocyte differentiation in the rat CA1. This idea is further supported by a parallel progression of the expression of certain cell surface molecules within these phenotypes (Zhou et al., 2006).

Another method of differentiating astrocytes is via the labeling of molecular markers. The cytoskeleton and process morphology of immature astrocytes are primarily made up of the intermediate filaments (IFs) vimentin and nestin (Eliasson et al., 1999). As astrocytes mature, vimentin and nestin are gradually replaced by another intermediate filament, glial fibrillary acidic protein (GFAP). Co-expression of these filament proteins

is common within a period of developmental overlap, but in the mature astrocyte, co-expression only occurs in astrocytes engaged in reactive gliosis (Eliasson et al., 1999). Accordingly, it is not uncommon for the identification of mature astrocytes in the CNS to be made by the presence GFAP (Eng et al., 1971). Using this technique, several subtypes of astrocytes have been described, including the fibrous astrocytes and a protoplasmic variety (Walz, 2000). Fibrous astrocytes, which have long, polarized processes, are mainly attributed to white matter areas, although these processes may extend into gray matter as well. Protoplasmic astrocytes have short, ramified processes and are localized to gray matter areas in the brain. Although both of these cells types have been shown to express GFAP, they do not always label as $GFAP^{(+)}$. Several explanations for this phenomenon have been put forth. One hypothesis is that GFAP expression may be so low within a subset of cells that it is virtually undetectable (Walz, 2000). Support for this argument comes from models of brain trauma and injury. Trauma-induced brain injury that leads to reactive gliosis is characterized by an increase in GFAP immunoreactivity without increasing cell proliferation, suggesting that existing cells that did not previously appear to express GFAP may, under these circumstances, do so (Walz, 2000; Wilhelmsson et al., 2006). Another explanation for $GFAP^{(-)}$ astrocyte-like cells is that immunohistological detection methods may only recognize GFAP that is incorporated into the astrocyte cytoskeleton and fail to stain the soluble subunits, thereby providing a false negative for the presence of GFAP (Stichel et al., 1991).

Using GFAP alone as an astrocytic marker can be problematic for several reasons, in part because not all astrocyte-like cells express GFAP and also because GFAP expression can change during development and under pathological conditions

(Steinhauser et al., 1994; Walz, 2000). Another difficulty with using immunohistological labeling of GFAP to identify astrocytes is that GFAP is predominantly located in astrocyte processes, which prevents visualization of the entire cell. Consequently, astrocytes have been mistakenly described as radial, star shaped cells (hence the name), which has inhibited the ability to study and understand the complete astrocyte landscape. Other immunohistochemical means of astrocyte identification can also be challenging. For instance, some studies have used the calcium binding protein S100β, which is expressed in mature astrocytes (Raponi et al., 2007). However, S100β only labels the cell body, and, therefore, is not optimal for identifying complete cellular morphology either. Furthermore, S100β is not expressed in astrocytes of the germinal zones, so it, too, only labels a subset of astrocytes (Raponi et al., 2007). Other studies have identified astrocytes using GLAST, a glutamate transporter, however, this method carries the same limitations as S100β labeling (Kimelberg, 2004; Nishiyama et al., 2005). Considering the diverse characteristics of astrocytes, it seems that, currently, the most accurate means of distinguishing them may be a combination of immunohistochemical labeling for several markers, including GFAP, S100β, GLAST and/or NG2 (in the case of synantocytes), along with morphological identification using a technique such as dye filling of astrocytes, which permits the entire cell to be visualized (Benediktsson et al., 2005).

Bushong *et al.* used the combined methods of immunolabeling for GFAP and S100β and dye-injecting individual astrocytes in the developing CA1 region of the rat hippocampus to demonstrate that astrocytes undergo several morphological changes throughout neurodevelopment (Bushong et al., 2004). Tracking the progress of

astrocytes across a broad developmental span revealed distinct age-related morphologies that corresponded to patterns of physiological changes that had been previously described. At pnd 7, a network of heterogeneous astrocytes with long, stringy, simplistic processes and some filopodial-like end feet were present. By pnd 14, the cells were much more homogeneous in appearance, with evidence of individual domains beginning to develop. Very fine astrocyte processes that were spongiform in nature became apparent by pnd 21, a time period when neighboring neuronal synapses also adopted a mature appearance. Based on these morphological studies, the shape of many mature astrocytes is now known to be reminiscent of a rounded cube rather than entirely star shaped (Bushong et al., 2004).

Astrocytes arrange themselves in space so that each cell occupies its own individual domain with boundaries that are defined by the spongiform processes. Cells arrange with respect to these domain-forming processes with a characteristic regularity termed "contact spacing", which was first described in the retina (Chan-Ling and Stone, 1991). It is notable that this contact spacing is not created by the more prominent GFAP-containing processes of the astrocyte. In fact, $GFAP^{(+)}$ processes are the minority in many brain regions, making up only about 15% of astrocyte mass in the rat hippocampus, for example (Bushong et al., 2002). Although astrocytes may reach as many as 100,000 synapses, there is very little overlap between the spongiform processes of adjacent astrocytes. In fact, astrocytes only show minor overlap between larger processes and at the boundary interface where finer astrocytic processes connect with one another via gap junctions to form a syncytium (Distler et al., 1991). This arrangement of astrocytes is consistent across brain regions and with other types of glia cells. The Bergmann glia of

the cerebellum, for example, also occupy distinct microdomains of space (Grosche et al., 1999).

The distribution of astrocytes within a given brain area occurs in a region-specific manner. Anatomical areas that are discernable by the distribution of neurons also show consistent patterns of astrocyte density within the same regional boundaries (Emsley and Macklis, 2006). Additionally, the arrangement of astrocytes in some brain regions demonstrate functional borders, such as seen in the hippocampus which has at least two functionally distinct populations of astrocytes (Zhou and Kimelberg, 2001; Zhou et al., 2006). Although the arrangement of astrocytes is regionally consistent and spatially distinct, morphology of individual cells may arise independent of region and be largely determined by the surrounding environment (Bushong et al., 2003).

These data demonstrate that astrocytes exhibit distinct morphological and physiological features during development and that these developmental sequences proceed in tandem and culminate in predictable cellular shape and arrangement. This suggests that astrocytes exhibit a set of characteristics that may be reliably determined. Furthermore, it describes a predictable arrangement of cells that is consistent with the various roles of astrocytes in maintaining brain function.

1.6.3 Biology and function of astrocytes.

Individual astrocytes can make contact with synapses numbering in the hundreds of thousands (Bushong et al., 2002). Within gray matter, astrocytes physically extend processes into synapses, while in white matter they wrap their processes around many axons. Additionally, astrocytes make physical contact with brain vasculature via endfoot

processes whereby they collect metabolic substrates and can induce vascular constriction via Ca^{2+}-dependent second messenger systems (Mulligan and MacVicar, 2004). Astrocytes are also in chemical contact with non-neuronal cell types in the brain, such as oligodendrocytes, and influence their anatomical positions and functions. In this way, astrocytes are able to monitor and respond in real time to an ever-changing environment by providing for the metabolic needs of neurons, regulating extracellular ion concentrations, influencing the number and stability of synaptic formations, synthesizing and recycling transmitter molecules, and facilitating cellular communication via the release of several transmitter molecules including Ca^{2+}, glutamate, and D-serine (Allen and Barres, 2005; Escartin et al., 2006).

Thus, neuronal functioning is irrevocably tied to astrocytes through numerous processes, and, as such, astrocytic dysfunction has the potential to directly and severely impact neuronal activity.

1.6.4 Nutrient metabolism.

Astrocytes monitor local brain activity and respond to activity-driven increases in cerebral blood flow by procuring glucose, the main source of energy in the brain, either from internal stores of glycogen or from nearby vasculature. Astrocytes are anatomically positioned to form a continuum between neurons and the vascular system (Simard et al., 2003; Zonta et al., 2003), making contact with the latter via end foot-bearing processes that express glucose transporter proteins (GLUT) 1 and 2 (Leloup et al., 1994; Leino et al., 1997) (Figure 1.3a). Additionally, this positioning allows astrocytes to participate in the regulation of cerebral blood flow by inducing vascular dilation or constriction as

needed. When astrocytes sense neuronal activity, glucose within the astrocyte undergoes glycolysis, a process that quickly produces lactic acid. Astrocytes release lactic acid which may be taken up by neurons and subsequently converted to energy yielding adenosine triphosphate (ATP) molecules via the tricarboxylic acid cycle (Pellerin and Magistretti, 2004). It has been hypothesized that another mode of delivering metabolic substrate is via the conversion of L-serine to pyruvate, a substance that can be used as a source of lactic acid. This process is performed by serine racemase, an enzyme found predominantly in astrocytes (Foltyn et al., 2005). In these ways, astrocytes rapidly provide neurons with replenishable energy sources. Conversely, disturbing astrocyte metabolism leads to increased vulnerability of neurons to neurotoxic damage (Lian and Stringer, 2004).

1.6.5 Ion metabolism.

Astrocytes participate in the regulation of ion concentrations in the whole brain as well as in the synapse. Bidirectional communication between astrocytes and neurons is accomplished by the release and uptake of ions such as Ca^{2+}, K^+, and H^+. K^+ released during presynaptic depolarization is taken up by adjacent astrocytes and redistributed across an expanse of several astrocytes via gap junction coupling, a process called spatial buffering (Laming et al., 2000) (Figure 1.3b). In this manner, the driving force of the K^+ equilibrium potential moves K^+ from an area of high concentration to an area of low concentration, away from the active neuron via astrocytes. The intra- and extracellular pH of a given area fluctuates during processes such as neuronal activity and metabolism. Astrocytes regulate pH through several processes, including ion exchange and transport.

One way that astrocytes respond to changes in metabolic need is by altering the gene expression of molecules involved in metabolism (Magistretti, 2006). Another method involves altering astrocyte morphology, as well as the morphology of surrounding neurons and synapses.

1.6.6 Signaling.

Another crucial way astrocytes monitor and respond to the environment is through the synthesis, release, and regulation of several signaling molecules such as (a) S100β, (b) Ca^{2+}, (c) glutamate, and (d) D-serine.

a. S100β.

S100β, named for its solubility in 100% saturated ammonium sulfate solution, is a molecule expressed by astrocytes that participates in a multitude of Ca^{2+}-dependent signaling cascades that influence the structure and function of both neurons and astrocytes (Rothermundt et al., 2003). During development, S100β regulates cellular proliferation, differentiation, and synaptogenesis (Rothermundt et al., 2004a), then in the mature CNS, S100β accumulates in astrocytic processes where it modulates enzymatic activities, transcription factors, and protein phosphorylation (Zimmer et al., 1995). S100β expression also influences energy metabolism, calcium homeostasis, and cell growth and differentiation, including the degree of astrocytic process extension, in part, by regulating GFAP phosphorylation-dependent incorporation into the cytoskeleton (Garbuglia et al., 1999; Mbele et al., 2002) (Figure 1.3c). S100β is secreted into the synapse by astrocytes where it is thought to play a role in synaptic plasticity (Nishiyama et al., 2005).

Inhibiting the expression of S100β in cultured astrocytes leads to altered morphology and proliferation of these cells (Selinfreund et al., 1990). Additionally, changes in the expression of S100β can alter neuronal axon and dendrite growth (Whitaker-Azmitia et al., 1997; Nishiyama et al., 2002). Conversely, in the absence of astrocytes, neuronal outgrowth *in vivo* and *in vitro* is severely limited, a phenomenon that may well depend upon S100β functionality (Nagler et al., 2001; Ullian et al., 2001; Ullian et al., 2004). Alterations to the expression of S100β can also induce behavioral changes. The over-expression of S100β in transgenic mice causes impaired spatial learning, decreased exploratory behavior, as well as a significant loss of neuronal branching (Gerlai et al., 1994; Whitaker-Azmitia et al., 1997; Winocura et al., 2001), whereas S100β-null mice, demonstrate enhanced synaptic plasticity, learning, and memory (Nishiyama et al., 2002). The far reaching effects of S100β expression and signaling within astrocytes make clear the importance of this molecule in maintaining astrocyte-related brain function.

b. Ca^{2+} signaling.

Although glia were identified prior to neurons, the study of these cells was quickly cast aside in favor of the electrically excitable, and, therefore, presumably more important neuronal counterparts. However, a renewed interest in astrocytes as active participants in brain signaling emerged in the early 1990s when it was shown that these cells were able to propagate Ca^{2+} waves (Cornell-Bell et al., 1990b; Charles et al., 1991). Astrocyte Ca^{2+} release in culture has been shown to travel about 20μm/s, including around 20-60 astrocytes per wave and affecting an area of about 50 to 300μm (Tian et al., 2006). Astrocytes can also detect changes in external environmental stimuli and set in

motion a series of intracellular responses via transient increases in internal Ca^{2+} levels (Verkhratsky et al., 1998).

The information processing abilities of these cells using internal and external Ca^{2+} are diverse and relatively specific. For example, hippocampal astrocytes can distinguish signaling from the specific axonal pathways and synaptic terminals of thousands of connections with extreme precision, assimilate multiple inputs that may serve to enhance and/or diminish Ca^{2+} signaling, and modulate spatiotemporal features of the signal (Perea and Araque, 2005). The arrangement of astrocytes into distinct microdomains allows internal Ca^{2+} fluctuations to propagate throughout a syncytium of astrocytes via uncoupled gap junctions (Cornell-Bell et al., 1990b; Saez et al., 2003). In this way, astrocytes can monitor, adjust, and respond to a variety of situations as an individual entity or as part of a functional unit. Additionally, astrocyte-to-astrocyte signaling, as well as idirectional signaling between astrocytes and neurons using extracellular Ca^{2+} waves have been shown (Hassinger et al., 1996). Briefly, synaptic release of several signaling molecules, including glutamate, activates astrocytes via various receptors, often ionotropic glutamate receptors or mGluRs. In the case of mGluR-mediated activation, intracellular Ca^{2+} levels increase following the activation of a G-protein coupled pathway involving phospholipase C and inositol 1,4,5-trisphosphate (IP_3), a signaling pathway that has multiple downstream effects (Hansson and Ronnback, 2003), including the release of glutamate and/or ATP that can bind P2X receptors on neighboring astrocytes and results in the further release of Ca^{2+} (Haydon and Carmignoto, 2006) (Figure 1.3d). This activation and extracellular release of Ca^{2+} is directly influenced by the intensity of the synaptic activity initiating the response (Wang et al., 2006).

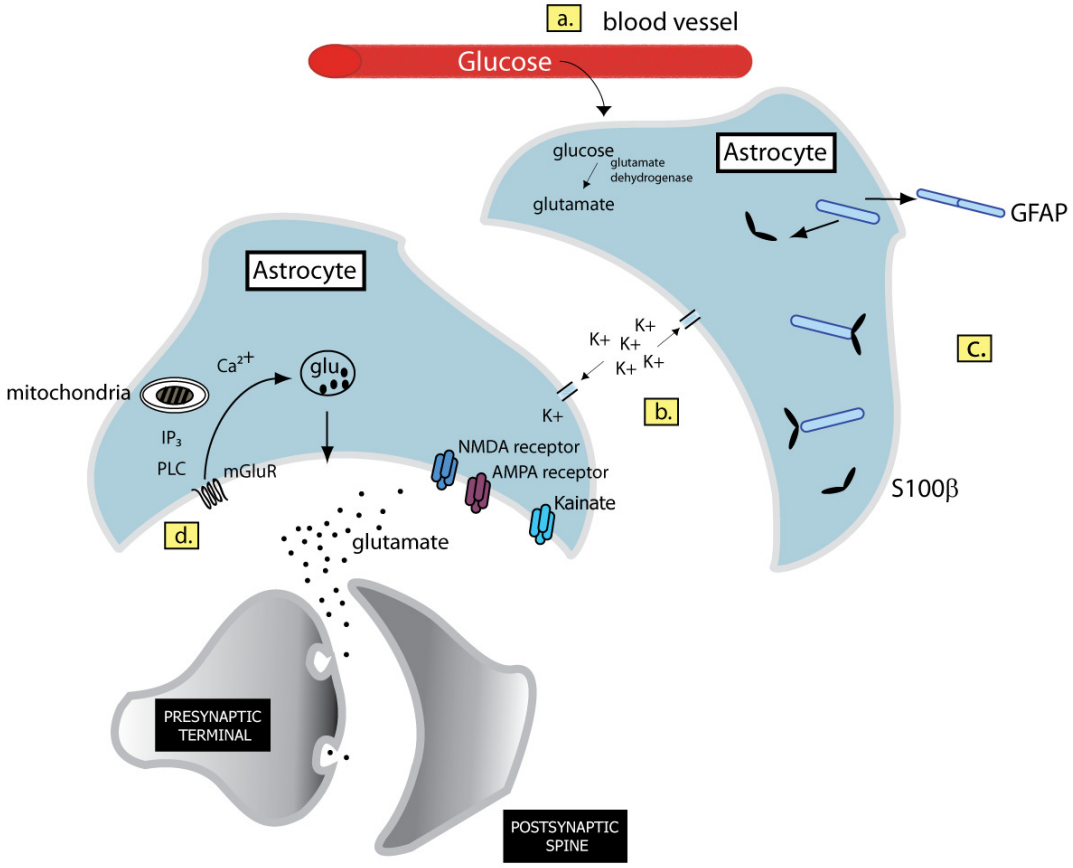

Figure 1.3 Selected functions of astrocytes. (a) Astrocytes respond to activity-driven increases in cerebral blood flow by procuring glucose from nearby vasculature via end foot-bearing processes that express GLUT 1 and 2. (b) Astrocytes regulate pH through several processes, including the ion exchange and transport of K^+ that is released during neuronal activity. (c) S100β expression influences energy metabolism, calcium homeostasis, and cell growth and differentiation, including the degree of astrocytic process extension, in part, by regulating GFAP phosphorylation-dependent incorporation into the cytoskeleton. (d) Glutamate activates various astrocytic receptors such as ionotropic glutamate receptors and mGluRs. During mGluR activation, intracellular Ca^{2+} levels increase via a G-protein coupled pathway involving phospholipase C and IP_3, a signaling pathway that can have multiple downstream cellular effects including the release of glutamate into the synapse.

c. Glutamate signaling.

In addition to bi-directional modulation of synaptic activity via Ca^{2+}, astrocytes can also respond to neuronal activation by releasing chemical transmitters, two of which, glutamate and the NMDA receptor co-agonist D-serine, are a part of a much bigger system involving astrocytes and glutamatergic functioning (Parpura et al., 1994; Schell et al., 1995; Montana et al., 2004; Mothet et al., 2005).

Glutamate is the primary excitatory neurotransmitter in the brain, and astrocytes play a pivotal role in regulating excitatory neurotransmission via the control of transmitter synthesis, release, reuptake, and recycling. Astrocytes can synthesize glutamate from glucose via the enzyme pyruvate carboxylase. Since neurons do not express this enzyme, they are dependent upon astrocytes to provide glutamate both as a metabolic substrate and neurotransmitter signaling molecule (Hertz and Zielke, 2004). Astrocytes also transport glutamate from the synapse following excitatory release via high affinity excitatory amino acid transporters (EAATs) types 1, 2, 3, and 4 (Conti et al., 1998; Sonnewald et al., 2002; Hu et al., 2003; Kim et al., 2003; Su et al., 2003) (Figure 1.4a). One fate of intracellular glutamate is conversion to glutamine by the astrocytic enzyme glutamine synthetase, an ATP-dependent enzyme detectable early in development in most species and in most tissue types that synthesize glutamine from glutamate (Suarez et al., 2002) (Figure 1.4b).

Within the brain, glutamine synthetase is primarily located in the cytoplasm of astrocytes where, in addition to maintaining the glutamate-glutamine cycle, it is responsible for nitrogen metabolism and the detoxification of ammonia. Furthermore, the activity of glutamine synthetase may modulate molecular correlates of memory, such as

long term potentiation. One study showed that when methionine sulfoximine is used to block the actions of glutamine synthetase in chicks, the ability to consolidate memories was abolished, a process that was preventable when the chicks were administered glutamine (Gibbs et al., 1996).

Glutamate that has been taken up into astrocytes from the synapse can be utilized by neurons both as metabolic fuel and as a transmitter molecule. Glutamate, however, cannot be transported directly to neurons via the synapse because of the threat of excitotoxicity and inadvertent activation of glutamatergic receptors (Danbolt, 2001). Glutamate must first be converted to glutamine via glutamine synthetase before being transported to nearby neurons for subsequent release (Had-Aissouni et al., 2002) (Figure 1.4b). Once glutamine is relocated back into the presynaptic neuron, it may be converted to glutamate by the enzyme phosphate-activated glutaminase and packaged into synaptic vesicles by vesicular transporters (Bellocchio et al., 2000; Takamori et al., 2000) (Figure 1.4c). The presynaptic neuron then possesses stored neurotransmitter that may be subsequently released, thus completing a cycle that is crucial for the survival of neurons, maintaining synaptic efficacy and diversifying signaling.

d. D-serine signaling.

The emergence of D-serine as an endogenous modulator of the NMDA receptor and as a putative "gliotransmitter" molecule was rather recent. Despite knowledge that a catabolizing enzyme for D-amino acids existed, proof of D-amino acids, aside from those related to dietary intake, was not available. However, a D-configuration of serine was eventually shown to exist in physiologically relevant quantities in the mammalian brain (Hashimoto et al., 1992). Subsequently, D-serine was found to bind with high affinity to

the glycine site on the NMDA receptor, with three times more potency than glycine (Matsui et al., 1995; Schell et al., 1995; Wolosker et al., 1999b).

The release of D-serine from astrocytes *in vitro* has been shown to be mediated by AMPA, kainate, and mGluR glutamate receptors (Schell et al., 1995; Martineau et al., 2005; Fujii et al., 2006). Evidence for vesicular storage and release of D-serine (as well as for glutamate and ATP) has been demonstrated to occur in astrocytes by a SNARE (soluble N-ethylmaleimide-sensitive factor attachment protein receptor)-dependent mechanism (Coco et al., 2003; Bezzi et al., 2004; Mothet et al., 2005). Release via gap-junction hemichannels or transmembrane transport systems are other methods of ATP, glutamate, and possibly D-serine release (Ribeiro et al., 2002; Volterra and Meldolesi, 2005). Increases in synaptic glutamate, the result of neuronal activation, trigger the release of D-serine from astrocytes into the synapse where, along with glutamate, it binds to and activates the NMDA receptor (Martineau et al., 2005). This role in NMDA receptor activation specifically implicates astrocytes and D-serine in NMDA receptor-mediated LTP that occurs in learning and memory (Kelley et al., 2003; Martineau et al., 2005). For example, learning and memory deficits in rats that demonstrate age-related alterations in LTP can be rescued by the addition of exogenous D-serine (Mothet et al., 2006). Conversely, in the absence of astrocytes, cultured hippocampal neurons are unable to elicit NMDA receptor-induced LTP (Yang et al., 2003).

D-serine appears to be removed from the synapse through a variety of transporters located on both neurons and astrocytes (Yamamoto et al., 2001; Javitt et al., 2002). The degradation of D-serine is accomplished, in part, by D-amino acid oxidase (DAAO), an enzyme expressed in astrocytes in a regionally distinct manner, mainly in the cerebellum

and hindbrain, that converts D-serine to α-keto acid, ammonia, and H_2O_2 (Moreno et al., 1999; Cook et al., 2002; Urai et al., 2002). Further evidence of the importance of D-serine to NMDA receptor activity is shown by the fact that in the presence of DAAO, NMDA receptor-mediated activation is inhibited (Hashimoto et al., 1993; Mothet et al., 2000). It is notable that the areas which express high levels of DAAO are relatively devoid of D-serine expression, which may suggest other regionally-specific methods of D-serine degradation.

D-serine is synthesized by the enzyme serine racemase, a predominantly astrocytic, PLP-dependent enzyme that co-localizes with D-serine (Schell et al., 1995; Schell et al., 1997; Wolosker et al., 1999a; Miranda et al., 2000; Yasuda E, 2001) (Figure 1.4d). This enzyme has been proposed as the main regulator of both intra- and extracellular D-serine concentrations in the forebrain via conversion of L-serine to D-serine following glutamate-induced activation of kainate and AMPA receptors and by an α, β-elimination reaction, respectively (Foltyn et al., 2005; Kim et al., 2005). The α, β-elimination of D-serine by serine racemase is the complement or perhaps competition to DAAO-mediated breakdown in the hindbrain. Nitric oxide (NO) has been shown to play a role in determining which of these methods predominates at a given synapse, inhibiting serine racemase mediated breakdown of D-serine and enhancing that of DAAO (Shoji et al., 2006a, b). Conversely, a feedback system for D-serine-mediated regulation of NO synthase, the synthesizing enzyme of NO, has also been proposed (Shoji et al., 2006a).

Yeast-two hybrid experiments suggest that the activity of serine racemase may be modulated, at least in part, by its binding with glutamate receptor interacting protein (GRIP) (Kim et al., 2005). GRIP, typically bound to AMPA receptors, is released by

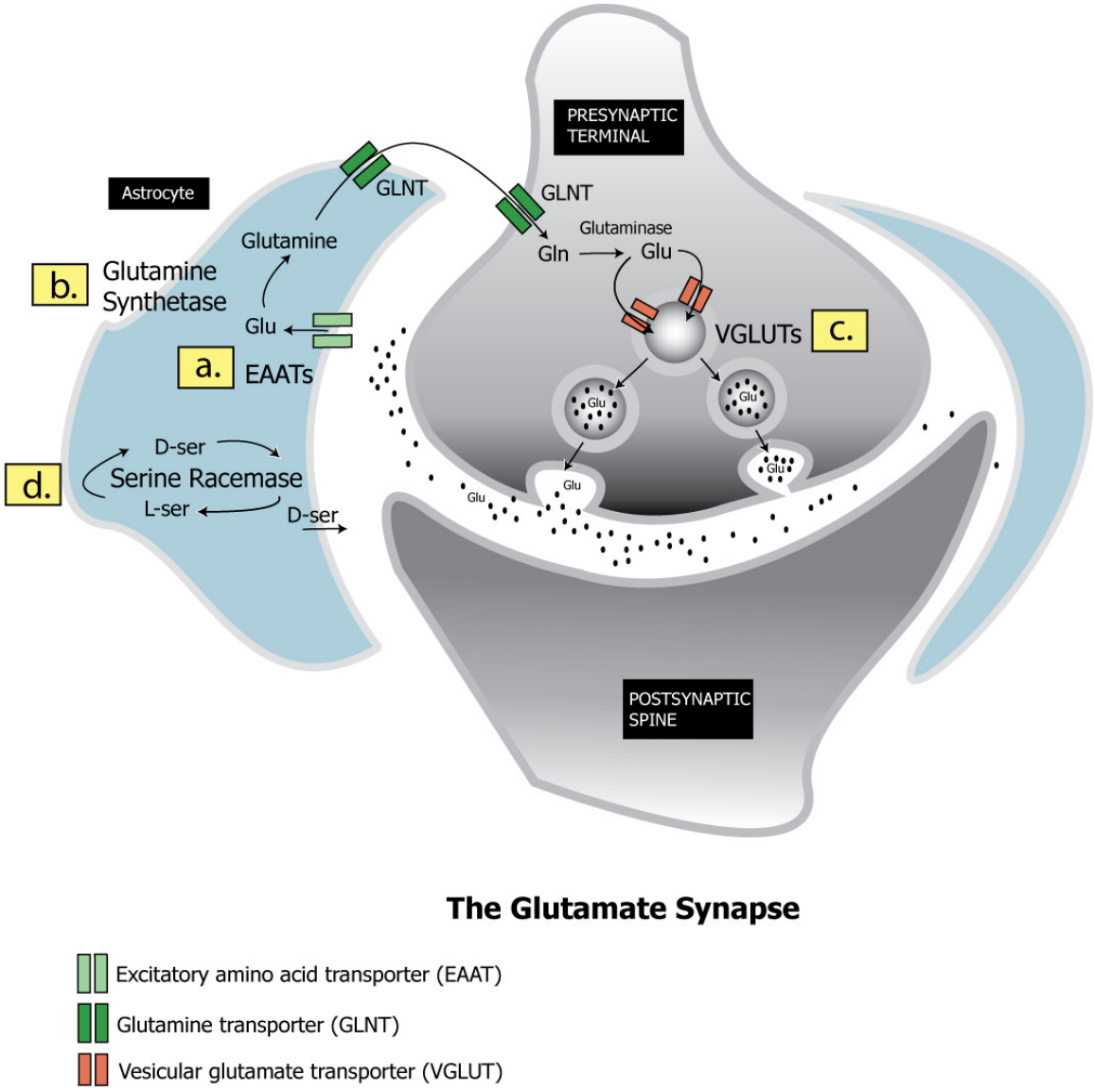

Figure 1.4 An astrocyte-mediated glutamate cycle. (a) Astrocytes transport glutamate from the synapse following excitatory release via high affinity EAAT1, 2, 3, and 4. Glutamate cannot be transported directly to neurons via the synapse because of the threat of excitotoxicity and inadvertent activation of glutamatergic receptors. (b) One fate of intracellular glutamate is conversion to glutamine by the astrocytic enzyme glutamine synthetase. (c) Once glutamine is relocated back into the presynaptic neuron, it may be converted to glutamate and packaged into synaptic vesicles by vesicular glutamate transporters (VGLUTs). (d) The NMDA receptor co-agonist, D-serine, is synthesized by the enzyme serine racemase.

receptor activation and made available to bind serine racemase, which in turn stimulates increased D-serine production. Another protein that interacts with serine racemase, possibly modulating its activity, is PICK1 (protein interacting with C-kinase) (Fujii et al., 2006). Both GRIP and PICK1 interactions with serine racemase occur via a PDZ protein interaction domain, but the effects of PICK1 binding are as yet unknown. Recently, it was shown that NO can also alter serine racemase activity by removing ATP via *S*-nitrosylation, a mechanism likely modulated by activation of the NMDA receptor (Mustafa et al., 2007).

Until recently, the synthesis of D-serine by serine racemase was thought to be astrocyte specific. More recent evidence shows that a subset of glutamatergic neurons also expresses this molecule, albeit in lesser quantities, in the cerebellum and hindbrain (Kartvelishvily et al., 2006; Williams et al., 2006). Additionally, primary cultures of rat neurons have been shown to express serine racemase protein and transcript at relatively high levels (Yoshikawa et al., 2006). However, cultured rat hippocampal neurons were unable to elicit NMDA receptor activation in the absence of astrocytes, even when supplemented with glial conditioned medium, suggesting that astrocytes are the primary source of D-serine in the brain (Yang et al., 2003). The reported distribution of D-serine in the brain has also been revised recently. Localization of D-serine containing astrocytes in the forebrain was thought to closely mirror that of the NMDA receptor. However, D-serine-expressing astrocytes have now been demonstrated in areas that express low levels of NMDA receptors, such as in white matter, opening up the possibility for as yet undiscovered functions of D-serine (Schell et al., 1997; Yasuda et al., 2001; Williams et al., 2006).

1.6.7 Morphology.

Every astrocyte-mediated function discussed so far requires that astrocytes respond to the changing needs around them, and this is mediated by the ability of astrocytes to alter process morphology. Astrocytes demonstrate considerable structural plasticity and will elongate processes to accommodate their environment, for example, by reaching toward blood vessels or extending processes in parallel with neuronal process extensions to monitor activity in adjacent synapses (Cornell-Bell et al., 1990a; Bushong et al., 2002). This plasticity is made possible by Ifs, such as GFAP, which are incorporated into the cytoskeleton to influence process morphology. GFAP molecules are often in a state of fluctuating polymerization and depolymerization. The assembly or disassembly of these filaments is regulated by kinases that phosphorylate GFAP at the N-terminus. The incorporation of GFAP into the cytoskeleton is mediated by several different signaling molecules, including Ca^{2+}, glutamate, and the calcium binding protein S100β (Rodnight et al., 1997; Kommers et al., 2002; Frizzo et al., 2004; Chang et al., 2005).

GFAP, arguably the most recognized cytoskeletal component of the astrocyte, is expressed throughout astrocytic processes and is used as the standard identifying marker for mature astrocytes in the CNS. Although the entire functional repertoire of GFAP and other intermediate filament proteins is not well understood, research shows that changes in its expression levels influence not only astrocytic and neuronal structure and function, but also animal behavior, suggesting that GFAP plays an important role in modulating brain physiology.

Mice devoid of GFAP due to a targeted deletion *in utero* develop and mature

normally, demonstrating normal astrocyte density and no compensatory upregulation of the IF vimentin (Pekny et al., 1995; McCall et al., 1996). While the animals retain the ability to elicit a response to brain trauma by reactive gliosis, it is mediated by vimentin rather than GFAP. Vimentin knock out mice ($Vim^{-/-}$), on the other hand, demonstrate disorganized assembly of GFAP filaments, a phenomenon that seems to affect only subsets of astrocytes. Gray matter astrocytes in the cerebellum of these mice were completely devoid of IFs and had abnormal morphology, while white matter astrocytes appeared normal and contained IFs (Galou et al., 1996). Immunostaining primary astrocyte cultures of $Vim^{-/-}$ cells demonstrated reduced GFAP levels and other studoes revealed that GFAP mRNA levels were also significantly reduced. While overall GFAP protein levels were normal, the soluble fraction of GFAP was significantly increased in $Vim^{-/-}$ cells. Taken together, these results suggest that both the transcription and cytoskeletal assembly of GFAP in astrocytes, or in a subset of astrocytes, may be regulated by vimentin (Galou et al., 1996).

When GFAP expression is decreased, astrocytes exhibit a profound loss of process extension. Astrocytes typically respond to the presence of neurons by extending processes toward them; however, studies have shown that blocking GFAP expression with antisense mRNA in human astrocytoma cells prevented this process extension (Weinstein et al., 1991), and that this ability was restored when GFAP was re-introduced to the cell line (Chen and Liem, 1994). Mice with disrupted GFAP expression have shorter astrocytic processes that are also smaller in diameter than those of wild type mice (McCall et al., 1996; Shibuki et al., 1996). Functionally, changes to GFAP expression, and therefore to astrocyte process morphology, may impact homeostatic functions such as

metabolism and ion regulation.

The survival and outgrowth of neurons has been shown to be under the influence of astrocytes (Goldberg and Barres, 2000). Interestingly, the loss of GFAP and vimentin in transgenic mice has been shown to *increase* neuronal survival and outgrowth. The loss of GFAP, but not vimentin, was shown to be responsible for the enhanced survival, a phenomenon mediated mainly by cell-to-cell contact and to a lesser extent by astrocytic releasing factors (Menet et al., 2001). Other studies have shown that loss of GFAP in transgenic mice results in white matter abnormalities, which, like symptoms of schizophrenia, emerge in adulthood (Liedtke et al., 1996). Recently, Alexander disease, a fatal, neurodegenerative condition, was found to be caused by missense mutations in GFAP (Brenner et al., 2001). These mutations result in abnormal cytoplasmic aggregations of GFAP within the astrocyte called Rosenthal fibers (Perng et al., 2006). In addition to neurodegeneration and abnormal aggregates of GFAP, Alexander disease is also characterized by a dramatic loss of myelination (Johnson, 2002). This suggests that astrocytes and more specifically GFAP expression can influence the survival and function of both neurons and oligodendrocytes.

Other animal models have suggested a role for astrocytes and GFAP expression in synaptic plasticity. Mice with disrupted GFAP expression exhibit important behavioral changes including impaired eyeblink conditioning, enhanced LTP, and reduced long term depression (LTD) in the cerebellum (McCall et al., 1996; Shibuki et al., 1996). The mechanism by which this took place was unclear until several studies demonstrated that polymerization of GFAP into the astrocyte cytoskeleton is regulated by phosphorylation induced by type II mGluRs in the hippocampus and NMDA receptors in the cerebellum

(Kommers et al., 1999; Kommers et al., 2002; Battu et al., 2005).

The role of cytoskeletal proteins as mediators of morphology has been expanded to also a role in glutamatergic transport. It has been shown that GFAP is required for the trafficking and expression of glutamate transporters in both astrocytes and neurons. Decreased expression of EAAT1 and EAAT3 has been demonstrated in the hippocampus of GFAP-null mice (Hughes et al., 2004) (Table 1.1). Additionally, the neuronal transporters in these mice were found to be localized in the cell body instead of within dendrites where they typically are expressed. Increased expression of the astrocytic transporter EAAT2 was found in the cerebellum of GFAP-null mice, which may account for previous reports of deficient LTD in these animals.

1.7 Glial dysfunction in schizophrenia

A developmental hypothesis of schizophrenia suggests that when paired with genetic vulnerability, a perturbation of the developmental processes of neurogenesis, differentiation, migration, and/or synapse formation may contribute to the structural and functional defects that are often present in this illness. However, neurons are not the only cell type affected either directly or indirectly when development is perturbed. In fact, a postmortem gene array profile established that the genes most frequently altered in schizophrenia were related to glial function (Sugai et al., 2005).

1.7.1 Oligodendrocyte abnormalities in schizophrenia.

Oligodendrocyte-related abnormalities associated with psychosis have been documented in diseases of demyelination and in literature describing changes to brain

volume, cellular density and morphology, and gene expression in schizophrenia. For example, diseases such as metachromatic leukodystrophy and velocardiofacial syndrome are characterized by loss of white matter and demyelination, and, like schizophrenia, result in psychosis during adolescence and early adulthood (Hyde et al., 1992; Walterfang M, 2005). Abnormal expression of myelin-related proteins, such as myelin basic protein, has been discovered in the brains of patients with schizophrenia and depressed suicide victims (Honer et al., 1999). Furthermore, *in vivo* imaging studies in schizophrenia reveal loss of white matter volume, which appears to be present at the first onset of illness that is associated with a predominance of negative symptoms (Buchanan et al., 1998; Cannon et al., 1998; Sanfilipo et al., 2000; Pagsberg et al., 2007).

At the cellular level, a study of postmortem brain tissue by Uranova *et al.* reported profound changes in oligodendrocytes that indicated that many of these cells were undergoing apoptosis or were already necrotic in schizophrenia (Uranova et al., 2001). Abnormalities included changes in cell morphology and key intracellular organelles such as nuclei, mitochondria, and endoplasmic reticulum, evidence of cell lysis near blood vessels, and loss or aberrant forms of myelination in the PFC and caudate nucleus. More recently, a 25% reduction in oligodendrocyte density in layer VI of the PFC was reported in schizophrenia, suggesting that apoptosis and overall degeneration of these cells may be characteristic of this illness (Uranova et al., 2004). Consistent with these data, another study demonstrated reduced oligodendrocyte density, as well as a reduction in oligodendrocyte to neuron ratio, in the thalamus in schizophrenia (Byne et al., 2006).

There are aslo studies that have revealed altered gene expression of molecules that provide and maintain myelination in schizophrenia. A study using DNA microarray

analysis showed that gene expression of five oligodendrocyte-related genes [myelin and lymphocyte protein (MAL), gelsolin, 2',3'-cyclic nucleotide 3'-phosphodiesterase (CNP), myelin-associated glycoprotein (MAG), transferrin, and Her3 (ErbB3)] were decreased in the PFC in schizophrenia (Hakak et al., 2001). These results are supported by McCullumsmith *et al.*, who found decreased transcript expression for several of the same proteins, including CNP, MAG, and transferrin, as well as the protein quaking, in white matter of the anterior cingulate cortex (ACC) in patients with schizophrenia (McCullumsmith et al., 2007). Dracheva *et al.* recently reported decreased mRNA and protein expression of molecules related to oligodendrocyte function in the ACC and hippocampus but not in the putamen in schizophrenia, indicating that these changes occur in a region-specific manner (Dracheva et al., 2006). New data are beginning to emerge which identify specific genes that may impart susceptibility to oligodendrocyte- and myelination-related dysfunction in schizophrenia. For example, oligodendrocyte lineage transcription factor 2 (OLIG2), a key molecule in developing and maintaining white matter functionality, has been identified as one of several genes implicated in this illness (Georgieva et al., 2006). Taken together, these data suggest that oligodendritic dysfunction is a prominent feature of the pathophysiology of schizophrenia. Whether this dysfunction arises during development, is secondary to neuronal abnormalities, or is a combination of multiple factors is unclear. Additional data suggest that other glial cells, such as astrocytes, may also play a role in this illness.

1.7.2 Astrocyte abnormalities in schizophrenia.

A developmental hypothesis for schizophrenia is a viable model; however, to

suggest that it would exclusively involve just one cell type, neurons, seems unrealistic. The involvement of astrocytes, especially in the two currently predominant hypotheses of schizophrenia, developmental and glutamatergic dysfunction, must be examined.

Radial glia, the early glial progenitor cells in the brain play an important role in early brain development. These cells regulate several processes, including neurogenesis, differentiation, and migration (Weissman et al., 2004; Gotz and Huttner, 2005). Studies suggest that alterations in the structure and function of radial glia could have a profound impact on the developing brain. For instance, early postnatal ablation of mouse brain astrocytes profoundly affects neuronal morphology, survival, and maturation (Delaney et al., 1996). Furthermore, a model of disturbed radial glia in the developing mouse brain demonstrates that abnormally shaped and irregularly organized glial cells contribute to a malformed cortical lamina (Ueda et al., 1997). The deleterious effects of ethanol on fetal brain development also appear to involve an impairment of radial glia function, which in turn disturbs neuronal migration (Guerri et al., 2001). At later developmental stages the astrocytes derived from these radial glial cells are morphologically and functionally disturbed, which further impairs brain function. Together these results confirm that malfunctioning radial glia can result in a compromised cortical lamina and that the already tenuous neurons that do survive may be further compromised by insufficient astrocyte support.

Signaling molecules expressed by astrocytes, such as S100β, have been examined in schizophrenia as a marker of astrocytic integrity. Multiple studies have provided evidence for increased S100β in the serum or cerebrospinal fluid (CSF) of patients with schizophrenia (Wiesmann et al., 1999; Lara et al., 2001; Rothermundt et al., 2001;

Schroeter et al., 2003; Rothermundt et al., 2004b; Rothermundt et al., 2004a; Schmitt et al., 2005; Rothermundt et al., 2007). These increased serum and CSF levels have been interpreted as a marker of structural damage, perhaps reflecting cells that are leaking S100β into the periphery, and alternatively as a sign of increased astrocyte activation (Wiesmann et al., 1999). On the other hand, increased S100β expression may occur due to genetic reasons, rather than being a result of the schizophrenia disease process. A S100β gene haplotype associated with schizophrenia has been shown to be involved with increased S100β expression (Liu et al., 2005). Another explanation for increased S100β levels in patients with schizophrenia may be treatment with antipsychotic medication. One study that compared recently medicated and non-medicated patients demonstrated that only medicated patients exhibited increased serum levels of S100β (Schroeter et al., 2003). However, increased S100β serum levels have also been demonstrated in individuals experiencing their first onset of the illness, never-before medicated patients, as well as in patients who were previously treated, but medication-free at the time of the study (Lara et al., 2001; Rothermundt et al., 2001; Steiner et al., 2006). Moreover, there is evidence that antipsychotic treatment can normalize S100β levels in some individuals. One study showed that six weeks of antipsychotic treatment significantly reduced S100β levels in half of the patients, although the other patients maintained significantly elevated levels compared to the nonpsychiatrically ill comparison group (Rothermundt et al., 2001). These conflicting results suggest that S100β expression is variable in schizophrenia, and evidence suggests that differences in S100β expression may delineate a particular subset of individuals in this illness. Multiple studies have shown a positive correlation between S100β levels and severity of thought disturbance and predominance

of negative symptoms in schizophrenia; positive symptoms, on the other hand, are not predictive of S100β levels (Lara et al., 2001; Rothermundt et al., 2001; Schroeter et al., 2003; Rothermundt et al., 2004b; Rothermundt et al., 2004a). Conversely, there is other evidence for a negative correlation between S100β levels and disease duration (Lara et al., 2001). These data for abnormal sera and CSF S100β levels, as well as the tendency for these changes to accompany negative symptomology, suggest S100β is an astrocytic molecule that may contribute to brain dysfunction in schizophrenia.

Additional support for the theory of disturbed astrocyte function playing a role in the pathophysiology of schizophrenia stems from the lack of gliosis in the brains of patients with this illness. This factor has typically been used to argue for developmental origins and against degeneration or post-maturational changes in schizophrenia. However, a lack of gliosis may simply portray a system that is significantly compromised and unable to respond in its normal manner. For instance, one postmortem study demonstrated that astrocyte-mediated gliosis in the brains of individuals with schizophrenia had a much weaker response to lobotomy-induced brain insult compared to a non-psychiatrically ill group (Niizato et al., 2001). There is evidence for situations in which prenatal viral exposure (most likely paired with genetic predisposition) can result in a psychiatric condition without evidence of gliosis (Sawa et al., 2004). Transgenic mice with glial cells selectively expressing Borna disease virus phosphoprotein (BDV-P) develop behavioral and structural brain abnormalities consistent with neurodevelopment insult without any evidence of gliosis (Kamitani et al., 2003).

In direct contrast to gliosis, early studies of astrocyte-related pathology in schizophrenia reported the loss of astrocytes in several cortical areas already known to

contain neuronal abnormalities, including the PFC, ACC, and motor cortex (Benes et al., 1986; Stark et al., 2004). Furthermore, localized astrocyte density reductions in layer VI of the ACC and layer V of the PFC suggest that some cortical regions demonstrate lamina-specific pathology in schizophrenia (Cotter et al., 2001; Cotter et al., 2002). Additionally, reports of altered astrocyte density around blood vessels in the PFC suggest a loss of astrocyte-mediated metabolic functioning in schizophrenia (Webster et al., 2001). These studies demonstrate regionalized astrocytic deficits and suggest that structural and functional abnormalities seen in schizophrenia may, in part, result from a loss of astrocytes.

1.7.3 Astrocyteinvolvement in glutamate system abnormalities.

Glutamate dysfunction is broadly implicated in schizophrenia; however, the majority of glutamatergic abnormalities reported in schizophrenia appear to target neurons. Astrocytes play numerous roles vital to glutamatergic transmission. As mentioned previously, a critical function of astrocytes at the glutamatergic synapse is the reuptake of glutamate, which is accomplished by high affinity EAATs. The disruption of glutamate reuptake, either by increased or decreased expression of these transporter molecules, could impart grave consequences for neuronal signaling and survival. Mice lacking the glutamate transporter GLT-1 (the equivalent of EAAT2 in humans) have been shown to exhibit increased extracellular glutamate levels and increased vulnerability to brain insult and excitotoxicity (Mitani and Tanaka, 2003) (Table 1.1). In humans, decreased glutamate transport caused by polymorphisms in the promoter region of EAAT2 has been associated with increased neurodegeneration following stroke (Mallolas

Table 1.1 Altered expression of excitatory amino acid transporters in schizophrenia

EAAT1				
thalamus	mRNA	increase		*(Smith, 2001)*

EAAT2				
PFC	mRNA	decrease	(cell level change only, not regional)	*(Ohnuma, 1998)*
PFC	mRNA	increase	(medication-free patients)	*(Matute, 2005)*
PFC	mRNA	decrease	(typical antipsych.)	*(Matute, 2005)*
Thalamus	mRNA	increase		*(Smith, 2001)*
HC	mRNA	decrease		*(Ohnuma, 2000)*
ACC	protein	increase	(EAAT2 interacting protein, GPS1)	*(Bauer, unpub.)*

EAAT3				
Thalamus	mRNA	increase	(EAAT3 interacting protein, JWA)	*(Huerta, 2006)*
ACC	mRNA	increase	(EAAT3 interacting protein, JWA)	*(Bauer, unpub.)*
ACC	protein	increased	(EAAT3 interacting protein, GPS1)	*(Bauer, unpub.)*

EAAT4				
Thalamus	mRNA	increase	(EAAT4 interacting protein, KIAA)	*(Huerta, 2006)*
DLPFC	mRNA	increase	(EAAT4 interacting protein, ARH)	*(Bauer, unpub.)*
ACC	mRNA	increase	(EAAT4 interacting proteins, ARH, KIAA)	*(Bauer, unpub.)*
ACC	protein	increase	(EAAT4 interacting protein,GPS1)	*(Bauer, unpub.)*

EAATs EXPRESSION INTERACTIONS					
Rat thalamus	mRNA	decrease	GPSI (EAAT2)	(clozapine)	*(Huerta, 2006)*
Rat thalamus	mRNA	decrease	JWA (EAAT3)	(clozapine)	*(Huerta, 2006)*
Rat thalamus	mRNA	increase	KIA (EAAT4)	(clozapine)	*(Huerta, 2006)*
Rat thalamus	mRNA	decrease	ARHGEF11 (EAAT4)	(clozapine)	*(Huerta, 2006)*
Rat Striatum	mRNA	decrease	EAAT2	(haloperidol, clz)	*(Schneider, 1998)*
Mouse CTX	protein	increase	EAAT1 (total)	(GFAP null)	*(Hughes, 2004)*
Mouse CTX	protein	decrease	EAAT1, 3 (synaptisomal)	(GFAP null)	*(Hughes, 2004)*
Mouse HC	protein	increase	EAAT2 (total)	(GFAP null)	*(Hughes, 2004)*
Mouse HC	protein	decrease	EAAT3 (total)	(GFAP null)	*(Hughes, 2004)*
Mouse CB	protein	increase	EAAT2 (synaptisomal)	(GFAP null)	*(Hughes, 2004)*
Cell culture	protein	decrease	EAAT1, 2	(mGluR (I) agonists)	*(Aronica, 2003)*
Cell culture	protein	increase	EAAT1, 2	(mGluR (II) agonists)	*(Aronica, 2003)*
Cell culture	protein	decrease	EAAT3	(mGluR (II) agonists)	*(Aronica, 2003)*

Abbreviations: anterior cingulate cortex (ACC), cerebellum (CB), cortex (CTX), dorsolateral prefrontal cortex (DLPFC), excitatory amino acid transporters (EAATs), hippocampus (HC), prefrontal cortex (PFC)

et al., 2006). In schizophrenia, there have been several studies that have document altered expression of astrocytic EAATs (Matute et al., 2005; Ohnuma et al., 1998; Ohnuma et al., 2000; Smith et al., 2001a). In the context of schizophrenia-related glutamate hypofunction, it is notable that astrocytes can play a role in the down-regulation of EAAT expression via treatment with antipsychotics. Synaptic glutamate activity can closely regulate the expression of EAAT molecules in astrocytes (Watanabe et al., 1999). This is accomplished, in part, by mGluRs and EAAT-interacting proteins, which have both been shown to be altered in schizophrenia (Ohnuma et al., 1998; Gupta et al., 2005; Huerta et al., 2006).

Other roles of astrocytes in the glutamate synapse are bidirectional signaling with neurons and mediation of neuronal synchrony via NMDA receptors (Fellin et al., 2004). Consistent with these roles, astrocytes have been implicated in dysfunction of the NMDA receptor in schizophrenia. Specifically, D-serine, an NMDA receptor co-agonist synthesized by astrocytes, has been implicated in the pathophysiology of schizophrenia. Recent studies document decreased levels of D-serine in the serum and CSF of patients with schizophrenia (Hashimoto et al., 2003; Bendikov et al., 2007). Additionally, patients with schizophrenia who concurrently received D-serine and standard antipsychotic treatment showed improvement in positive and negative symptoms as well as in cognition (Tsai et al., 1998; Tsai et al., 1999; Heresco-Levy et al., 2005). The prominent association of glutamatergic dysfunction in schizophrenia with D-serine also implicates the synthesizing enzyme serine racemase. Recently, an allele for the gene encoding serine racemase was associated with schizophrenia that suggests reduced serine racemase expression can distinguishs a subtype of patients with paranoid schizophrenia

(Morita et al., 2007).

Another molecule linking D-serine with NMDA receptor dysfunction is the catabolizing enzyme DAAO. Several studies have found significant linkage between schizophrenia and genes that both encode as well as regulate D-amino acid oxidase, including D-amino acid oxidase activator (DAOA) (previously referred to in the literature as G72), and an associated gene, G30 (Chumakov et al., 2002; Korostishevsky et al., 2004; Schumacher et al., 2004). Alterations of these genes are consistent with a hypothesis of abnormal NMDA receptor activity in schizophrenia, as oxidation of D-serine by DAAO has been shown to reduce NMDA receptor activity, while increased NMDA receptor activity has been shown to stimulate transcript expression of DAAO (Mothet et al., 2000; Yoshikawa et al., 2004; Almond et al., 2006).

These data, together with studies demonstrating that gene expression changes in schizophrenia are predominantly comprised of those related to glia cell function, argue that astrocytes are compromised in schizophrenia, and furthermore, may be dynamically contributing to an already severely affected system.

1.8 Conclusions

There is a body of literature that points to a developmental hypothesis of schizophrenia. Genetic and environmental factors may work together to dysregulate normal neurodevelopmental processes and thereby give rise to disorganized connectivity across several brain regions. Furthermore, while this disturbed neural connectivity may only initially give rise to subtle functional and behavioral abnormalities, it may be particularly vulnerable to intense synaptic pruning that occurs during adolescence. As a

result, since circuits were not functioning optimally to begin with, a dramatic reduction of synaptic connections may precipitate a sudden magnification of dysfunction and the emergence of schizophrenic symptomology.

While the majority of studies to date have focused on neuronal pathology and possible perturbations of neuronal development in schizophrenia, more recent work points to developmental alterations that could also impact glial structure and function. Astrocytes provide a multitude of crucial functions in the brain and there is growing evidence to suggest that astrocytes have a contributing role in the pathophysiology of schizophrenia. Examining the molecular components of astrocytes that facilitate brain function will help determine whether there is a relationship between the expression and function of astrocytes and the dysfunction seen in schizophrenia. Deriving a disease pathway that involves altered expression and/or activity of molecules governing astrocyte-mediated brain function will require a reappraisal of the existing neuro-centric disease model of schizophrenia. *Consequently, the major aim of this dissertation research is to examine whether key structural and functional molecules expressed by astrocytes are altered in the brain in schizophrenia, and, furthermore, to determine whether astrocytes contribute to glutamatergic dysfunction in schizophrenia.*

1.9 Goals of dissertation research

I hypothesize that there is an astrocytic component to the pathophysiology of schizophrenia and that key structural and functional molecules expressed by astrocytes are abnormally expressed in this illness. Previous research suggests that glutamatergic function that is prominent in schizophrenia may have an underlying astrocytic

component. Furthermore, I hypothesize that schizophrenia may be characterized by a global astrocytic lesion. This dissertation work is comprised of three sets of experiments designed to examine the transcript and protein expression of specific structural and functional astrocytic molecules in multiple brain regions, including the dorsolateral prefrontal, anterior cingulate, superior temporal, and primary visual cortices, as well as in the hippocampus.

Chapters two and three will describe findings for the transcript and protein expression of GFAP, S100β, and glutamine synthetase, molecules linked to astrocyte structure and function. Chapter three also details the protein expression of several of these molecules in haloperidol treated rats, to determine whether any expression changes found in postmortem studies could be attributed to a medication effect. Chapter four will detail a multi-regional study of the expression of an enzyme only recently linked to schizophrenia, serine racemase.

The ability of astrocytes to monitor and respond to changes in the environment depends, in part, upon the ability to alter process morphology. Therefore, I hypothesize that cytoskeletal proteins such as GFAP and the molecules that regulate incorporation into the cytoskeleton, such as S100β, are abnormally expressed in schizophrenia. The impetus for studying S100β, besides its role in regulating GFAP, comes from genetic studies implicating increased S100β expression in schizophrenia. In addition, individuals with schizophrenia have altered S100β levels which positively correlate with symptom severity. Surprisingly, in light of these factors linking S100β to schizophrenia, this protein has been relatively unexamined. The study of GFAP expression in schizophrenia has already uncovered several abnormalities; however, only a limited number of brain

regions have been investigated. My intention is to determine whether GFAP expression changes will replicate those already published, and to expand the existing literature to include several brain regions in which GFAP had not yet been examined.

Alterations of multiple molecules involved in glutamatergic function have been demonstrated in schizophrenia. Historically, studies have focused on neuronal dysfunction. Despite the pivotal role of glutamine synthetase in maintaining the ongoing function and survival of neurons and astrocytes through a tightly regulated glutamate-glutamine cycle, surprisingly few studies have examined this molecule in schizophrenia. Likewise, serine racemase, which modulates NMDA receptor function via the synthesis of D-serine, is an important molecule to study in an illness wrought with glutamatergic dysfunction.

These molecules govern several overlapping functional aspects of astrocytes and alterations in their expression may span multiple brain regions. Based on the patterns that emerge from these studies, we can begin to define the role of astrocytes in regional dysfunction in schizophrenia. For instance, decreased expression of numerous molecules might indicate a global astrocytic lesion in schizophrenia, while, distinct regional expression changes could be indicative of the loss of function in a specific subset of astrocytes. Ultimately, these studies will help determine whether astrocytes significantly contribute to schizophrenia, and, if so, delineate some of the specific regions affected and the functional implications.

CHAPTER 2

TRANSCRIPT EXPRESSION OF GFAP, S100β, AND GLUTAMINE SYNTHETASE IN THE DORSOLATERAL PREFRONTAL AND ANTERIOR CINGULATE CORTICIES IN SCHIZOPHRENIA

2.1 Introduction

As discussed previously, the recent understanding of the complexity of astrocyte involvement in brain function has lead to this cell type being implicated in psychiatric illnesses such as schizophrenia. Several studies have found decreased astrocyte density in cortical areas compromised in schizophrenia, including prefrontal, anterior cingulate, and motor cortices (Benes et al., 1986; Cotter et al., 2001; Cotter et al., 2002; Stark et al., 2004). Evidence now suggests a role for those components of astrocytes involved with cytoskeletal integrity, signaling, and enzymatic activity in the pathophysiology of schizophrenia.

As critical component of the astrocyte cytoskeleton, GFAP is expressed throughout cell processes and is used as a standard to identify mature astrocytes in the CNS. The expression of GFAP can influence not only cellular structure and function, but also behavior (Weinstein et al., 1991; Shibuki et al., 1996), suggesting a role for this molecule in modulating brain physiology. In schizophrenia, studies have shown decreases in phosphorylated isoforms of GFAP and a decreased immunoreactivity of this

protein (Rajkowska et al., 1998; Johnston-Wilson et al., 2000; Cotter et al., 2001; Knable et al., 2001; Webster et al., 2001; Rajkowska et al., 2002; Clark et al., 2006). Despite numerous studies measuring protein expression, there is little published about the transcript expression of GFAP in schizophrenia.

Like GFAP, the calcium binding protein S100β is a molecule expressed by astrocytes that influences cellular structure and function (Selinfreund et al., 1990; Whitaker-Azmitia et al., 1997; Winocura et al., 2001). Due to its involvement in multiple signaling cascades, abnormal expression can impact energy metabolism, calcium homeostasis, and cell growth and differentiation. Additionally, S100β modulates the ability of astrocytes to extend processes by regulating the incorporation of GFAP into the cytoskeleton (Garbuglia et al., 1999; Mbele et al., 2002). Multiple studies have found increased S100β in the serum and CSF of patients with schizophrenia, and furthermore, these increases correlate with negative symptoms and illness duration (Wiesmann et al., 1999; Lara et al., 2001; Rothermundt et al., 2001; Schroeter et al., 2003; Rothermundt et al., 2004b; Rothermundt et al., 2004a; Schmitt et al., 2005). Although S100β has been studied in depth in peripheral fluids in schizophrenia, few reports on the brain expression of this molecule have been published.

One of the major components of the pathophysiology of schizophrenia is glutamate system dysfunction (Goff and Coyle, 2001). Studies have reported abnormal expression of molecules involved in the synthesis, transport, binding, reuptake, and recycling of glutamate--most of which are neuronal (Laruelle et al., 2003). Astrocytes also mediate several aspects of glutamate functioning, as such an astrocytic component to glutamatergic dysfunction is emerging (Burbaeva et al., 1999; Anderson and Swanson,

2000; Ghose et al., 2004). A tightly regulated glutamate-glutamine cycle is crucial to normal brain function (Hertz and Zielke, 2004). Glutamine synthetase is an astrocytic enzyme that converts glutamate taken up from the synapse into glutamine. This is important for a few reasons. First, neurons depend on recycled glutamate as a metabolic intermediate and neurotransmitter molecule. Second, excess glutamate in the synapse interferes with receptor activation and, at high levels, can result in excitotoxicity and cell death. Studies have shown that thalamic transcript and cortical protein expression of glutamine synthetase are altered in schizophrenia (Burbaeva et al., 2003; Bruneau et al., 2005). Studies measuring glutamine synthetase transcript expression in schizophrenia have not yet been performed.

Accumulating evidence suggests that structural and functional components of astrocytes may be compromised in schizophrenia. I hypothesize that the expression of cytoskeletal, signaling, and enzymatic molecules expressed by astrocytes is altered in schizophrenia. Accordingly, I used *in situ* hybridization to examine GFAP, S100β, and glutamine synthetase in two brain areas associated with complex cognitive functions that are often impaired in schizophrenia, the DLPFC and ACC.

2.2 Methods

2.2.1 Subjects

Twenty seven subjects from the Mount Sinai Medical Center and Bronx Veterans Administration Medical Center were utilized for this study, consisting of eleven non-psychiatrically ill individuals and sixteen patients with schizophrenia. Consent for autopsy and use of brain tissue for research was obtained from the legal next of kin of

each donor. Subjects were diagnosed with schizophrenia if the following criteria were fulfilled: 1) the presence of schizophrenic symptoms could be documented before age 40; 2) the medical records contained evidence of psychotic symptoms and at least 10 years of psychiatric hospitalization with a diagnosis of schizophrenia; 3) a DSM-III-R diagnosis of schizophrenia was agreed upon by two experienced clinicians; and 4) neuropathologic examination did not reveal Alzheimer's disease or other discernable neuropathologic abnormalities. There were no significant differences between diagnostic groups for age, postmortem interval, sex, tissue pH, or hemisphere studied. Brains were obtained at autopsy and the left hemisphere was cut coronally into 1 cm slabs and frozen. Fresh frozen blocks of DLPFC and ACC, as identified by gross anatomical landmarks, were cryostat-sectioned (14μm). Sections were mounted on Superfrost Plus microscopic slides (Fisher Scientific, Pittsburgh, PA, USA) and stored at -80^{o} C until use. Two slides were studied per subject. Within each region of cortex studied, the number of subjects varied, this information, as well as other subject characteristics are shown in Table 2.1.

2.2.2 In situ hybridization.

The expression of mRNA was measured by *in situ* hybridization using subclones generated by amplifying unique segments of GFAP (Genebank accession no. NM_002055; nucleotide coding region 1093-1311), S100β (NM_006272; 105-412), and glutamine synthetase (NM_002065, 214-530) from an unamplified human adult brain cDNA library (Edge Biosystems, Gaithersburg, MD, USA) and by Polymerase Chain Reaction (PCR). PCR-amplified cDNA segments were extracted (QIAquick Gel Extraction Kit, Qiagen, Valencia, CA, USA), subcloned (Zero Blunt TOPO PCR cloning

Table 2.1 Subject characteristics for Mount Sinai and Bronx Veterans Administration Medical Centers cohort – transcript study

Diagnosis	DLPFC	ACC	Sex	Age	PMI	pH	Cause of Death
Comparison Group		x	F	79	3.0	6.3	cardiopulmonary failure
n=11	x	x	F	96	3.3	6.7	cardiopulmonary failure
	x	x	F	90	4.2	6.0	cardiopulmonary failure
	x	x	M	69	4.3	6.3	information not available
	x	x	F	64	19.1	6.1	pulmonary edema
	x	x	M	93	19.0	6.4	congestive heart failure
	x	x	F	102	7.1	6.5	acute Myocardial Infarction
		x	F	73	3.4	6.3	acute Myocardial Infarction
	x	x	F	79	7.7	6.5	acute Myocardial Infarction
		x	F	84	18.5	6.2	information not available
		x	M	101	4.7	6.8	coronary artery disease
means + SD			**3M, 8F**	**85 ± 13**	**8.5 ± 6.8**	**6.4 ± 0.2**	
Schizophrenia	x	x	F	86	6.9	5.8	respiratory insufficiency, renal failure
n=16	x	x	F	84	15.6	6.2	information not available
		x	M	69	4.5	6.4	cardiac infarction, renal failure
	x	x	F	69	13.7	6.2	cardiopulmonary failure
	x	x	M	87	11.2	6.5	cardiopulmonary failure
	x	x	M	68	5.6	6.8	cardiopulmonary failure
	x	x	M	85	5.3	6.3	cardiopulmonary arrest
	x	x	M	73	7.9	6.5	cardiorespiratory failure
	x	x	M	66	12.1	6.5	acute cardiac failure
	x	x	F	76	21.2	6.1	cardiogenic shock
	x	x	M	97	9.3	6.5	cardiopulmonary arrest
	x	x	M	66	8.4	6.7	cardiopulmonary arrest
		x	F	82	18.8	6.6	cardiopulmonary arrest
	x	x	F	79	9.9	6.8	cardiac Arrest
	x	x	M	68	17.3	6.6	cardiopulmonary arrest
	x		F	79	20.4	7.1	cardiopulmonary failure, pancreatic cancer
means + SD			**9M, 7F**	**77 ± 9**	**11.7 ± 5.5**	**6.5 ± 0.3**	

kit; Invitrogen, Carlsbad, CA, USA), and confirmed by nucleotide sequencing. Sense and antisense probes for *in situ* hybridization were synthesized using 100 µCi of dried [^{35}S] UTP, 2.0 µl 5x transcription buffer (40mM Tris, 6mM $MgCl_2$, 2mM spermidine,10mM NaCl, pH 7.9); 1.0µl each of 10mM ATP, CTP, and GTP; 1.0µg linearized plasmid DNA; 0.5µl RNAse inhibitor; and 1.5µl SP6 or T7 RNA polymerase. After incubation for two hours at 37˚C, 1.0µl DNAse (RNAse-free) was added and incubated for 15 minutes at room temperature. [^{35}S] Labeled cRNA was purified with spin columns (Micro Bio-Spin P-30 Tris Spin Columns, Bio-Rad Laboratories, Hercules, CA, USA), diluted to 100µl final volume, and 1.0µl of 1M dithiothreitol (DTT) was added to a final concentration of 0.01M.

Sections were fixed with 4% (weight:volume) formaldehyde for one hour at room temperature. Next, sections were rinsed three times in 2X SSC (300mM NaCl / 30mM sodium citrate, pH 7.2) and incubated on a stir plate in 0.1M triethanolamine, pH 8.0 / acetic anhydride, 1:400 (volume/volume) for ten minutes at room temperature. Sections were then washed in 2X SSC for ten minutes at room temperature, and dehydrated though graded alcohols and air-dried. [^{35}S] labeled riboprobe (3-5x10^6 cpm) was applied in 80µl of 50% formamide buffer (50% formamide, 10% dextran sulfate, 3X SSC, 50mM Na_2HPO_4, pH 7.4, 1X Denhardt's solution, 100µg/ml yeast tRNA) with 0.1% of 1M DTT per each slide. The slides were then covered with glass coverslips and stored in humidified chambers saturated with 50% formamide at 55˚C overnight. The next day, the cover slips were removed and the sections were washed in 2X SSC for two minutes at room temperature, 2X SSC for ten minutes at room temperature, and then incubated with RNAse A (200mg/ml in 10mM Tris, pH 8.0 / 0.5M NaCl) at 37˚C for 30 minutes. Slides

were then washed at room temperature two times for ten minutes in 2X SSC, ten minutes in 1X SSC, five minutes in 0.5X SSC, for 30-90 minutes in 0.1X SSC at 55˚C and ten minutes in 0.1X SSC at room temperature. The sections were then dehydrated in graded alcohol solutions and air-dried. Finally, the slides were placed in X-ray cassettes, apposed to Kodak BioMax MR autoradiographic film (Eastman Kodak Co., Rochester, NY, USA) and developed after six days (GFAP), nineteen days (S100β), or four days (glutamine synthetase).

2.2.3 Data analysis.

Images of each slide were captured with a CCD based imaging system using Scion Imaging software 4.0.3 (Scion Corporation, Fredrick, MD, USA). Each transcript showed a distinct pattern of laminar distribution across the cortical thickness, and gray-scale values were obtained from bands with densities different from adjacent bands (isodense bands). Isodense bands did not correspond exactly with traditionally defined cortical layers. Gray scale values from isodense bands were corrected for tissue background, and then converted to optical density (OD). OD values were converted to amount of radioactivity bound (nCi/g) determined from calibrated [^{14}C] microscale standards (Amersham Biosciences, Piscataway, NJ, USA) (Miller, 1991). The amount of bound probe was expressed as concentration of target mRNA per gram of tissue (fmol/g) by taking into account the specific activity of [^{35}S] and the number of uridine residues in each probe.

2.2.4 Statistical analysis.

Statistical analysis was performed using factorial analysis of variance (ANOVA) with diagnosis and isodense bands as categorical independent variables. In cases where age, postmortem interval (PMI), or pH was found to correlate significantly with a dependent variable, analysis of covariance (ANCOVA) was used. For all tests $\alpha = 0.05$.

2.3 Results

I used *in situ* hybridization to measure transcript expression of three astrocytic molecules: GFAP, S100β, and glutamine synthetase in the DLPFC and ACC. Sense and antisense probes were synthesized and tested, and only antisense probes produced specific labeling. I detected each of the three molecules in each brain region (Figure 2.1).

2.3.1 GFAP transcript expression.

Transcript expression of GFAP in the DLPFC and ACC was heterogeneously distributed across cortical lamina, exhibiting four isodense bands in the gray matter and distinct labeling in white matter (Figure 2.1a, b). In the DLPFC, there were no associations between GFAP expression and age, PMI, or pH. I found a main effect for diagnosis using ANOVA, which revealed that GFAP transcript was significantly decreased in the DLPFC in schizophrenia ($F(1, 87) = 3.98$; $p < 0.05$) (Figure 2.2a). In the ACC, I detected an association between GFAP expression and age ($r = 0.25$; $p < 0.01$). Using ANCOVA, I found a main effect for diagnosis for increased GFAP expression in schizophrenia ($F(1, 96) = 11.97$; $p < 0.01$) (Figure 2.2b). There was no diagnosis by isodense band interaction in either cortical area.

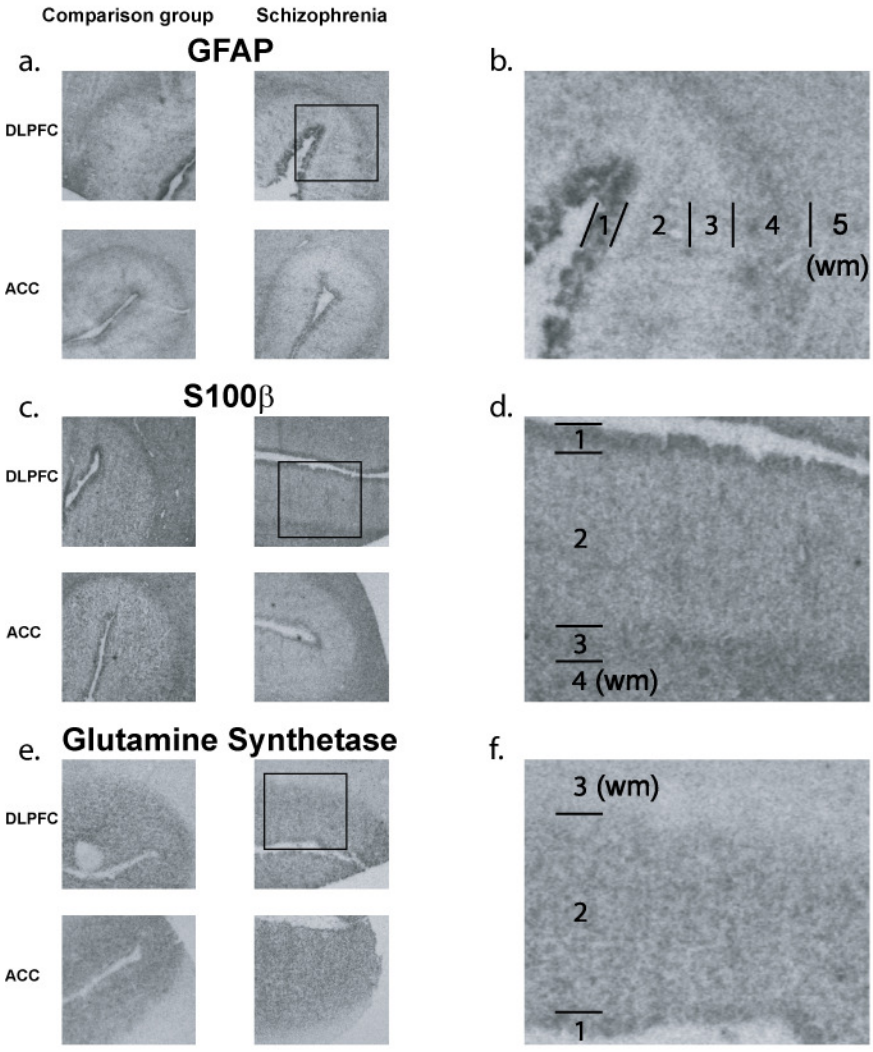

Figure 2.1 GFAP, S100β, and glutamine synthetase mRNA expression in the DLPFC and ACC. Transcript expression of astrocytic molecules in the DLPFC and ACC of representative subjects with schizophrenia and a comparison group: GFAP (a, b), S100β (c, d), and glutamine synthetase (e, f) mRNAs. Between two and four isodense bands were identified in the gray matter, and labeling in the white matter (wm) was also noted, resulting in 3-5 quantifiable isodense bands.

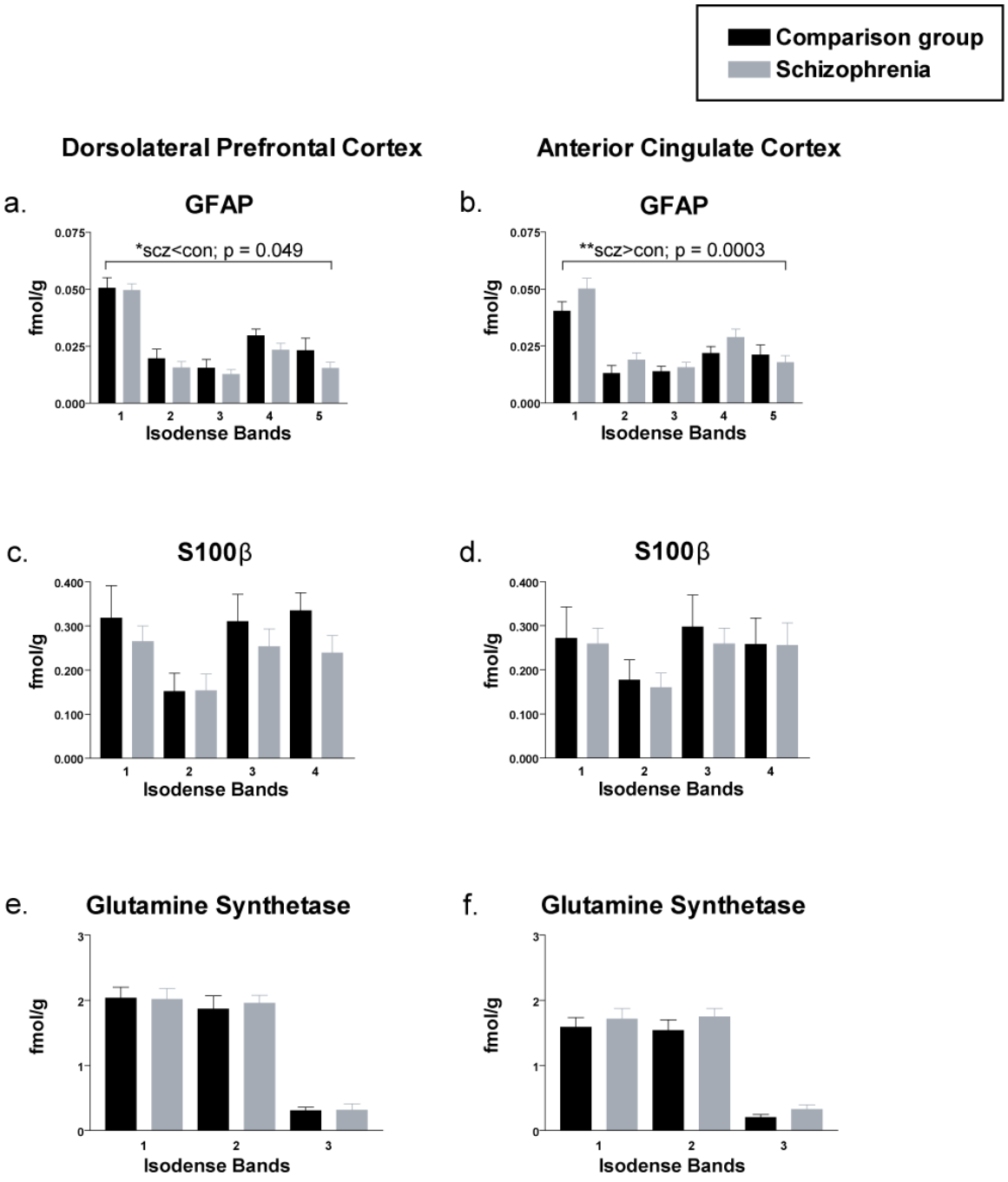

Figure 2.2 Transcript expression of astrocytic molecules in schizophrenia. Graphs depict transcript expression (fmol/g) in the DLPFC and ACC for GFAP (a, b), S100β (c, d), and glutamine synthetase (e, f) analyzed by diagnosis (comparison group and schizophrenia). GFAP was decreased in the DLPFC ($F(1, 87) = 3.98$; $^{*}p < 0.05$) and increased in the ACC ($F(1, 96) = 11.97$; $^{**}p < 0.01$) in schizophrenia.

Given a past literature on the effects of normal aging on GFAP expression, I performed a secondary analysis examining the effect of age on GFAP mRNA expression in the DLPFC and ACC (Figure 2.3). In the DLPFC, there was no significant effect for age on GFAP expression when all subjects were pooled (Figure 2.3a). When I separated the subjects based on diagnosis there was a significant effect for age on GFAP expression in the comparison group ($r = 0.40$, $p < 0.05$), but not in schizophrenia (Figure 2.3a). There was a significant effect for age on GFAP expression in the ACC when all subjects were analyzed together ($r = 0.25$, $p < 0.01$) (Figure 2.3b). This effect remained significant for the comparison group, but not for schizophrenia when groups were analyzed separately ($r = 0.42$, $p < 0.01$) (Figure 2.3b).

2.3.2 S100β transcript expression.

The transcript expression of S100β in the DLPFC and ACC was heterogeneously distributed across cortical lamina and exhibited labeling in three isodense bands in the gray matter, and white matter labeling was also apparent (Figure 2.1c, d). In the DLPFC, I detected a significant association between age ($r = 0.28$; $p < 0.05$) and PMI ($r = -0.40$; $p < 0.01$) and S100β expression. I did not find a main effect for diagnosis in the DLPFC using ANCOVA (Figure 2.2c). In the ACC, I detected an association between age ($r = 0.54$; $p < 0.01$) and PMI ($r = -0.27$; $p < 0.05$) and S100β transcript expression. ANCOVA revealed a trend for decreased S100β transcript in the ACC in schizophrenia ($F(1, 81) = 3.21$; $p = 0.077$) (Figure 2.2d). There was no diagnosis by isodense band interactions in either cortical area.

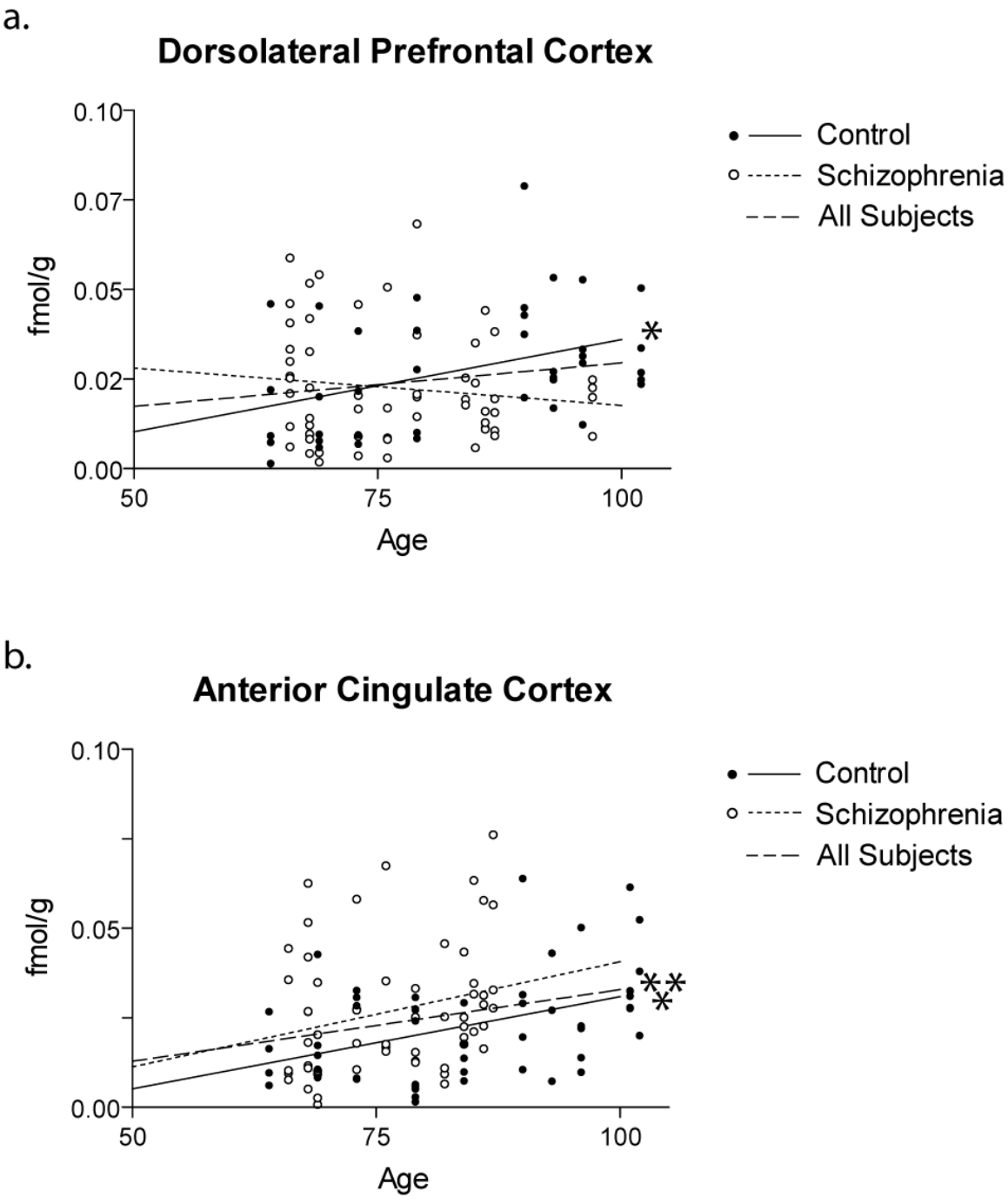

Figure 2.3 Bivariate plots of GFAP mRNA expression (fmol/g) versus age. Regression lines are shown for schizophrenia and comparison subjects plotted separately, as well as pooled. (a) In the DLPFC, there was a significant effect for age on GFAP expression in the comparison group (*$p < 0.05$). (b) In the ACC, there was an effect for age on GFAP expression when all subjects were analyzed together (**$p < 0.01$), as well as for the comparison subjects alone (*$p < 0.005$).

2.3.3 Glutamine Synthetase transcript expression.

I detected glutamine synthetase transcript expression in two isodense bands in gray matter, and white matter labeling was also present (Figure 2.1e, f). Correlation analysis did not reveal an association between glutamine synthetase expression, age, and PMI in the DLPFC or ACC. Using ANCOVA, there was no effect for diagnosis and no diagnosis by isodense band interaction for glutamine synthetase expression in cortical area (Figure 2.2e, f).

2.4 Discussion

I tested the hypothesis that structural and functional components of astrocytes are compromised in schizophrenia by examining the transcript expression of the cytoskeletal, signaling, and enzymatic molecules (GFAP, S100β, and glutamine synthetase) in the DLPFC and ACC. GFAP, an astrocytic intermediate-filament protein and component of the astrocytic cytoskeleton important for cellular structure and function, was decreased in the DLPFC. Decreased transcript expression in the DLPFC, particularly in the context of a literature showing deficits in GFAP protein expression in schizophrenia could be due to a loss of astrocytic activation, process branching, and/or cellular density (Rajkowska et al., 1998; Johnston-Wilson et al., 2000; Cotter et al., 2001; Knable et al., 2001; Webster et al., 2001; Rajkowska et al., 2002; Clark et al., 2006).

In the ACC of the same subjects, I found a significant increase in GFAP mRNA expression, indicating that GFAP is differentially regulated in frontal cortical regions. This finding is in contrast to the only other report on GFAP transcripts in the ACC in schizophrenia, which found decreased expression (Webster et al., 2005). It is unlikely

that this increase I observed is due to enhanced activation of astrocytes, as reactive gliosis has not been described in this illness (Arnold et al., 1996; Falkai et al., 1999; Damadzic et al., 2001). This increase is more likely a compensatory upregulation, perhaps in response to loss of astrocytes as has been reported previously in the ACC (Cotter et al., 2001). I hoped to determine whether these abnormalities were due to transcript changes in individually identified cells, so I emulsion-dipped and processed the slides for cell-level studies of mRNA expression. Although our laboratory has successfully used this technique in the past to identify neuronal transcripts at a cellular level, I was unable to consistently detect expression of this and other astrocytic molecules above background levels. The relatively small size of astrocytes and extended process branching beyond the cell soma likely contributed to the inability to complete cell-level studies (Kohama et al., 1995).

Both GFAP mRNA and protein expression have been shown to routinely increase with age (Hansen et al., 1987; Nichols et al., 1993; Kohama et al., 1995; Yoshida et al., 1996; David et al., 1997). These age-related increases are thought to occur without an accompanying increase in astrocyte cell density (Nichols et al., 1993; Kohama et al., 1995). I did not detect any significant correlations between GFAP expression and age in the DLPFC when I analyzed all of the subjects pooled together. However, I found that when grouped by diagnosis, GFAP expression in the comparison subjects positively correlated with age, which is consistent with earlier reports. In contrast, GFAP expression was not correlated with age in schizophrenia. This suggests that my finding of decreased GFAP in the DLPFC in schizophrenia is likely illness-related, as it occurs despite evidence of age-related increases in comparison subjects. In the ACC, I found a

positive correlation between GFAP expression and age in both the pooled subjects and the comparison group alone. Although this correlation was limited to the comparison group, GFAP expression was significantly increased in schizophrenia. Reports of increased GFAP expression in schizophrenia are rare, however, one study found increase protein expression in elderly patients with schizophrenia also exhibiting dementia (Arnold et al., 1996). This, taken with my present data in elderly subjects, suggests that GFAP expression varies not only by region and diagnosis, but may also delineate subgroups of persons with schizophrenia particularly associated with advanced age and end stage schizophrenia. Supporting this hypothesis, several studies that have used younger subjects found GFAP expression unchanged in schizophrenia, although most of these studies did not seek to examine the effects of age on GFAP expression (Perrone-Bizzozero et al., 1996; Karson et al., 1999; Fatemi et al., 2004; Dean et al., 2006). Recently, a study finding increased GFAP immunoreactivity in the prefrontal cortex was published (Toro et al., 2006). However, since these results were drawn from the combined analysis of tissue from multiple Brodmann areas it is difficult to reconcile with my data given the region-specific differences in GFAP expression that I noted.

A potentially confounding variable is that the majority of the schizophrenia subjects were chronically treated with typical antipsychotic medications. Typical antipsychotics predominantly act at D2-like dopamine receptors. Ligand binding data from rodent, primate, and human brains indicate that approximately 35% of cortical D2 receptor binding may be associated with astrocyte expression (Khan et al., 2001). Nonetheless, double immunofluorescence labeling for c-*fos* and GFAP in rats astrocyte activation (Ma et al., 2003). Increased glial density has been reported in primates

chronically treated with typical antipsychotics (Selemon et al., 1999). However, studies of elderly patients with schizophrenia do not find increased astrocyte density as the result of chronic antipsychotic treatment (Nishioka and Arnold, 2004). A recent study revealed a correlation between GFAP protein expression in the prefrontal cortex and cumulative lifetime dose of antipsychotics (Toro et al., 2006). The effect of medication on GFAP expression remains unresolved. While it is possible that my findings of altered GFAP expression are secondary to treatment with medications, it seems unlikely given that I found changes in opposite directions in DLPFC and ACC.

I also measured transcript expression of the calcium binding protein S100β. S100β has been examined in schizophrenia and other psychiatric disorders as a marker of astrocytic integrity. Recently, cortical transcript and protein expression of S100β was described; although changed in bipolar disorder, this molecule was found to be unaltered in schizophrenia (Dean et al., 2006). Consistent with this study, I found S100β mRNA levels unchanged in the DLPFC and ACC in schizophrenia. These results do not necessarily conflict with other data finding increased S100β in serum and CSF of patients with schizophrenia (Wiesmann et al., 1999; Lara et al., 2001; Rothermundt et al., 2001; Schroeter et al., 2003; Rothermundt et al., 2004b; Rothermundt et al., 2004a; Schmitt et al., 2005). One hypothesis is that increased S100β in these fluids is a sign of damaged cells leaking the protein. This would not necessarily coincide with changes in transcript expression in the brain, however. One could speculate that treatment with antipsychotic medication could affect the expression of S100β, but increased S100β is found in sera and CSF of first time, unmedicated patients with schizophrenia. Furthermore, increased treated with haloperidol did not show co-localization, suggesting a lack of antipsychotic-

induced S100β persists following antipsychotic treatment; therefore, medication-related changes to peripheral S100β seem unlikely (Rothermundt et al., 2001; Rothermundt et al., 2004a; Schmitt et al., 2005). A predictive factor for increased S100β in sera and CSF is a predominance of negative symptoms (Rothermundt et al., 2001; Schroeter et al., 2003; Rothermundt et al., 2004a). It is unclear whether cortical S100β expression is associated with any specific characteristics of this illness or whether it is affected by antipsychotic treatment.

Finally, I measured the astrocytic enzyme glutamine synthetase. Previously, our laboratory found increased glutamine synthetase transcript expression in the thalamus in a similar group of subjects (Bruneau et al., 2005). In the present study, I did not detect any changes in either of the cortical regions studied. In light of these results, transcript expression of this molecule, like GFAP, appears to be affected regionally rather than globally in this illness. So far, none of the published studies of glutamine synthetase expression in schizophrenia have found changes that can be related to medication (Burbaeva et al., 2003; Toro et al., 2006) and there are no studies directly testing the effect of antipsychotics on this enzyme.

This study examined structural and functional components of astrocytes including cytoskeletal, signaling, and enzymatic molecules in schizophrenia. I found that GFAP, a molecule crucial for maintaining normal astrocyte structure and function is abnormally expressed in both the DLPFC and the ACC. Although further work is needed to decipher whether these changes are associated with altered cell density, diminished process extension, or compensatory mechanisms secondary to this illness, altered expression of GFAP suggests that there are abnormalities in astrocytes that contribute to cortical

dysfunction in schizophrenia.

CHAPTER 3

CORTICAL EXPRESSION OF GLIAL FIBRILLARY ACIDIC PROTEIN AND GLUTAMINE SYNTHETASE IS DECREASED IN SCHIZOPHRENIA

3.1 Introduction

As discussed in chapter one, alterations in astrocyte density, function and gene expression in schizophrenia suggest a role for these cells in the pathophysiology of this illness. Astrocytes are connected to multiple synapses, blood vessels, and multiple types of cells by extensive process branching. This enables astrocytes to support the metabolic needs of neurons, regulate extracellular ions, and facilitate cell to cell communication and neuroplasticity through the release of signaling molecules (Cornell-Bell et al., 1990b; Charles et al., 1991; Parpura et al., 1994; Schell et al., 1995; Allen and Barres, 2005). Due to the extensive role of astrocytes in brain physiology, changes to critical components of astrocytes involving structure and function may underlie brain dysfunction in schizophrenia.

Studies reporting decreased density of astrocytes in cortical regions known to be compromised in schizophrenia, including prefrontal, anterior cingulate, and motor cortices (Benes et al., 1986; Cotter et al., 2002; Stark et al., 2004), as well as studies measuring the response of astrocytes to mechanical injury suggest an underlying vulnerability of this cell type in schizophrenia (Niizato et al., 2001).

Glutamatergic dysfunction has been widely reported in schizophrenia (Goff and Coyle, 2001). Astrocytes orchestrate a series of events critical to normal glutamate function, and therefore are hypothesized in this dysfunction. Within the glutamatergic synapse, presynaptic neurons package glutamate into vesicles for release into the synapse where it may bind to and activate receptors found on pre- and postsynaptic neurons and astrocytes (Hollmann and Heinemann, 1994; Bellocchio et al., 2000; Takamori et al., 2000). The subsequent removal of glutamate from the synapse, an event critical to regulating synaptic activity and preventing excitotoxicity, is accomplished primarily by astrocytes which express high affinity EAATs types 1, 2, 3, and 4 (Conti et al., 1998; Sonnewald et al., 2002; Hu et al., 2003; Kim et al., 2003). Once recovered by astrocytes, glutamate may be converted to glutamine by the enzyme glutamine synthetase, and subsequently transported back to the presynaptic neuron for use as a metabolic substrate or transmitter molecule (Rothman et al., 1999). The central role of glutamine synthetase in regulating a glutamate-glutamine cycle makes it a high yield target for investigating glutamate dysfunction in schizophrenia.

The role of astrocytes in normal brain functioning is made possible, in part, by extensive process branching. Astrocyte processes contain the cytoskeletal molecule GFAP, which when immunohistochemically labeled is used as a measure of cell density. The assembly of GFAP subunits into a functional cytoskeleton facilitates process extension that allows astrocytes to respond to changes in the microenvironment and contribute to synaptic plasticity (Rodnight et al., 1997; Ullian et al., 2001; Ullian et al., 2004; Allen and Barres, 2005). *In vivo* and *in vitro* experiments of altered GFAP expression demonstrate that loss of this cytoskeletal protein leads to decreased branching,

prevention of normal synaptic functioning, disruption of myelination, and aberrant behavior (Weinstein et al., 1991; Chen and Liem, 1994; Liedtke et al., 1996; McCall et al., 1996; Shibuki et al., 1996). Abnormal assembly of the cytoskeleton caused by mutations in GFAP, as seen in Alexander disease, may lead to neurodegeneration and premature death (Mignot et al., 2004; Perng et al., 2006). Since GFAP is necessary for cytoskeletal integrity and process branching, abnormalities in GFAP expression could lead to marked dysfunction.

Astrocytes express molecules that are critical to normal brain function, including enzymes involved in the glutamate-glutamine cycle and cytoskeletal proteins which enable branching of astrocytic processes. Abnormal expression of these molecules may be a factor in the pathophysiology of schizophrenia. I hypothesize that glutamine synthetase and GFAP protein are abnormally expressed in this illness. I measured these molecules in an elderly cohort with schizophrenia and a non-psychiatrically ill comparison group using Western blot analysis in the DLPFC, ACC, STG, PVC, and hippocampus.

3.2 Methods

3.2.1 Acquisition and processing of human brain tissue

Fifty subjects from the Mount Sinai Medical Center and Bronx Veterans Administration Medical Center were utilized for this study, consisting of 27 non-psychiatrically ill individuals and 23 patients with schizophrenia (Table 3.1). Consent for autopsy and use of brain tissue for research was obtained from the legal next of kin of each donor. Subjects were diagnosed with schizophrenia if the following criteria were

Table 3.1 Subject characteristics for Mount Sinai and Bronx Veterans Administration Medical Centers cohort – protein study

Diagnosis	Sex	Age	PMI (hours)	pH	Cause of Death
Comparison Group	F	62	7.0	6.6	acute myocardial infarction
	F	73	3.4	6.3	acute myocardial infarction
	F	74	3.0	6.0	cardiopulmonary arrest
	F	75	6.5	6.0	cardiopulmonary arrest
	F	80	4.8	6.2	sepsis
	F	82	5.7	6.1	cardiopulmonary arrest
	F	83	6.2	6.8	cardiopulmonary arrest
	F	84	18.5	6.2	myocardial infarction
	F	86	4.7	6.5	acute myocardial infarction
	F	88	5.1	6.4	chronic obstructive pulmonary disease
	F	89	2.3	6.7	bronchopneumonia, chronic obstructive pulmonary disease
	F	90	4.2	6.0	pneumonia
	F	98	1.4	6.6	myocardial infarction, aortic aneurysm
	M	59	20.4	6.7	coronary arterial disease
	M	60	28.8	6.6	acute myocardial infarction
	M	64	4.2	6.4	acute myocardial infarction
	M	65	3.8	6.8	renal failure
	M	66	7.6	6.6	acute myocardial infarction
	M	69	4.3	6.3	cancer of lung
	M	69	7.4	6.7	septic shock
	M	74	16.6	6.7	cardiopulmonary arrest
	M	76	2.9	6.3	bronchopneumonia
	M	85	5.3	6.5	transitional cell cancer of bladder
	M	92	20.0	6.4	arrhythmia
	M	93	4.2	6.3	acute myocardial infarction
	M	95	4.1	6.5	chronic renal failure
	M	101	4.7	6.8	congestive heart failure
	14M, 13F	**79 ± 12**	**7.7 ± 6.8**	**6.5 ± 0.2**	
Schizophrenia	F	69	13.7	6.2	cardiopulmonary arrest
	F	74	7.0	6.3	Cardiopulmonary Arrest
	F	77	9.7	6.0	cardiopulmonary arrest
	F	79	9.9	6.8	cardiac arrest
	F	81	12.5	5.9	acute myocardial infarction
	F	81	15.1	6.7	cardiopulmonary arrest
	F	83	20.4	7.1	cardiopulmonary arrest, cancer of pancreas
	M	52	29.5	5.9	cardiopulmonary arrest
	M	56	13.5	6.5	cardiopulmonary arrest
	M	57	30.3	6.1	expired during open heart surgery
	M	57	21.4	6.4	small cell cancer of lung
	M	58	6.7	6.2	cardiopulmonary arrest
	M	58	13.3	6.9	cardiopulmonary arrest
	M	63	6.2	6.3	cardiopulmonary arrest
	M	68	17.3	6.6	congestive heart failure, coronary artery disease
	M	69	40.2	6.7	acute renal failure
	M	73	7.9	6.5	cardiopulmonary arrest
	M	76	16.6	6.7	coronary artery disease, congestive heart failure
	M	84	6.2	6.5	cardiopulmonary arrest
	M	85	5.3	6.3	chronic obstructive pulmonary disease, pneumonia
	M	86	7.0	6.3	cardiopulmonary arrest
	M	86	14.1	6.7	artheroschlerotic heart disease
	M	87	11.2	6.5	cardiopulmonary arrest
	16M, 7F	**72 ± 12**	**14.6 ± 8.9**	**6.5 ± 0.3**	

fulfilled: 1) the presence of schizophrenic symptoms could be documented before age 40; 2) the medical records contained evidence of psychotic symptoms and at least 10 years of psychiatric hospitalization with a diagnosis of schizophrenia; 3) a DSM-III-R diagnosis of schizophrenia was agreed upon by two experienced clinicians; and 4) neuropathologic examination did not reveal Alzheimer's disease or other discernable neuropathologic abnormalities.

Brains obtained at autopsy were divided mid-sagittally at the time of extraction. The right half was fixed in 4% formaldehyde and used for neuropathological characterization. The left half was sectioned in 6-8 mm coronal slabs, snap-frozen, and stored at –80^{o}C. The areas of interest were identified by gross anatomical landmarks and dissected from the frozen slabs from the following areas: dorsolateral prefrontal cortex, primary visual cortex, superior temporal gyrus, anterior cingulate cortex, and hippocampus (Dracheva et al., 2004; Katsel et al., 2005; Dracheva et al., 2006). Dissected, never-thawed tissues were pulverized at –190^{o}C into a fine powder and aliquoted into individual Eppendorf tubes, and stored at –80^{o}C.

3.2.2 Acquisition and processing of rodent brain tissue

Twenty-two adult, male Sprague-Dawley rats (250g) were housed two-three to a cage, with food and water ad libitum. Animals were treated with daily intramuscular injections of haloperidol dissolved in dimethyl sulfoxide (DMSO) (Fisher Scientific, Fair Lawn, NJ, USA) (1 mg/kg/day, n=11), or vehicle (DMSO) (n=11) for 29 days. Twenty-four hours after the last injection, the animals were sacrificed and brains were immediately removed, dissected, and frozen in isopentane. The frontal, cingulate,

occipital, and temporal cortices, and hippocampus were dissected according to *The Rat Brain in Stereotaxic Coordinates* (Paxinos and Watson, 1986). The brains were stored at −80°C until assayed. All animal experiments were approved by the University Committee on the Use and Care of Animals at the University of Michigan and were performed according to the guidelines for The Care and Use of Laboratory Animals of the National Institutes of Health.

3.2.3 Tissue preparation

Tissue specimens (human 50mg, rodent 20mg) were homogenized in 5mM Tris–HCl (pH 7.4), containing Complete, mini, EDTA-free protease inhibitor cocktail tablets (Roche Applied Science, Indianapolis, IN, USA) (1 tablet/10mls) for 30 seconds with a PowerGen 125 homogenizer (Fisher Scientific International, Inc., Hampton NH, USA). Total protein concentration was determined with a BCA Protein Assay Kit (Pierce Biotechnology, Inc., Rockford IL, USA), and homogenates were stored at −80°C.

3.2.4 Western blot analysis

Samples were prepared by combining tissue homogenate with sample buffer (62.5mM Tris-HCl, 20% glycerol, 2% sodium dodecyl sulfate, 5% β-mercaptoethanol, pH 6.8) and heated at 95°C for four minutes. Samples were then loaded onto pre-cast 10% polyacrylamide Tris-HCl gels (Bio-Rad) in duplicate (20µg protein/well) and run in SDS/Tris/glycine buffer (25mM Tris-HCl, 192mM glycine, 0.1% SDS, pH 8.3) at 130 mV for about one hour. Following transblotting to nitrocellulose membranes in Tris-glycine buffer (25mM Tris, 192mM glycine, pH 8.3), blots were blocked with powdered

milk in 5% TBS with 0.01% Tween-20 (TBST) (Fisher Scientific, Fair Lawn, NJ, USA) (pH 7.4) for one hour at room temperature and then agitated with mouse anti-glutamine synthetase monoclonal antibody (BD Biosciences, San Diego, CA) (1:1000), or mouse anti-glial fibrillary acidic protein monoclonal antibody (1:500,000) (Chemicon International, Temecula, CA, USA) in 5% powdered milk in TBST for two hours. Blots were washed four times in TBST and incubated with a horseradish peroxidase-coupled goat anti-mouse secondary antibody (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) (1:5000) for two hours on a shaker at room temperature. Following four washes in TBST and two washes in distilled water, enhanced chemiluminescence (ECL) was used for detection. Blots were saturated with ECL reagent (Amersham, Piscataway, NJ, USA), covered in plastic wrap and exposed to ECL film (Amersham, Piscataway, NJ, USA). Film was developed and digitally captured with a CCD based imaging system using Scion Imaging software 4.0.3. Gray scale values were obtained for protein bands at the expected molecular weights, membrane background was subtracted, and the adjusted gray scale values from duplicate samples in adjacent lanes were averaged and converted to optical density. Membranes were stripped and re-blotted for β-Tubulin (Upstate, lake Placid, NY, USA) as a loading control, and the mean optical density ratio of glutamine synthetase or GFAP/β-Tubulin was used for data analysis.

3.2.5 Statistical Analysis

Statistical analysis was performed with Statistical analysis was performed using Statistica software (StatSoft, Tulsa, OK). Analysis of variance (ANOVA) with diagnosis as the independent variable and mean optical density ratio as the dependent variable was

used. In cases where age, postmortem interval (PMI), or pH were found to significantly correlate with a dependent variable, analysis of covariance (ANCOVA) was used. For all tests $\alpha = 0.05$.

3.3 Results

3.3.1 Protein expression of glutamine synthetase and GFAP in Schizophrenia

I used Western blot analysis to measure the expression of glutamine synthetase and GFAP in the DLPFC, ACC, STG, PVC, and hippocampus. Using antibodies specific for these molecules, I detected prominent bands at the expected molecular weights of 45 kDa for glutamine synthetase, and 51 kDa for GFAP (Figure 3.1).

Regression analysis showed no associations between glutamine synthesis expression and age, postmortem interval or pH in any of the areas examined. Using ANOVA, I found a main effect for diagnosis for glutamine synthetase expression in the STG ($F(1, 30) = 6.44$; $p = 0.02$) and ACC ($F(1, 30) = 6.99$; $p = 0.01$) (Figure 3.2), but not in the DLPFC, PVN, or hippocampus. Glutamine synthetase expression in the STG was reduced by 32% in schizophrenia versus the comparison group. In the ACC, there was a 19% decrease in the expression of glutamine synthetase in schizophrenia.

Regression analysis showed no associations between GFAP expression and age, postmortem interval or pH in the DLPFC, ACC, or PVC. In the STG and hippocampus, there was an association between GFAP expression, age, and PMI (age: $r = 0.443$, $p = 0.003$; PMI: $r = 0.465$, $p = .0002$), and hippocampus (age: $r = 0.329$, $p = 0.041$; PMI: $r = 0.409$, $p = 0.009$). Using ANCOVA, I did not detect an effect for diagnosis on GFAP expression in either the STG or hippocampus. Using ANOVA, I found a main effect for

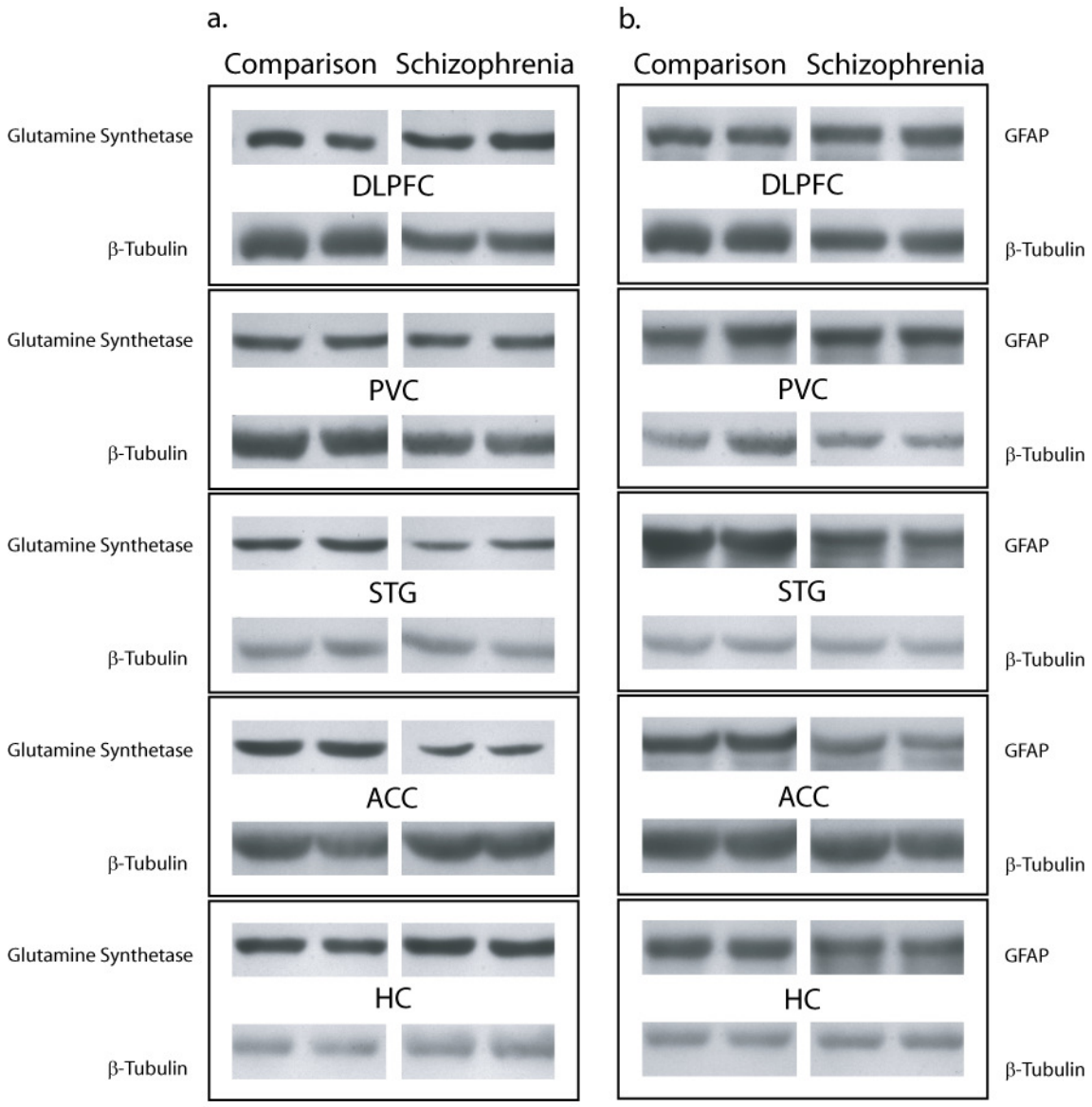

Figure 3.1 Western blots of astrocytic molecules from representative subjects with schizophrenia and a nonpsychiatrically ill comparison group. Blots show protein expression of (a) glutamine synthetase, and (b) GFAP in the DLPFC, PVC, STG, ACC, and hippocampus. β-Tubulin was measured as a loading control.

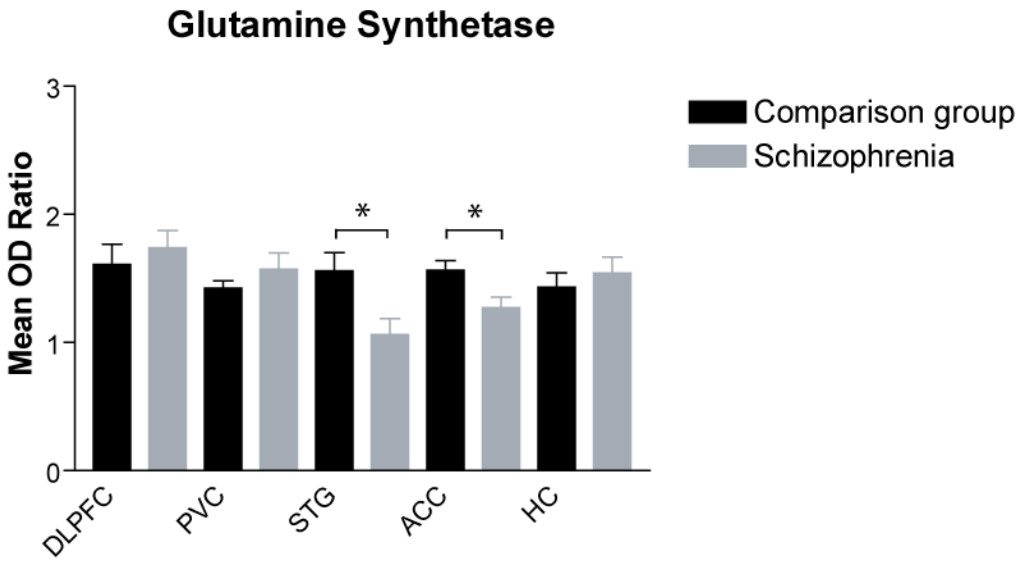

Figure 3.2 Glutamine synthetase protein expression in the DLPFC, PVN, STG, ACC, and hippocampus. Protein expression was analyzed by diagnosis (comparison group and schizophrenia). Glutamine synthetase was significantly decreased in the STG ($F(1,30) = 6.44$; $^*p < 0.05$ and ACC ($F(1,30) = 6.99$; $p < 0.05$) in schizophrenia.

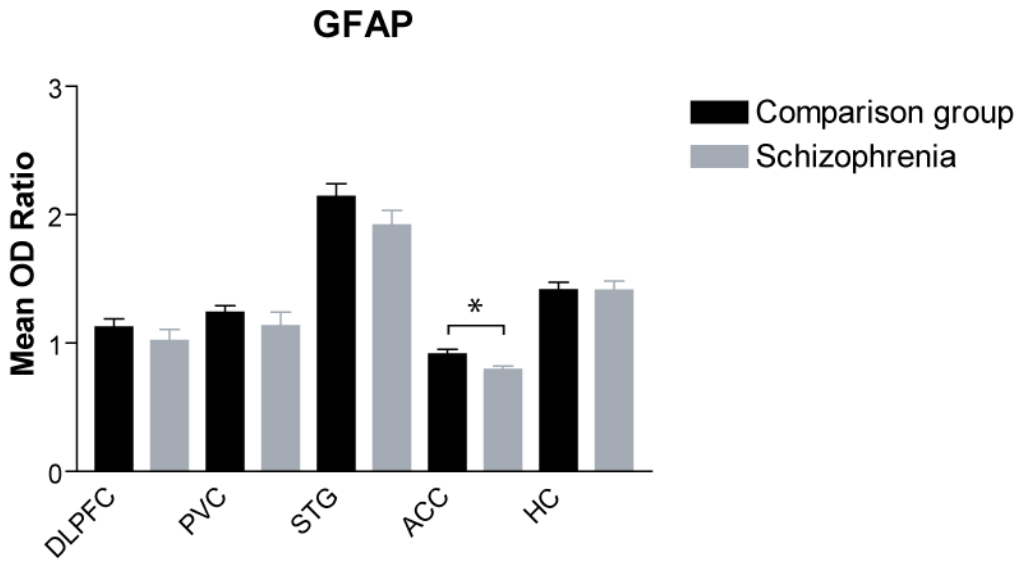

Figure 3.3 GFAP protein expression in the DLPFC, PVN, STG, ACC, and hippocampus. Protein expression was analyzed by diagnosis (comparison group and schizophrenia). Expression is shown as mean OD ratio for β-Tubulin with GFAP. There was a significant decreased in GFAP expression in the ACC ($F(1,43) = 5.68$; $p < 0.05$) in schizophrenia.

diagnosis for GFAP expression in the ACC ($F(1, 43) = 5.68$; $p = 0.02$), but not in the DLPFC, or PVC (Figure 3.3). The expression of GFAP in the ACC was reduced in schizophrenia by 13% versus the comparison group.

I performed regression analysis to examine the effect of age on protein expression of glutamine synthetase and GFAP in these five brain regions. I did not detect a significant effect for age on glutamine synthetase expression in any of the regions studied, whether data were analyzed by diagnosis or pooled (data not shown). GFAP expression was not associated with age in any of the brain areas when subjects from each diagnostic group where analyzed separately. When data were pooled, there was a significant association between GFAP expression and age in the STG ($p = 0.0029$), and hippocampus ($p = 0.0406$) (Figure 3.4). Using pooled data, there was no association between age and GFAP expression in the DLPFC, PVC, or ACC.

3.3.2 Effects of haloperidol treatment on protein expression of glutamine synthetase and GFAP in the rat brain

Most of the patients with schizophrenia in this study were treated with typical antipsychotics. I used Western blot analysis to measure the expression of these astrocytic molecules in rats chronically treated (2mg/kg/day) for 28 days with haloperidol. I did not detect differences in protein expression for glutamine synthetase or GFAP in any of the brain regions studied (Figure 3.5).

3.4 Discussion

I tested the hypothesis that critical components of astrocytes, including molecules

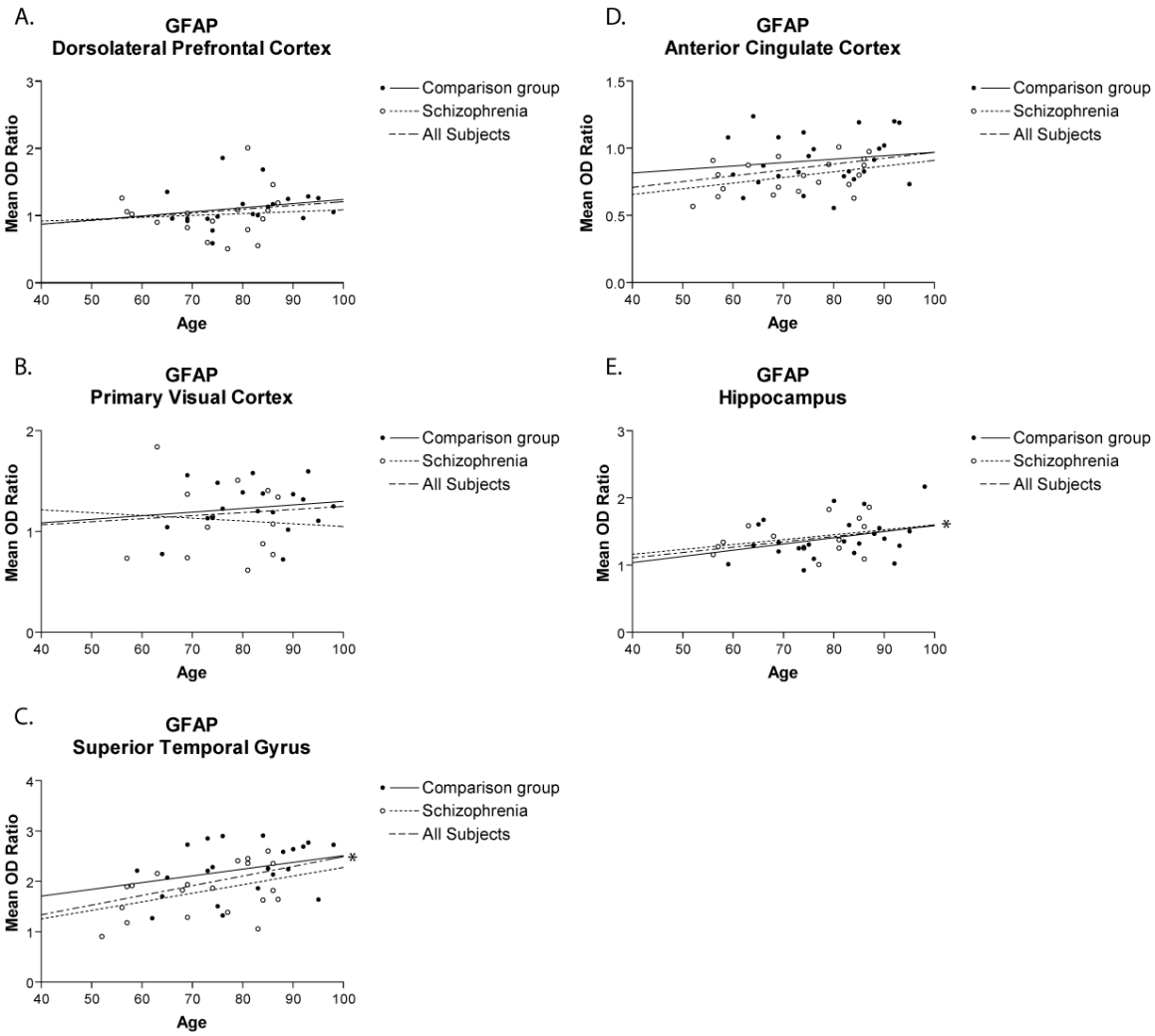

Figure 3.4 Bivariate plots of GFAP protein expression versus age. Regression lines are shown for diagnosis (schizophrenia and comparison group) plotted separately and pooled. There was a significant interaction for age and GFAP expression when all subjects were pooled in the (c) STG ($p < 0.001$), and (e) hippocampus ($p < 0.05$), and a trend for an effect in the (d) ACC ($p = 0.06$).

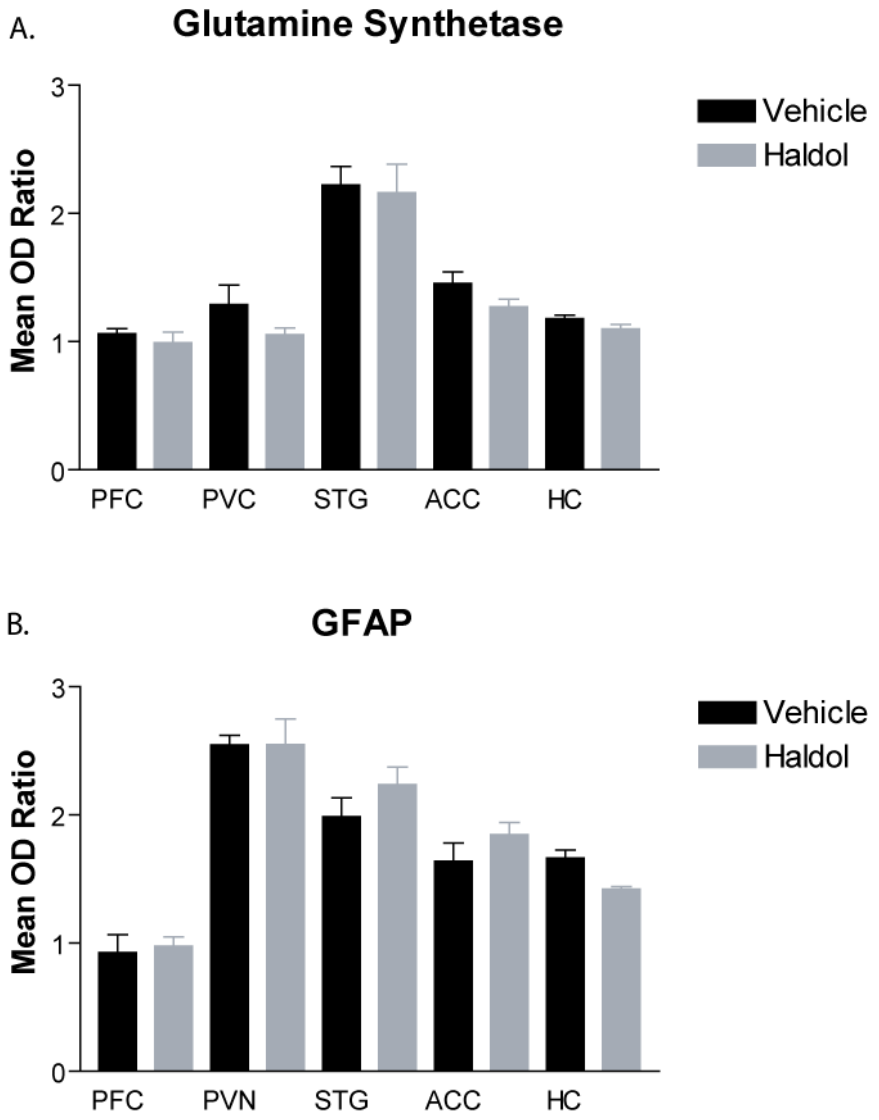

Figure 3.5 Protein expression of astrocytic molecules were measured in the PFC, PVC, STG, ACC, and hippocampus of rats treated chronically treated with haloperidol or vehicle. Expression is shown as mean OD ratio for β-Tubulin with: (a) glutamine synthetase and (b) GFAP in the PFC, PVC, STG, ACC, and hippocampus of rats treated chronically treated with haloperidol or vehicle. There were no treatment effects detected in any of the brain regions studied.

involved with the glutamate cycle and cytoskeletal structure, are abnormally expressed in schizophrenia. Astrocytes are involved in a cycle of synthesis and degradation of glutamate. The enzyme glutamine synthetase contributes to this cycle by converting recovered glutamate to glutamine, which is then shuttled back to the presynaptic terminal. Thus, altered expression and/or function of this enzyme will have profound effects on the glutamate-glutamine cycle and potentially on glutamatergic transmission. I found decreased protein expression of this enzyme in the ACC and STG in schizophrenia, suggesting a decreased capacity to cycle glutamate in these regions.

The cytoskeletal integrity of astrocytes is vital for normal brain functioning. Changes in GFAP expression leading to abnormal structure and function of astrocytes, including decreased process branching, have been linked to impairments in synaptic functioning and abnormal behavior. I found decreased GFAP protein in the ACC in schizophrenia, suggesting that astrocyte-mediated brain functions are impaired in schizophrenia.

3.4.1 Expression of glutamine synthetase in schizophrenia

There are few studies of glutamine synthetase expression in schizophrenia. However, my results are consistent with a study that demonstrated deceased protein expression in area 10 of the PFC in schizophrenia (Burbaeva et al., 2003). Consistent with reports of multiple isoforms of glutamine synthetase (Chakrabarti et al., 1995; Boksha et al., 2000), the study by Burbaeva *et al.* showed that protein expression of an enzyme similar in activity and immunoreactivity to glutamine synthetase, termed glutamine synthetase like protein (GSLP), was increased in this brain region. In addition

to protein levels, this study measured the transferase activity of glutamine synthetase and GSLP; however, no changes were detected. Taken together, unchanged enzyme activity and expression changes in the opposite direction suggest that glutamine synthetase and GSLP may be differentially regulated in disease states by astrocytes. Furthermore, these data suggest that the study of glutamine synthetase may be confounded by the presence of multiple distinct isoforms and that past reports of unchanged glutamine synthetase activity may have been due to a summation of activity from multiple isoforms (Gluck et al., 2002).

The activity of glutamine synthetase is, in part, controlled by NMDA receptor activation (Watts et al., 2005). Following presynaptic glutamate release and subsequent receptor activation neurons release nitric oxide (NO), a key biological messenger and neurotransmitter, which diffuses to bordering astrocytes and decreases glutamine synthetase activity (Kosenko et al., 2003). Conversely, decreased NMDA receptor activation lessens inhibition of glutamine synthetase. Abnormalities of NMDA receptor expression, assembly, cellular localization, and binding have been shown in the ACC and STG in schizophrenia (Humphries et al., 1996; Grimwood et al., 1999; Corre et al., 2000; Kristiansen et al., 2006), so it is possible that illness-related changes in NMDA receptor activation could keep glutamine synthetase in a chronically active state. In turn, this could signal astrocytes to decrease production of glutamine synthetase or to increase protein disassembly and degradation to correct for abnormally high activity (Kosenko et al., 2003).

Abnormal glutamine synthetase expression in the ACC and STG may be due to other brain regions implicated in glutamatergic dysfunction, such as the thalamus. The

thalamus is a relay station of multiple sensory inputs that processes information from multiple cortical regions. Some of the sensory and cognitive disturbances seen in schizophrenia are consistent with glutamatergic dysfunction of thalamic circuitry (Gao et al., 2000; Ibrahim et al., 2000; Smith et al., 2001a; Smith et al., 2001b; Clinton et al., 2006; Huerta et al., 2006). Transcript expression studies of glutamatergic molecules in the thalamus by our laboratory have revealed many changes in this illness: increased vesicular glutamate transporters, decreased NMDA receptor subunits and proteins involved in receptor trafficking, increased astrocytic EAAT1 and 2 and increased EAAT interacting proteins (for review Clinton et al., 2003; Clinton and Meador-Woodruff, 2004). Our laboratory has also demonstrated that glutamine synthetase transcript levels in the thalamus are increased in several nuclei, including those that project to the ACC and STG (Bruneau et al., 2005). Increased transcript expression of thalamic glutamine synthetase may be a compensatory response to decreased glutamine synthetase protein or to abnormal assembly leading to decreased enzyme activity. Reduced glutamate recycling in the thalamus could cause abnormal glutamatergic transmission and potentially affect astrocyte gene expression in areas that receive thalamic efferent projections, such as the ACC and STG.

Alternatively, decreased glutamine synthetase expression, especially in areas exhibiting decreases in GFAP, may reflect region-specific loss of astrocytes. I suggest that my findings of decreased glutamine synthetase protein in the ACC and STG may be related to: 1) abnormal enzyme activity, 2) glutamatergic dysfunction spanning several brain regions, and/or 3) loss of astrocyte density.

3.4.2 Expression of GFAP in Schizophrenia

My data showing decreased expression of GFAP in the ACC are consistent with a literature that finds reduced expression of this cytoskeletal protein. GFAP expression in the PFC, as measured by immunohistochemical labeling, has been shown to be significantly reduced in schizophrenia (Knable et al., 2001). Additionally, studies demonstrating lamina-specific reductions in the area occupied by GFAP labeled cells have been interpreted as evidence of decreased processes branching in this illness (Rajkowska et al., 1998; Rajkowska et al., 2002). Consistent with loss of process branching, phosphorylated isoforms of GFAP protein are also decreased, suggesting that mechanisms regulating GFAP assembly and disassembly may also be abnormal (Johnston-Wilson et al., 2000). Additionally, immunohistochemical labeling showed a reduction in GFAP-positive cells around blood vessels in the DLPFC, suggesting that reduced process branching may impede delivery of nutrients to neurons (Webster et al., 2001). Taken together, these studies demonstrate that loss of process branching due to decreased GFAP expression may be contributing to astrocytic dysfunction, effectively causing wide-spread dysfunction in schizophrenia.

In a previous study examining a similar subject cohort, I reported decreased transcript expression in the DLPFC; however, in the present study I did not find protein changes in this region. Conversely, I previously demonstrated increased transcript expression of GFAP in the ACC, and now report reduced expression in this region. These data suggest that regulation of transcript and protein expression is region-specific. Furthermore, these findings suggest that astrocytes in some brain regions, such as the ACC, may exhibit a compensatory upregulation of transcript because of decreased

protein expression.

Several studies have demonstrated that GFAP mRNA and protein increase with age without an accompanying change in astrocyte density (Hansen et al., 1987; Nichols et al., 1993; Kohama et al., 1995; David et al., 1997). Consistent with these findings, my analysis of pooled data, which included both diagnostic groups, showed that GFAP expression increased with age in all of the brain regions studied (Fig. 3.4). There was a positive correlation between GFAP expression and age that reached statistical significance in the STG ($r = 0.443$, $p = 0.003$) and hippocampus ($r = 0.329$, $p = 0.041$), and was marginally significant in the ACC ($r = 0.285$, $p = 0.058$). However, when subjects were analyzed separately by diagnosis group none of the measures reached statistical significance (Fig. 3.4).

My findings in the ACC demonstrate that decreased GFAP in schizophrenia may be evidence of: 1) a potential loss of process branching, 2) region-specific compensatory gene regulation and, 3) illness-related expression changes inconsistent with predicted changes caused by aging. It is worth mentioning the possibility that a population of astrocytes with decreased GFAP expression, and therefore, loss of process branching, could contribute to reduced glutamine synthetase expression by limiting the reuptake of synaptic glutamate. In turn, this could result in a decreased capacity to cycle glutamate causing wide-spread dysfunction.

3.4.3 Effects of Haloperidol on expression of glutamine synthetase and GFAP in the rat brain

The majority of the subjects with schizophrenia were treated with typical

antipsychotics, which could potentially affect the expression of glutamine synthetase and/or GFAP. I investigated the protein expression of these molecules in rats chronically administered haloperidol using a schedule of 1 mg/kg/day for 28 days (Spurney et al., 1999; Halim et al., 2004; Schmitt et al., 2004). I did not find any changes in glutamine synthetase expression in any of the brain regions of the treated rats, suggesting that my findings of decreased glutamine synthetase protein are not due to a medication effect.

There are few reports describing the effects antipsychotic treatment on the expression of glutamine synthetase. Studies measuring brain glutamine synthetase protein levels do not find changes that can be related to antipsychotic treatment (Burbaeva et al., 2003; Toro et al., 2006). However, recently it was reported that peripheral protein levels of GSLP, an isoform of glutamine synthetase, may be altered by treatment with antipsychotics. Burbaeva *et al.* demonstrated that increased platelet expression of GSLP in schizophrenia is reduced following treatment with the *atypical* antipsychotic, olanzapine (Burbaeva et al., 2006). The activity of this enzyme may also be under the influence of antipsychotic medication. A study found that glutamine synthetase activity was increased in various regions of the rat brain after a single dose of the typical antipsychotic chlorpromazine, and was increased in the cerebral cortex after long-term administration (Chandrakala et al., 1987).

I did not find changes in GFAP expression in rats treated with haloperidol either. This is consistent with a recent study that failed to detect changes in the GFAP protein expression in rat frontal cortex following chronically administered haloperidol (Dean et al., 2006). The literature surrounding acute and chronic treatment with haloperidol does not resolve whether changes to astrocyte density or to the expression of astrocytic

molecules such as GFAP can be attributed to antipsychotics. Reportedly, rats given a single dose of haloperidol did not demonstrate astrocyte activation as measured by double immunofluorescence labeling of c-*fos* and GFAP (Ma et al., 2003). Another study reported that MK801-induced astrocyte density and GFAP immunoreactivity increases in the rat cingulate and retrosplenial cortices can be prevented by the atypical antipsychotic clozapine, but not by haloperidol (Arif et al., 2007). It remains uncertain whether haloperidol influences glutamine synthetase or GFAP expression, and whether any one animal model parallels this treatment in humans.

In this study, I report decreased glutamine synthetase in ACC and STG in schizophrenia, and decreased GFAP protein in the ACC. Further study is needed to clarify whether these changes are associated with altered cell density, altered process branching, or with compensatory mechanisms secondary to this illness. My data support the hypothesis that astrocytes contribute to the pathophysiology of schizophrenia, and that astrocytic molecules involved in glutamatergic function and cytoskeletal integrity are compromised in this illness.

CHAPTER 4

SERINE RACEMASE PROTEIN EXPRESSION IN THE CORTEX AND HIPPOCAMPUS IN SCHIZOPHRENIA

4.1 Introduction

As discussed in previous chapters, glutamate neurotransmission has been hypothesized to be abnormal in schizophrenia. This is based in part on pharmacological data indicating that antagonists of the NMDA receptor, such as PCP, can induce schizophreniform symptoms in non-psychiatrically ill individuals, and exacerbate these symptoms in patients with schizophrenia (Javitt, 1987). Our laboratory has reported changes in the expression of numerous molecules associated with the transport, reuptake and recycling of glutamate in schizophrenia, including multiple studies demonstrating altered expression of NMDA receptor, subunits and binding sites, as well as associated postsynaptic density proteins that facilitate intracellular signaling (McCullumsmith and Meador-Woodruff, 2002; Clinton and Meador-Woodruff, 2004; Mueller and Meador-Woodruff, 2004; Bruneau et al., 2005; Kristiansen and Meador-Woodruff, 2005).

The vast majority of reported schizophrenia-associated abnormalities are of neuronal molecules. Astrocytes, a specialized type of glial cell, are also present at the excitatory synapse and are intimately involved with glutamatergic transmission and NMDA receptor function. In particular, the synthesis of the NMDA receptor co-agonist

D-serine is via serine racemase, a PLP-dependent enzyme expressed predominantly in astrocytes that co-localize to areas of the brain replete with NMDA receptors (Schell et al., 1995; Wolosker et al., 1999a; Miranda et al., 2000). Serine racemase has been proposed as the main regulator of both intra- and extracellular D-serine concentrations in the forebrain by conversion of L-serine to D-serine following glutamate-induced activation of kainate and α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors, and by an α, β-elimination reaction, respectively (Kim et al., 2005; Strisovsky et al., 2005).

Co-localization of serine racemase and D-serine-containing astrocytes with the NMDA receptor complex suggests a functional relationship (Schell et al., 1995). D-serine binds with a high affinity to the modulatory strychnine-insensitive glycine site on the NMDA receptor NR1 subunit; thus, D-serine producing astrocytes directly modulate NMDA receptor activity (Hashimoto et al., 1993; Matsui et al., 1995; Mothet et al., 2000).

Recent studies have reported decreased levels of D-serine in the serum and CSF of patients with schizophrenia (Hashimoto et al., 2003; Hashimoto et al., 2005). Additionally, patients with schizophrenia who received D-serine along with antipsychotic treatment showed improvement in both positive and negative symptoms, as well as in cognition (Tsai et al., 1998; Heresco-Levy et al., 2005). Over-expression of D-amino acid oxidase, the enzyme responsible for catabolizing D-serine, reduces NMDA receptor activity (Mothet et al., 2000). Genetic evidence suggests linkage in a subset of patients with schizophrenia for polymorphisms in functionally related genes that encode and regulate D-amino acid oxidase (Chumakov et al., 2002).

Serine racemase has not yet been studied in the brain in schizophrenia, although the distribution of NMDA receptors and serine racemase-expressing astrocytes in the hippocampus, cortex and other cerebral structures coincides with areas of glutamatergic dysfunction in this illness (Goff and Coyle, 2001a; Harrison et al., 2003). I hypothesize that there are alterations of serine racemase expression in schizophrenia in brain regions that have in the past demonstrated NMDA receptor abnormalities. Accordingly, I measured protein expression of serine racemase using Western blot analysis in elderly subjects with schizophrenia and a non-psychiatrically ill comparison group in the DLPFC, ACC, STG, and hippocampus. Additionally, I studied the PVC, an area not as closely implicated in glutamatergic abnormalities in schizophrenia, to determine specificity of findings.

4.2 Methods

4.2.1 Acquisition and processing of human brain tissue.

Fifty subjects from the Mount Sinai Medical Center and Bronx Veterans Administration Medical Center were used for this study, consisting of 27 non-psychiatrically ill individuals and 23 patients with schizophrenia. Subjects were diagnosed with schizophrenia if the following criteria were fulfilled: 1) the presence of schizophrenic symptoms could be documented before age 40; 2) the medical records contained evidence of psychotic symptoms and at least 10 years of psychiatric hospitalization with a diagnosis of schizophrenia; 3) a DSM-III-R diagnosis of schizophrenia was agreed upon by two experienced clinicians; 4) neuropathologic examination did not reveal Alzheimer's disease or other discernable neuropathologic

Table 4.1 Subject characteristics for Mount Sinai and Bronx Veterans Administration Medical Centers cohort – serine racemase study

Diagnosis	Sex	Age	PMI (hours)	pH	Cause of Death
Comparison Group	F	62	7.0	6.6	acute myocardial infarction
	F	73	3.4	6.3	acute myocardial infarction
	F	74	3.0	6.0	cardiopulmonary arrest
	F	75	6.5	6.0	cardiopulmonary arrest
	F	80	4.8	6.2	sepsis
	F	82	5.7	6.1	cardiopulmonary arrest
	F	83	6.2	6.8	cardiopulmonary arrest
	F	84	18.5	6.2	myocardial infarction
	F	86	4.7	6.5	acute myocardial infarction
	F	88	5.1	6.4	chronic obstructive pulmonary disease
	F	89	2.3	6.7	bronchopneumonia, chronic obstructive pulmonary disease
	F	90	4.2	6.0	pneumonia
	F	98	1.4	6.6	myocardial infarction, aortic aneurysm
	M	59	20.4	6.7	coronary arterial disease
	M	60	28.8	6.6	acute myocardial infarction
	M	64	4.2	6.4	acute myocardial infarction
	M	65	3.8	6.8	renal failure
	M	66	7.6	6.6	acute myocardial infarction
	M	69	4.3	6.3	cancer of lung
	M	69	7.4	6.7	septic shock
	M	74	16.6	6.7	cardiopulmonary arrest
	M	76	2.9	6.3	bronchopneumonia
	M	85	5.3	6.5	transitional cell cancer of bladder
	M	92	20.0	6.4	arrhythmia
	M	93	4.2	6.3	acute myocardial infarction
	M	95	4.1	6.5	chronic renal failure
	M	101	4.7	6.8	congestive heart failure
	14M, 13F	**79 ± 12**	**7.7 ± 6.8**	**6.5 ± 0.2**	
Schizophrenia	F	69	13.7	6.2	cardiopulmonary arrest
	F	74	7.0	6.3	Cardiopulmonary Arrest
	F	77	9.7	6.0	cardiopulmonary arrest
	F	79	9.9	6.8	cardiac arrest
	F	81	12.5	5.9	acute myocardial infarction
	F	81	15.1	6.7	cardiopulmonary arrest
	F	83	20.4	7.1	cardiopulmonary arrest, cancer of pancreas
	M	52	29.5	5.9	cardiopulmonary arrest
	M	56	13.5	6.5	cardiopulmonary arrest
	M	57	30.3	6.1	expired during open heart surgery
	M	57	21.4	6.4	small cell cancer of lung
	M	58	6.7	6.2	cardiopulmonary arrest
	M	58	13.3	6.9	cardiopulmonary arrest
	M	63	6.2	6.3	cardiopulmonary arrest
	M	68	17.3	6.6	congestive heart failure, coronary artery disease
	M	69	40.2	6.7	acute renal failure
	M	73	7.9	6.5	cardiopulmonary arrest
	M	76	16.6	6.7	coronary artery disease, congestive heart failure
	M	84	6.2	6.5	cardiopulmonary arrest
	M	85	5.3	6.3	chronic obstructive pulmonary disease, pneumonia
	M	86	7.0	6.3	cardiopulmonary arrest
	M	86	14.1	6.7	artheroschlerotic heart disease
	M	87	11.2	6.5	cardiopulmonary arrest
	16M, 7F	**72 ± 12**	**14.6 ± 8.9**	**6.5 ± 0.3**	

abnormalities. A summary of subject characteristics is shown in Table 4.1.

Brains obtained at autopsy were divided mid-sagittally at the time of extraction. The right half was fixed in 4% formaldehyde and used for neuropathological characterization. The left half was sectioned in 6-8 mm coronal slabs, immediately snap-frozen, and stored at –80^{o}C. The areas of interest were dissected from the frozen slabs (Dracheva et al., 2005). Dissected never-thawed tissues were pulverized at –190^{o}C into a fine powder and aliquoted into individual Eppendorf tubes and stored at –80^{o}C.

4.2.2 Acquisition and processing of rodent brain tissue.

Twenty-two adult, male Sprague-Dawley rats (250g) were housed two-three to a cage, with food and water ad libitum. Animals were treated with daily intramuscular injections of haloperidol dissolved in dimethyl sulfoxide (DMSO) (Fisher Scientific, Fair Lawn, NJ, USA) (1 mg/kg/day, *n*=11), or vehicle (DMSO) (*n*=11) for 29 days. Twenty-four hours after the last injection, the animals were sacrificed and brains were immediately removed, dissected, and frozen in isopentane. The frontal, cingulate, occipital, and temporal cortices, and hippocampus were dissected according to *The Rat Brain in Stereotaxic Coordinates* (Paxinos and Watson, 1986). The brains were stored at −80°C until assayed. All animal experiments were approved by the University Committee on the Use and Care of Animals at the University of Michigan and were performed according to the guidelines for The Care and Use of Laboratory Animals of the National Institutes of Health.

4.2.3 Tissue preparation.

Tissue specimens (human 50mg, rodent 20mg) were homogenized in 1ml of 5mM Tris–HCl (pH 7.4), containing Complete, mini, EDTA-free protease inhibitor cocktail tablets (Roche Applied Science, Indianapolis, IN) (1 tablet/10mls) for 30 seconds with a PowerGen 125 homogenizer (Fisher Scientific). Total protein concentration was determined with a BCA Protein Assay Kit (Pierce) and fluorescence was measured on a SpectraCount absorbance microplate reader (Packard) at 540 nm. Homogenates were stored at -80^{o}C until assay.

4.2.4 Western blot analysis.

Samples were prepared by combining tissue homogenate with sample buffer (62.5mM Tris-HCl, 20% glycerol, 2% sodium dodecyl sulfate, 5% β-mercaptoethanol, pH 6.8) and heated at 95^{o} C for four minutes. Samples were loaded onto pre-cast 10% polyacrylamide Tris-HCl gels (Bio-Rad) in duplicate (20μg protein/well) and run in SDS/Tris/glycine buffer (25mM Tris-HCl, 192mM glycine, 0.1% SDS, pH 8.3) at 130 mV for about one hour. Following transblotting to nitrocellulose membranes in Tris-glycine buffer (25mM Tris, 192mM glycine, pH 8.3), blots were blocked with 1% powdered milk in PBS (pH 7.4), and agitated for two hours at room temperature and then overnight at 4^{o}C with a monoclonal antibody directed against mouse serine racemase (BD Biosciences, San Diego, CA) at a dilution of 1:500 in 1% powdered milk in PBS. Next, blots were washed in PBS and incubated with a horseradish peroxidase-coupled goat anti-mouse secondary antibody (Santa Cruz Biotechnology, INC.) for three hours on a shaker at room temperature. Following four washes in PBS and two washes in distilled water,

enhanced chemiluminescence (ECL) was used for detection. Blots were saturated with ECL reagent (Amersham, Piscataway, NJ), covered in plastic wrap and exposed to ECL film (Amersham, Piscataway, NJ). Film was developed and digitally captured with a CCD based imaging system using Scion Imaging software 4.0.3. Gray scale values were obtained for protein bands at the expected molecular weight (38kDa), membrane background was subtracted, and the adjusted gray scale values from duplicate samples in adjacent lanes were averaged and converted to optical density. Membranes were stripped and re-blotted for β-Tubulin (Upstate, lake Placid, NY), as a loading control, and the mean ratio of serine racemase /β-Tubulin optical density was used for data analysis.

4.2.5 Statistical analysis.

Statistical analysis was performed with Statistica (StatSoft, Tulsa, OK). Correlation analysis was performed to investigate possible associations between protein expression and age, postmortem interval, and tissue pH. I analyzed data from each region by one-way ANOVA, with diagnosis as the independent variable and mean optical density ratio (serine racemase/β-Tubulin) as the dependent variable. For all tests $\alpha = 0.05$.

4.3 Results

4.3.1 Protein expression of serine racemase in schizophrenia

I used Western blot analysis to measure the expression of serine racemase in the brain in schizophrenia. Serine racemase was detected in all five of the brain regions studied, with a band at the expected molecular weight of 38kDa. Correlation analysis

showed no associations between serine racemase expression and age, postmortem interval, or pH in any of the areas examined. I found a main effect for diagnosis for increased serine racemase expression in the hippocampus in schizophrenia ($F(1, 44) = 5.199$; $p = 0.028$) (Figure 4.1a). There was no effect of diagnosis on serine racemase expression in any of the cortical areas studied (Figure 4.1b-e).

4.3.2 Effects of haloperidol treatment on protein expression of serine racemase in the rat brain

Most of the patients with schizophrenia in this study were treated with typical antipsychotics. I used Western blot analysis to measure the expression of serine racemase in rats chronically treated (2mg/kg/day) for 28 days with haloperidol. There was a significant effect for treatment in the PVC ($F(1, 19) = 7.52$; $p = 0.013$). Treatment with haloperidol decreased serine racemase expression in the PVC of the rat brain. I did not detect differences in protein expression for serine racemase in any of the other brain regions studied (Figure 4.2).

4.4 Discussion

Linking astrocytic function to schizophrenia represents a divergence from work that has historically focused on changes primarily attributable to neurons. Based on the coordinated activity of neuronal and astrocytic molecules in glutamatergic transmission and NMDA receptor function, I used human postmortem tissue to study brain regions implicated as areas of glutamatergic dysfunction in schizophrenia: the hippocampus and four cortical regions including the DLPFC, ACC, STG, and PVC. I detected a

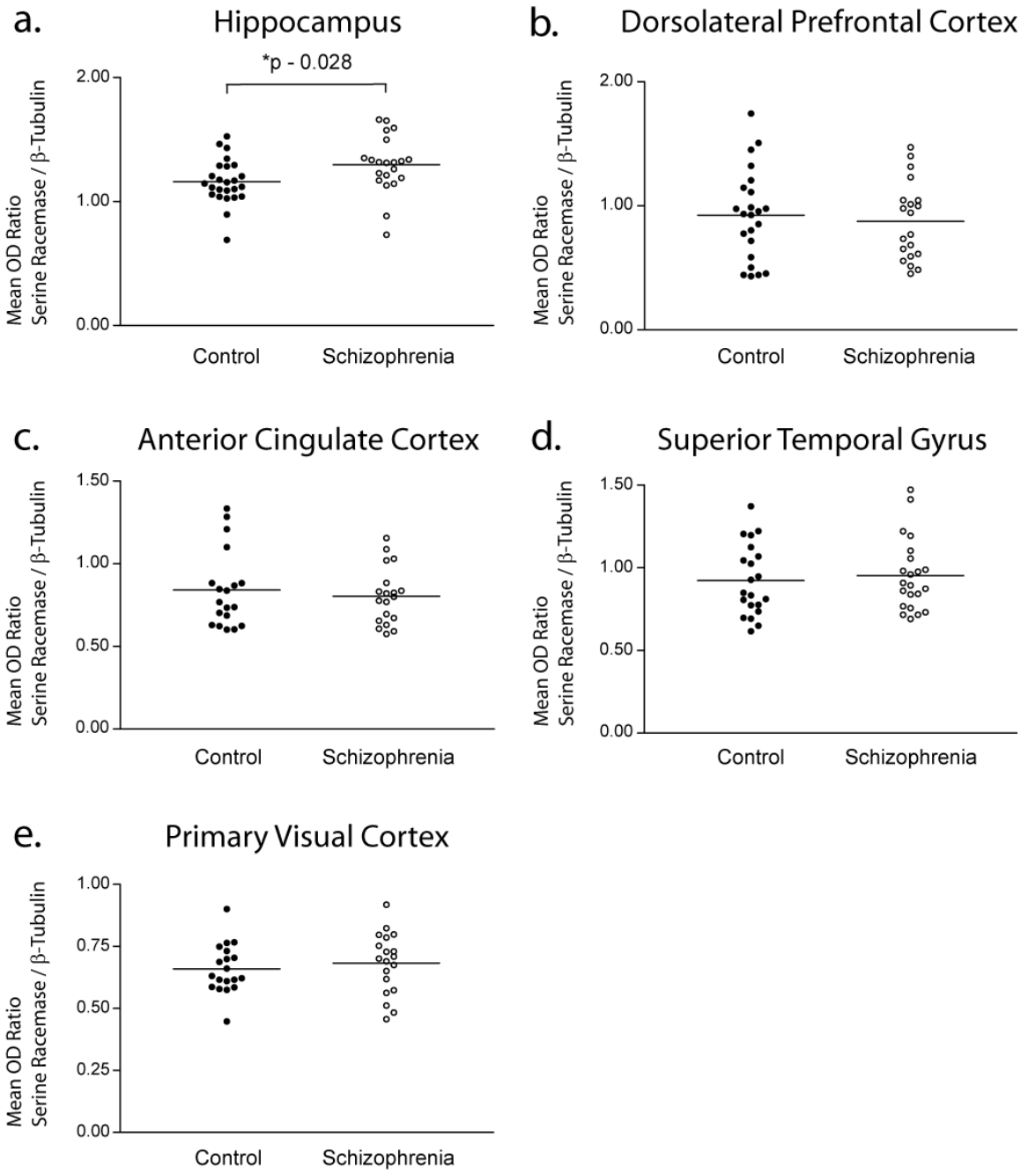

Figure 4.1 Serine racemase protein expression in the hippocampus, DLPFC, ACC, STG, PVC in schizophrenia. Expression is shown as mean OD ratio for β-Tubulin with serine racemase. (a) Serine racemase protein expression is increased in the hippocampus in schizophrenia ($F(1, 44) = 5.199$; $p=0.028$), (b-e) but not in the other cortical regions studied.

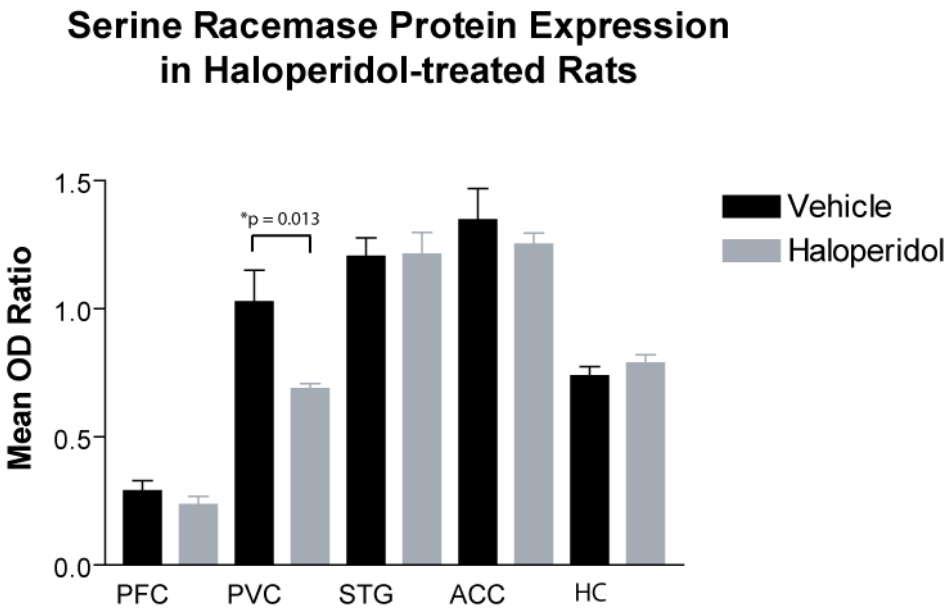

Figure 4.2 Protein expression of serine racemase in the PFC, PVC, STG, ACC, and hippocampus of rats chronically treated with haloperidol or vehicle. There was a significant effect for treatment in the PVC ($F(1, 19) = 7.52$; $p = 0.013$). Treatment with haloperidol decreased serine racemase expression in the PVC of the rat brain. There were no treatment effects detected in any of the other regions studied.

significant increase in the expression of serine racemase protein in the hippocampus in schizophrenia.

The hippocampus plays an important role in learning and in verbal and spatial memory via its connections with virtually all association cortices. Additionally, it plays a role in long term potentiation, a component of memory specifically linked to NMDA receptor activity. Diminished cognitive processing, often a feature of schizophrenia, and evidence for structural and functional hippocampal pathology, have implicated the hippocampus, which is a brain region of particular sensitivity to insult in this illness (Harrison, 2004).

NMDA receptor antagonists such as (+)-5-methyl-10, 11-dihydro-5H-dibenzo [a, d] cyclohepten-5, 10-imine (MK-801) and PCP, have been shown to alter various components of the glutamatergic neurotransmitter system, including those associated with astrocytes. These results include evidence that NMDA receptor function influences the transcript expression of serine racemase in the hippocampus and other brain areas (Yoshikawa et al., 2004). Whether changes in the expression of serine racemase are the result of neuronal or astrocytic NMDA receptor blockade, or both, my data demonstrate alterations of an astrocyte-derived rapid response system that is sensitive to changes potentially affecting multiple components of the glutamatergic synapse. It should be noted that although there are no direct linkages to serine racemase reported, an association may exist upstream of serine racemase for a regulatory gene in a pathway that modulates the expression and/or activity of this enzyme.

Several studies have identified abnormal expression of glutamatergic molecules in schizophrenia. The present findings provide evidence for an astrocytic component to

these abnormalities which should be replicated in other relevant schizophrenia cohorts. Furthermore, this study indicates that brain expression of the neuromodulator D-serine would be an appropriate target for further study in schizophrenia.

CHAPTER 5

DISCUSSION

5.1 Summary of findings

Numerous studies demonstrate the major supportive roles that astrocytes and astrocyte-related molecules play in brain function, including contributions to energy metabolism, regulation of ion concentrations, intracellular signaling, and glutamatergic neurotransmission. Four astrocytic molecules, in particular - GFAP, S100β, glutamine synthetase, and serine racemase - have emerged as being especially important for serving these functions (Figure 5.1). Therefore, a breakdown in the structure or function of these molecules could have profound effects on brain function. While GFAP is a major component of the astrocytic cytoskeleton, only a small portion of its functions are known. GFAP has been shown to regulate process extension, enabling astrocytes to monitor and respond to changes in the environment (Weinstein et al., 1991; Chen and Liem, 1994), and its expression may have a reciprocal relationship with glutamatergic transmission (Porter and McCarthy, 1995; Kommers et al., 2002; Hu et al., 2003). I found that GFAP transcript expression was decreased in the DLPFC and increased in the ACC in schizophrenia, and that GFAP protein expression was decreased in the ACC in schizophrenia. S100β, a signaling molecule with a multitude of functions, modulates astrocyte morphology as well as neuronal morphology and survival. My studies did not

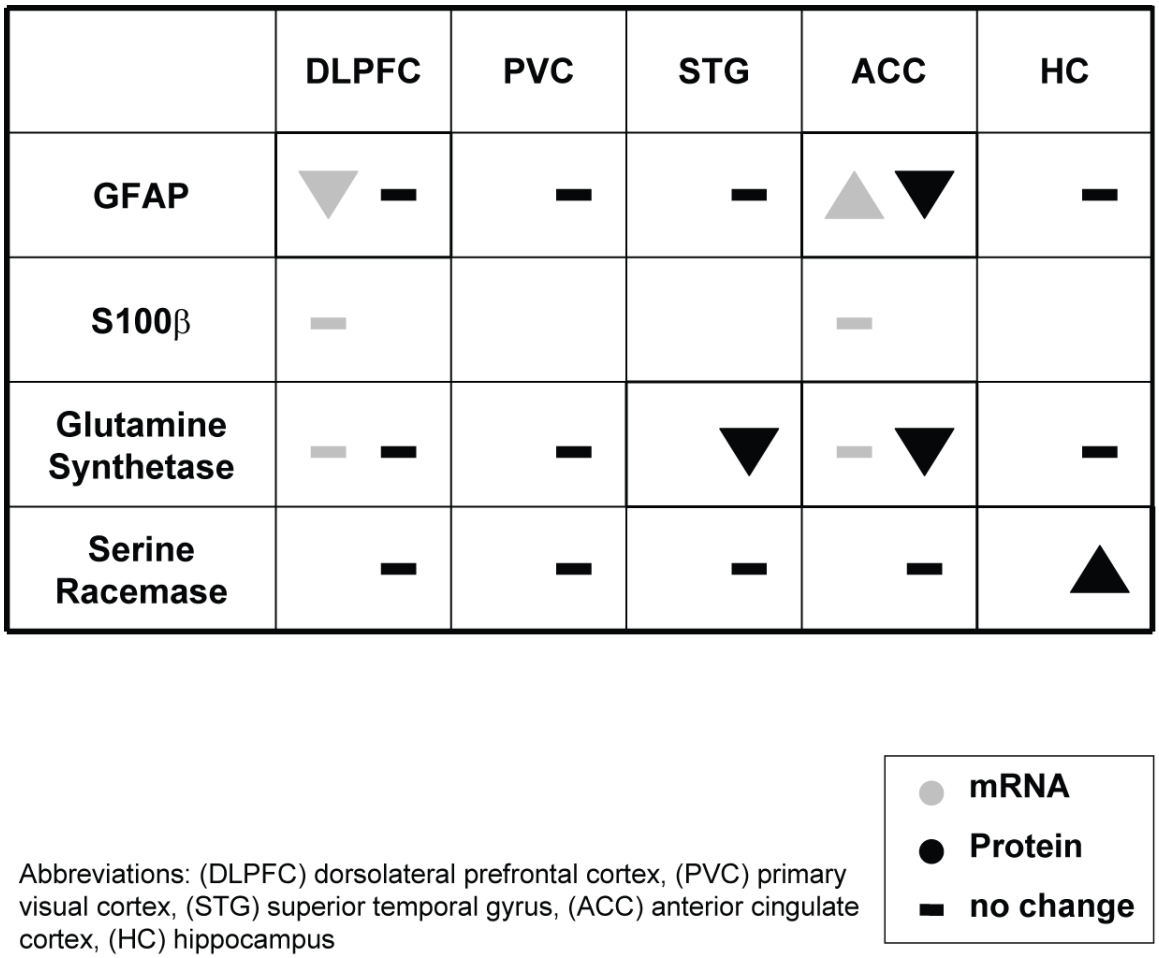

Figure 5.1 Summary of results. The transcript and protein expression of the astrocytic molecules GFAP, S100β, glutamine synthetase, and serine racemase were measured in schizophrenia. GFAP transcript expression was decreased in the DLPFC and increased in the ACC, and GFAP protein expression was decreased in the ACC. Glutamine synthetase protein was decreased in the STG and ACC. Serine racemase protein was increased in the hippocampus in schizophrenia.

detect any changes in S100β transcript expression in schizophrenia. The astrocytic enzyme glutamine synthetase maintains the tightly regulated glutamate-glutamine cycle, which in turn controls levels of metabolic substrates, neurotransmitter levels, and also protects cells from excitotoxicity. I found decreased protein expression of glutamine synthetase in the STG and ACC in schizophrenia; however, I did not detect any changes to glutamine synthetase transcript expression in the ACC. Finally, serine racemase is the enzyme responsible for synthesizing D-serine, an NMDA receptor co-agonist that facilitates glutamatergic signaling, learning and memory-mediated LTP. My findings show that serine racemase protein levels are increased in the hippocampus in schizophrenia.

5.2 Altered expression of astrocyte-related molecules in schizophrenia: global astrocytic lesion or discrete regional and molecular defects

The study of potential astrocytic abnormalities in schizophrenia represents a relatively new line of investigation, as the majority of work to date has focused on neuronal dysfunction in this illness. One of the preliminary findings that suggested a role for astrocytes in schizophrenia was altered cell density in several brain regions thought to be involved in the pathophysiology of the illness. Thus, it was hypothesized that a loss of astrocytes, or compromised astrocytic functioning in those particular brain regions, could contribute to brain dysfunction in schizophrenia. Although, subsequent research has failed to show prominent changes in astrocyte density in select brain regions in schizophrenia, an alternate hypothesis suggests that a global astrocytic lesion may give rise to brain abnormalities observed in the illness. Specifically, this theory posits that

widespread expression changes in structural and functional astrocytic molecules may contribute to prominent dysfunction of neural circuits in the brains of patients with schizophrenia. Thus, the current body of work sought to determine whether the expression of four major astrocytic molecules – GFAP, S100β, glutamine synthetase, and serine racemase was collectively altered across multiple brain regions in schizophrenia.

5.2.1 Regional transcript and protein expression changes in the brain in schizophrenia.

Within these studies, no individual brain region emerged as being a focal point of acute altered astrocytic marker expression; instead, several regional expression deficits were found. I identified three brain regions, the DLPFC, STG, and hippocampus, which showed altered expression of astrocytic markers; however, each area exhibited altered expression for just one out of the four molecules measured. Each region had been previously implicated in schizophrenia, with evidence of structural and/or functional deficits. Based on data for the four molecules that I studied, astrocytic contribution to brain dysfunction in these areas is discrete. The ACC appeared to be the most affected region, with two different molecules (GFAP and glutamine synthetase) showing altered expression. The literature regards the ACC as an area having structural, functional, and gene expression changes in schizophrenia, and my data suggest that astrocytic abnormalities may play a role in ACC dysfunction. In contrast to the other four regions studied, I did not find altered transcript or protein expression for any of the molecules studied in the PVC. This was not entirely unexpected; as mentioned previously this area has often been used as a "control region" to compare with other commonly affected areas. My data do not support comprehensive dysfunction of astrocytes in schizophrenia;

rather, these results suggest that changes in the expression of astrocytic molecules are localized to specific areas.

5.2.2 Direction of transcript and protein expression change of astrocytic molecules in schizophrenia.

There were no consistent patterns in the direction of change for transcript or protein expression for any of the molecules studied. A predominance of decreased expression of astrocytic molecules, both transcript and protein, could suggest a net loss of astrocytes overall, or loss of a specific subset of astrocytes that expressed particular molecular markers. Meanwhile, an overall increase in expression might suggest a wide-spread compensatory upregulation, a faulty feedback mechanism chronically signaling for increased expression, or support gliosis and an increase in astrocytes. Instead of any uniform changes, I observed opposite patterns of GFAP transcription changes in the DLPFC versus ACC. Despite reports of altered protein expression of GFAP in schizophrenia, there is little information regarding transcript expression in the literature with which to compare and interpret these results. GFAP mRNA levels were decreased in the DLPFC in schizophrenia, which may suggest that events that modulate gene transcription, such as astrocyte activation, are abnormal in schizophrenia. I did not detect changes in GFAP protein expression in this region, however, which may indicate a compensatory mechanism where astrocytes decrease GFAP protein turnover by inhibiting phosphorylation-mediated disassembly of this cytoskeletal protein.

In contrast to findings in the DLPFC, GFAP transcript expression was significantly increased in the ACC. This is a divergence from protein expression findings

that predominantly show decreases in GFAP (Johnston-Wilson et al., 2000; Cotter et al., 2001; Knable et al., 2001; Webster et al., 2001a). Increased GFAP transcript expression in the ACC may be due to altered gene splicing during transcription, either by the improper removal of exons or by introns that remain unspliced, either of which could result in an unstable and short-lived protein product. Alterations in the rates of synthesis and breakdown of astrocytic proteins has been hypothesized to exist in schizophrenia (Burbaeva et al., 2007), and would offer an interesting explanation for the discrepant transcript and protein data. My findings of increased transcript expression of GFAP in the ACC may reflect a compensatory upregulation in response to increased recycling of protein.

I also found altered protein levels of two other molecules, glutamine synthetase (in the ACC and STG) and serine racemase (in the hippocampus). These expression changes were not uniform in direction either. Glutamine synthetase protein expression was decreased in the ACC and STG in schizophrenia, whereas serine racemase protein levels were increased in the hippocampus. By virtue of their heterogeneous nature, these results argue that transcript and protein expression are not altered in a manner suggestive of a global astrocytic lesion, but rather, changes are discrete and molecule-specific.

5.3 The expression of astrocytic structural and functional molecules is altered in schizophrenia: analysis by molecule

5.3.1 Analysis by molecule: S100β

All of the molecules studied showed some level of expression change, either transcript, protein, or both, except for S100β. This finding was unexpected given a

literature depicting S100β alterations in CSF and serum of patients with schizophrenia. However, since I only measured S100β transcript expression, I cannot exclude the possibility of protein expression changes. There is only one other study reporting brain expression of S100β, and it showed that S100β protein expression was unchanged in the DLPFC in schizophrenia (Dean et al., 2006). Whether or not these results are of particular relevance to the present findings is unclear, but there is support for consistency across studies by the fact that they, too, did not detect GFAP protein expression changes in the DLPFC.

Altered CSF and serum S100β levels in patients with schizophrenia have been interpreted as a sign of either increased astrocyte activation or of cells leaking contents into peripheral fluids. However, the notion of leaking cells is not supported by a recent study which showed that patients with schizophrenia did not exhibit increased levels of GFAP or myelin basic protein along with increased levels of S100β in serum and CSF (Steiner et al., 2006). Other work showing that S100β serum levels increased simultaneously with levels of myo-inositol, a product of the phosphatidylinositol second messenger system, in patients with schizophrenia suggests that increased S100β may instead correlate with astrocyte activation (Malhi et al., 2002). Thus, the scarcity of reports on S100β brain expression makes CSF and serum level data difficult to interpret. More studies integrating these measures are needed to clarify the pathophysiological significance of these findings.

5.3.2 Analysis by molecule: GFAP

Earlier reports speculated that altered GFAP transcript expression may result in an

unstable and short-lived protein. Protein function of this molecule is mediated by the phosphorylation of several sites on the N-terminal head of the protein, and a site localized to the tail end of the filament. During GFAP transcription, incorrect splicing patterns could result in the loss of function mediated by phosphorylation.

Within the astrocyte, GFAP undergoes assembly and disassembly in accordance with the needs and functional states of the cell. Phosphorylated GFAP exists as unassembled subunits in a soluble pool that are interchangeable with polymerized counterparts. Normally, equilibrium of phosphorylated soluble GFAP is maintained with GFAP within the cytoskeleton (Takemura et al., 2002). The phosphorylation of GFAP is stimulated by the presence of glutamate and by the absence of Ca^{2+} (Rodnight et al., 1997; Kommers et al., 2002). The mechanisms of glutamate-mediated phosphorylation, in part, include synaptic targeting and activation of group II mGluRs expressed by astrocytes (Oppelt et al., 2004; Battu et al., 2005). This activation inhibits the entry of Ca^{2+}, possibly through L-type channels, and decreases cytosolic Ca^{2+}, thereby downregulating Ca^{2+}-dependent dephosphorylation and increasing polymerization (Oppelt et al., 2004). The phosphorylation state of GFAP also appears to be regulated by internal Ca^{2+}, which, like the presence of extracellular Ca^{2+}, causes the dephosphorylation of GFAP.

Phosphorylation takes place via a group of kinases, such as cyclic AMP-dependent protein kinase (PKA), Ca^{2+}/calmodulin-dependent kinase II, (CaMK II), protein kinase C (PKC), and Cdc2 kinase, at various sites, five localized to the N-terminal head of the protein, and one localized to the tail end of the filament (Rodnight et al., 1997). Phosphorylation results in the disassembly of preformed GFAP and prevents

unassembled subunits from incorporating into the astrocytic cytoskeleton (Rodnight et al., 1997). Immunohistochemical and proteomic studies have show that phosphorylated isoforms of GFAP are decreased in schizophrenia, suggesting altered function of this molecule (Johnston-Wilson et al., 2000; Webster et al., 2001b).

Alternatively, molecules regulating the expression of GFAP may be abnormal in schizophrenia. Vimentin, another IF protein expressed by astrocytes, has been shown to modulate the expression and structure of GFAP filaments (Galou et al., 1996). In the absence of vimentin, GFAP expression is decreased and its incorporation into the cytoskeleton is altered. Therefore, a change in GFAP could actually reflect a more wide-spread abnormality for cytoskeletal proteins in schizophrenia. There have been few studies examining the expression of vimentin in schizophrenia, and so far, none have detected any changes (Arnold et al., 1996; Miyamae et al., 1998).

5.3.3 Analysis by molecule: Glutamine synthetase

The expression and activity of glutamine synthetase, which is hypothesized to be a component of glutamatergic dysfunction in schizophrenia (Bruneau et al., 2005; Burbaeva et al., 1999; Goff and Coyle, 2001), is influenced by downstream events that take place prior to glutamate recycling, for example by molecules regulating the length of time glutamate is in the synapse. This is accomplished by the expression and function of EAATs. Altered EAATs expression has been shown in several brain regions in schizophrenia, including the thalamus and cortex (Smith et al., 2001; Matute et al., 2005). Although the direction of change for EAAT expression is not consistent, these data, along with findings that proteins involved in the trafficking and expression of EAATs are

increased in schizophrenia (Bauer, unpublished data), suggest that glutamate reuptake is abnormal in schizophrenia. The release of aracadonic acid, as a result of NMDA receptor activity, can affect astrocytic reuptake of glutamate by inhibiting EAAT expression (Volterra et al., 1994). Therefore, it could be hypothesized that NMDA receptor hypofunction could abnormally increase EAAT expression. These factors, in turn, could cause downstream changes to the expression and/or activity of glutamine synthetase.

It is notable that the cycling of glutamate is affected by the expression of GFAP. GFAP-null mice are able to transport glutamate normally, but they exhibit increased glutamine synthetase activity and levels of glutamine (Pekny et al., 1999). While I did not detect any glutamine synthetase transcript expression changes, I did find decreased protein expression in the ACC and STG in schizophrenia. This finding, along with that of decreased GFAP in the ACC in schizophrenia, may indicate abnormal regulation of glutamine synthetase under conditions demanding increased glutamate recycling. Additionally, I found decreased protein expression of this enzyme in the STG, suggesting that a decreased capacity of astrocytes to cycle glutamate may be a common manifestation in schizophrenia. On the other hand, decreased glutamine synthetase expression could be a normal response to a system that is exhibiting decreased reuptake of synaptic glutamate. These conditions could result in prolonged activation of synapses, which is consistent with evidence for decreased deactivation reported in the ACC and STG in schizophrenia (Assaf et al., 2006; Walter et al., 2007).

5.3.4 Analysis by molecule: Serine racemase

The expression and function of serine racemase in the brain adjusts according to

the changing needs of the astrocyte and surrounding neurons (Martineau et al., 2006). Therefore, a straightforward interpretation of my finding for increased serine racemase protein expression in the hippocampus is that of a compensatory upregulation in response to NMDA receptor hypofunction. Serine racemase expression and activity can directly influence neuronal signaling by altering the synthesis of the NMDA receptor co-agonist, D-serine. Conversely, NMDA receptor antagonists such as MK-801 have been shown to upregulate serine racemase mRNA expression (Yoshikawa et al., 2004). The exact mechanisms for such activation are not well understood, however. Evidence from cell culture experiments show that activation of AMPA and kainate receptors increases the activity of serine racemase and results in the release of D-serine (Schell et al., 1995). However, these results have not yet been confirmed *in vivo*. In fact, studies in the rat striatum showed that treatment with glutamatergic agonists and antagonists did not result in D-serine release (Ciriacks and Bowser, 2006). There is evidence for NMDA receptor-mediated D-serine release, although it is unclear whether this is due to neuronal or astrocytic NMDA receptors (Martineau et al., 2006).

The hippocampus, because of the high density of NMDA receptors and D-serine expression, plays a prominent role in learning, memory, and induction of LTP (Schell et al., 1997). As such, the hippocampus, which exhibits cytoarchitectural abnormalities in schizophrenia, including decreased expression of synaptic proteins, may be particularly affected by altered NMDA receptor function in this illness (Newell et al., 2007). Abnormalities in NMDA receptor function have been definitively linked to D-serine levels, which suggests that altered expression and activities of enzymes regulating D-serine could, in turn, contribute to NMDA receptor hypofunction. Indeed, associations

for genes involved in D-serine metabolism have been linked to schizophrenia. In particular, DAOA, a gene linked to enhanced activity of DAAO, has been shown to correlate with decreased activity in the hippocampus during working memory tasks (Goldberg et al., 2006). Increased activity of DAAO, leading to decreased availability of synaptic D-serine, may be a predominant component of NMDA receptor hypofunction in schizophrenia, and may in turn stimulate the upregulation of hippocampal serine racemase expression.

A newly published postmortem study has found altered expression of several molecules involved in D-serine activity. They report decreased serine racemase protein expression in the hippocampus, and showed that a subset of chronically ill subjects exhibited increased protein expression of DAAO (Bendikov et al., 2007). These findings bring up several interesting points. These data support my findings of altered serine racemase expression in the hippocampus in schizophrenia; however, it is notable that the two studies report an opposite pattern of expression changes. This discrepancy could be due to an age effect, as my subject cohort was substantially older than the other (mean of 72 years compared to 39 years). It is not known whether serine racemase expression or activity is effected by aging in humans, but, aged rats show a significant loss of serine racemase mRNA and protein expression as well as D-serine levels in the hippocampus, accompanied by decreased capacities for learning, memory, and LTP (Mothet et al., 2006). Furthermore, neither cognitive deficits nor decreased D-serine levels could be attributed to age-related changes in NMDA receptor binding capacity or to altered levels of DAAO in these rats, suggesting that serine racemase is directly responsible for these hippocampal alterations. Considering this age-related decrease of serine racemase in rats,

one might expect to find decreased serine racemase in the elderly subjects I studied, but yet another factor to consider is the effect of illness duration on serine racemase expression. Evidence shows that schizophrenia can initially be characterized by a rapid and progressive degeneration of both brain volume and cognitive capacities. It may be the case, then, that younger patients and/or those with shorter illness duration may have impairments that change over time as compensatory measures for NMDA receptor dysfunction are established. In support of this theory, studies show that PCP administration in rats leads to acute increases of NMDA receptor binding activity, however, in the long-term, NMDA receptor binding activity significantly drops, and remains low for weeks following drug exposure (Newell et al., 2007). This interpretation may explain the discrepant findings between this work and that of Bendikov *et al.* in these studies; however, it will be interesting to see if it holds true as more reports of serine racemase expression in schizophrenia are published.

It seems unlikely that antipsychotic treatment played a role in either of these two reports of serine racemase expression changes, as the majority of subjects in both studies were treated with typical antipsychotics, yet showed expression changes in opposite directions. The only explanation then would be that antipsychotics might exert dissimilar effects on serine racemase expression according to age and illness duration.

The issue of serine racemase activity has not been addressed in the protein expression studies, but it is an important facet of understanding the role of serine racemase in schizophrenia. The characterization of serine racemase, as well as a purported role for this enzyme in schizophrenia, is relatively new. As such, the mechanisms of activity and expression are not yet well understood; although there is

evidence to suggest that serine racemase-interacting proteins, such as PICK1 and GRIP, participate in the regulation of activity (Kim et al., 2005; Fujii et al., 2006). An assay capable of accurately determining serine racemase activity in human postmortem tissue would therefore be of great value. Details of a proposed experimental design can be found in the *Future Directions* section.

5.4 The expression of serine racemase, glutamine synthetase, and GFAP impact excitatory transmission and plasticity in the brain.

This dissertation work examined astrocytic molecules in five brain regions in schizophrenia. No immediate qualitative pattern emerged that indicated widespread abnormalities either across brain regions, or across multiple astrocyte-related molecules, thus my findings do not support the idea of a global astrocytic lesion giving rise to the pathophysiology of schizophrenia. The transcript and protein expression of several molecules were altered, but the directions of expression changes and the regions in which the changes took place varied, so it does not seem that loss of astrocyte density is a defining characteristic of schizophrenia, at least in the elderly cohort of patients that were examined in these studies. However, within these results, one major theme does arise--all of the astrocytic molecules that demonstrated altered expression in schizophrenia can be linked to glutamatergic function. Furthermore, these results show that altered expression of serine racemase, glutamine synthetase, and GFAP individually can reciprocally impact the expression and function of the entire group of molecules.

Glutamatergic dysfunction and specifically hypofunction of the NMDA receptor have been demonstrated in schizophrenia (Coyle et al., 2003). Studies show that under

typical conditions the NMDA receptor co-agonist binding site is nearly saturated by D-serine (Panatier et al., 2006). However, in response to altered availability of transmitter molecules, astrocytes positioned within close proximity of the synapse can alter serine racemase expression and activity. Compensatory upregulation of serine racemase has been shown in several brain regions in rats following treatment with the NMDA receptor antagonist MK-801, including the hippocampus (Yoshikawa et al., 2004). Increased D-serine improves cognitive function in rats with PCP-induced NMDA receptor hypofunction (Andersen and Pouzet, 2004), which is consistent with D-serine-mediated improvements in patients with schizophrenia (Tsai et al., 1998; Tsai et al., 1999; Heresco-Levy et al., 2005). Thus, my postmortem finding of increased serine racemase in schizophrenia is consistent with the notion of compensatory increases in serine racemase expression occurring in response to NMDA receptor-mediated glutamatergic dysfunction in the hippocampus.

NMDA receptor hypofunction, through compromised aracadonic acid release, can result in decreased glutamate release, which, in turn, may influence the expression of astrocytic EAATs. Such alterations in EAAT expression may lead to decreased glutamine synthetase protein expression, since without synaptic signaling and glutamate reuptake, glutamine synthetase expression and activity could be down-regulated. Thus, my findings for decreased glutamine synthetase are consistent with the hypothesis of NMDA receptor hypofunction in schizophrenia. But, what about GFAP expression changes, how does this molecule fit into an astrocytic model of glutamatergic dysfunction?

The expression of GFAP, like serine racemase and glutamine synthetase, has also

been shown to have reciprocal influence with glutamatergic transmission (Porter and McCarthy, 1995; Kommers et al., 2002; Hu et al., 2003). Astrocytic processes are closely associated with glutamatergic synapses where they have a direct role in NMDA receptor activation via the synthesis and release of gliotransmitters such as D-serine and in the reuptake and cycling of synaptic glutamate via EAATs and glutamine synthetase (Figure 5.2). The ability of astrocytes to change process morphology in response to changing synaptic conditions is mediated by GFAP expression. Therefore, my findings of decreased GFAP expression in schizophrenia may signify that altered process morphology contributes to glutamatergic dysfunction in this illness. Alternatively, it may demonstrate a compensatory response by astrocytes to abnormal glutamatergic transmission.

A dramatic example of GFAP expression changes altering glutamatergic transmission can found in the supraoptic nucleus (SON) of the hypothalamus. This region is interspersed with neurons that synthesize and release oxytocin and vasopressin, two neuroendocrine molecules associated with events following parturition, such as lactation, and with maintaining water balance, respectively. It is an area also highly populated by astrocytes that cover an estimated 99% of neuronal synapses with their processes during basal conditions (Hatton, 1997). However, under certain conditions astrocytes in the SON undergo an interesting transformation. At the onset of lactation in the rat, astrocytic processes that normally ensheath synapses exhibit a decrease in the expression of GFAP and begin to physically retract away from the synapse. As processes are pulled away several changes mediating enhanced synaptic functioning occur. First, in the absence of astrocytic processes the concentration of synaptic K^+ is increased.

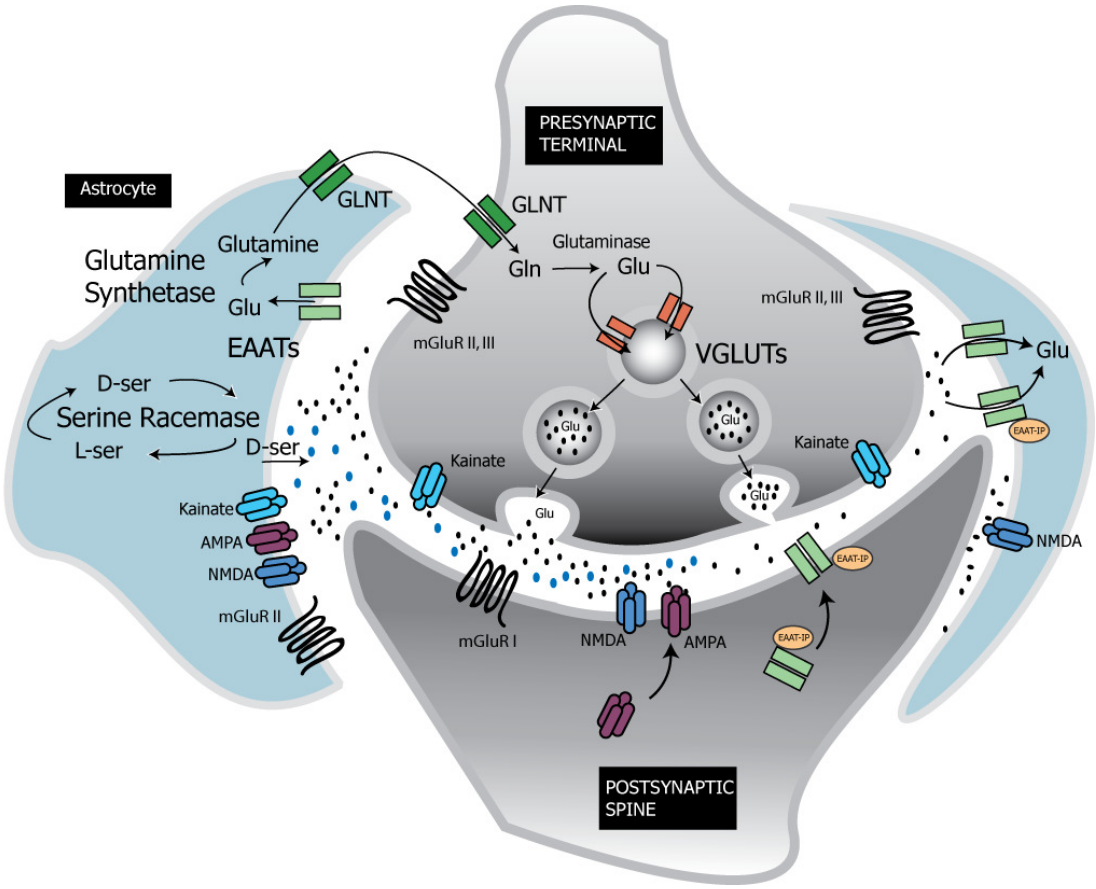

Figure 5.2 Astrocytic processes are closely associated with glutamatergic synapses. Astrocytes play a direct role in NMDA receptor activation via the synthesis and release of gliotransmitters such as D-serine, by the reuptake of synaptic glutamate by EAATs and the cycling of glutamate by glutamine synthetase. NMDA receptors are typically located within the synapse and are activated by presynaptic glutamate release. Glutamate release activates postsynaptic AMPARs causing membrane depolarization via Na^+ influx. This, along with the binding of glutamate and D-serine, results in the removal of Mg^+ from the receptor channel and allows influx of Ca^{2+}. Upon activation, these receptors set into motion a series of events, including increased AMPA receptor trafficking and CREB and protein kinase activation leading to gene transcription. If a series of these depolarizations or excitatory post synaptic potentials (EPSPs) occur it leads to LTP.

Second, both the concentration and the period of available synaptic glutamate increase in the absence of EAAT-bearing astrocytic processes (Piet et al., 2004) (Figure 5.3). Third, physical barriers around the synapse once created by astrocytes are removed allowing neurons to make otherwise unattainable contacts. What follows is an increase in the clustering and chemical coupling of neurons and an increase in the formation of new synapses (Hatton, 1997). Additionally, the removal of astrocytic processes permits signaling to occur over larger distances through the spillover of extrasynaptic glutamate. The end result is an "inter-synaptic crosstalk" between neurons that creates a temporally and spatially synchronized signal. In the lactating rat, these actions shift cells out of a slow basal level of irregular neuronal firing to a strong and rhythmic synaptic response that result in the release of oxytocin and milk letdown. Mechanistically, the retraction of processes allows extrasynaptic glutamate to begin a tonic activation of presynaptic mGluRs (Oliet et al., 2001; Piet et al., 2004). Studies indicate that this activation results in decreased spontaneous release of presynaptic glutamate that in turn inhibits GABAergic synapses (Piet et al., 2004). This silencing of GABAergic synapses, along with increased neuron excitability mediated by increased extracellular $[K^+]$ and increased synaptic clustering, augments regional excitability. Thus, by altering process morphology during lactation astrocytes regulate glutamatergic transmission. The presence of astrocytic processes can also alter the amount of stimulation needed to induce synaptic plasticity (LTP versus LTD), thereby changing the potentiation of individual synapses (Panatier et al., 2006).

These are remarkable roles for GFAP in excitatory transmission and synaptic plasticity. However, of what relevance are they to schizophrenia? It is a lengthy leap

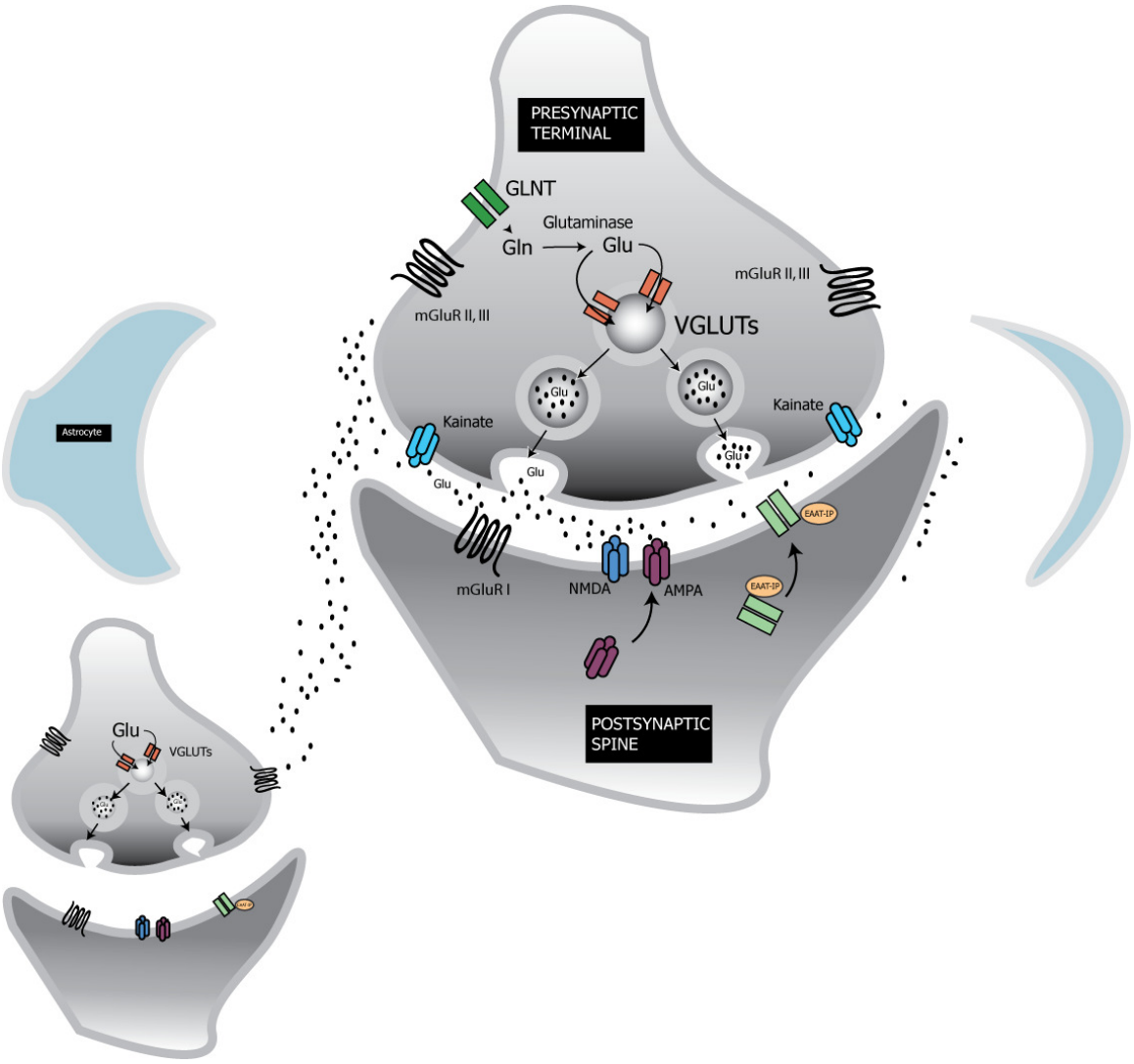

Figure 5.3 The retraction of astrocyte processes enables inter-synaptic crosstalk. The removal of astrocytic processes allows synaptic glutamate to activate distant synapses which are otherwise prevented by the physical presence of astrocytes and by astrocyte-mediated glutamate reuptake via EAATs.

from the lactating rat to psychiatric illness. To create a workable model in which astrocyte process retraction, mediated by GFAP expression changes, contributes to glutamatergic dysfunction in schizophrenia, some basic criteria should be discussed. Evidence should show that astrocyte-mediated changes to excitatory transmission mediated by GFAP expression: (1) occurs in circumstances other than lactation, (2) involves molecules implicated in glutamatergic dysfunction in schizophrenia, and (3) accounts for abnormal brain function reported in schizophrenia. If these criteria are met, it would suggest a distinct role for GFAP, as well as for astrocytes, in glutamatergic dysfunction in schizophrenia.

5.4.1 Astrocyte-mediated changes to excitatory transmission via GFAP expression occur during states of dehydration and stress.

The circumstances surrounding lactation are distinct, including the brain regions and signaling molecules involved, and the resulting behavior. Examining other circumstances in which astrocytes influence excitatory transmission by retracting processes from the synapse may uncover a novel facet of GFAP involvement in glutamatergic dysfunction in schizophrenia. During lactation, the retraction of astrocytic processes and decreases in GFAP immunoreactivity has been shown to occur in a matter of hours. These events have also been observed in rats during states of dehydration and under physical and psycho-social stressors (Hatton, 1997). In the SON of dehydrated rats, in addition to process retraction which has been confirmed by reports of decreased astrocyte process surface area, astrocytes have been shown to undergo a 90^{o} change in orientation relative to the pial surface (Bobak and Salm, 1996). This reversible

reorganization of the astrocyte cytoskeleton and decrease in GFAP immunoreactivity, interestingly, is accompanied by an increase in GFAP mRNA (Hawrylak et al., 1998; Lally et al., 2005). The absence of GFAP has already been shown to result in an inability to extend processes toward neurons in cultured astrocytes (Weinstein et al., 1991). These examples in the lactating and dehydrated rat confirm this phenomenon *in vivo*. In culture, re-introducing GFAP restores the ability to extend processes (Chen and Liem, 1994). Likewise, a reversible structural relationship where GFAP expression leads to reestablishment of synaptic coverage by astrocytes exists in the rat brain (Hawrylak et al., 1998).

In addition, stress induced activation of the hypothalamic-pituitary-adrenal (HPA) system is hypothesized to play a role in astrocyte-mediated synaptic plasticity. During restraint stress, rats exhibit structural reorganizations in the SON similar to those under conditions of lactation and dehydration, and adrenalectomy lessens astrocyte process retraction (Miyata et al., 1994; Hatton, 1997). Interestingly, it was shown long ago that behavior can induce astrocyte-mediated synaptic plasticity as well. Studies show that non-lactating (virgin) rats that engage in maternal behavior show qualitatively similar changes in the SON (Salm et al., 1988).

Together, these data demonstrate that in addition to the case of lactation, astrocytes regulate excitatory transmission by altering the expression of GFAP in a variety of situations including dehydration, stress, and behavioral manipulations.

5.4.2 Astrocyte-mediated changes to excitatory transmission via GFAP expression in the SON involves glutamatergic molecules implicated in schizophrenia.

The SON is not an area associated with schizophrenia. It is, however, an optimal target site for the study of the effects and mechanisms of astrocytic modulation of excitatory transmission. Because the SON expresses molecules that are implicated in glutamatergic dysfunction in other brain regions in schizophrenia, it could also be a relevant model of glutamatergic dysfunction in schizophrenia. Studies show that the SON contains neurons and astrocytes that express the same molecules that synthesize, transport, cycle, and bind glutamate in brain regions implicated in glutamatergic dysfunction in schizophrenia. These molecules include glutamate receptors, such as mGluR, NMDA and AMPA receptors, and the glutamate transporters VGLUTs and EAATs (Ponzio et al., 2006). In addition, the SON similarly contains a large population of astrocytes expressing enzymes such as glutamine synthetase and serine racemase (Panatier et al., 2006).

Furthermore, brain regions in the present body of work exhibit environmental similarities to those found in the SON. For example, like the SON, the hippocampus is an area that is densely covered by astrocytic processes that also undergoes synaptic plasticity mediated by glutamate function, which suggests that astrocyte-mediated excitatory transmission in the SON may be a relevant model of glutamatergic dysfunction in brain regions such as the hippocampus.

5.4.3 Astrocyte-mediated changes to excitatory transmission via GFAP expression can account for abnormal brain function in schizophrenia.

Abnormal structure and function have been shown in various regions in the brain in schizophrenia. These include volumetric changes, abnormal activation, and aberrant

functional connectivity (Assaf et al., 2006; Garrity et al., 2007), all of which can be attributed to altered GFAP expression.

Loss of brain volume in schizophrenia likely has an astrocytic component. One study found an 81% increase in $GFAP^{(+)}$ cell bodies, but a 32% reduction in the area occupied by these cells, a phenomenon that has been interpreted as evidence of decreased processes branching in this illness (Rajkowska et al., 1998; Rajkowska et al., 2002). Likewise, abnormal activation in schizophrenia can be linked to astrocytes through GFAP expression changes. As has been described previously, decreased GFAP expression leading to the retraction of astrocytic processes from synapses can result in region-specific increases in excitability and can alter the potentiation threshold of synapses. In theory then, astrocytes can respond to regions of abnormally high or low activation by altering the degree of process extension within the synapse. Conceivably, my findings of altered GFAP expression in schizophrenia may represent a compensatory retraction of processes in response to glutamatergic hypofunction. Alternatively, decreased GFAP expression may be a marker for brain regions that are unable to regulate excitatory transmission in schizophrenia such as the STG and ACC.

In the ACC, I have detected *increased* GFAP mRNA and *decreased* protein. These discordant expression changes are the same as those reported in the SON of dehydrated rats (Hawrylak et al., 1998; Lally et al., 2005). So, ACC glutamatergic dysfunction in schizophrenia may be associated with changes in the expression of GFAP made to compensate for glutamatergic hypofunction. Furthermore, I detected decreased GFAP expression in the STG, which is consistent with studies showing abnormal deactivation during working memory tasks in patients with schizophrenia (Walter et al.,

2007). This suggests that in some brain regions altered GFAP expression may underlie glutamatergic dysfunction in schizophrenia.

Whether causal or compensatory, these results suggest that astrocyte process retraction mediating changes to excitatory transmission and synaptic plasticity may also occur under illness-related conditions. Furthermore, if GFAP expression changes are indicative of the type of astrocyte-mediated plasticity observed in the SON, it would implicate astrocytes in dysfunctional connectivity seen across multiple brain regions in this illness.

5.5 Future Directions

In situ hybridization and Western blot analysis are useful means of analyzing transcript and protein expression changes within a brain region; however, neither technique is designed to determine whether discrete populations of astrocytes are exhibiting expression changes. Astrocytes are a heterogeneous cell type displaying different phenotypes and physiologies, thus, it would be advantageous to identify the specific population(s) of astrocytes involved in the pathophysiology of schizophrenia. For example, several studies identify serine racemase-expressing astrocytes as being $GFAP^{(+)}$ and acknowledge the existence of astrocytes which co-express GFAP, glutamine synthetase, and serine racemase (Schell et al., 1995; Wolosker et al., 1999; Xia et al., 2004; Williams et al., 2006). However, the localization of serine racemase has also been attributed to $GFAP^{(-)}$ cells (Schell et al., 1995). The techniques used were not designed to identify whether serine racemase expression abnormalities are found in the same population of astrocytes that were found to exhibit altered expression of GFAP and

glutamine synthetase, but cell level expression studies, though they have proved technically challenging when studying these astrocytic molecules in the past, would help clarify the source of expression changes.

This dissertation work was composed of human postmortem studies that identified molecular abnormalities specific to astrocytes in schizophrenia. I found that three key astrocytic molecules involved in glutamatergic dysfunction in schizophrenia are abnormally expressed, therefore, further studies examining transcript and protein expression could identify other intracellular abnormalities specific to astrocytes and further clarify the role of these cells in brain dysfunction. For example, because of its role in modulating the phosphorylation of GFAP and multiple reports of altered CSF and serum levels in patients with schizophrenia, S100β is an interesting molecule for further study. The expression of GFAP has been shown to alter astrocyte process morphology and in turn glutamatergic function, but the mechanisms regulating the assembly and disassembly of GFAP, such as interactions with the signaling molecule S100β, have not yet been studied in schizophrenia. Techniques such as the co-immunoprecipitation of GFAP and S100β could help determine whether abnormal association of these two molecules occurs in schizophrenia. Preliminary studies indicate that "pulling down" one molecule from brain homogenate using protein A/G beads and then measuring protein expression interactions using Western blot analysis could be successfully employed (Figure 5.4). The IF molecule vimentin has also been shown to affect GFAP expression and organization, however, few studies have examined this molecule in schizophrenia. Additionally, measuring the expression of phosphorylated isoforms of GFAP could reveal whether this molecule is abnormally regulated in schizophrenia. While there are

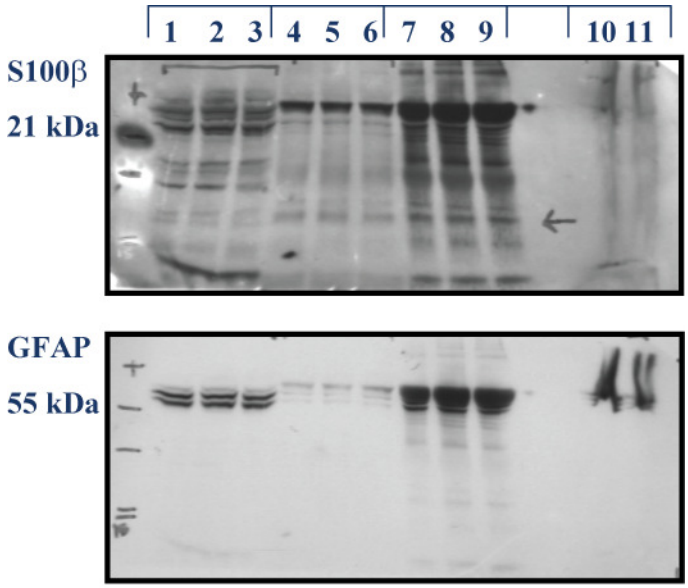

Figure 5.4 Western blots of GFAP and S100β co-immunoprecipitation experiments. The interactions between GFAP and S100β can be studied using co-immunoprecipitation. Preliminary studies demonstrate that each molecule can be extracted from human brain homogenate using A/G protein beads and detected using Western blot analysis.

commercial antibodies available to identify phosphorylated isoforms of GFAP, I failed to detect a signal in preliminary studies using Western blot analysis. Further study is needed to determine whether GFAP phosphorylation is maintained in human postmortem tissue during the homogenization process.

A role for GFAP expression in astrocyte-mediated excitatory transmission has been described. The degree of astrocyte process extension can influence aspects of glutamatergic function, such as the synthesis and release of D-serine in proximity of the glutamatergic synapse and the reuptake of synaptic glutamate via EAATs. However, the type of plasticity mediated by GFAP that, in the SON, results in altered potentiation and increased neuronal excitability has not been described in schizophrenia. Since NMDA receptor hypofunction can account for all categories of symptoms in schizophrenia and studies have concluded that NMDA receptor antagonism, when occurring long term, may be a relevant pharmacological model of this illness (Newell et al., 2007), it would be interesting to determine whether PCP administration in rats would alter GFAP expression and process retraction in the SON and other brain regions.

The plastic changes seen in the SON during lactation, dehydration, and stress involve the neuroendocrine molecules oxytocin and vasopressin. There is some evidence that increased levels of oxytocin and vasopressin in CSF and plasma occur in both medicated and non-medicated patients with schizophrenia (Legros et al., 1992). The relationship between these molecules and schizophrenia are unclear, but studies suggest that altered levels of oxytocin and vasopressin in peripheral fluids may be secondary to dopaminergic abnormalities (Meltzer et al., 2001). Tying astrocyte-mediated dysfunction to other neurotransmitter and endocrine functions may uncover novel avenues of

pharmacological treatment in this illness.

The study of serine racemase is relatively new and my finding of increased serine racemase expression in the hippocampus was the first published in the schizophrenia literature. Examining serine racemase expression in multiple brain regions and in specific populations of astrocytes is an obvious next step. Additionally, further explorations of serine racemase using an assay to determine whether the activity of this enzyme is altered in schizophrenia and co-immunoprecipitation experiments to examine protein interactions of serine racemase could be performed. I attempted to measure the activity of serine racemase from human postmortem brain homogenates using an assay described by Cook *et al.* that quantifies a breakdown product of D-serine (Cook et al., 2002) (Figure 5.5). Although this assay has been successfully used with purified serine racemase, measuring activity levels in brain homogenate proved technically challenging.

Recent literature demonstrates astrocytic expression of AMPA receptors and interacting proteins which facilitate receptor trafficking and expression (Kim et al., 2005; Fujii et al., 2006). I performed some preliminary experiments in which I immunoprecipitated serine racemase from human brain homogenate and probed for proteins that associated with it. Consistent with other reports, I detected GRIP1 and PICK1. However, I also detected neurofilament light chain (NFL), a neuronal marker being used as a negative control. One interpretation for the association of NFL with serine racemase is that while these molecules do not exhibit intracellular interactions, they may do so during the homogenation process. In light of new information demonstrating serine racemase expression in neurons (Kartvelishvily et al., 2006; Williams et al., 2006), it is possible that my findings represent a neuronal sample of

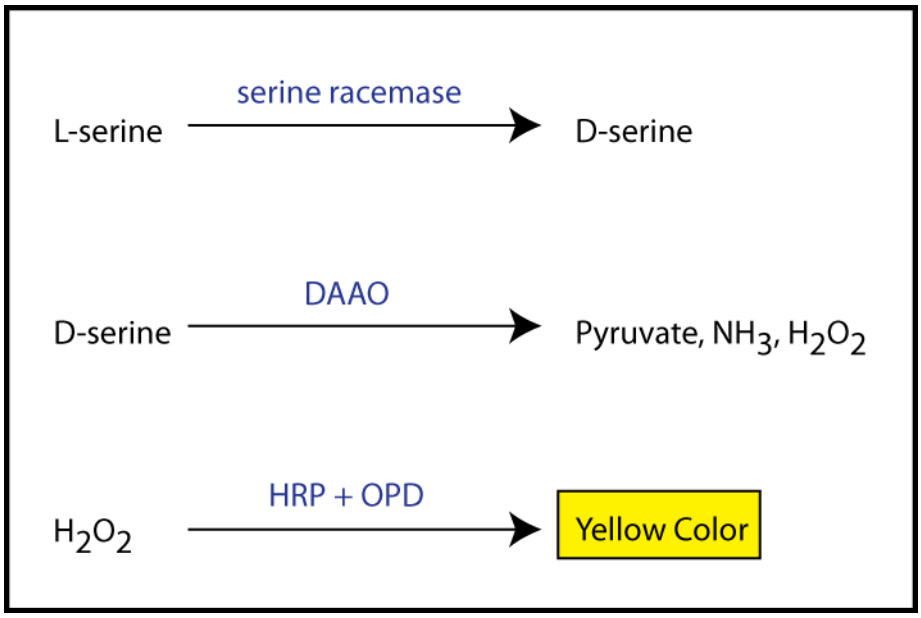

Figure 5.5 Enzymatic assay for serine racemase activity. In the presence of DAAO, D-serine is converted to α-keto acid, ammonia, and H_2O_2. Hydrogen peroxide can be measured using a horseradish peroxidase-based assay and the peroxidase substrate o-phenylenediamine, the oxidation of which produces a quantifiable yellow color

serine racemase. However, this seems unlikely as serine racemase expression in neurons has only been shown in measurable quantities in the cerebellum and hindbrain, whereas I used cortical tissue in these co-immunoprecipitation experiments. Additionally, other neuronal molecules such as PSD95 were not detected in association with serine racemase suggesting that neuronal origin of the serine racemase is unlikely (Figure 5.6).

In addition to serine racemase, other astrocytic enzymes involved in glutamatergic function, such as glutamine synthetase, warrant further study. The literature describing the activity of glutamine synthetase in schizophrenia so far has shown mixed results and suggests that multiple isoforms of glutamine synthetase may be involved in the abnormal expression and perhaps activity of this enzyme in schizophrenia.

5.6 Conclusions

Schizophrenia is an illness that emerges as a result of complex interactions between genetics and environment. Developmental abnormalities are hypothesized to underlie illness-related brain abnormalities. Historically, neuronal dysfunction has been the main hallmark of schizophrenia; however, an increasing awareness of the diverse role played by astrocytes also implicates these cells. Specifically, astrocytes play a prominent role in glutamate function. Astrocytes mediate synaptic transmission at the glutamate synapse in three fundamental ways: 1) the expression of serine racemase determines D-serine occupancy of the co-agonist site on NMDA receptors, which helps ensure receptor activation during input stimulation; 2) EAAT-mediated uptake of synaptic glutamate and recycling by glutamine synthetase provides metabolic substrate and transmitter molecules to neurons and prevents extrasynaptic glutamate spillover leading to subsequent

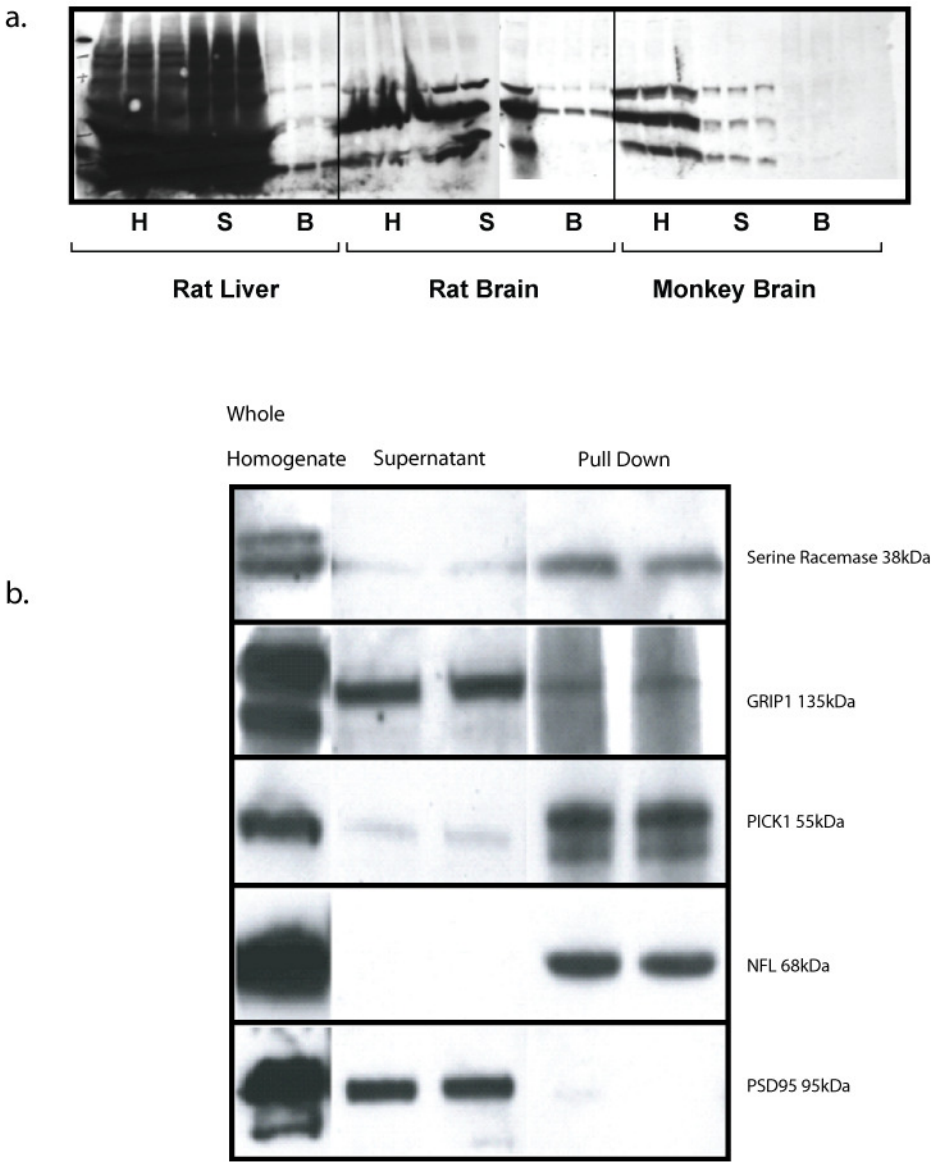

Figure 5.6 Serine racemase co-immunoprecipitation experiment. I successfully immunoprecipitated and detected serine racemase using protein A/G beads and Western blot analysis from (a) rat liver and brain, and from monkey brain tissue. I performed the same technique with (b) human brain homogenate and probed for proteins that co-immunoprecipitated. I detected the AMPA receptor interacting proteins GRIP1 and PICK1, as well as a neuronal molecule, NFL. I did not detect the neuronal molecule PSD95 in association with serine racemase.

activation of distant synapses and excitotoxicity; and 3) GFAP expression and the retraction of processes from typically ensheathed synapses can result in a spatially and temporally coordinated activation of synapses.

Our findings suggest that astrocytes do contribute to the pathophysiology of schizophrenia, and that astrocytic molecules involved in cytoskeletal integrity and glutamatergic function are compromised in this illness. A complex illness such as schizophrenia requires a reappraisal of the existing neuro-centric model to include an astrocytic hypothesis of dysfunction, which could lead to a more complete understanding of schizophrenia, and perhaps provide a novel target for treatment strategies in this illness.

References

Abi-Dargham A, Guillin O (2007) Alterations of serotonin transmission in schizophrenia. In: International review of neurobiology, pp 133-164: Academic Press.

Allardyce J, Boydell J (2006) Review: The wider social environment and schizophrenia. Schizophrenia Bulletin 32:592-598.

Allen N, Barres B (2005) Signaling between glia and neurons: Focus on synaptic plasticity. Current Opinion in Neurobiology 15:542-548.

Almond S, Fradley R, Armstrong E, Heavens R, Rutter A, Newman R, Chiu C, Konno R, Hutson P, Brandon N (2006) Behavioral and biochemical characterization of a mutant mouse strain lacking D-amino acid oxidase activity and its implications for schizophrenia. Molecular and Cellular Neurosciences 32:324-334.

Alvarez-Buylla A, Garcia-Verdugo J (2002) Neurogenesis in adult subventricular zone. Journal of Neuroscience 22:629-634.

Alvarez-Buylla A, Seri B, Doetsch F (2002) Identification of neural stem cells in the adult vertebrate brain. Brain Research Bulletin 57:751-758.

Anderson C, Swanson R (2000) Astrocyte glutamate transport: Review of properties, regulation, and physiological functions. Glia 32:1-14.

Andersen J, Pouzet B (2004) Spatial memory deficits induced by perinatal treatment of rats with PCP and reversal effect of D-serine. Neuropsychopharmacology 29:1080-1090.

Anthony T, Klein C, Fishell G, Heintz N (2004) Radial glia serve as neuronal progenitors in all regions of the central nervous system. Neuron 41:881-890.

Arif M, Chikuma T, Ahmed M, Yoshida S, Kato T (2007) Suppressive effect of clozapine but not haloperidol on the increases of neuropeptide-degrading enzymes and glial cells in MK-801-treated rat brain regions. Neuroscience Research 57:248-258.

Arnold S, Franz B, Trojanowski J, Moberg P, Gur R (1996) Glial fibrillary acidic protein-immunoreactive astrocytosis in elderly patients with schizophrenia and dementia. Acta Neuropathologica 91:269-277.

Assaf M, Rivkin P, Kuzu C, Calhoun V, Kraut M, Groth K, Yassa M, Jr JH, Pearlson G (2006) Abnormal object recall and anterior cingulate overactivation correlate with formal thought disorder in schizophrenia. Biological Psychiatry 59:452-459.

Baiano M, David A, Versace A, Churchill R, Balestrieri M, Brambilla P (2007) Anterior cingulate volumes in schizophrenia: A systematic review and a meta-analysis of MRI studies. Schizophrenia Research. (In press).

Barres B, Raff M (1999) Axonal control of oligodendrocyte development. The Journal of Cell Biology 147:1123-1128.

Battu C, Godinho G, Thomazi A, Almeida Ld, Goncalves C, Kommers T, Wofchuk S (2005) Ontogenetic changes in glial fibrillary acid protein phosphorylation, glutamate uptake and glutamine synthetase activity in olfactory bulb of rats. Neurochemical Research 30:1101-1108.

Bearden C, Erp Tv, Thompson P, Toga A, Cannon T (2007) Cortical mapping of genotype-phenotype relationships in schizophrenia. Human Brain Mapping. (In press)

Bellocchio E, Reimer R, Fremeau R, Edwards R (2000) Uptake of glutamate into synaptic vesicles by an inorganic phosphate transporter. Science 289:957-960.
Bendikov I, Nadri C, Amar S, Panizzutti R, Miranda JD, Wolosker H, Agam G (2007) A CSF and postmortem brain study of D-serine metabolic parameters in schizophrenia. Schizophrenia Research 90:41-51.
Benediktsson A, Schachtele S, Green S, Dailey M (2005) Ballistic labeling and dynamic imaging of astrocytes in organotypic hippocampal slice cultures. Journal of Neuroscience Methods 141:41-53.
Benes F, Davidson J, Bird E (1986) Quantitative cytoarchitectural studies of the cerebral cortex of schizophrenics. Archives of General Psychiatry 43:31-35.
Benes F, Vincent S, Todtenkopf M (2001) The density of pyramidal and nonpyramidal neurons in anterior cingulate cortex of schizophrenic and bipolar subjects. Biological Psychiatry 50:395-406.
Benes F, McSparren J, Bird E, Vincent S (1991) Deficits in small interneurons in prefrontal and cingulate cortices of schizophrenic and schizoaffective patients. Archives of General Psychiatry 48:996-1001.
Bertolino A, Roffman J, Lipska B, Gelderen Pv, Olson A, Weinberger D (2002) Reduced n-acetylaspartate in prefrontal cortex of adult rats with neonatal hippocampal damage. Cerebral Cortex 12:983-990.
Bezzi P, Gundersen V, Galbete J, Seifert G, Steinhauser C, Pilati E, Volterra A (2004) Astrocytes contain a vesicular compartment that is competent for regulated exocytosis of glutamate. Nature Neuroscience 7:613-620.
Bobak J, Salm A (1996) Plasticity of astrocytes of the ventral glial limitans subjacent to the supraoptic nucleus. Journal of Comparative Neurology 376:188-197.
Boksha I, Tereshkina E, Burbaeva GS (2000) Glutamine synthetase and glutamine synthetase-like protein from human brain. Purification and comparative characterization. Journal of Neurochemistry 75:2574-2582.
Boyer P, Phillips J, Rousseau F, Ilivitsky S (2007) Hippocampal abnormalities and memory deficits: New evidence of a strong pathophysiological link in schizophrenia. Brain Research Reviews 54:92-112.
Brenner M, Johnson A, Boespflug-Tanguy O, Rodriguez D, Goldman J, Messing A (2001) Mutations in GFAP, encoding glial fibrillary acidic protein, are associated with Alexander disease. Nature Genetics 27:117-120.
Bruneau E, McCullumsmith R, Haroutunian V, Davis K, Meador-Woodruff J (2005) Increased expression of glutaminase and glutamine synthetase mRNA in the thalamus in schizophrenia. Schizophrenia Research 75:27-34.
Buchanan R, Vladar K, Barta P, Pearlson G (1998) Structural evaluation of the prefrontal cortex in schizophrenia. American Journal of Psychiatry 155:1049-1055.
Burbaeva G, Boksha I, Turishcheva M, Vorobyeva E, Savushkina O, Tereshkina E (2003) Glutamine synthetase and glutamate dehydrogenase in the prefrontal cortex of patients with schizophrenia. Progress in Neuro-Psychopharmacology and Biological Psychiatry 27:675-680.
Burbaeva G, Boksha I, Tereshkina E, Savushkina O (1999) Biochemical approaches to the brain glutamate metabolism study in schizophrenia. Schizophrenia Research 36:70.

Burbaeva G, Boksha I, Tereshkina E, Savushkina O, Turishcheva M, Starodubtseva L, Brusov O, Morozova M (2006) Effect of olanzapine treatment on platelet glutamine synthetase-like protein and glutamate dehydrogenase immunoreactivity in schizophrenia. World Journal of Biological Psychiatry 7:75-81.

Bushong E, Martone M, Ellisman M (2003) Examination of the relationship between astrocyte morphology and laminar boundaries in the molecular layer of adult dentate gyrus. The Journal of Comparative Neurology 462:241-251.

Bushong E, Martone M, Ellisman M (2004) Maturation of astrocyte morphology and the establishment of astrocyte domains during postnatal hippocampal development. International Journal of Developmental Neuroscience 22:73-86.

Bushong E, Martone M, Jones Y, Ellisman M (2002) Protoplasmic astrocytes in CA1 stratum radiatum occupy separate anatomical domains. Journal of Neuroscience 22:183-192.

Butt A, Kiff J, Hubbard P, Berry M (2002) Synantocytes: New functions for novel NG2 expressing glia. Journal of Neurocytology 31:551-565.

Butt A, Hamilton N, Hubbard P, Pugh M, Ibrahim M (2005) Synantocytes: The fifth element. Journal of Anatomy 207:695-706.

Byne W, Kidkardnee S, Tatusov A, Yiannoulos G, Buchsbaum M, Haroutunian V (2006) Schizophrenia-associated reduction of neuronal and oligodendrocyte numbers in the anterior principal thalamic nucleus. Schizophrenia Research 85:245-253.

Cannon T, Erp Tv, Huttunen M, Lonnqvist J, Salonen O, Valanne L, Poutanen V, Standertskjold-Nordenstam C, Gur R, Yan M (1998) Regional gray matter, white matter, and cerebrospinal fluid distributions in schizophrenic patients, their siblings, and controls. Archives of General Psychiatry 55:1084-1091.

Cannon T, Huttunen M, Lonnqvist J, Tuulio-Henriksson A, Pirkola T, Glahn D, Finkelstein J, Hietanen M, Kaprio J, Koskenvuo M (2000) The inheritance of neuropsychological dysfunction in twins discordant for schizophrenia. The American Journal of Human Genetics 67:369-382.

Cannon T, Erp Tv, Bearden C, Loewy R, Thompson P, Toga A, Huttunen M, Keshavan M, Seidman L, Tsuang M (2003) Early and late neurodevelopmental influences in the prodrome to schizophrenia: Contributions of genes, environment, and their interactions. Schizophrenia Bulletin 29:653-669.

Carlsson A, Waters N, Holm-Waters S, Tedroff J, Nilsson M, Carlsson M (2001) Interactions between monoamines, glutamate, and GABA in schizophrenia: New evidence. Annals of the New York Acadamy of Sciences ual Reveiw of Pharmacology and Toxicology 41:237-260.

Casanova M, Stevens J, Kleinman J (1990) Astrocytosis in the molecular layer of the dentate gyrus: A study in Alzheimer's disease and schizophrenia. Psychiatry Research 35:149-166.

Chakrabarti R, McCracken J, Chakrabarti D, Souba W (1995) Detection of a functional promoter/enhancer in an intronless human gene encoding a GS-like enzyme. Gene 153:163-169.

Chan-Ling T, Stone J (1991) Factors determining the morphology and distribution of astrocytes in the cat retina: A contact-spacing model of astrocyte interaction. The Journal of Comparative Neurology 303:387-399.

Chandrakala M, Marcus S, Nadiger H, Sadasivudu B (1987) Acute and long-term effects of chlorpromazine on glutamine synthetase and glutaminase in rat brain. Journal of Neurochemistry 49:32-34.

Chang M, Ariah L, Marks A, Azmitia E (2005) Chronic gliosis induced by loss of S100b: Knockout mice have enhanced GFAP-immunoreactivity but blunted response to a serotonin challenge. Brain Research 1031:1-9.

Charles A, Merrill J, Dirksen E, Sandersont M (1991) Intercellular signaling in glial cells: Calcium waves and oscillations in response to mechanical stimulation and glutamate. Neuron 6:983-992.

Chen W, Liem R (1994) Reexpression of glial fibrillary acidic protein rescues the ability of astrocytoma cells to form processes in response to neurons. Journal of Cell Biology 127:813-823.

Chumakov I, Blumenfeld M, Guerassimenko O, Cavarec L, Palicio M, Abderrahim H, Bougueleret L, Barry C, Tanaka H, Rosa PL, Puech A, Tahri N, Cohen-Akenine A, Delabrosse S, Lissarrague S, Picard F, Maurice K, Essioux L, Millasseau P, Grel P, Debailleul V, Simon A, Caterina D, Dufaure I, Malekzadeh K, Belova M, Luan J, Bouillot M, Sambucy J, Primas G, Saumier M, Boubkiri N, Martin-Saumier S, Nasroune M, Peixoto H, Delaye A, Pinchot V, Bastucci M, Guillou S, Chevillon M, Sainz-Fuertes R, SMeguenni, Aurich-Costa J, Cherif D, Gimalac A, Duijn CV, Gauvreau D, Ouellette G, Fortier I, Raelson J, Sherbatich T, Riazanskaia N, Rogaev E, Raeymaekers P, Aerssens J, Konings F, Luyten W, Sham FMP, Straub R, Weinberger D, Cohen N, Cohen D (2002) Genetic and physiological data implicating the new human gene G72 and the gene for D-amino acid oxidase in schizophrenia. Proceedings of the National Academy of Sciences 99:13675-13680.

Ciriacks C, Bowser M (2006) Measuring the effect of glutamate receptor agonists on extracellular D-serine concentrations in the rat striatum using online microdialysis-capillary electrophoresis. Neuroscience Letters 393:200-205.

Clark D, Dedova I, Cordwell S, Matsumoto I (2006) A proteome analysis of the anterior cingulate cortex gray matter in schizophrenia. Molecular Psychiatry 11:459-470.

Clinton S, Meador-Woodruff J (2004) Abnormalities of the NMDA receptor and associated itracellular molecules in the thalamus in schizophrenia and bipolar disorder. Neuropsychopharmacology 29:1353-1362.

Clinton S, Haroutunian V, Meador-Woodruff J (2006) Up-regulation of NMDA receptor subunit and post-synaptic density protein expression in the thalamus of elderly patients with schizophrenia. Journal of Neurochemistry 98:1114-1125.

Clinton S, Haroutunian V, Davis K, Meador-Woodruff J (2003) Altered transcript expression of NMDA receptor-associated postsynaptic proteins in the thalamus of subjects with schizophrenia. The American Journal of Psychiatry 160:1100-1109.

Coco S, Calegari F, Pravettoni E, Pozzi D, Taverna E, Rosa P, Matteoli M, Verderio C (2003) Storage and release of ATP from astrocytes in culture. Journal of Biological Chemistry 278:1354-1362.

Conti F, DeBiasi S, Minelli A, Rothstein J, Melone M (1998) EAAC1, a high-affinity glutamate tranporter, is localized to astrocytes and GABAergic neurons besides pyramidal cells in the rat cerebral cortex. Cerebral Cortex 8:108-116.

Cook S, Galve-Roperh I, Martínez del Pozo A, Rodríguez-Crespo I (2002) Direct calcium binding results in activation of brain serine racemase. Journal of Biological Chemistry 277:27782-27792.
Cornell-Bell A, Thomas P, Smith S (1990a) The excitatory neurotransmitter glutamate causes filopodia formation in cultured hippocampal astrocytes. Glia 3:322-334.
Cornell-Bell A, Finkbeiner S, Cooper M, Smith S (1990b) Glutamate induces calcium waves in cultured astrocytes: Long-range glial signaling. Science 247:470-473.
Corre SL, Harper C, Lopez P, Ward P, Catts S (2000) Increased levels of expression of an NMDAri splice variant in the superior temporal gyrus in schizophrenia. Neuroreport 11:983-986.
Cotter D, Pariante C (2002) Stress and the progression of the developmental hypothesis of schizophrenia. British Journal of Psychiatry 181:363-365.
Cotter D, Mackay D, Landau S, Kerwin R, Everall I (2001) Reduced glial cell density and neuronal size in the anterior cingulate cortex in major depressive disorder. Archives of General Psychiatry 58:545-553.
Cotter D, Mackay D, Chana G, Beasley C, Landau S, Everall I (2002) Reduced neuronal size and glial cell density in area 9 of the dorsolateral prefrontal cortex in subjects with major depressive disorder. Cerebral Cortex 12:386-394.
Coyle J (1996) The glutamatergic dysfunction hypothesis for schizophrenia. Harvard Review of Psychiatry 3:21-36.
Coyle J (2004) The GABA-glutamate connection in schizophrenia: Which is the proximate cause? Biochemical Pharmacology 68:1507-1514.
Coyle J (2006) Glutamate and schizophrenia: Beyond the dopamine hypothesis. Cellular and Molecular Neurobiology 26:365-384.
Coyle J, Schwarcz R (2000) Mind glue: Implications of glial cell biology for psychiatry. Archives of General Psychiatry 57:90-93.
Coyle J, Tsai G, Goff D (2003) Converging evidence of NMDA receptor hypofunction in the pathophysiology of schizophrenia. Annals of the New York Acadamy of Sciences als of the New York Acadamy of Sciences 1003:318-327.
Damadzic R, Bigelow L, Krimer L, Goldenson D, Saunders R, Kleinman J, Herman M (2001) A quantitative immunohistochemical study of astrocytes in the entorhinal cortex in schizophrenia, bipolar disorder and major depression: Absence of significant astrocytosis. Brain Research Bulletin 55:611-618.
Danbolt N (2001) Glutamate uptake. Progress in Neurobiology 65:1-105.
David J, Ghozali F, Fallet-Bianco C, Wattez A, Delaine S, Boniface B, Menza CD, Delacourte A (1997) Glial reaction in the hippocampal formation is highly correlated with aging in human brain. Neuroscience Letters 235:53-56.
Davis K, Kahn R, Ko G, Davidson M (1991) Dopamine in schizophrenia: A review and reconceptualization. American Journal of Psychiatry 148:1474-1486.
Dean B, Gray L, Scarr E (2006) Regionally specific changes in levels of cortical S100 beta in bipolar 1 disorder but not schizophrenia. Australian and New Zealand Journal of Psychiatry 40:217-224.
Delaney C, Brenner M, Messing A (1996) Conditional ablation of cerebellar astrocytes in postnatal transgenic mice. Journal of Neuroscience 16:6908-6918.

Desco M, Gispert J, Reig S, Sanz J, Pascau J, Sarramea F, Benito C, Santos A, Palomo T, Molina V (2003) Cerebral metabolic patterns in chronic and recent-onset schizophrenia. Psychiatry Research 122:125-135.

Distler C, Dreher Z, Stone J (1991) Contact spacing among astrocytes in the central nervous system: An hypothesis of their structural role. . Glia 4:484-494.

Dorph-Petersen K, Pierri J, Wu Q, Sampson A, Lewis D (2007) Primary visual cortex volume and total neuron number are reduced in schizophrenia. Journal of Comparative Neurology 501:290-301.

Dracheva S, Marras S, Elhakem S, Kramer F, Davis K, Haroutunian V (2001) N-methyl-d-aspartic acid receptor expression in the dorsolateral prefrontal cortex of elderly patients with schizophrenia. American Journal of Psychiatry 158:1400-1410.

Dracheva S, Elhakem S, McGurk S, Davis K, Haroutunian V (2004) GAD67 and GAD65 mRNA and protein expression in cerebrocortical regions of elderly patients with schizophrenia. Journal of Neuroscience Research 76:581-592.

Dracheva S, McGurk S, Haroutunian V (2005) Mrna expression of ampa receptors and ampa receptor binding proteins in the cerebral cortex of elderly schizophrenics. Journal of Neuroscience Research 79:868-878.

Dracheva S, Davis K, Chin B, Woo D, Schmeidler J, Haroutunian V (2006) Myelin-associated mRNA and protein expression deficits in the anterior cingulate cortex and hippocampus in elderly schizophrenia patients. Neurobiology of Disease 21:531-540.

Dupree J, Mason J, Marcus J, Stull M, Levinson R, Matsushima G, Popko B (2005) Oligodendrocytes assist in the maintenance of sodium channel clusters independent of the myelin sheath. Neuron Glia Biology 1:1-14.

Eastwood S, Harrison P (2000) Hippocampal synaptic pathology in schizophrenia, bipolar disorder and major depression: A study of complexin mRNAs. Molecular Psychiatry 5:425-432.

Eliasson C, Sahlgren C, Berthold C, Stakeberg J, Celis J, Betsholtz C, Eriksson J, Pekny M (1999) Intermediate filament protein partnership in astrocytes. Journal of Biological Chemistry 274:23996-24006.

Emsley J, Macklis J (2006) Astroglial heterogeneity closely reflects the neuronal-defined anatomy of the adult murine CNS. Neuron Glia Biology 2:175-186.

Eng L, Vanderhaeghen J, Bignami A, Gerstl B (1971) An acidic protein isolated from fibrous astrocytes. Brain Research 28:351-354.

Escartin C, Valette J, Lebon V, Bonvento G (2006) Neuron-astrocyte interactions in the regulation of brain energy metabolism: A focus on NMR spectroscopy. Journal of Neurochemistry 99:393-401.

Ever L, Gaiano N (2005) Radial 'glial' progenitors: Neurogenesis and signaling. Current Opinion in Neurobiology 15:29-33.

Falkai P, Honer W, David S, Bogerts B, Majtenyi C, Bayer T (1999) No evidence for astrogliosis in brains of schizophrenic patients. A post-mortem study. Neuropathology and Applied Neurobiology 25:48-53.

Falls D (2003) Neuregulins: Functions, forms, and signaling strategies. Experimental Cell Research 284:14-30.

Fatemi S, Laurence J, Araghi-Niknam M, Stary J, Schulz S, Lee S, Gottesman I (2004) Glial fibrillary acidic protein is reduced in cerebellum of subjects with major depression, but not schizophrenia. Schizophrenia Research 69:317-323.

Fellin T, Pascual O, Gobbo S, Pozzan T, Haydon P, Carmignoto G (2004) Neuronal synchrony mediated by astrocytic glutamate through activation of extrasynaptic NMDA receptors. Neuron 43:729-743.

Foltyn V, Bendikov I, Miranda JD, Panizzutti R, Dumin E, Shleper M, Li P, Toney M, Kartvelishvily E, Wolosker H (2005) Serine racemase modulates intracellular D-serine levels through an alpha,beta-elimination activity. Journal of Biological Chemistry 280:1754-1763.

Frizzo J, Tramontina F, Bortoli E, Gottfried C, Leal R, Lengyel I, Donato R, Dunkley P, Goncalves C (2004) S100b-mediated inhibition of the phosphorylation of GFAP is prevented by trtk-12. Neurochemical Research 29:735-740.

Fujii K, Maeda K, Hikida T, Mustafa A, Balkissoon R, Xia J, Yamada T, Ozeki Y, Kawahara R, Okawa M, Huganir R, Ujike H, Snyder S, Sawa A (2006) Serine racemase binds to PICK1: Potential relevance to schizophrenia. Molecular Psychiatry 11:150-157.

Galou M, Colucci-Guyon E, Ensergueix D, Ridet J, Ribotta MGy, Privat A, Babinet C, Dupouey P (1996) Disrupted glial fibrillary acidic protein network in astrocytes from vimentin knockout mice. Journal of Cell Biology 133:853-863.

Gao X, Sakai K, Roberts R, Conley R, Dean B, Tamminga C (2000) Ionotropic glutamate receptors and expression of N-methyl-D-aspartate receptor subunits in subregions of human hippocampus: Effects of schizophrenia. American Journal of Psychiatry 157:1141-1149.

Garbuglia M, Verzini M, Sorci G, Bianchi R, Giambanco I, Agneletti A, Donato R (1999) The calcium-modulated proteins, S100a1 and S100b, as potential regulators of the dynamics of type III intermediate filaments. Brazilian Journal of Medical and Biological Research 32:1177-1185.

Garrity A, Pearlson G, McKiernan K, Lloyd D, Kiehl K, Calhoun V (2007) Aberrant "Default mode" Functional connectivity in schizophrenia. American Journal of Psychiatry 164:450-457.

Georgieva L, Moskvina V, Peirce T, Norton N, Bray N, Jones L, Holmans P, Macgregor S, Zammit S, Wilkinson J, Williams H, Nikolov I, Williams N, Ivanov D, Davis K, Haroutunian V, Buxbaum J, Craddock N, Kirov G, Owen M, O'Donovan M (2006) Convergent evidence that oligodendrocyte lineage transcription factor 2 (olig2) and interacting genes influence susceptibility to schizophrenia. Proceedings of the National Academy of Sciences 103:12469-12474.

Gerlai R, Marks A, Roder J (1994) T-maze spontaneous alternation rate is decreased in S100 beta transgenic mice. Behavioral Neuroscience 108:100-106.

Ghose S, Weickert C, Colvin S, Coyle J, Herman M, Hyde T, Kleinman J (2004) Glutamate carboxypeptidase II gene expression in the human frontal and temporal lobe in schizophrenia. Neuropsychopharmacology 29:117-125.

Gibbs M, O'Dowd B, Hertz L, Robinson S, Sedman G, Ng K (1996) Inhibition of glutamine synthetase activity prevents memory consolidation. Cognitive Brain Research 4:57-64.

Gluck M, Thomas R, Davis K, Haroutunian V (2002) Implications for altered glutamate and GABA metabolism in the dorsolateral prefrontal cortex of aged schizophrenic patients. American Journal of Psychiatry 159:1165-1173.
Goff D, Coyle J (2001) The emerging role of glutamate in the pathophysiology and treatment of schizophrenia. American Journal of Psychiatry 158:1367-1377.
Goldberg J, Barres B (2000) The relationship between neuronal survival and regeneration. Annals of the New York Acadamy of Sciences ual Review of Neuroscience 23:579-612.
Goldberg T, Straub R, Callicott J, Hariri A, Mattay V, Bigelow L, Coppola R, Egan M, Weinberger D (2006) The G72/G30 gene complex and cognitive abnormalities in schizophrenia. Neuropsychopharmacology 31:2022-2032.
Gothelf D, Soreni N, Nachman R, Tyano S, Hiss Y, Reiner O, Weizman A (2000) Evidence for the involvement of the hippocampus in the pathophysiology of schizophrenia. European Neuropsychopharmacology 10:389-395.
Gotz M, Huttner W (2005) The cell biology of neurogenesis. Nature Reviews Molecular Cell Biology 6:777-788.
Griffiths I, Klugmann M, Anderson T, Yool D, Thomson C, Schwab M, Schneider A, Zimmermann F, McCulloch M, Nadon N, Nave K (1998) Axonal swellings and degeneration in mice lacking the major proteolipid of myelin. Science 280:1610-1613.
Grimwood S, Slater P, Deakin J, Hutson P (1999) NR2b-containing NMDA receptors are up-regulated in temporal cortex in schizophrenia. Neuroreport 10:461-465.
Grosche J, Matyash V, Moller T, Verkhratsky A, Reichenbach A, Kettenmann H (1999) Microdomains for neuron-glia interaction: Parallel fiber signaling to Bergmann glial cells. Nature Neuroscience 2:139-143.
Guerri C, Pascual M, Renau-Piqueras J (2001) Glia and fetal alcohol syndrome. Neurotoxicology 22:593-599.
Guillet B, Velly L, Canolle B, Masmejean F, Nieoullon A, Pisano P (2005) Differential regulation by protein kinases of activity and cell surface expression of glutamate transporters in neuron-enriched cultures. Neurochemistry International 46:337-346.
Guillin O, Abi-Dargham A, Laruelle M (2007) Neurobiology of dopamine in schizophrenia. In: International review of neurobiology, pp 1-39: Academic Press.
Gupta D, McCullumsmith R, Beneyto M, Haroutunian V, Davis K, Meador-Woodruff J (2005) Metabotropic glutamate receptor protein expression in the prefrontal cortex and striatum in schizophrenia. Synapse 57:123-131.
Had-Aissouni L, Re D, Nieoullon A, Kerkerian-Le Goff L (2002) Importance of astrocytic inactivation of synaptically released glutamate for cell survival in the central nervous system--are astrocytes vulnerable to low intracellular glutamate concentrations? Journal of Physiology, Paris 96:317-322.
Hakak Y, Walker J, Li C, Wong W, Davis K, Buxbaum J, Haroutunian V, Fienberg A (2001) Genome-wide expression analysis reveals dysregulation of myelination-related genes in chronic schizophrenia. Proceedings of the National Academy of Sciences 98:4746-4751.

Halim N, Weickert C, McClintock B, Weinberger D, Lipska B (2004) Effects of chronic haloperidol and clozapine treatment on neurogenesis in the adult rat hippocampus. Neuropsychopharmacology 29:1063-1069.
Hambrecht M, Hafner H (1996) Substance abuse and the onset of schizophrenia. Biological Psychiatry 40:1155-1163.
Hansen L, Armstrong D, Terry R (1987) An immunohistochemical quantification of fibrous astrocytes in the aging human cerebral cortex. Neurobiology of Aging 8:1-6.
Hansson E, Ronnback L (2003) Glial neuronal signaling in the central nervous system The FASEB Journal 17:341-348.
Harrison P (1999) The neuropathology of schizophrenia. A critical review of the data and their interpretation. Brain 122:593-624.
Harrison P (2004) The hippocampus in schizophrenia: A review of the neuropathological evidence and its pathophysiological implications. Psychopharmacology 174:151-162.
Harrison P, Weinberger D (2005) Schizophrenia genes, gene expression, and neuropathology: On the matter of their convergence. Molecular Psychiatry 10:40-68.
Harrison P, Law A (2006) Neuregulin 1 and schizophrenia: Genetics, gene expression, and neurobiology. Biological Psychiatry 60:132-140.
Harrison P, Law A, Eastwood S (2003) Glutamate receptors and transporters in the hippocampus in schizophrenia. Annals of the New York Acadamy of Sciences als of the New York Acadamy of Sciences 1003:94-101.
Hashimoto A, Nishikawa T, Oka T, Takahashi K (1993) Endogenous D-serine in rat brain: N-methyl-D-aspartate receptor-related distribution and aging. Journal of Neurochemistry 60:783-786.
Hashimoto A, Nishikawa T, Hayashi T, Fujii N, Harada K, Oka T, Takahashi K (1992) The presence of free D-serine in rat brain. FEBS Letters 296:33-36.
Hashimoto K, Engberg G, Shimizu E, Nordin C, Lindstrom L, Iyo M (2005) Reduced D-serine to total serine ratio in the cerebrospinal fluid of drug naive schizophrenic patients. Progress in Neuro-Psychopharmacology and Biological Psychiatry 29:767-769.
Hashimoto K, Fukushima T, Shimizu E, Komatsu N, Watanabe H, Shinoda N, Nakazato M, Kumakiri C, Okada S, Hasegawa H, Imai K, Iyo M (2003) Decreased serum levels of D-serine in patients with schizophrenia. Archives of General Psychiatry 60:572-576.
Hassinger T, Guthrie P, Atkinson P, Bennett M, Kater S (1996) An extracellular signaling component in propagation of astrocytic calcium waves. Proceedings of the National Academy of Sciences 93:13268-13273.
Hatton G (1997) Funtion-related plasticity in the hypothalamus. Annals of the New York Acadamy of Sciences ual Review of Neuroscience 20:375-397.
Hawrylak N, Fleming J, Salm A (1998) Dehydration and rehydration selectively and reversibly alter glial fibrillary acidic protein immunoreactivity in the rat supraoptic nucleus and subjacent glial limitans. Glia 22:260-271.
Haydon P, Carmignoto G (2006) Astrocyte control of synaptic transmission and neurovascular coupling. Physiological Reviews 86:1009-1031.

Heckers S (2001) Neuroimaging studies of the hippocampus in schizophrenia. Hippocampus 11:520-528.
Henquet C, Murray R, Linszen D, van Os J (2005) The environment and schizophrenia: The role of cannabis use. Schizophrenia Bulletin 31:608-612.
Heresco-Levy U, Javitt D, Ebstein R, Vass A, Lichtenberg P, Bar G, Catinari S, Ermilov M (2005) D-serine efficacy as add-on pharmacotherapy to risperidone and olanzapine for treatment-refractory schizophrenia. Biological Psychiatry 57:577-585.
Hertz L, Zielke H (2004) Astrocytic control of glutamatergic activity: Astrocytes as stars of the show. Trends in Neuroscience 27:735-743.
Ho B, Andreasen N, Nopoulos P, Arndt S, Magnotta V, Flaum M (2003) Progressive structural brain abnormalities and their relationship to clinical outcome: A longitudinal magnetic resonance imaging study early in schizophrenia. Archives of General Psychiatry 60:585-594.
Hollmann M, Heinemann S (1994) Cloned glutamate receptors. Annals of the New York Acadamy of Sciences ual Review of Neuroscience 17:31-108.
Honer W, Falkai P, Chen C, Arango V, Mann J, Dwork A (1999) Synaptic and plasticity-associated proteins in anterior frontal cortex in severe mental illness. Neuroscience 91:1247-1255.
Hu W, Walters W, Xia X, Karmally S, Bethea J (2003) Neuronal glutamate transporter EAAT4 is expressed in astrocytes. Glia 44:13-25.
Huerta I, McCullumsmith R, Haroutunian V, Gimenez-Amaya J, Meador-Woodruff J (2006) Expression of excitatory amino acid transporter interacting protein transcripts in the thalamus in schizophrenia. Synapse 59:394-402.
Hughes E, Maguire J, McMinn M, Scholz R, Sutherland M (2004) Loss of glial fibrillary acidic protein results in decreased glutamate transport and inhibition of pka-induced EAAT2 cell surface trafficking. Molecular Brain Research 124:114-123.
Humphries C, Mortimer A, Hirsch S, de Belleroche J (1996) NMDA receptor mRNA correlation with antemortem cognitive impairment in schizophrenia. Neuroreport 7:2051-2055.
Hyde T, Ziegler J, Weinberger D (1992) Psychiatric disturbances in metachromatic leukodystrophy. Insights into the neurobiology of psychosis. Archives of Neurology 49:401-406.
Ibrahim H, Hogg Jr AJ, Healy D, Haroutunian V, Davis K, Meador-Woodruff J (2000) Ionotropic glutamate receptor binding and subunit mRNA expression in thalamic nuclei in schizophrenia. American Journal of Psychiatry 157:1811-1823.
Ishibashi T, Dakin K, Stevens B, Lee P, Kozlov S, Stewart C, Fields R (2006) Astrocytes promote myelination in response to electrical impulses. Neuron 49:823-832.
Itoh K, Stevens B, Schachner M, Fields R (1995) Regulated expression of the neural cell adhesion molecule l1 by specific patterns of neural impulses. Science 270:1369-1372.
Jakob H, Beckmann H (1985) Prenatal developmental disturbances in the limbic allocortex in schizophrenics. Journal of Neural Transmission 65:303-326.
Javitt D (1987) Negative schizophrenic symptomatology and the PCP (phencyclidine) model of schizophrenia. Hillside Journal of Clinical Psychiatry 9:12-35.

Javitt D, Balla A, Sershen H (2002) A novel alanine-insensitive D-serine transporter in rat brain synaptosomal membranes. Brain Research 941:146-149.
Johnson A (2002) Alexander disease: A review and the gene. International Journal of Developmental Neuroscience 20:391-394.
Johnston-Wilson NL, Sims CD, Hofmann J-P, Anderson L, Shore AD, Torrey EF, Yolken RH (2000) Disease-specific alterations in frontal cortex brain proteins in schizophrenia, bipolar disorder, and major depressive disorder. Molecular Psychiatry 5:142-149.
Kamitani W, Ono E, Yoshino S, Kobayashi T, Taharaguchi S, Lee B, Yamashita M, Kobayashi T, Okamoto M, Taniyama H, Tomonaga K, Ikuta K (2003) Glial expression of borna disease virus phosphoprotein induces behavioral and neurological abnormalities in transgenic mice. Proceedings of the National Academy of Sciences 100:8968-8974.
Karson C, Mrak R, Schluterman K, Sturner W, Sheng J, Griffin W (1999) Alterations in synaptic proteins and their encoding mRNAs in prefrontal cortex in schizophrenia: A possible neurochemical basis for 'hypofrontality'. Molecular Psychiatry 4:39-45.
Kartvelishvily E, Shleper M, Balan L, Dumin E, Wolosker H (2006) Neuron-derived D-serine release provides a novel means to activate N-methyl-D-aspartate receptors Journal of Biological Chemistry 281:14151-14162.
Katsel P, Davis K, Gorman J, Haroutunian V (2005) Variations in differential gene expression patterns across multiple brain regions in schizophrenia. Schizophrenia Research 77:241-252.
Kelley A, Andrzejewski M, Baldwin A, Hernandez P, Pratt W (2003) Glutamate-mediated plasticity in corticostriatal networks: Role in adaptive motor learning. Annals of the New York Acadamy of Sciences als of the New York Academy of Sciences 1003:159-168.
Kendler K, Diehl S (1993) The genetics of schizophrenia: A current, genetic-epidemiologic perspective. Schizophrenia Bulletin 19:261-285.
Khan Z, Koulen P, Rubinstein M, Grandy D, Goldman-Rakic P (2001) An astroglia-linked dopamine D2-receptor action in prefrontal cortex. Proceedings of the National Academy of Sciences 98:1964-1969.
Kim P, Aizawa H, Kim P, Huang A, Wickramasinghe S, Kashani A, Barrow R, Huganir R, Ghosh A, Snyder S (2005) Serine racemase: Activation by glutamate neurotransmission via glutamate receptor interacting protein and mediation of neuronal migration. Proceedings of the National Academy of Sciences 102:2105-2110.
Kim S, Choi S, Chao W, Volsky D (2003) Transcriptional regulation of human excitatory amino acid transporter 1 (EAAT1): Cloning of the EAAT1 promoter and characterization of its basal and inducible activity in human astrocytes. Journal of Neurochemistry 87:1485-1498.
Kimelberg H (2004) The problem of astrocyte identity. Neurochemistry International 45:191-202.
Knable M, Torrey E, Webster M, Bartko J (2001) Multivariate analysis of prefrontal cortical data from the Stanley Foundation Neuropathology Consortium. Brain Research Bulletin 55:651-659.

Kohama S, Goss J, Finch C, McNeill T (1995) Increases of glial fibrillary acidic protein in the aging female mouse brain. Neurobiology of Aging 16:59-67.
Kommers T, Rodnight R, Oppelt D, Oliveira D, Wofchuk S (1999) The mGluR stimulating GFAP phosphorylation in immature hippocampal slices has some properties of a group II receptor. Neuroreport 10:2119-2123.
Kommers T, Rodnight R, Boeck C, Vendite D, Oliveira D, Horn J, Oppelt D, Wofchuk S (2002) Phosphorylation of glial fibrillary acidic protein is stimulated by glutamate via NMDA receptors in cortical microslices and in mixed neuronal/glial cell cultures prepared from the cerebellum. Developmental Brain Research 137:139-148.
Korostishevsky M, Kaganovich M, Cholostoy A, Ashkenazi M, Ratner Y, Dahary D, Bernstein J, Bening-Abu-Shach U, Ben-Asher E, Lancet D, Ritsner M, Navon R (2004) Is the G72/G30 locus associated with schizophrenia? Single nucleotide polymorphisms, haplotypes, and gene expression analysis. Biological Psychiatry 56:169-176.
Kosenko E, Llansola M, Montoliu C, Monfort P, Rodrigo R, Hernandez-Viadel M, Erceg S, Sanchez-Perez A, Felipo V (2003) Glutamine synthetase activity and glutamine content in brain: Modulation by NMDA receptors and nitric oxide. Neurochemistry International 43:493.
Kristensen K, Cadenhead K (2007) Cannabis abuse and risk for psychosis in a prodromal sample. Psychiatry Research 151:151-154.
Kristiansen L, Meador-Woodruff J (2005) Abnormal striatal expression of transcripts encoding NMDA interacting PSD proteins in schizophrenia, bipolar disorder and major depression. Schizophrenia Research 78:87-93.
Kristiansen L, Beneyto M, Haroutunian V, Meador-Woodruff J (2006) Changes in NMDA receptor subunits and interacting PSD proteins in dorsolateral prefrontal and anterior cingulate cortex indicate abnormal regional expression in schizophrenia. Molecular Psychiatry 11:737-747.
Kristiansen L, Huerta I, Beneyto M, Meador-Woodruff J (2007) NMDA receptors and schizophrenia. Current Opinion in Pharmacology 7:48-55.
Lally B, Albrecht P, Levison S, Salm A (2005) Divergent glial fibrillary acidic protein and its mRNA in the activated supraoptic nucleus. Neuroscience Letters 380:295-299.
Lahti A, Koffel B, LaPorte D, Tamminga C (1995) Subanesthetic doses of ketamine stimulate psychosis in schizophrenia. Neuropsychopharmacology 13:9-19.
Laming P, Kimelberg H, Robinson S, Salm A, Hawrylak N, Muller C, Roots B, Ng K (2000) Neuronal-glial interactions and behaviour. Neuroscience and Behavioral Reviews 24:295-340.
Lara D, Gama C, Belmonte-de-Abreu P, Portela L, Goncalves C, Fonseca M, Hauck S, Souza D (2001) Increased serum S100β protein in schizophrenia: A study in medication-free patients. Journal of Psychiatric Research 35:11-14.
Laruelle M, Kegeles L, Abi-Dargham A (2003) Glutamate, dopamine, and schizophrenia: From pathophysiology to treatment. Annals of the New York Acadamy of Sciences 1003:138-158.
Legros J, Gazzotti C, Carvelli T, Franchimont P, Timsit-Berthier M, von Frenckell R, Ansseau M (1992) Apomorphine stimulation of vasopressin- and oxytocin-

neurophysins. Evidence for increased oxytocinergic and decreased vasopressinergic function in schizophrenics. Psychoneuroendocrinology 17:611-617.
Leino R, Gerhart D, Bueren Av, McCall A, Drewes L (1997) Ultrastructural localization of GLUT 1 and GLUT 3 glucose transporters in rat brain. Journal of Neuroscience Research 49:617-626.
Leloup C, Arluison M, Lepetit N, Cartier N, Marfaing-Jallat P, Ferre P, Penicaud L (1994) Glucose transporter 2 (GLUT 2): Expression in specific brain nuclei. Brain Research 638:221-226.
Lian X, Stringer J (2004) Energy failure in astrocytes increases the vulnerability of neurons to spreading depression. European Journal of Neuroscience 19:2446-2454.
Liedtke W, Edelmann W, Bieri P, Chiu F, Cowan N, Kucherlapati R, Raine C (1996) GFAP is necessary for the integrity of CNS white matter architecture and long-term maintenance of myelination. Neuron 17:607-615.
Lipska B (2004) Using animal models to test a neurodevelopmental hypothesis of schizophrenia. Journal of Psychiatry and Neuroscience 29:282-286.
Liu J, Shi Y, Tang J, Guo T, Li X, Yang Y, Chen Q, Zhao X, He G, Feng G, Gu N, Zhu Z, Liu H, He L (2005) SNPs and haplotypes in the S100β gene reveal association with schizophrenia. Biochemical and Biophysical Research Communications 328:335-341.
Ma J, Ye N, Lange N, Cohen B (2003) Dynorphinergic GABA neurons are a target of both typical and atypical antipsychotic drugs in the nucleus accumbens shell, central amygdaloid nucleus and thalamic central medial nucleus. Neuroscience 121:991-998.
Magistretti P (2006) Neuron-glia metabolic coupling and plasticity. The Journal of Experimental Biology 209:2304-2311.
Malatesta P, Hartfuss E, Gotz M (2000) Isolation of radial glial cells by fluorescent-activated cell sorting reveals a neuronal lineage. Development 127:5253-5263.
Malhi G, Valenzuela M, Wen W, Sachdev P (2002) Magnetic resonance spectroscopy and its applications in psychiatry. The Australian and New Zealand Journal of Psychiatry 36:31-43.
Mallolas J, Hurtado O, Castellanos M, Blanco M, Sobrino T, Serena J, Vivancos J, Castillo J, Lizasoain I, Moro M, Davalos A (2006) A polymorphism in the EAAT2 promoter is associated with higher glutamate concentrations and higher frequency of progressing stroke. Journal of Experimental Medicine 203:711-717.
Manoach D (2003) Prefrontal cortex dysfunction during working memory performance in schizophrenia: Reconciling discrepant findings. Schizophrenia Research 60:285-298.
Maragakis N, Rothstein J (2006) Mechanisms of disease: Astrocytes in neurodegenerative disease. Nature Clinical Practice Neurology 2:679-689.
Martineau M, Baux G, Mothet J (2005) Gliotransmission at central glutamatergic synapses: D-serine on stage. Journal of Physiology, Paris 99:103-110.
Matsui T, Sekiguchi M, Hashimoto A, Tomita U, Nishikawa T, Wada K (1995) Functional comparison of D-serine and glycine in rodents: The effect on cloned

NMDA receptors and the extracellular concentration. Journal of Neurochemistry 65:454-458.
Matthias K, Kirchhoff F, Seifert G, Huttmann K, Matyash M, Kettenmann H, Steinhauser C (2003) Segregated expression of AMPA-type glutamate receptors and glutamate transporters defines distinct astrocyte populations in the mouse hippocampus. Journal of Neuroscience 23:1750-1780.
Matute C, Melone M, Vallejo-Illarramendi A, Conti F (2005) Increased expression of the astrocytic glutamate transporter GLT-1 in the prefrontal cortex of schizophrenics. Glia 49:451-455.
Mbele G, Deloulme J, Gentil B, Delphin C, Ferro M, Garin J, Takahashi M, Baudier J (2002) The zinc- and calcium-binding S100β interacts and co-localizes with IQGAP1 during dynamic rearrangement of cell membranes. The Journal of Biological Chemistry 277:49998-50007.
McCall M, Gregg R, Behringer R, Brenner M, Delaney C, Galbreath E, Zhang C, Pearce R, Chiu S, Messing A (1996) Targeted deletion in astrocyte intermediate filament (GFAP) alters neuronal physiology. Proceedings of the National Academy of Sciences 93:6361-6366.
McCullumsmith R, Meador-Woodruff J (2002) Striatal excitatory amino acid transporter transcript expression in schizophrenia, bipolar disorder, and major depressive disorder. Neuropsychopharmacology 26:368-375.
McCullumsmith R, Gupta D, Beneyto M, Kreger E, Haroutunian V, Davis K, Meador-Woodruff J (2007) Expression of transcripts for myelination-related genes in the anterior cingulate cortex in schizophrenia. Schizophrenia Research 90:15-27.
Meador-Woodruff J, Davis K, Haroutunian V (2001) Abnormal kainate receptor expression in prefrontal cortex in schizophrenia. Neuropsychopharmacology 24:545-552.
Meador-Woodruff J, Clinton S, Beneyto M, McCullumsmith R (2003) Molecular abnormalities of the glutamate synapse in the thalamus in schizophrenia. Annals of the New York Academy of Sciences 1003:75-93.
Meltzer H, Lee M, Jayathilake K (2001) The blunted plasma cortisol response to apomorphine and its relationship to treatment response in patients with schizophrenia. Neuropsychopharmacology 24:278-290.
Menet V, Ribotta MGy, Chauvet N, Drian M, Lannoy J, Colucci-Guyon E, Privat A (2001) Inactivation of the glial fibrillary acidic protein gene, but not that of vimentin, improves neuronal survival and neurite growth by modifying adhesion molecule expression Journal of Neuroscience 21:6147-6158.
Meyer-Lindenberg A, Olsen R, Kohn P, Brown T, Egan M, Weinberger D, Berman K (2005) Regionally specific disturbance of dorsolateral prefrontal-hippocampal functional connectivity in schizophrenia. Archives of General Psychiatry 62:379-386.
Mignot C, Boespflug-Tanguy O, Gelot A, Dautigny A, Pham-Dinh D, Rodriguez D (2004) Alexander disease: Putative mechanisms of an astrocytic encephalopathy Cellular and Molecular Life Sciences 61:369-385.
Miller J (1991) The calibration of 35S or 32P with 14C-labeled brain paste or 14C-plastic standards for quantitative autoradiography using LKB Ultrofilm or Amersham Hyperfilm. Neuroscience Letters 121:211-214.

Miranda JD, Santoro A, Engelender S, Wolosker H (2000) Human serine racemase: Moleular cloning, genomic organization and functional analysis. Gene 265:183-188.
Mitani A, Tanaka K (2003) Functional changes of glial glutamate transporter GLT-1 during ischemia: An in vivo study in the hippocampal CA1 of normal mice and mutant mice lacking GLT-1. The Journal of Neuroscience 23:7176-7182.
Miyamae Y, Nakamura Y, Kashiwagi Y, Tanaka T, Kudo T, Takeda M (1998) Altered adhesion efficiency and fibronectin content in fibroblasts from schizophrenic patients. Psychiatry and Clinical Neurosciences 52:345-352.
Miyata S, Itoh T, Matsushima O, Nakashima T, Kiyohara T (1994) Not only osmotic stress but also repeated restraint stress causes structural plasticity in the supraoptic nucleus of the rat hypothalamus. Brain Research Bulletin 33:669-675.
Montana V, Ni Y, Sunjara V, Hua X, Parpura V (2004) Vesicular glutamate transporter-dependent glutamate release from astrocytes. The Journal of Neuroscience 24:2633-2642.
Moreno S, Nardacci R, Cimini A, Ceru M (1999) Immunocytochemical localization of D-amino acid oxidase in rat brain. Journal of Neurocytology 28:169-185.
Morita Y, Ujike H, Tanaka Y, Otani K, Kishimoto M, Morio A, Kotaka T, Okahisa Y, Matsushita M, Morikawa A, Hamase K, Zaitsu K, Kuroda S (2007) A genetic variant of the serine racemase gene is associated with schizophrenia. Biological Psychiatry (In press).
Mothet J, Pollegioni L, Ouanounou G, Martineau M, Fossier P, Baux G (2005) Glutamate receptor activation triggers a calcium-dependent and snare protein-dependent release of the gliotransmitter D-serine. Proceedings of the National Academy of Sciences 102:5606-5611.
Mothet J, Parent A, Wolosker H, Brady R, Linden D, Ferris C, Rogawski M, Snyder S (2000) D-serine is an endogenous ligand for the glycine site of the N-methyl-D-aspartate receptor. Proceedings of the National Academy of Sciences 97:4926-4931.
Mothet J, Rouaud E, Sinet P, Potier B, Jouvenceau A, Dutar P, Videau C, Epelbaum J, Billard J (2006) A critical role for the glial-derived neuromodulator D-serine in the age-related deficits of cellular mechanisms of learning and memory. Aging Cell 5:267-274.
Mueller H, Meador-Woodruff J (2004) NR3A NMDA receptor subunit mRNA expression in schizophrenia, depression and bipolar disorder. Schizophrenia Research 71:361-370.
Mulligan S, MacVicar B (2004) Calcium transients in astrocyte endfeet cause cerebrovascular constrictions. Nature 431:195-199.
Mustafa A, Kumar M, Selvakumar B, Ho G, Ehmsen J, Barrow R, Amzel L, Snyder S (2007) Nitric oxide S-nitrosylates serine racemase, mediating feedback inhibition of D-serine formation. Proceedings of the National Academy of Sciences 104:2950-2955.
Nagler K, Mauch D, Pfrieger F (2001) Glia-derived signals induce synapse formation in neurones of the rat central nervous system. Journal of Physiology 533:665-679.
Newell K, Zavitsanou K, Huang X (2007) Short and long term changes in NMDA receptor binding in mouse brain following chronic phencyclidine treatment.

Journal of Neural Transmission (In press).
Nichols N, Day J, Laping N, Johnson S, Finch C (1993) GFAP mRNA increases with age in rat and human brain. Neurobiology of Aging 14:421-429.
Niizato K, Iritani S, Ikeda K, Arai H (2001) Astroglial function of schizophrenic brain: A study using lobotomized brain. Neuroreport 12:1457-1460.
Nishioka N, Arnold S (2004) Evidence for oxidative DNA damage in the hippocampus of elderly patients with chronic schizophrenia. American Journal of Geriatric Psychiatry 12:167-175.
Nishiyama A, Yang Z, Butt A (2005) Astrocytes and NG2-glia: What's in a name? Journal of Anatomy 207:687-693.
Nishiyama H, Knöpfel T, Endo S, Itohara S (2002) Glial protein S100β modulates long-term neuronal synaptic plasticity. Proceedings of the National Academy of Sciences 99:4037-4042.
Ohnuma T, Augood S, Arai H, McKenna P, Emson P (1998) Expression of the human excitatory amino acid transporter 2 and metabotropic glutamate receptors 3 and 5 in the prefrontal cortex from normal individuals and patients with schizophrenia. Brain Research Molecular Brain Research 56:207-217.
Ohnuma T, Tessler S, Arai H, Faull R, McKenna P, Emson P (2000) Gene expression of metabotropic glutamate receptor 5 and excitatory amino acid transporter 2 in the schizophrenic hippocampus. Brain Research Molecular Brain Research 85:24-31.
Oliet S, Piet R, Poulain D (2001) Control of glutamate clearance and synaptic efficacy by glial coverage of neurons. Science 292:923-926.
Oppelt D, Rodnight R, Horn J, Fitarelli D, Kommers T, Oliveira D, Wofchuk S (2004) Role of intracellular calcium stores on the effect of metabotropic glutamate receptors on phosphorylation of glial fibrillary acidic protein in hippocampal slices from immature rats. Neurochemical Research 29:1541-1545.
Pagsberg A, Baare W, Christensen AR, Fagerlund B, Hansen M, Labianca J, Krabbe K, Aarkrog T, Paulson O, Hemmingsen R (2007) Structural brain abnormalities in early onset first-episode psychosis. Journal of Neural Transmission 114:489-498.
Panatier A, Theodosis D, Mothet J, Touquet B, Pollegioni L, Poulain D, Oliet S (2006) Glia-derived D-serine controls NMDA receptor activity and synaptic memory. Cell 125:775-784.
Pantelis C, Yucel M, Wood S, Velakoulis D, Sun D, Berger G, Stuart G, Yung A, Phillips L, McGorry P (2005) Structural brain imaging evidence for multiple pathological processes at different stages of brain development in schizophrenia. Schizophrenia Bulletin 31:672-696.
Parpura V, Basarsky TA, Liu F, Jeftinija K, Jeftinija S, Haydon PG (1994) Glutamate-mediated astrocyte-neuron signalling. Nature 369:744-747.
Paxinos G, Watson C (1986) The rat brain in stereotaxic coordinates. Second Edition. New York: Academic Press.
Pekny M, Leveen P, Pekna M, Eliasson C, Berthold C, Westermark B, Betsholtz C (1995) Mice lacking glial fibrillary acidic protein display astrocytes devoid of intermediate filaments but develop and reproduce normally. European Molecular Biology Organization Journal 14:1590-1598.
Pekny M, Eliasson C, Siushansian R, Ding M, Dixon S, Pekna M, Wilson J, Hamberger A (1999) The impact of genetic removal of GFAP and/or vimentin on glutamine

levels and transport of glucose and ascorbate in astrocytes. Neurochemical Research 24:1357-1362.
Pellerin L, Magistretti P (2004) Neuroenergetics: Calling upon astrocytes to satisfy hungry neurons. The Neuroscientist 10:53-62.
Perea G, Araque A (2005) Properties of synaptically evoked astrocyte calcium signal reveal synaptic information processing by astrocytes. The Journal of Neuroscience 25:2192-2203.
Perlstein W, Carter C, Noll D, Cohen J (2001) Relation of prefrontal cortex dysfunction to working memory and symptoms in schizophrenia. American Journal of Psychiatry 58:1105-1113.
Perng M, Su M, Wen S, Li R, Gibbon T, Prescott A, Brenner M, Quinlan R (2006) The Alexander disease–causing glial fibrillary acidic protein mutant, R416W, accumulates into rosenthal fibers by a pathway that involves filament aggregation and the association of αb-crystallin and HSP27. The American Journal of Human Genetics 79:197-213.
Perrone-Bizzozero N, Sower A, Bird E, Benowitz L, Ivins K, Neve R (1996) Levels of the growth-associated protein GAP-43 are selectively increased in association cortices in schizophrenia. Proceedings of the National Academy of Sciences 93:14182-14187.
Peters A (2004) A fourth type of neuroglial cell in the adult central nervous system. Journal of Neurocytology 33:345-357.
Piet R, Vargova L, Sykova E, Poulain D, Oliet S (2004) Physiological contribution of the astrocytic environment of neurons to intersynaptic crosstalk. Proceedings of the National Academy of Sciences 101:2151-2155.
Ponzio T, Ni Y, Montana V, Parpura V, Hatton G (2006) Vesicular glutamate transporter expression in supraoptic neurones suggests a glutamatergic phenotype. Journal of Neuroendocrinology 18:253-265.
Porter J, McCarthy K (1995) GFAP-positive hippocampal astrocytes in situ respond to glutamatergic neuroligands with increases in $[Ca^{2+}]_i$. Glia 13:101-112.
Rajarethinam R, DeQuardo J, Nalepa R, Tandon R (2000) Superior temporal gyrus in schizophrenia: A volumetric magnetic resonance imaging study. Schizophrenia Research 41:303-312.
Rajarethinam R, Sahni S, Rosenberg D, Keshavan M (2004) Reduced superior temporal gyrus volume in young offspring of patients with schizophrenia. American Journal of Psychiatry 161:1121-1124.
Rajkowska G, Selemon L, Goldman-Rakic P (1998) Neuronal and glial somal size in the prefrontal cortex: A postmortem morphometric study of schizophrenia and Huntington disease. Archives of General Psychiatry 55:215-224.
Rajkowska G, Miguel-Hidalgo J, Makkos Z, Meltzer H, Overholser J, Stockmeier C (2002) Layer-specific reductions in GFAP-reactive astroglia in the dorsolateral prefrontal cortex in schizophrenia. Schizophrenia Research 57:127-138.
Raponi E, Agenes F, Delphin C, Assard N, Baudier J, Legraverend C, Deloulme J (2007) S100b expression defines a state in which GFAP-expressing cells lose their neural stem cell potential and acquire a more mature developmental stage. Glia 55:165-177.

Rapoport J, Addington A, Frangou S, Psych M (2005) The neurodevelopmental model of schizophrenia: Update 2005. Molecular Psychiatry 10:434-449.
Ribeiro C, Reis M, Panizzutti R, Miranda Jd, Wolosker H (2002) Glial transport of the neuromodulator D-serine. Brain Research 929:202-209.
Roberts G, Colter N, Lofthouse R, Johnstone E, Crow T (1987) Is there gliosis in schizophrenia? Investigation of the temporal lobe. Biological Psychiatry 22:1459-1468.
Roberts G, Colter N, Lofthouse R, Bogerts B, Zech M, Crow T (1986) Gliosis in schizophrenia: A survey. Biological Psychiatry 21:1043-1050.
Rodnight R, Goncalves C, Wofchuk S, Leal R (1997) Control of the phosphorylation of the astrocyte marker glial fibrillary acidic protein (GFAP) in the immature rat hippocampus by glutamate and calcium ions: Possible key factor in astrocytic plasticity. Brazilian Journal of Medical and Biological Research 30:325-338.
Roth B, Hanizavareh S, Blum A (2003) Serotonin receptors represent highly favorable molecular targets for cognitive enhancement in schizophrenia and other disorders. Psychopharmacology 174:17-24.
Rothermundt M, Peters M, Prehn J, Arolt V (2003) S100b in brain damage and neurodegeneration. Microscopy Research and Technique 60:614-632.
Rothermundt M, Ponath G, Glaser T, Hetzel G, Arolt V (2004a) S100β serum levels and long-term improvement of negative symptoms in patients with schizophrenia. Neuropsychopharmacology 29:1004-1011.
Rothermundt M, Missler U, Arolt V, Peters M, Leadbeater J, Wiesmann M, Rudolf S, Wandinger K, Kirchner H (2001) Increased S100β blood levels in unmedicated and treated schizophrenic patients are correlated with negative symptomatology. Molecular Psychiatry 6:445-449.
Rothermundt M, Ohrmann P, Abel S, Siegmund A, Pedersen A, Ponath G, Suslow T, Peters M, Kaestner F, Heindel W, Arolt V, Pfleiderer B (2007) Glial cell activation in a subgroup of patients with schizophrenia indicated by increased S100b serum concentrations and elevated myo-inositol. Progress in neuro-psychopharmacology & Biological Psychiatry 31:361-364.
Rothermundt M, Falkai P, Ponath G, Abel S, Burkle H, Diedrich M, Hetzel G, Peters M, Siegmund A, Pedersen A, Maier W, Schramm J, Suslow T, Ohrmann P, Arolt V (2004b) Glial cell dysfunction in schizophrenia indicated by increased S100β in the CSF. Molecular Psychiatry 9:897-899.
Rothman D, Sibson N, Hyder F, Shen J, Behar K, Shulman R (1999) In vivo nuclear magnetic resonance spectroscopy studies of the relationship between the glutamate--glutamine neurotransmitter cycle and functional neuroenergetics. Philosophical Transactions of the Royal Society B: Biological Sciences 354:1165-1177.
Saez J, Contreras J, Bukauskas F, Retamal M, Bennett M (2003) Gap junction hemichannels in astrocytes of the CNS. Acta Physiologica Scandinavica 179:9-22.
Salm A, Modney B, Hatton G (1988) Alterations in supraoptic nucleus ultrastructure of maternally behaving virgin rats. Brain Research Bulletin 21:685-691.
Sanfilipo M, Lafargue T, Rusinek H, Arena L, Loneragan C, Lautin A, Feiner D, Rotrosen J, Wolkin A (2000) Volumetric measure of the frontal and temporal lobe

regions in schizophrenia: Relationship to negative symptoms. Archives of General Psychiatry 57:471-480.
Sarter M, Nelson C, Bruno J (2005) Cortical cholinergic transmission and cortical information processing in schizophrenia. Schizophrenia Bulletin 31:117-138.
Sawa A, Pletnikov M, Kamiya A (2004) Neuron-glia interactions clarify genetic-environmental links in mental illness. Trends in Neuroscience 27:294-297.
Schell M, Molliver M, Snyder S (1995) D-serine, an endogenous synaptic modulator: Localization to astrocytes and glutamate-stimulated release. Proceedings of the National Academy of Sciences 92:3948-3952.
Schell M, Brady R, Molliver M, Snyder S (1997) D-serine as a neuromodulator: Regional and developmental localizations in rat brain glia resemble NMDA receptors. Journal of Neuroscience 17:1604-1615.
Schmitt A, Weber S, Jatzko A, Braus D, Henn F (2004) Hippocampal volume and cell proliferation after acute and chronic clozapine or haloperidol treatment. Journal of Neural Transmission 111:91-100.
Schmitt A, Bertsch T, Henning U, Tost H, Klimke A, Henn F, Falkai P (2005) Increased serum S100β in elderly, chronic schizophrenic patients: Negative correlation with deficit symptoms. Schizophrenia Research 80:305-313.
Schroeter M, Abdul-Khaliq H, Fruhauf S, Hohne R, Schick G, Diefenbacher A, Blasig I (2003) Serum S100β is increased during early treatment with antipsychotics and in deficit schizophrenia. Schizophrenia Research 62:231-236.
Schumacher J, Jamra R, Freudenberg J, Becker T, Ohlraun S, Otte A, Tullius M, Kovalenko S, Bogaert A, Maier W, Rietschel M, Propping P, Nothen M, Cichon S (2004) Examination of G72 and D-amino acid oxidase as genetic risk factors for schizophrenia and bipolar affective disorder. Molecular Psychiatry 9:203-207.
Sei Y, Ren-Patterson R, Li Z, Tunbridge E, Egan M, Kolachana B, Weinberger D (2007) Neuregulin1-induced cell migration is impaired in schizophrenia: Association with neuregulin1 and catechol-o-methyltransferase gene polymorphisms. Molecular Psychiatry (In press).
Selemon L, Goldman-Rakic P (1999) The reduced neuropil hypothesis: A circuit based model of schizophrenia. Biological Psychiatry 45:17-25.
Selemon L, Rajkowska G, Goldman-Rakic P (1995) Abnormally high neuronal density in the schizophrenic cortex. A morphometric analysis of prefrontal area 9 and occipital area 17. Archives of General Psychiatry 52:805-818.
Selemon L, Lidow M, Goldman-Rakic P (1999) Increased volume and glial density in primate prefrontal cortex associated with chronic antipsychotic drug exposure. Biological Psychiatry 46:161-172.
Selinfreund R, Barger S, Welsh M, Van Eldik L (1990) Antisense inhibition of glial S100 beta production results in alterations in cell morphology, cytoskeletal organization, and cell proliferation. Journal of Cell Biology 111:2021-2028.
Sherwood C, Stimpson C, Raghanti M, Wildman D, Uddin M, Grossman L, Goodman M, Redmond J, Bonar C, Erwin J, Hof P (2006) Evolution of increased glia-neuron ratios in the human frontal cortex. Proceedings of the National Academy of Sciences 103:13606-13611.
Shibuki K, Gomi H, Chen L, Bao S, Kim J, Wakatsuki H, Fujisaki T, Fujimoto K, Katoh A, Ikeda T (1996) Deficient cerebellar long-term depression, impaired eyeblink

conditioning, and normal motor coordination in GFAP mutant mice. Neuron 16:587-599.
Shoji K, Mariotto S, Ciampa A, Suzuki H (2006a) Regulation of serine racemase activity by D-serine and nitric oxide in human glioblastoma cells. Neuroscience Letters 392:75-78.
Shoji K, Mariotto S, Ciampa A, Suzuki H (2006b) Mutual regulation between serine and nitric oxide metabolism in human glioblastoma cells. Neuroscience Letters 394:163-167.
Simard M, Arcuino G, Takano T, Liu Q, Nedergaard M (2003) Signaling at the gliovascular interface. Journal of Neuroscience 23:9254-9262.
Smith R, Haroutunian V, Davis K, Meador-Woodruff J (2001a) Expression of excitatory amino acid transporter transcripts in the thalamus of subjects with schizophrenia. American Journal of Psychiatry 158:1393-1399.
Smith R, Haroutunian V, Davis K, Meador-Woodruff J (2001b) Vesicular glutamate transporter transcript expression in the thalamus in schizophrenia. Neuroreport 12:2885-2887.
Sokolov B, Tcherepanov A, Haroutunian V, Davis K (2000) Levels of mRNAs encoding synaptic vesicle and synaptic plasma membrane proteins in the temporal cortex of elderly schizophrenic patients. Biological Psychiatry 48:184-196.
Song H, Stevens C, Gage F (2002) Astroglia induce neurogenesis from adult neural stem cells. Nature 417:39-44.
Sonnewald U, Qu H, Aschner M (2002) Pharmacology and toxicology of astrocyte-neuron glutamate transport and cycling. Journal of Pharmacology and Experimental Therapeutics 301:1-6.
Spurney C, Baca S, Murray A, Jaskiw G, Kleinman J, Hyde T (1999) Differential effects of haloperidol and clozapine on ionotropic glutamate receptors in rats. Synapse 34:266-276.
Stark A, Uylings H, Sanz-Arigita E, Pakkenberg B (2004) Glial cell loss in the anterior cingulate cortex, a subregion of the prefrontal cortex, in subjects with schizophrenia. American Journal of Psychiatry 161:882-888.
Steen R, Mull C, McClure R, Hamer R, Lieberman J (2006) Brain volume in first-episode schizophrenia: Systematic review and meta-analysis of magnetic resonance imaging studies. British Journal of Psychiatry 188:510-518.
Steiner J, Bielau H, Bernstein H, Bogerts B, Wunderlich M (2006) Increased cerebrospinal fluid and serum levels of S100b in first-onset schizophrenia are not related to a degenerative release of glial fibrillar acidic protein, myelin basic protein and neurone-specific enolase from glia or neurones. The Journal of Neurology, Neurosurgery, and Psychiatry 77:1284-1287.
Steinhauser C, Jabs R, Kettenmann H (1994) Properties of GABA and glutamate responses in identified glial cells of the mouse hippocampal slice. Hippocampus 4:19-35.
Stephane M, Barton S, Boutros N (2001) Auditory verbal hallucinations and dysfunction of the neural substrates of speech. Schizophrenia Research 50:61-78.
Stevens C, Altshuler L, Bogerts B, Falkai P (1988) Quantitative study of gliosis in schizophrenia and Huntington's chorea Biological Psychiatry 24:697-700.

Stevens J (1982) Neuropathology of schizophrenia. Archives of General Psychiatry 39:1131-1139.
Stichel C, Muller C, Zilles K (1991) Distribution of glial fibrillary acidic protein and vimentin immunoreactivity during rat visual cortex development. Journal of Neurocytology 20:97-108.
Strisovsky K, Jiraskova J, Mikulova A, Rulisek L, Konvalinka J (2005) Dual substrate and reaction specificity in mouse serine racemase: Identification of high-affinity dicarboxylate substrate and inhibitors and analysis of the beta-eliminase activity. Biochemistry 44:13091-13100.
Su Z, Leszczyniecka M, Kang D, Sarkar D, Chao W, Volsky D, Fisher P (2003) Insights into glutamate transport regulation in human astrocytes: Cloning of the promoter for excitatory amino acid transporter 2 (EAAT2). Proceedings of the National Academy of Sciences 100:1955-1960.
Suarez I, Bodega G, Fernandez B (2002) Glutamine synthetase in brain: Effect of ammonia. Neurochemistry International 41:123-142.
Sugai T, Kawamura M, Iritani S, Araki K, Makifuchi T, Imai C, Nakamura R, Kakita A, Takahashi H, Nawa H (2005) Prefrontal abnormality of schizophrenia revealed by DNA microarray: Impact on glial and neurotrophic gene expression. Annals of the New York Acadamy of Sciences 1025:84-91.
Sun X, Takahashi S, Cui C, Zhang R, Sakata-Haga H, Sawada K, Fukui Y (2002) Normal and abnormal neuronal migration in the developing cerebral cortex. The Journal of Medical Investigation 49:97-110.
Takamori S, Rhee J, Rosenmund C, Jahn R (2000) Identification of a vesicular glutamate transporter that defines a glutamatergic phenotype in neurons. Nature 407:189-194.
Takemura M, Nishiyama H, Itohara S (2002) Distribution of phosphorylated glial fibrillary acidic protein in the mouse central nervous system. Genes to Cells 7:295-307.
Tamminga C, Holcomb H (2005) Phenotype of schizophrenia: A review and formulation. Molecular Psychiatry 10:27-39.
Tamminga C, Vogel M, Gao X, Lahti A, Holcomb H (2000) The limbic cortex in schizophrenia: Focus on the anterior cingulate. Brain Research Brain Research Reviews 31:364-370.
Thompson P, Vidal C, Giedd J, Gochman P, Blumenthal J, Nicolson R, Toga A, Rapoport J (2001) Mapping adolescent brain change reveals dynamic wave of accelerated gray matter loss in very early-onset schizophrenia. Proceedings of the National Academy of Sciences 98:11650-11655.
Tian G, Takano T, Lin J, Wang X, Bekar L, Nedergaard M (2006) Imaging of cortical astrocytes using 2-photon laser scanning microscopy in the intact mouse brain. Advanced Drug Delivery Reviews 58:773-787.
Toro C, Hallak J, Dunham J, Deakin J (2006) Glial fibrillary acidic protein and glutamine synthetase in subregions of prefrontal cortex in schizophrenia and mood disorder. Neuroscience Letters 404:276-281.
Tsai G, Yang P, Chung L, Lange N, Coyle J (1998) D-serine added to antipsychotics for the treatment of schizophrenia. Biological Psychiatry 44:1081-1089.

Tsai G, Yang P, Chung L, Tsai I, Tsai C, Coyle J (1999) D-serine added to clozapine for the treatment of schizophrenia. American Journal of Psychiatry 156:1822-1825.
Ueda S, Saitoh Y, Koibuchi N, Ishizuya-Oka A (1997) Local disturbance of neuronal migration in the S100 beta-retarded mutant mouse. Cell and Tissue Research 289:547-551.
Ullian E, Christopherson K, Barres B (2004) Role for glia in synaptogenesis. Glia 47:209-216.
Ullian E, Sapperstein S, Christopherson K, Barres B (2001) Control of synapse number by glia. Science 291:657-661.
Urai Y, Jinnouchi O, Kwak K, Suzue A, Nagahiro S, Fukui K (2002) Gene expression of D-amino acid oxidase in cultured rat astrocytes: Regional and cell type specific expression. Neuroscience Letters 324:101-104.
Uranova N, Vostrikov V, Orlovskaya D, Rachmanova V (2004) Oligodendroglial density in the prefrontal cortex in schizophrenia and mood disorders: A study from the Stanley Neuropathology Consortium. Schizophrenia Research 67:269-275.
Uranova N, Orlovskaya D, Vikhreva O, Zimina I, Kolomeets N, Vostrikov V, Rachmanova V (2001) Electron microscopy of oligodendroglia in severe mental illness. Brain Research Bulletin 55:597-610.
Verkhratsky A, Orkand R, Kettenmann H (1998) Glial calcium: Homeostasis and signaling function. Physiological Reviews 78:99-141.
Volterra A, Meldolesi J (2005) Astrocytes, from brain glue to communication elements: The revolution continues. Nature Reviews Neuroscience 6:626-640.
Volterra A, Trotti D, Racagni G (1994) Glutamate uptake is inhibited by arachidonic acid and oxygen radicals via two distinct and additive mechanisms. Molecular Pharmacology 46:986-992.
Walter H, Vasic N, Hose A, Spitzer M, Wolf R (2007) Working memory dysfunction in schizophrenia compared to healthy controls and patients with depression: Evidence from event-related fMRI. Neuroimage 35:1551-1561.
Walterfang M, Wood S, Velakoulis D, Copolov D, Pantelis C (2005) Diseases of white matter and schizophrenia-like psychosis. The Australian and New Zealand Journal of Psychiatry 39:746-756.
Walz W (2000) Controversy surrounding the existence of discrete functional classes of astrocytes in adult gray matter. Glia 31:95-103.
Wang X, Lou N, Xu Q, Tian G, Peng W, Han X, Kang J, Takano T, Nedergaard M (2006) Astrocytic Ca2+ signaling evoked by sensory stimulation in vivo. Nature Neuroscience 9:816-823.
Wassef A, Baker J, Kochan L (2003) Gaba and schizophrenia: A review of basic science and clinical studies. Journal of Clinical Psychopharmacology 23:601-640.
Watanabe T, Morimoto K, Hirao T, Suwaki H, Watase K, Tanaka K (1999) Amygdala-kindled and pentylenetetrazole-induced seizures in glutamate transporter glast-deficient mice. Brain Research 845:92-96.
Watts J, Fowler L, Whitton P, Pearce B (2005) Release of arginine, glutamate and glutamine in the hippocampus of freely moving rats: Involvement of nitric oxide. Brain Research Bulletin 65:521.

Webster M, O'Grady J, Kleinman J, Weickert C (2005) Glial fibrillary acidic protein mRNA levels in the cingulate cortex of individuals with depression, bipolar disorder and schizophrenia. Neuroscience 133:453-461.

Webster M, Knable M, Johnston-Wilson N, Nagata K, Inagaki M, Yolken R (2001) Immunohistochemical localization of phosphorylated glial fibrillary acidic protein in the prefrontal cortex and hippocampus from patients with schizophrenia, bipolar disorder, and depression. Brain, Behavior, and Immunity 15:388-400.

Weickert C, Hyde T, Lipska B, Herman M, Weinberger D, Kleinman J (2003) Reduced brain-derived neurotrophic factor in prefrontal cortex of patients with schizophrenia. Molecular Psychiatry 8:592-610.

Weinberger D, Berman K (1996) Prefrontal function in schizophrenia: Confounds and controversies. Philosophical Transactions of the Royal Society B: Biological Sciences 351:1495-1503.

Weinberger D, Berman K, Zec R (1986) Physiologic dysfunction of dorsolateral prefrontal cortex in schizophrenia. I. Regional cerebral blood flow evidence. Archives of General Psychiatry 43:114-124.

Weinstein D, Shelanski M, Liem R (1991) Suppression by antisense mRNA demonstrates a requirement for the glial fibrillary acidic protein in the formation of stable astrocytic processes in response to neurons. Journal of Cell Biology 112:1205-1213.

Weissman T, Riquelme P, Ivic L, Flint A, Kriegstein A (2004) Calcium waves propagate through radial glial cells and modulate proliferation in the developing neocortex. Neuron 43:647-661.

Whitaker-Azmitia P, Wingate M, Borella A, Gerlai R, Roder J, Azmitia E (1997) Transgenic mice overexpressing the neurotrophic factor S100β show neuronal cytoskeletal and behavioral signs of altered aging processes: Implications for Alzheimer's disease and Down's Syndrome. Brain Research 776:51-60.

Wiesmann M, Wandinger K, Missler U, Eckhoff D, Rothermundt M, Arolt V, Kirchner H (1999) Elevated plasma levels of S100β protein in schizophrenic patients. Biological Psychiatry 45:1508-1511.

Wilhelmsson U, Bushong E, Price D, Smarr B, Phung V, Terada M, Ellisman M, Pekny M (2006) Redefining the concept of reactive astrocytes as cells that remain within their unique domains upon reaction to injury. Proceedings of the National Academy of Sciences 103:17513-17518.

Williams S, Diaz C, Macnab L, Sullivan R, Pow D (2006) Immunocytochemical analysis of D-serine distribution in the mammalian brain reveals novel anatomical compartmentalizations in glia and neurons. Glia 53:401-411.

Winocura G, Roder J, Lobaugh N (2001) Learning and memory in S100b transgenic mice: An analysis of impaired and preserved function. Neurobiology of Learning and Memory 75:230-243.

Winterer G, Coppola R, Egan M, Goldberg T, Weinberger D (2003) Functional and effective frontotemporal connectivity and genetic risk for schizophrenia. Biological Psychiatry 54:1181-1192.

Wolf R, Vasic N, Hose A, Spitzer M, Walter H (2007) Changes over time in frontotemporal activation during a working memory task in patients with schizophrenia. Schizophrenia Research 91:141-150.

Wolosker H, Blackshaw S, Snyder S (1999a) Serine racemase: A glial enzyme synthesizing D-serine to regulate glutamate-N-methyl-D-aspartate neurotransmission. Proceedings of the National Academy of Sciences 96:13409-13414.

Wolosker H, Sheth K, Takahashi M, Mothet J, Brady R, Ferris C, Snyder S (1999b) Purification of serine racemase: Biosynthesis of the neuromodulator D-serine. Proceedings of the National Academy of Sciences 96:721-725.

Woo T, Crowell A (2005) Targeting synapses and myelin in the prevention of schizophrenia. Schizophrenia Research 73:193-207.

Yamamoto N, Tomita U, Umino A, Nishikawa T (2001) Uptake of D-serine by synaptosomal P2 fraction isolated from rat brain. Synapse 42:84-86.

Yang Y, Ge W, Chen Y, Zhang Z, Shen W, Wu C, Poo M, Duan S (2003) Contribution of astrocytes to hippocampal long-term potentiation through release of D-serine. Proceedings of the National Academy of Sciences 100:15194-15199.

Yasuda E, Ma N, Semba R (2001) Immunohistochemical evidences for localization and production of D-serine in some neurons in the rat brain. Neuroscience Letters 299:162-164.

Ye Z, Sontheimer H (2002) Modulation of glial glutamate transport through cell interactions with the extracellular matrix. International Journal of Developmental Neuroscience 20:209-217.

Yoshida T, Goldsmith S, Morgan T, Stone D, Finch C (1996) Transcription supports age-related increases of GFAP gene expression in the male rat brain. Neuroscience Letters 215:107-110.

Yoshikawa M, Kobayashi T, Oka T, Kawaguchi M, Hashimoto A (2004) Distribution and MK-801-induced expression of serine racemase mRNA in rat brain by real-time quantitative PCR. Brain Research Molecular Brain Research 128:90-94.

Yoshikawa M, Nakajima K, Takayasu N, Noda S, Sato Y, Kawaguchi M, Oka T, Kobayashi H, Hashimoto A (2006) Expression of the mRNA and protein of serine racemase in primary cultures of rat neurons. European Journal of Pharmacology 548:74-76.

Zhou M, Kimelberg H (2001) Freshly isolated hippocampal CA1 astrocytes comprise two populations differing in glutamate transporter and AMPA receptor expression. Journal of Neuroscience 21:7901-7908.

Zhou M, Schools G, Kimelberg H (2006) Development of GLAST(+) astrocytes and NG2(+) glia in rat hippocampus CA1: Mature astrocytes are electrophysiologically passive. Journal of Neurophysiology 95:134-143.

Zimmer D, Cornwall E, Landar A, Song W (1995) The S100 protein family: History, function, and expression. Brain Research Bulletin 37:417-429.

Zonta M, Angulo M, Gobbo S, Rosengarten B, Hossmann K, Pozzan T, Carmignoto G (2003) Neuron-to-astrocyte signaling is central to the dynamic control of brain microcirculation. Nature Neuroscience 6:43-50.