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Alterations in neuromodulators of GABAergic transmission in the cerebellar cortex of patients with schizophrenia.

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ALTERATIONS IN NEUROMODULATORS OF GABAERGIC TRANSMISSION IN THE CEREBELLAR CORTEX OF PATIENTS WITH SCHIZOPHRENIA

BY

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DISSERTATION

Submitted in Partial Fulfillment of the
Requirements for the Degree of

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Dedication

This dissertation is dedicated to the two most important women in my life -- my wife Angelique and my mother Peggy.

This dissertation is also dedicated to my son Austin. You are my future!

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I wish to express my appreciation to the staff of the Neuroscience Department at UNM. You perform all the tasks that allow this department, and each person within, to be functional. Thank you for "the small things" that are so important.

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ABSTRACT OF DISSERTATION

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Abstract

One of the most consistent findings in schizophrenia is the dysfunction of specific subsets of GABAergic interneurons in distributed brain regions including the prefrontal cortex and cerebellum. Analyses of post-mortem tissue from cerebellar hemispheres from 13 schizophrenic patients and 13 matched controls by quantitative real-time PCR (qRT-PCR) revealed that the mRNA levels of both the 67 kDa and 65 kDa isoforms of glutamic acid decarboxylase (GAD_{67} and GAD_{65}) are decreased. Additionally, the presynaptic GABA transporter GAT-1 and the Golgi cell specific neuromodulator metabotropic glutamate receptor 2 (mGluR2) were decreased in the schizophrenic group. Postsynaptic upregulation of $GABA_A$-α6 and δ along with downregulation of neuronal nitric oxide synthase (nNOS), a negative modulator of GABA release, were also seen, suggesting a

compensatory mechanism to counteract deficits in GABAergic transmission. In addition to GABAergic alterations in schizophrenia, N-methyl-D-aspartate (NMDA) receptor dysfunction has been proposed. To investigate this possibility, we measured NMDA receptor subunit mRNAs in the same samples and found that the NR2B subunit showed a near-significant decrease ($p=0.0681$) in the patients. In contrast, the kainic acid receptor subunits GluR6 and KA2 were upregulated in the granule cell layer. In an effort to understand the mechanisms for these gene expression changes, we examined adult rats chronically exposed to NMDA receptor antagonist phencyclidine (PCP, 2.58 mg/kg/day, i.p.), which elicits schizophrenia-like symptoms in both humans and animal models. Analyses of PCP-treated rat cerebellar hemispheres demonstrated similar decreases in all GABAergic marker mRNAs as seen in patients, as well as a decrease in Golgi cell GAD_{67} as shown by quantitative *in situ* hybridization (qISH). Additionally, decreases in both NR2B and NR2D transcripts, which are present in Golgi cells and colocalize extra-synaptically, were seen. Since low doses of PCP preferentially block NMDA receptors in GABAergic interneurons, chronic PCP administration could preferentially affect Golgi cells in the cerebellum. Deficits in these GABAergic interneurons may lead to disinhibited firing of cerebellar granule cells, as suggested by our previous studies of increased activity-dependent gene expression in these neurons. In conclusion, our results support the notion that GABA deficits are key elements in the pathophysiology of schizophrenia.

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Chapter 1

Introduction

1.1 Schizophrenia

Schizophrenia is a complex and devastating neuropsychiatric disorder affecting approximately 1% of the world's population. An estimated $58 million is spent annually dealing with the direct and indirect consequences of schizophrenia in the United States alone (Uhl and Grow, 2004). Both patients and their families are affected by the damaging constellation of symptoms caused by the disease. Schizophrenia is characterized by positive (hallucinations, delusions, thought disorders), negative (flat affect, alogia, avolition) and cognitive symptoms (impairments in learning and specific areas of memory, i.e. working memory) (American Psychiatric Association, 2000; Lewis and Lieberman, 2000). These symptoms tend to manifest during late adolescence to the mid-twenties. They may be overwhelming and may contribute to suicide committed by 10% of schizophrenic patients.

Both genetic and environmental factors contribute to the complex pathophysiology underlying schizophrenia. Approximately 50% of monozygotic twins with schizophrenia will have an affected twin and approximately 17% of dizygotic twins and first-degree relatives are also affected (Austin, 2005). Genes such as neuregulin (NRG1), dysbindin, D-amino acid oxidase (DAAO), G72, catechol-o-methyl transferase (COMT), regulator of G protein signaling 4 (RSG4), and disrupted in schizophrenia 1 (DISC1) have been identified as susceptibility genes in schizophrenia (Rapoport et al., 2005; Duan et al., 2007). These genes confer a risk, but seemingly need environmental insults to trigger

disease onset. Environmental factors may include a range of aspects from prenatal stress to viral infection *in utero,* but are more correlative than predictive (Austin, 2005). Typically, patients start to manifest changes in behavior during the prodromal phase, before their first psychotic episode. After this first-episode, subtle structural changes in the brain are seen, such as increased ventricular size and reduction in cortical grey matter and related areas (Potkin et al., 2002). This is due primarily to an overall reduction in cell size and synaptic connectivity, not as a reduction in neuronal numbers (Lewis and Lieberman, 2000).

Classical treatments for schizophrenia involve the administration of typical or atypical antipsychotics. Typical antipsychotics, such as haloperidol and chlorpromazine, bind primarily to dopamine (DA) D_2 receptors to antagonize receptor effects. Atypical antipsychotics, such as clozapine and risperidone, bind to a broader range of receptors including D4, 5-HT2A and noradrenergic receptors among others (Kapur and Remington, 2001). While typical antipsychotics are effective at relieving the positive symptoms mediated by D2 receptor blockade (Snyder, 2006), they are not effective in ameliorating the negative and cognitive symptoms (Lieberman et al., 2005; Ross et al., 2006). These aspects of schizophrenia are better indicators of the severity of the disease than are the positive symptoms and they show gradual deterioration as the disease progresses. Additionally, typical and atypical antipsychotics may produce unwanted side effects, including extrapyramidal side effects, prolactin increase, and weight gain to name a few (Lewis and Lieberman, 2000; Kapur and Remington, 2001; Freedman, 2005). While

these medications manage positive symptomology effectively, further research is needed in order to effectively treat the negative and cognitive symptoms.

1.2 Role of Glutamate and NMDA Receptors in Schizophrenia

While dopaminergic dysfunction in schizophrenia is widely accepted, a growing body of evidence suggests the involvement of glutamate and γ-aminobutyric acid (GABA) alterations in this illness (Akbarian and Huang, 2006; Kristiansen et al., 2007). The glutamate hypothesis of schizophrenia states that symptoms are related to hypofunction of N-methyl-D-aspartate (NMDA) receptors. Glutamate activates ionotropic NMDA receptors allowing for the influx of Na+ and Ca2+ leading to excitation and cellular signaling. These channels have been implicated in processes encompassing development, long-term potentiation (LTP), and excitotoxicity (Llansola et al., 2005). Each channel consists of an obligatory NR1 subunit and co-assembles with any of the NR2A-D subunits, each conferring upon the channel distinct electrophysiological and biochemical properties. Channels containing NR2A and NR2B are thought to be high conductance channels while NR2C and NR2D containing channels are low conductance channels (Misra et al., 2000). NR2C and NR2D are more prone to NMDA receptor antagonism due to less Mg^{2+} block and longer open channel time than either NR2A or NR2B (Monyer et al., 1994; Grunze et al., 1996). These subunits localize more to interneurons that express γ-amino butyric acid (GABA) in the hippocampus (Monyer et al., 1994). GABAergic interneurons in other brain regions express different combinations of NR2 subunits in NMDA channels, such as seen in Golgi cells in the cerebellum, which express the NR2B and NR2D subunits (Misra et al., 2000; Brickley et al., 2003). Furthermore,

the NR2B subunit is more prone to antagonism than is the NR2A subunit as seen in pharmacological studies of prepulse inhibition and locomotor activity in rats (Chaperon et al., 2003). Decreased expression of NMDA receptor components or selective block by NMDA receptor antagonists, such as MK-801 or phencyclidine (PCP), will alter the functional properties of cells containing NMDA receptors. These alterations may lead to downstream gene expression alterations, diminished LTP, or aberrant development, all important in the pathophysiology underlying schizophrenia.

NMDA receptor dysfunction has been characterized in different brain regions in schizophrenic patients (Kristiansen et al., 2007). The levels of different receptor subunits were shown to be altered in the prefrontal cortex (Akbarian et al., 1996b; Dracheva et al., 2001) and limbic areas (Kristiansen et al., 2006; McCullumsmith et al., 2007), and SPET studies have demonstrated decreased NMDA receptor binding in the hippocampus in schizophrenics (Pilowsky et al., 2006). Studies of NMDA receptor antagonists further implicate hypofunction of these channels in schizophrenia (Coyle, 2006). Administration of these agents elicits symptoms similar to that of schizophrenia in healthy adults, with negative and cognitive symptoms as well as overt psychosis (Adler et al., 1999), and exacerbation of symptoms in schizophrenic patients. NMDA receptor dysfunction, particularly on specific subsets of GABAergic interneurons, may be central to schizophrenia (Lewis and Gonzalez-Burgos, 2000; Coyle, 2006). NMDA receptor antagonists have been shown to selectively block NMDA channels located on GABAergic interneurons (Grunze et al., 1996; Rujescu et al., 2006; Homayoun and Moghaddam, 2007). These findings may be of great importance since GABAergic

interneurons are known to be an essential component in modulating the synchronization of neuronal activity necessary for highly demanding cognitive tasks (Traub et al., 1996).

1.3 Role of GABA in Schizophrenia

GABA is synthesized by the 65kDa and 67kDa forms of glutamic acid decarboxylase (GAD_{65} and GAD_{67}), each the product of different genes (Erlander et al., 1991). These enzymes remove the carboxyl group from L-glutamate to produce GABA. GAD_{65} protein is present in greater abundance in axon terminals while GAD_{67} localizes preferentially to the soma. However, mRNA for both is localized to cell bodies of GABA positive cells (Esclapez et al., 1994). Additionally, GAD_{67} expression is more activity dependent than GAD_{65} as seen by increased levels in models of epilepsy (Sloviter et al., 1996; Freichel et al., 2006). Once synthesized, GABA is released mainly by interneurons throughout the brain. These interneurons modulate the activity by either synchronizing and/or disabling excitatory cell signaling. The presynaptic GABA reuptake transporter (GAT-1) removes any remaining GABA from the synaptic cleft, effectively ending GABAergic neurotransmission. Therefore GAD_{65}, GAD_{67}, and GAT-1 are crucial to control GABA mediated transmission and overall brain function.

GABA dysfunction in certain subsets of GABAergic interneurons is one of the most consistent findings in the study of schizophrenia (Woo et al., 2004; Lewis et al., 2005; Torrey et al., 2005; Akbarian and Huang, 2006). Reductions in mRNA and protein levels of GAD_{67} were reported in the prefrontal cortex (PFC) (Volk et al., 2000; Hashimoto et al., 2003; Lewis et al., 2004), the hippocampus (Benes and Berretta, 2001; Heckers et al.,

2002), and the cerebellum (Guidotti et al., 2000; Fatemi et al., 2005). In addition to decreased expression of GAD_{67}, chandelier and basket interneurons in the PFC show decreased mRNA levels for GAT-1 (Volk et al., 2001) and the calcium binding protein parvalbumin (Lewis et al., 2001) (**Figure 1.1**).

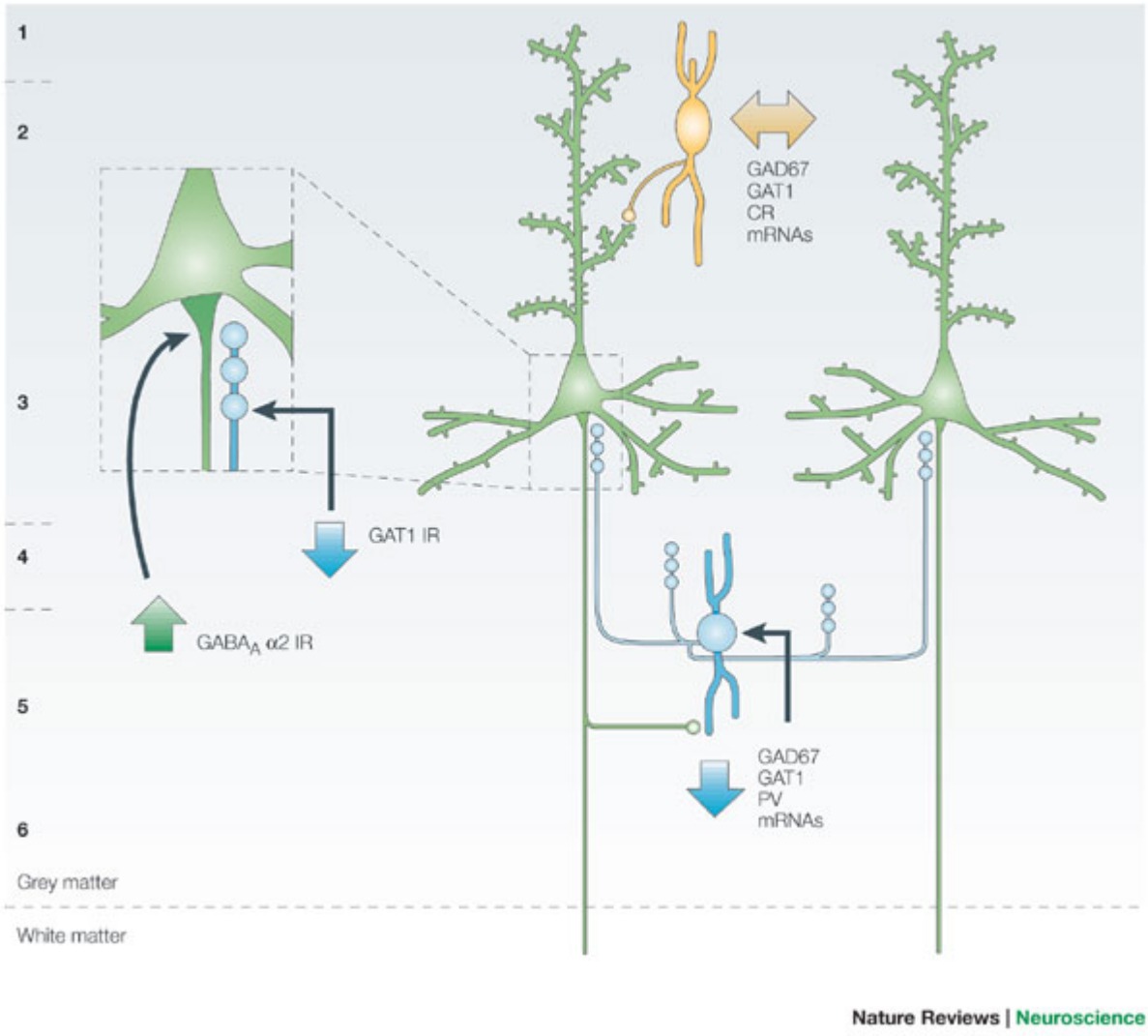


Figure 1.1: Representation of prefrontal circuitry showing deficits in specific subsets of GABAergic interneurons. Chandelier and basket cells in patients with schizophrenia show decreased mRNA levels of GAD_{67}, GAT-1, and parvalbumin as well as increased immunoreactivity in pyramidal $GABA_A$-α2. Reproduced from Lewis, et al., 2005.

Postsynaptic changes such as increases in $GABA_A$-α2 receptor density and $GABA_A$ receptor radioligand binding in the PFC and anterior cingulate cortex were also observed (Benes et al., 1992; Benes et al., 1996; Volk et al., 2002). Additionally, single nucleotide polymorphisms in the promoter region of the GAD_{67} gene were shown to be associated with reductions in grey matter in patients with childhood-onset schizophrenia (Addington et al., 2005) and with decreased cognitive functioning in adult patients (Straub et al., 2007). Considering the role of GABAergic interneurons in the modulation of excitatory output, it can be hypothesized that dysfunction in these cells may mediate some of the positive, negative, and cognitive (Spencer et al., 2004) symptoms seen in schizophrenia.

1.4 Cerebellar Contributions to Schizophrenia

Traditionally most of the research performed in the field of schizophrenia has focused on brain regions directly implicated in the symptomology of the disease, mainly the prefrontal cortex and limbic areas. However, Andreasen *et al* (1997) have implicated the cerebellum as an affected region in schizophrenia through its connections in the cortico-cerebellar-thalamic-cortical circuit (CCTCC). Dysfunction in one area of this circuit will affect all other areas of the circuit. As a component of the CCTCC, the lateral hemispheres of the cerebellum have been implicated in cognitive (posterior cerebellar lobe) and emotional (cerebellar vermis) functioning (Schmahmann and Sherman, 1998). Retroviral tracing and neuroimaging studies have shown connections between cerebellum-prefrontal (Middleton and Strick, 2001) and cerebellar-parietal areas (Allen et al., 2005). These connections have major implications in cognitive dysfunction in schizophrenia. Intrinsic to these connections, a forward modeling system of the

cerebellum has been proposed where information from the motor cortex or the PFC is transferred to the cerebellum and the cerebellum acts as a predictor of the outcome for both motor (anterior cerebellar lobe) and cognitive functioning. Connections to the dorsolateral PFC (DLPFC) were shown to localize to the lateral hemispheres of the cerebellum, particularly in crus I and crus II of lobule VII (Kelly and Strick, 2003; Ramnani, 2006). Therefore, lateral cerebellar hemisphere connections to higher cognitive areas of the brain implicate the cerebellum in cognitive functioning and subsequently in schizophrenia.

Clinically, patients exhibit cerebellar neurological signs (Ho et al., 2004), deficits in eyeblink conditioning (Sears et al., 2000), and shortfalls in timing responses (Brown et al., 2005). Neuroimaging studies have shown increases in blood flow and in glucose consumption in the cerebellum of schizophrenic patients relative to that of other brain regions (Andreasen et al., 1997; Kim et al., 2000b; Potkin et al., 2002; Malaspina et al., 2004). In accordance with increased cerebellar activity, our laboratory found that activity dependent genes expressed by granule cells, GAP-43 and BDNF, are upregulated in the cerebellum (Paz et al., 2006). Additional molecular studies have shown decreased expression of the development markers reelin and semaphorin 3A as well as GABA synthesizing enzymes GAD_{65} and GAD_{67} (Guidotti et al., 2000; Eastwood et al., 2003; Fatemi et al., 2005). This is interesting considering the fact that GABA deficits in Golgi interneurons of the cerebellum may lead to aberrant cerebellar output due to disinhibition of granule cell firing. This in turn could account for the increases seen in blood flow,

glucose utilization, and the increased expression of activity dependent genes. These changes ultimately impact cerebellar contributions to cognitive (and motor) functioning.

Apart from the cognitive aspects of the cerebellum, other factors make this region particularly interesting for the study of schizophrenia. The local circuitry of the cerebellum with the granule cell-Golgi cell interaction can be compared morphologically and physiologically with the pyramidal cell-chandelier cell interaction in the PFC implicated in schizophrenia (Lewis et al., 2005). The excitatory granule cell of the cerebellum receives input from pontine mossy fibers. Activation by mossy fibers excites granule cells and causes them to activate Purkinje cells, which are the sole output of the cerebellum. This interaction is modulated in a phasic manner by Golgi cells, which function in a negative feedback loop with granule cells (Watanabe et al., 1998; Hirano et al., 2002) (**Figure 1.2**), in a structural and functional unit named the cerebellar glomerulus. GABA function in the glomerulus is regulated by various factors, including postsynaptic $GABA_A$ receptors, presynaptic

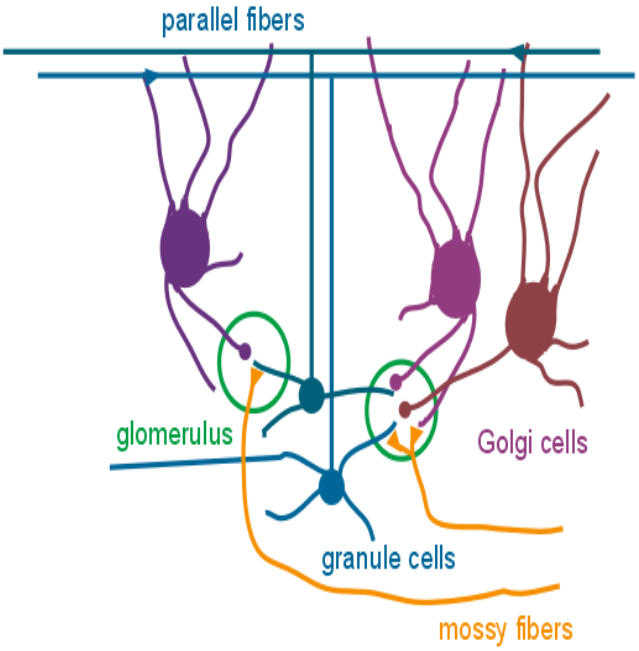


Figure 1.2: Golgi cell circuit and the cerebellar glomerulus (green). Granule cells (GrC; blue) are excited by pontine mossy fibers (MF; yellow). GrC parallel fibers (PF) synapse on Purkinje (PC; not shown), basket (BC; not shown), stellate (SC; not shown), and Golgi cells (GoC; purple). The GoC provide inhibitory feedback to the GrC for synchronized activity . Reproduced from De Schutter, 2002.

metabotropic glutamate receptors (Geurts et al., 2003), pre- and post-synaptic kainate receptors (Fiszman et al., 2007; Mathew et al., 2008), and retrograde nitric oxide (NO) synthesized by neuronal nitric oxide synthase (nNOS) (Wall, 2003).

Golgi cells normally impart GABAergic tone on the granule cell (Hamann et al., 2002; Geurts et al., 2003; Rossi et al., 2003; Chadderton et al., 2004) through intrinsic autorhythmic firing (Forti et al., 2006). Therefore, dysfunctions of Golgi cells in this circuit leads to disinhibited firing of the granule cells, potentially accounting for the increased activity and the subsequent changes seen by neuroimaging (Andreasen et al., 1997; Kim et al., 2000b; Potkin et al., 2002; Malaspina et al., 2004) and molecular studies (Paz et al., 2006). Golgi cells express GABA synthesizing enzymes GAD_{65} and GAD_{67} as well as GAT-1 (Takayama and Inoue, 2005) and the NMDA receptor subunits NR2B and NR2D (Misra et al., 2000; Brickley et al., 2003). These subunits are preferentially targeted by NMDA receptor antagonists such as PCP (Grunze et al., 1996; Rujescu et al., 2006). Altogether these data implicate Golgi cells as the affected interneuron in the cerebellar GABAergic dysfunction seen in schizophrenia.

In addition to Golgi and Purkinje cells, basket and stellate interneurons are the other major GABAergic cells in the cerebellum. Basket cells and stellate cells both provide feedforward inhibitory modulation to Purkinje cells. Basket cells synapse on the proximal dendrite, soma, and axon of the Purkinje cell while stellate cells synapse on the distal dendritic tree (Takayama and Inoue, 2005). Both cell types help the Purkinje cell, which also receives input via inferior olive climbing fibers, integrate granule cell

information and modulate Purkinje cell output (Midtgaard et al., 1993). Basket and stellate cells both express GAT-1 (Takayama and Inoue, 2005) while Purkinje cells only express GAT-1 transiently during development (Yan and Ribak, 1998). While Golgi cells influence Purkinje cell function by modulating granule cells, decreased basket and/or stellate cells activity directly contributes to unregulated Purkinje cell firing. This unregulated activity of Purkinje cells mediated by GABAergic interneuron dysfunction may also contribute to cerebellar pathology in schizophrenia.

Of further interest, the lateral hemispheres of the cerebellum do not contain any direct dopaminergic input (Schweighofer and Doya, 2003), making this system more purely glutamate/GABA than is seen in other brain regions with dopaminergic input, such as the PFC. Therefore, studying the cerebellum could be useful in determining the direct pathophysiological consequences of schizophrenia with minimal effects from medication. In addition, the cerebellum has intrinsic neuroprotective pathways mediated by low level basal NMDA receptor activation leading to BDNF release and subsequent TrkB activation (Jiang et al., 2003; Wu et al., 2005; Xifro et al., 2005). Native cerebellar neuroprotection may be accountable for the increase in blood flow and glucose consumption seen in neuroimaging studies and in increased expression of activity dependent genes. Taken as a whole these findings indicate the cerebellum participates in the pathophysiology of schizophrenia.

1.5 Medication Effects on Gene Expression

Antipsychotic medication by itself can differentially affect gene expression in distributed brain regions. Typical antipsychotics include such drugs as chlorpromazine, thioridazine, and haloperidol, the prototypical typical antipsychotic. Haloperidol is thought to increase GAD_{67} mRNA levels through signaling mechanisms mediated through blockade of D2 receptors (Lipska et al., 2003). Increased levels of GAD_{67} and GAT-1 were reported in the medial frontal cortex, striatum, and nucleus accumbens in rats (Laprade and Soghomonian, 1995; Lipska et al., 2003; Zink et al., 2004a; Zink et al., 2004b). Conversely, Volk *et al* (2000, 2001) report no change in expression of these levels in non-human primates in the PFC. Furthermore, magnetic resonance spectroscopy (MRS) studies found that typical antipsychotics do not alter the levels of glutamate, glutamine, or N-acetylaspartate (NAA) (Bustillo et al., 2006), which is considered a putative marker of cellular integrity. However, medication effects have not been studied in the cerebellum and merit further consideration.

Atypical antipsychotics have been shown to upregulate the GABAergic markers GAD_{67} and GAT-1 in prefrontal and limbic areas through unknown mechanisms (Zink et al., 2004a; Zink et al., 2004b). Also, anti-apoptotic genes such as Bcl-2 were shown to be upregulated by atypical antipsychotics, seemingly imparting neuroprotection (Bai et al., 2004). These same drugs also showed alterations in NMDA receptor subunit expression (Hanaoka et al., 2003) and reversed the functional hyperactivity produced by NMDA receptor antagonists such as PCP (Ninan et al., 2003). In light of these data, medication effects seem to be significant contributors to the overall cellular and molecular changes

observed in patients. Since the vast majority of patients are currently on neuroleptics these effects need to be researched for understanding their effects in treating schizophrenia.

1.6 Phencyclidine

Phencyclidine (PCP) is a non-competitive NMDA receptor antagonist (for review see Morris et al., 2005). Initially used as a surgical anesthetic, its use was discontinued because it caused symptoms very similar to schizophrenia (Jentsch and Roth, 1999; Morris et al., 2005; Hajszan et al., 2006), but is still continued to be used illicitly known on the street as "angel dust" or "hog". Like MK-801, PCP binds in the pore of open NMDA channels near the ion selectivity filter (Ferrer-Montiel et al., 1998), blocking cation flow though the channel (**Figure 1.3**). It acts in a dose dependent manner (Morris et al., 2005) and selectively blocks NMDA channels located on GABAergic interneurons (Grunze et al., 1996; Homayoun and Moghaddam, 2007). Thus, PCP disrupts GABAergic interneurons from modulating the activity of glutamatergic cells with which they synapse leading to

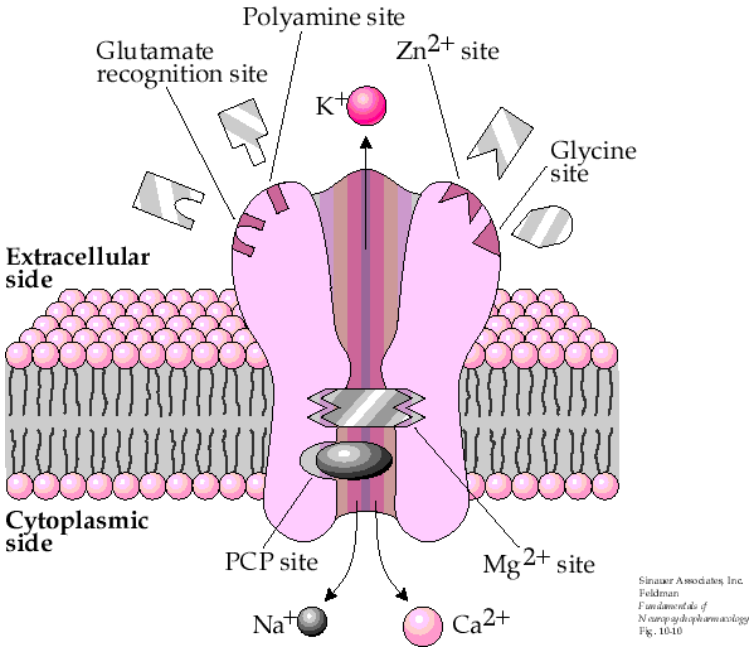


Figure 1.3: An NMDA channel illustrating the binding sites for neurotransmitters, binding factors, Mg^{2+}, and PCP. PCP is a non-competitive antagonist. Reproduced from Feldman and Quenzer, 1984.

increased glutamate release (Jackson et al., 2004). This, in turn, could lead to downstream excitotoxic effects, such as neuronal damage and/or cell death. Because of the selective effect of PCP on GABAergic interneurons, areas more susceptible to excitotoxic damage, such as the PFC, are more vulnerable to the effects of PCP administration whereas areas such as the cerebellum, with its neuroprotective mechanisms, are not (Jiang et al., 2003; Wu et al., 2005; Xifro et al., 2005). Therefore, NMDA receptor hypofunction may be accountable for the neuroimaging findings of hypofrontality (Andreasen et al., 1992; Wolkin et al., 1992). Animal models utilizing NMDA receptor blockade through PCP may be an effective tool in studying the pathophysiological processes cardinal to schizophrenia.

1.7 Chronic Low-Dose PCP Administration in Rats

Animal models exploiting the GABA/glutamate system have proven useful in studying the underlying pathophysiology of schizophrenia. Models include picrotoxin induced antagonism of $GABA_A$ receptors in rats (Berretta et al., 2001), genetic knockdown of NMDA NR1 subunit in mice (Mohn et al., 1999), and NMDA receptor blockade by NMDA receptor antagonists, such as PCP and MK-801, in both rodents and non-human primates (Jentsch et al., 1997b; Jentsch et al., 1997a; Jentsch and Roth, 1999; Cochran et al., 2003; Morris et al., 2005; Rujescu et al., 2006). All these models affect the same system in different manners, but only the PCP model has reproduced GABAergic and NMDA receptor changes seen in schizophrenic patients (Cochran et al., 2003; Lindahl and Keifer, 2004). PCP elicits in humans many of the symptoms inherent to schizophrenia. Studies are now being conducted focusing on administering the

compound to rodents and primates to simulate a schizophrenic phenotype in the animals. Acute and chronic dosing regimens show differential and often opposing effects in rodents (Jentsch and Roth, 1999). Immediately after administration of PCP, neurons of the PFC show an initial paradoxical excitation as seen by activation of early immediate genes (Gao et al., 1998). This is followed by a period of cortical depression as described in glucose utilization studies (Gao et al., 1993), presumably because glutamate is depleted. Acute PCP also produces schizophrenia-like symptoms including social withdrawal (Sams-Dodd, 1997), impaired sensory motor gating (Mansbach and Geyer, 1989) and cognitive dysfunction (Jentsch et al., 1997b; Jentsch et al., 1997a). Chronic low dose administration of PCP in rodents showed decreased metabolic activity in the medial frontal cortex PFC, auditory cortex, hippocampus, and reticular nucleus of the thalamus (Cochran et al., 2003), all regions affected in schizophrenia. Along with decreased metabolic function, decreased parvalbumin expression was also seen in the PFC and reticular nucleus of the thalamus (Cochran et al., 2003). This mirrors the chandelier and basket cell dysfunction seen in the prefrontal cortex of schizophrenic patients (Hashimoto et al., 2003). Additionally, levels of NAA and its metabolite n-acetylaspartylglutamate (NAAG) are also altered with chronic PCP similar to that seen in schizophrenic patients (Reynolds et al., 2005; Bustillo et al., 2006). Taken together, the data suggest that the model Cochran *et al* (2003) proposed of chronic intermittent exposure to low levels of PCP is the most functionally and neurochemically relevant animal model based on GABAergic interneuron dysfunction.

1.8 Goals of this Study

GABAergic dysfunction and NMDA receptor hypofunction have been characterized in schizophrenia in multiple brain regions (Torrey et al., 2005; Akbarian and Huang, 2006; Kristiansen et al., 2007). However, these changes have not been as extensively studied in the cerebellum. This study is designed to assess the presence and localization of GABA signaling deficits in the cerebellum of patients with schizophrenia. Experiments in this study sought to test the overall hypothesis that *dysfunction of certain GABAergic interneurons, specifically Golgi cells, underlies the pathophysiology of schizophrenia in the cerebellum.*

Initial experiments involved characterizing both the transcript levels of GABAergic markers, NMDA receptor subunits, and neuromodulators in the lateral cerebellar hemisphere of post-mortem tissue from patients with schizophrenia versus pair matched controls using qRT-PCR analysis. Given the fact that other brain regions showed deficits in GABAergic gene expression, these studies examined the hypothesis that *GABA deficits exist in the cerebellum of patients*. Gene expression effects of typical and atypical medication were evaluated by examining mRNA levels of the same transcripts in rats treated with either haloperidol or clozapine.

Subsequent experiments compared gene expression in the cerebellum of a proposed animal model of chronic intermittent low-dose PCP to that seen in patients. Considering that PCP elicits many of the symptoms seen in patients with schizophrenia in both humans and animal models, these studies tested the hypothesis that *chronic intermittent*

administration of low doses of PCP in rats will reproduce the gene expression changes seen in the cerebellum of patients with schizophrenia. qRT-PCR was also used to examine this hypothesis.

Finally, we localized changes in the main GABA synthesizing GAD_{67} to specific interneurons in the cerebellum of PCP treated rats. Since GABAergic interneurons are preferentially affected by PCP administration and PCP rats reproduced the GABA deficits seen in patients with schizophrenia, we tested the hypothesis that *Golgi cells, the interneurons responsible for negative feedback, are deficient in* GAD_{67} *expression*. This hypothesis was evaluated using quantitative *in situ* hybridization.

Overall, these studies support the hypothesis that specific subsets of GABAergic interneurons are deficient in patients with schizophrenia. Additionally, these experiments will also help validate the chronic low dose PCP treatment paradigm in rats as an animal model for GABAergic dysfunction related to schizophrenia. Ultimately, this project will provide important information to support (or disprove) the notion that the cerebellum is an affected component in the brain of patients with schizophrenia.

Chapter 2

Methods

2.1 Human Subjects

Cerebellar tissue from 13 patients with a diagnosis of schizophrenia according to DSM–IV criteria and 13 sex-, age- and postmortem interval (PMI)-matched comparison subjects was acquired from the Maryland Brain Collection (Table 2.1). All subjects were male between 25 and 65 years of age at the time of death with a PMI lower than 24 hours. None of the subjects had a history of alcohol abuse or dependence. No significant differences were found between patients with schizophrenia and control subjects in mean age (43.5±11 years vs. 40.9±8 years), PMI (12.1±5.1 hours vs. 16.5±5.4 hours) or RIN (6.53±0.2 vs. 6.50±0.1). Six of the patients were receiving typical antipsychotics and seven were receiving atypical antipsychotics at the time of death. Neither patients nor control subjects were receiving medications for mood disorders. Tissue used in this study originated from the lateral cerebellar hemisphere corresponding to crus I of lobule VIIA, which was dissected at –20^{o}C and frozen at –80^{o}C until use.

2.2 Haloperidol Treated Rats

RNA from the cerebellum of haloperidol treated rats was acquired from a previous study (Bustillo et al., 2006). Briefly adult male Sprague-Dawley rats (n=16) were injected i.m. either with 38 mg/kg/month haloperidol-depo (Novaplus), corresponding to ~1/mg/kg/day, or with sesame oil (vehicle). These rats were pair housed and kept on a 12:12 light:dark cycle. After a 7 day acclimation period the rats were injected once a month for 6 months. The rats were anesthetized using isoflurane and decapitated one

TABLE 2.1: Postmortem Characteristics of Schizophrenia and Comparison Subjects

Group	IDN	Gender	Race	Age (years)	Postmortem interval (hours)	RIN	Treatment	Cause of Death
Schizophrenia subjects[a]								
1	42a	M	Black	33	12	7.40	Olanzapine	Appendicitis
2	39*	M	Black	31	14	8.30	Clozapine	ASCVD
3	7s	M	White	32	7	8.00	Clozapine	Suicide
4	41	M	White	37	14	7.30	Risperidone	Suicide
5	3s	M	Black	38	6	7.80	Fluphenazine	Asphyxia
6	11s	M	Black	38	5	8.50	Chlorpromazine	Pneumonia
7	5s	M	Black	42	21	7.80	Perphenazine	Tuberculosis
8	18	M	White	45	6	8.40	Olanzapine	Suicide
9	31	M	White	46	20	8.50	Olanzapine	ASCVD
10	5	M	White	49	16	7.50	Thioridazine	Intoxication
11	36	M	White	59	13	8.40	Haloperidol	Embolism
12	1s	M	White	53	11	7.90	Perphenazine	ASCVD
13	1	M	Black	62	12	8.00	Olanzapine	ASCVD
Comparison subjects[b]								
1	35	M	Black	33	14	8.60		M. Obesity
2	8s	M	Black	27	14	7.80		Asthma
3	12s	M	White	31	16	7.80		Polytrauma
4	34*	M	White	35	16	7.70		ASCVD
5	4s	M	White	38	24	7.70		Asphyxia
6	4*	M	Black	38	12	7.50		CMP
7	40	M	White	43	20	7.50		ASCVD
8	12	M	White	47	6	8.30		ASCVD
9	10s	M	White	46	24	7.00		Arrhythmia
10	6s	M	White	47	17	7.50		Arrhythmia
11	17	M	White	48	19	8.00		Sub-archn
12	21*	M	White	49	10	7.30		ASCVD
13	2s	M	White	50	22	7.80		ASCVD

[a] Mean age was 43.5 years (range=31-62); mean postmortem interval was 12.1 hours (range=5-21); mean RIN was 7.98 (range=7.40-8.50).
[b] Mean age was 40.9 years (range=31-50); mean postmortem interval was 16.5 hours (range=6-24); mean RIN was 7.73 (range=7.00-8.60).
[1-13] Pairs matched according to age and PMI.

month after the last injection. The cerebellum was removed intact, frozen on dry ice, and stored at -80^{o}C.

2.3 Clozapine Treated Rats

Adult male Long-Evans rats (n=20) were injected either with 10 mg/kg/day clozapine (Novartis, Princeton, NJ) dissolved in 0.4% acetic acid in 0.9% saline, or with 0.4% acetic acid in 0.9% saline i.p. for 21 days. This paradigm was modified from methods described by (Bai et al., 2004), which suggested maximum D2 receptor occupancy for clozapine. Eighteen hours following the last injection, the cerebellum of control and clozapine treated rats was removed and the lateral hemispheres dissected and frozen at -80^{o}C.

2.4 PCP Treated Rats

For quantitative real-time PCR (qRT-PCR) experiments, pair housed adult male Long-Evans rats (n=20) were injected intraperitoneally with 2.58 mg/kg/day of either PCP dissolved in saline or saline alone (Cochran et al., 2003). After an acclimation period of 7 days the rats were injected once a day for the first five days, then injected on days 8, 10, 12, 15, 17, 19, 22, 24, and 26. The rats were anesthetized using isoflurane and sacrificed after a washout period of 72 hours. The lateral hemisphere of the cerebellum was removed and frozen on dry ice. Samples were stored at -80^{o}C.

For *in situ* hybridization and immunohistochemistry studies, a separate group of rats (n=10) were injected as previously described. The cerebellum was removed and flash

frozen in isopentane that was cooled at -40°C using methanol/dry ice. Samples were stored at -80°C.

2.5 Sectioning of PCP Treated Rat Tissue

Frozen rat cerebellar tissue was cut to 10 μm thick sections on a cryostat. Each slide had one coronal section from a PCP treated rat and its pair-housed saline control rat. A total of five slides per rat pair were taken, each slide with one PCP and one saline control rat. Slides were stored at -80°C.

2.6 Quantitative Real-Time PCR (qRT-PCR)

All experimental procedures and data analyses were performed blind to the diagnosis. Human and rat tissue was homogenized using a Polytron homogenizer (Brinkmann Instruments, Inc.; Westbury, NY) and total RNA was isolated using TriReagent (Sigma; St. Louis, MO). The integrity of samples was validated using a Bioanalyzer 2100 (Agilent Technologies; Santa Clara, CA). Samples were used for analysis only if RNA integrity number (RIN) $\geq$7.0 for human samples and $\geq$8.0 for rat samples. cDNA was synthesized from 4μg total RNA using M-MLV reverse transcriptase (Promega; Madison, WI). qRT-PCR reactions were run on an Applied Biosystems 7000, 7300, or 7500 Fast quantitative Real-Time PCR machine. Gene expression levels in humans, PCP treated rats, and neuroleptic-treated rats were examined using SYBR Green (Applied Biosystems; Foster City, CA) with the exception of $GABA_A$-α6 and $GABA_A$-δ, which were analyzed using TaqMan® Assays-on-Demand probes (Applied Biosystems), all according to the manufacturer's protocols. Exon spanning primer pairs (Operon;

Huntsville, AL) specific to GABAergic markers $GABA_A$-β3, GAD_{67}, GAD_{65}, Parvalbumin (human only), and GAT-1, NMDA receptor subunits NR1, NR2A, NR2B, NR2C, and NR2D, and cerebellar neuromodulators GluR6, KA2, mGluR2, mGluR3, and nNOS were designed with Primer Express 3.0 (Applied Biosystems) (Table 2.2). Genes of interest were normalized by the housekeeping genes β-actin and cyclophilin. Dissociation curves were examined for all SYBR® green primer pairs and validated against housekeeping genes with a linear slope <|0.1|. Samples were run in triplicate in three separate plates and compared to housekeeping gene on the same plate. The relative levels of expression of each mRNA was corrected by β-actin and reconfirmed by cyclophilin (Supplementary Figures A.4.1 – A.4.3) as previously described (Paz et al., 2006) and the relative levels of transcript in patients vs. controls (human) or treatment vs. vehicle (rat) were calculated using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001).

2.7 qRT-PCR Data and Statistical Analysis

The averaged values per sample were entered into Prism 4.0 (GraphPad Software; San Diego, CA) and analyzed using the Wilcoxon Signed Rank analysis for pair-matched human subjects (see Appendix A.3 for unpaired data analyzed by *t*-tests) and *t*-tests for rat samples. Values were expressed as a ratio of schizophrenics/controls (S/C), drug/vehicle (D/V), or PCP/Saline (P/S). SSPS 15.0 for Windows (SPSS Inc.; Chicago, IL) was used for further analysis of human subjects, which included analyses of covariance (ANCOVA) to determine the effect of age, PMI, and RIN on each gene's transcript expression and bivariate Spearman's ρ correlation analysis to examine putative gene-gene interactions. Any statistical

TABLE 2.2: Human and Rat Primer Pairs for Quantitative Real-Time PCR

Gene	Amplicon Size (bp)	Human	Amplicon Size (bp)	Rat
SYBR® Green Primer Pairs[b]				
β-actin	242	5'-GGA CTT CGA GCA AGA GAT GG-3' 5'-AGC ACT GTG TTG GCG TAC AG-3'	120	5'-GAG GCC CCT CTG AAC CCT AA-3' 5'-ACC AGA GGC ATA CAG GGA CAA-3'
	120	5'-GCC CTG AGG CAC TCT TCC A-3' 5'-TGT TGG CGT ACA GGT CTT TGC-3'		
Cyclophilin A	225	5'-GCC GAG GAA AAC CGT GTA CTA TT-3' 5'-GTC ACC ACC CTG ACA CAT AAA CC-3'		
$GABA_A$-β3	120	5'-CCA CGA CAG CAG CAT GCA T-3' 5'-CGC CTC GCC AGT AAA ACT CA-3'	120	5'-GCT CGA GTT GCC CTA GGG ATT-3' 5'-CGA AGC AAC CCA TCA GGT ACA-3'
GAD_{65}	226	5'-GCC TCC TAC CTC TTT CAG CA-3' 5'-TCC CAT CAA ACA CCA TCT CA-3'	120	5'-CGG AAC AGA CAG CGT GAT TCT-3' 5'-GCA CTC ACC AGG AAA GGA ACA-3'
GAD_{67}	291	5'-TGG CAC GAC TGT TTA TGG AG-3' 5'-TAC TGC TTG TCT GGC TGG AA-3'	120	5'-CTG CCA TCC TGG TCA AGG AA-3' 5'-GAA TCG CCT TGT CCC CTG TA-3'
GAT-1	283	5'-TGC ATC TGG TGG AAA CTC TG-3' 5'-GCT GCT CAG GAC CAT TCT CT-3'	132	5'-GCC CTG GAC TGG CAT TCT T-3' 5'-AGC CCT CCA CGG TAC AGA ACT-3'
Parvalbumin	202	5'-CTA CCG ACT CCT TCG ACC AC-3' 5'-GCC ATC AGC ATC TTG GTT TC-3'		
NR1	123	5'-CCA GGC GGA GAG ACA GAG AA-3' 5'-CTC CTT GCA TGT CCC ATC ACT-3'	128	5'-AAT GTG ACG GCT CTG CTG ATG-3' 5'-TAC CCA GAG CCC GTC ATG TT-3'
NR2A	125	5'-ATG GGA AAA GGT GGG CAA GT-3' 5'-CCT CCA GGG TGA CGA TGC T-3'	120	5'-GCC TCC GTC TGG GTG ATG AT-3' 5'-GGG AGC TTT CCC TTT GGC TAA-3'
NR2B	120	5'-TGG AGG AGG CAC CAT TTG TC-3' 5'-CCG GCT CCT CGT CTG TTT TA-3'	120	5'-ACC GGC TAT GGC ATT GCT ATC-3' 5'-GCC AGT GAG CCA GAG AGC TT-3'
NR2C	123	5'-GCA CAC CCA CAT GGT CAA GTT-3' 5'-TCC TTG CCT GCC ATG TAG TTG-3'	120	5'-TGC ACA CCC ACA TGG TCA AG-3' 5'-TGC CCG CCA TGT AGT TGA G-3'
NR2D	120	5'-GGC TCA GTG ACC GCA AGT TC-3' 5'-AGC TGT GCA TGT CGG GAT AGT-3'	134	5'-TGG CAT GAT TGG TGA GGT GTT-3' 5'-GTG CCA CCA TGA CGC TGA T-3'
GluR6	123	5'-GTG GCC GTT CAA CAG TTT CC-3' 5'-TGT GAG GCC TTC CCA ATG TG-3'	125	5'-ACC CTT GCA ACC CTG ACT CA-3' 5'-CCT GGT GGA GAG TGC TTT GG-3'
KA2	127	5'-GGA GGA GGA CCA TCG AGC TAA-3' 5'-CCT CCG TGT GGA CCA TAT GAA-3'	120	5'-GGC GGA TCA GAC CAA CAT TG-3' 5'-GGC TGC TTC GAC TGC ATG TAA-3'
mGluR2	132	5'-TCA ACG AGG CCA AGT TCA TTG-3' 5'-GGC TGA CTG ACA CGC ACA TG-3'	110	5'-CAT CAT CTG GCT GGC TTT CC-3' 5'-CAA GCA CCA CAG AGC CAC TGA-3'
mGluR3	120	5'-CAC TGG GCA GAA ACC TTA TCG-3' 5'-CCC TGG TTG CAT ATT CTT CAT TT-3'	121	5'-GCA TTG CTC GCA TCT TCG AT-3' 5'-ACG GAC ACC ATC ACA ATT TGC-3'
nNOS	120	5'-GGA GTC ACC CTG CGA ACG T-3' 5'-GGC AAG AGG GTC CAG TTA GGA-3'	121	5'-TGC TGC CTC TCC TGC TTC AG-3' 5'-AGC CCC AGG TCC TTA AAC CA-3'
TaqMan® Probe Sets[c]				
β-actin	171	Catalog # Hs99999903_m1	91	Catalog # Rn00667869_m1
$GABA_A$-α6	108	Catalog # Hs00181301_m1	144	Catalog # Rn00573029_m1
$GABA_A$-δ	71	Catalog # Hs00181309_m1	75	Catalog # Rn00568740_m1

Primer pairs listed according to species with forward primer above reverse primer. Includes amplicon size in bp. Amplicon sizes of gene of interest and housekeeping genes were matched based on primer pairs and species. Probe set data may be obtained from Applied Biosystems.

calculation with $p<0.05$ was considered significant. Values from rat experiments exceeding ± 2 SD from the mean were excluded from further analysis.

2.8 Quantitative *In Situ* Hybridization (qISH) and Immunohistochemistry (IHC)

GAD_{67} cRNA sense and antisense transcripts with incorporated ^{35}S-UTP were synthesized from cloned plasmids obtained from Niranjala Tillakaratne (UCLA). Riboprobes were transcribed using T3 RNA polymerase (Promega; Madison, WI), purified using RNeasy (Qiagen; Valencia, CA) and kept at -80^{o}C until slides were treated.

Frozen slides were placed at -20^{o}C for 1 hour prior to fixation. ISH was performed according to methods developed by Simmons, *et al*. (1989). Slides were hybridized with 1.5 x 10^6 cpm in a volume of 75 μL hybridization buffer and hybridized overnight at 55^{o}C for 16 hours. After hybridization, slides were treated with RNase A and then washed in decreasing SSC (20X stock solution; 0.3M sodium citrate, pH 7.0, 3M NaCl) concentrations. Slides were then exposed to film for 24 hours to monitor adequate hybridization and to determine proper exposure time to emulsion.

Following ISH, the slides were preincubated for 1 hour in 10% normal horse serum (NHS) in TBS (50 mM Tris Base, pH 7.4, 0.9% NaCl), then incubated with mGluR2 monoclonal antibodies (AbCam; Cambridge, MA) at a 1:750 dilution in 1% NHS in TBS for 16 hours at 4^{o}C. Slides were washed and then incubated for 2 hours with biotinylated horse-anti-mouse antibodies (Vector Labs; Burlingame, CA) at a 1:400 dilution in 1%

NHS in TBS at room temperature. After another wash, the slides were treated with Vector ABC Elite kit (Vector Labs) according to manufacturer's directions and developed using DAB as the chromogen.

Following ISH and IHC, slides were dipped in Kodak NTB2 emulsion (Kodak; Rochester, NY) and exposed for 6 days at 4^{o}C. Slides were then developed using Kodak D-19 developer and fixer.

2.9 Imaging Autoradiographic Slides

After development of emulsion slides half of the slides were counterstained using hematoxylin and eosin. Slides were photographed using an Olympus DP71 camera (Olympus America Inc.; Center Valley, PA) attached to an Olympus BX60 microscope. To count the number of cells per area, images were acquired using either a 10X objective (for Golgi cell and Purkinje cell measurements) or at 20X (for basket/stellate cell measurements). Images were acquired using a 60X objective to measure the number of grains per cell.

2.10 ISH Data and Statistical Analysis

Pictures from slides were analyzed using ImagePro® Plus 4.0 (Media Cybernetics; Bethesda, MD). Cell number per area was determined by counting cells at 10X for Golgi and Purkinje cells, and 20X for basket/stellate cells by manually defining the region of interest and counting the cells within that region. Area covered by grains per cell was determined by creating a circle with a predetermined diameter, placing the circle over the

cell of interest, and quantitating the area covered by grains within the area of the circle. Diameters were set at 13 μm for basket/stellate cells, 25 μm for Golgi cells, and 30 μm for Purkinje cells. More than 300 cells were counted per area and more than 25 cells were evaluated for area covered by grains per cell. Values from slides were averaged independently, entered into Prism 4.0 (GraphPad Software; San Diego, CA), and analyzed using *t*-tests with a $p<0.05$ considered significant.

Chapter 3

Results

3.1 Expression Data from Lateral Cerebellar Hemisphere of Patients with Schizophrenia

3.1.1 GABAergic Marker Expression

Given the evidence of GABA deficits in schizophrenia, we sought to characterize the condition of inhibitory interneurons in the cerebellum of patients. The reasons behind these studies were two-fold: first to investigate the mechanisms underlying cerebellar abnormalities in the patients and second to examine the nature and extent of GABA dysfunction in different neuronal populations. Initial studies examined the expression of 7 markers of GABA function in the lateral cerebellar hemisphere of 13 pairs of patients with schizophrenia and matched control subjects. Comparisons of transcript levels in patients and matched control subjects by the Wilcoxon Signed Rank test revealed significant decreases in the mRNA levels of GAD_{67} (S/C=0.62; p=0.0134), GAD_{65} (S/C=0.74; p=0.0171), and GAT-1 (S/C=0.57; p=0.0024) by 38%, 26% and 43%, respectively, but not in Parvalbumin (Figure 3.1, Table 3.1). Patients with schizophrenia also showed significant increases in the mRNAs for the granule cell extrasynaptic $GABA_A$ receptor subunits $\alpha 6$ (S/C=1.44; p=0.0046) and δ (S/C=1.53; p=0.0002), relative to β-actin mRNA, but no changes in the $GABA_A$-$\beta 3$ subunit mRNA, which is expressed in various cells. ANCOVA analyses demonstrated that the changes in GAD_{65} transcript levels were affected by age, PMI, and RIN as a group (p=0.1456; Table 3.2) and age independently (p=0.508; Table 3.2). No other effects of age, PMI, or RIN were seen.

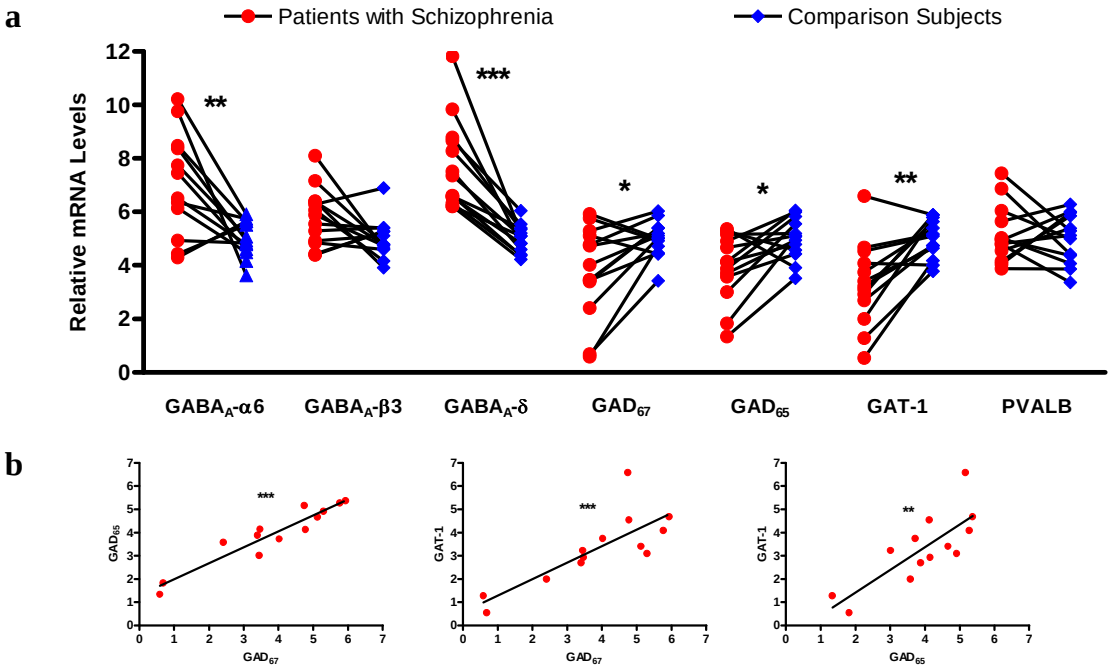


Figure 3.1 **a)** Relative expression levels of GABAergic markers in the cerebellum of patients with schizophrenia versus comparison subjects. Postsynaptic $GABA_A$ receptors α6 and δ are significantly increased in patients while GAD_{67}, GAD_{65}, and GAT-1 are significantly decreased. Expression levels are normalized to β-actin. **b)** Correlation of presynaptic GABA gene expression markers among patients showing high correlations between GAD_{67}, GAD_{65}, and GAT-1. *p<0.05, **p<0.01, ***p<0.005.

TABLE 3.1: Summary of Gene Expression Changes in Patients with Schizophrenia and Neuroleptic Treated Rats

	Patients with Schizophrenia			Haloperidol Treated Rats			Clozapine Treated Rats		
Gene of Interest	*Change (%)*	*S/C ratio*	*p value*	*Change (%)*	*D/V ratio*	*p value*	*Change (%)*	*D/V ratio*	*p value*
GABAergic Markers									
$GABA_A$-α6	+44	1.44	**0.0046	*nd*	*nd*	*nd*	+25	1.25	***<0.0001
$GABA_A$-β3	+16	1.16	0.1099	+33	1.33	*0.0126	+35	1.35	***<0.0001
$GABA_A$-δ	+52	1.52	***0.0002	*nd*	*nd*	*nd*	+25	1.25	***<0.0001
GAD_{65}	-26	0.74	*0.0171	-19	0.81	*0.0114	+28	1.28	**0.0023
GAD_{67}	-38	0.62	*0.0134	+20	1.20	**0.0012	+31	1.31	***<0.0001
GAT-1	-43	0.57	**0.0024	+17	1.17	*0.0205	-23	0.77	***<0.0001
NMDA Receptor Subunits									
NR1	-5	0.95	0.4973	+13	1.13	*0.0389	+34	1.34	***<0.0001
NR2A	-6	0.94	0.2734	+23	1.23	0.0803	-24	0.76	***<0.0001
NR2B	-16	0.84	0.0681	+34	1.34	0.1792	-20	0.80	**0.0035
NR2C	+7	1.07	0.5417	-15	0.85	0.2776	-19	0.81	***0.0005
NR2D	+6	1.06	0.2734	-49	0.51	***<0.0001	-10	0.90	0.1009
Neuromodulators									
GluR6	+23	1.23	*0.0327	+26	1.26	*0.0304	+24	1.24	***0.0005
KA2	+29	1.29	***0.0002	-10	0.90	0.1271	-23	0.77	***<0.0001
mGluR2	-27	0.73	**0.0024	+32	1.32	***0.0008	+10	1.10	0.6163
mGluR3	-5	0.95	0.5417	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*
nNOS	-28	0.72	*0.0327	+11	1.11	0.3364	-19	0.81	*0.0149

Summary of gene expression data from patients with schizophrenia and rats treated with either haloperidol or clozapine. Deficits in GABAergic markers and neuromodulator alterations are shown in patients with schizophrenia and represented as % change and Schizophrenic/Control ratio. Both haloperidol and clozapine show positive effects on GAD_{67} expression with clozapine showing a more favorable profile for treating GABA deficits in patients. Differences in human expression determined by pair-matched Wilcoxon Signed Rank test while rat differences analyzed by *t* tests - **p<0.05, **p<0.01, ***p<0.0001.*

Table 3.2: ANCOVA Analysis of Gene Expression Data from Patients with Schizophrenia versus Comparison Subjects

	Covariates											
	Age, PMI, and RIN			Age			PMI			RIN		
	df	*F*	p-*value*	*df*	*F*	p-*value*	*df*	*F*	p-*value*	*df*	*F*	p-*value*
$GABA_A$-α6	4	3.9411	0.0154	2	7.2276	0.0037	2	6.4922	0.0058	2	6.3237	0.0065
$GABA_A$-β3	4	1.5887	0.2142	2	2.6718	0.0905	2	3.0024	0.0694	2	2.6754	0.0902
$GABA_A$-δ	4	8.1258	0.0004	2	16.0097	0.0000	2	15.5406	0.0001	2	17.0303	0.0000
GAD_{67}	4	4.9587	0.0057	2	8.4089	0.0018	2	8.0940	0.0022	2	9.5165	0.0010
GAD_{65}	4	1.9133	*0.1456	2	3.3992	*0.0508	2	3.4659	0.0483	2	3.6836	0.0409
GluR6	4	2.6269	0.0636	2	4.7911	0.0182	2	5.5030	0.0111	2	4.9390	0.0164
KA2	4	6.4746	0.0015	2	13.8144	0.0001	2	14.1600	0.0001	2	13.7862	0.0001
NR1	4	0.4002	0.8062	2	0.2360	0.7916	2	0.6097	0.5520	2	0.5387	0.5907
NR2A	4	0.8853	0.4897	2	0.8429	0.4433	2	0.6725	0.5202	2	1.8562	0.1789
NR2B	4	3.3833	*0.0275	2	2.6036	0.0957	2	1.3834	0.2708	2	3.4896	*0.0474
NR2C	4	0.4675	0.7589	2	0.4478	0.6445	2	0.9940	0.3854	2	0.4438	0.6470
NR2D	4	0.7347	0.5786	2	1.0169	0.3774	2	0.6777	0.5176	2	0.4655	0.6336
mGluR2	4	1.9597	*0.1378	2	3.3063	*0.0546	2	3.6760	0.0412	2	3.5922	0.0439
mGluR3	4	1.3178	0.2958	2	0.4890	0.6195	2	1.1630	0.3303	2	0.8296	0.4488
nNOS	4	3.0461	0.0398	2	3.9291	0.0340	2	4.6694	0.0199	2	4.4273	0.0236
Parvalbumin	4	0.3418	0.8467	2	0.1210	0.8866	2	0.1603	0.8529	2	0.7257	0.4947
GAT-1	4	3.8619	0.0167	2	7.1040	0.0040	2	6.4269	0.0061	2	6.7414	0.0050

Summary of analysis of covariance (ANCOVA) with condition as a fixed factor and age, PMI, and RIN as covariates. The covariates were analyzed as a group and independently. Changes in GAD_{65} and mGluR2 were shown to be non-significant while NR2B was shown to be significant when age, PMI, and RIN were accounted for. When analyzed independently, GAD65 and mGluR2 showed effects of age while NR2B was influenced by RIN.

To examine possible interactions in patterns of gene expression in patients with schizophrenia and control subjects, mRNA levels were analyzed by correlating transcript expression per group using Spearman's ρ. In the schizophrenic group, positive correlations included GAD_{67} with GAD_{65} (r=0.922; p <0.001) and GAT-1 (r=0.797; p=0.001), GAD_{65} with GAT-1 (r=0.770; p=0.002) (Figure 3.1), and $GABA_A$-α6 with $GABA_A$-β3 (r=0.610; p=0.027). Control subjects also showed a correlation in GAD_{67} with GAT-1 (r=0.646; p=0.017). No significant negative correlations were observed among the GABAergic markers studied.

3.1.2 NMDA Receptor Subunit Expression

In addition to GABA deficits, NMDA dysfunction has been reported in patients with schizophrenia (Akbarian et al., 1996b). Analyses of the levels of the NR1 and NR2A – NR2D subunits of the NMDA receptor revealed no significant changes (Table 3.1). The only observed change was a trend towards decrease in the Golgi cell selective subunit NR2B (Scherzer et al., 1997) (S/C=0.84; p=0.0681). Furthermore, the levels of NR2B in the schizophrenic population were positively correlated with NR1 (r=0.553; p=0.050), NR2A (r=0.553; p=0.050), and NR2D (r=0.663; p=0.014) (Figure 3.2). ANCOVA analyses showed significant contributions of age, PMI, or RIN to NR2B as a group (p=0.0275; Table 3.2) while RIN affected NR2B independently (p=0.0474; Table 3.2).

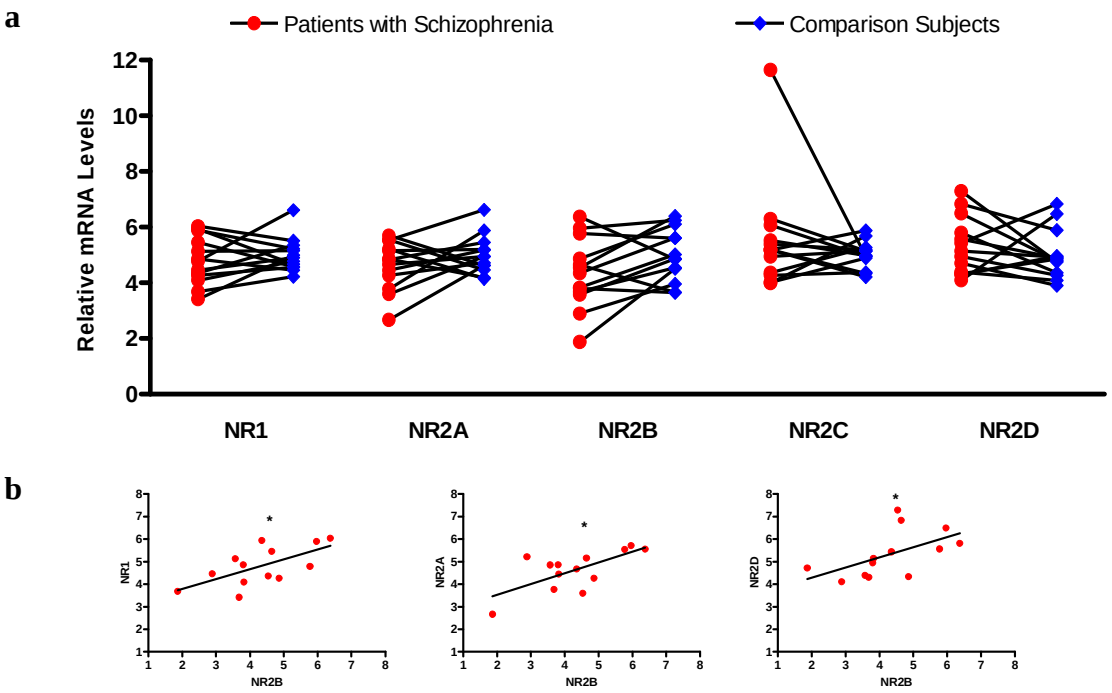


Figure 3.2 **a)** Relative expression levels of NMDA receptor subunits in the cerebellum of patients with schizophrenia versus comparison subjects. No significant changes are seen in any of the subunits examined. Expression levels are normalized to β-actin. **b)** Correlation of NMDA receptor subunits among patients showing high correlations between NR2B and NR1, NR2A, and NR2D. **p*<0.05.

3.1.3 Cerebellar Neuromodulator Expression

GABA release onto cerebellar granule cells is regulated at the level of the cerebellar glomerulus, where pontine mossy fiber and Golgi cell outputs synapse onto granule cell dendrites in a closed structural and functional unit. We therefore, examined major modulatory factors in this unit that could contribute to decreases in GABA neurotransmission on granule cells. Of these factors, we observed significant decreases in transcript levels of neuronal nitric oxide synthase (nNOS) (S/C=0.72; p=0.0327), present in granule cells (Baader and Schilling, 1996), and the Golgi cell specific metabotropic glutamate receptor mGluR2 (Berthele et al., 1999) (S/C=0.73; 0.0024), but not in the ubiquitously-expressed metabotropic glutamate receptor mGluR3 (Figure 3.3). Interestingly, both NO production and presynaptic mGluR2 receptor activation modulates GABA release from Golgi cells (Geurts et al., 2003; Wall, 2003). Moreover, we found significantly increased levels of the glutamatergic kainate receptor subunits GluR6 (S/C=1.23; p=0.0327) and the granule cell-specific subunit KA2 (Porter et al., 1997) (S/C=1.29; 0.0002) and a positive correlation between mGluR2 and GluR6 mRNA levels (r=0.709; p=0.007) in the schizophrenic patient group. ANCOVA analyses showed that the changes in transcript levels are of mGluR2 are affected by age, PMI, and RIN as a group (p=0.1378; Table 3.2) and age independently (p=0.0546; Table 3.2).

3.1.4 Correlations of Transcripts Levels of Golgi Cell Genes, Granule Cell Genes, and Local Cerebellar Circuitry Components

To investigate further the functional integrity of the cerebellar glomerulus in schizophrenia, interactions between specific markers for Golgi cells, granule cells, or the

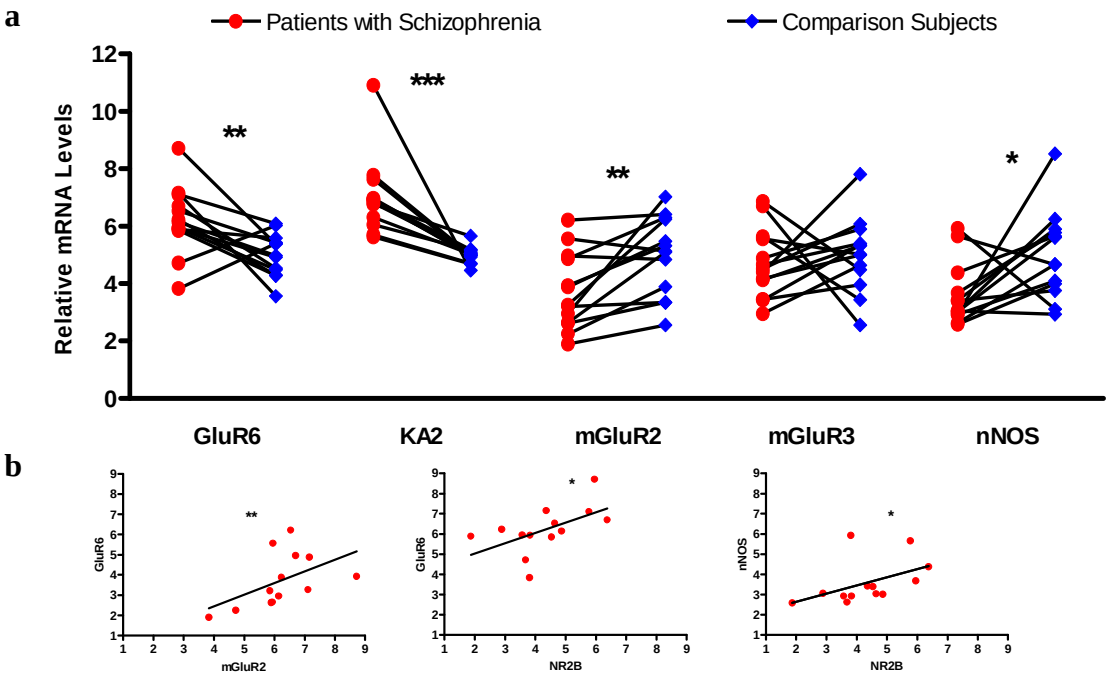


Figure 3.3 **a)** Relative expression levels of cerebellar neuromodulators of GABA release at the cerebellar glomerulus in patients with schizophrenia versus comparison subjects. Significant increases were seen in kainate receptor subunits Golgi and granule cell GluR6 and granule cell specific KA2 while significant decreases were shown in Golgi cell specific mGluR2 and granule cell nNOS. Expression levels are normalized to β-actin. **b)** Correlation analysis between markers expressed in the Golgi-granule cell circuit show high correlations between GluR6 with mGluR2 and NR2B and between nNOS and NR2B. ** $p<0.05$, **$p<0.01$, ***$p<0.001$.*

Golgi-granule cell circuit were analyzed. Examining genes from the schizophrenic population that are expressed in Golgi cells, we found positive correlations between the Golgi cell selective NMDA receptor subunit NR2B with GAD_{67} ($r=0.922$; $p<0.001$), GAD_{65} ($r=0.854$; $p<0.001$), and GAT-1 ($r=0.671$; $p=0.012$), and mGluR2 with GAD_{65} ($r=0.575$; $p=0.040$), all of which were decreased in the patients. In addition, transcripts expressed in granule cells showed correlations between NR2C with $GABA_A$-$\alpha6$ ($r=0.639$; $p=0.019$), $GABA_A$-$\beta3$ ($r=0.722$; $p=0.005$), $GABA_A$-δ ($r=0.652$; $p=0.016$), and KA2 ($r=0.726$; $p=0.005$), which were all increased in the patient population. Finally, we found that nNOS correlated with $GABA_A$-$\beta3$ ($r=0.757$; $p=0.003$), $GABA_A$-$\alpha6$ with GluR6 ($r=0.659$; $p=0.014$), and KA2 with $GABA_A$-δ ($r=0.600$; $p=0.030$).

Positive correlations between components of granule cell-Golgi cell circuitry included $GABA_A$-$\alpha6$ with mGluR2 ($r=0.797$; $p<0.001$) and NR2B with $GABA_A$-$\beta3$ ($r=0.600$; $p=0.030$), GluR6 ($r=0.594$, $p=0.032$), and nNOS ($r=0.624$; $p=0.023$). Significant negative correlations were observed for GAT-1 with NR2C ($r=-0.553$, $p=0.050$) and KA2 ($r=-0.779$; $p=0.002$), presumably reflecting a compensatory mechanism to increase GABA neurotransmission in the synaptic cleft.

3.2 Medication Effects on Gene Expression in Haloperidol and Clozapine Treated Rats

All patients were taking antipsychotics at the time of death (Table 2.1). Analysis of gene expression levels between patients who were administered atypical versus typical antipsychotics showed a significant increase in GAD_{67} ($p=0.0334$ using Mann-Whitney

Rank Sum test; Supplementary Figure A.5.4) and NR2A (p=0.0140; Supplementary Figure A.5.9) gene expression. These findings suggested that medication type (typical versus atypical) may have an effect on gene expression levels in patients. Therefore, we analyzed the levels of the same mRNAs in the cerebellum of rats treated with haloperidol, the classic typical neuroleptic, given at a dose of about 1 mg/kg/day for six months. As shown in Table 3.1, we found significant increases in $GABA_A$-β3, GAD_{67}, and GAT-1 in these rats. On the other hand, a significant decrease was seen in the expression of GAD_{65}. NMDA receptor subunit expression showed only a significant increase in NR1 and a significant decrease in NR2D transcript levels. Expression levels in cerebellar neuromodulators GluR6 and mGluR2 showed significant increases. In contrast, KA2 and nNOS mRNA levels were not different between control and treated groups.

To examine the effects of atypical medications, rats were treated with clozapine, administered for 21 days at a dose of 10/mg/kg/day. As shown in Table 3.1, these animals showed increased cerebellar mRNA levels of GABAergic markers $GABA_A$-β3, GAD_{67}, and GAD_{65}, while GAT-1 expression was significantly decreased. For NMDA receptor subunit expression, NR1 was significantly increased whereas NR2A, NR2B, and NR2C were all decreased, and NR2D showed no change between groups. Finally, the cerebellar neuromodulator GluR6 was significantly increased, in contrast to decreased in KA2 and nNOS, but not mGluR2 mRNAs. Overall, our results indicate that the antipsychotic medication could compensate, in part, for the decreased level of GABA

synthesizing enzymes and Golgi cell-specific marker mGluR2 that we observed in the patient samples.

3.3 Expression Data from Lateral Cerebellar Hemisphere of PCP Treated Rats

3.3.1 GABAergic Marker Expression

Given the evidence that PCP elicits many of the behaviors seen in schizophrenia (for review see Morris et al., 2005), we sought to characterize GABA dysfunction in inhibitory interneurons from the cerebella of these rats. Expression of six markers of GABA function in the lateral cerebellar hemisphere of PCP treated rats and paired saline control rats were initially examined using qRT-PCR. Transcript levels of presynaptic GABA signaling components GAD_{67} (P/S=0.89; p=0.0060), GAD_{65} (P/S=0.82; p=0.0023), and GAT-1 (P/S=0.82; p=0.0242) were shown to be significantly decreased in PCP rats versus controls by 11%, 18%, and 17% respectively (Figure 3.4, Table 3.3) using *t*-tests. Contrastingly, postsynaptic GABA receptor subunits $GABA_A$-α6 (P/S=1.55; p=0.0001), $GABA_A$-β3 (P/S=1.28; p=0.0024), and $GABA_A$-δ (P/S=1.55; p=0.0002) were significantly upregulated in the PCP rats. The overall GABAergic expression profile in the cerebellum showed a decrease in marker expression presynaptically while postsynaptic expression of markers was increased.

3.3.2 NMDA Receptor Subunit Expression

PCP acts as an open channel NMDA receptor antagonist (for review see Morris et al., 2005) and preferentially antagonizes GABAergic interneurons

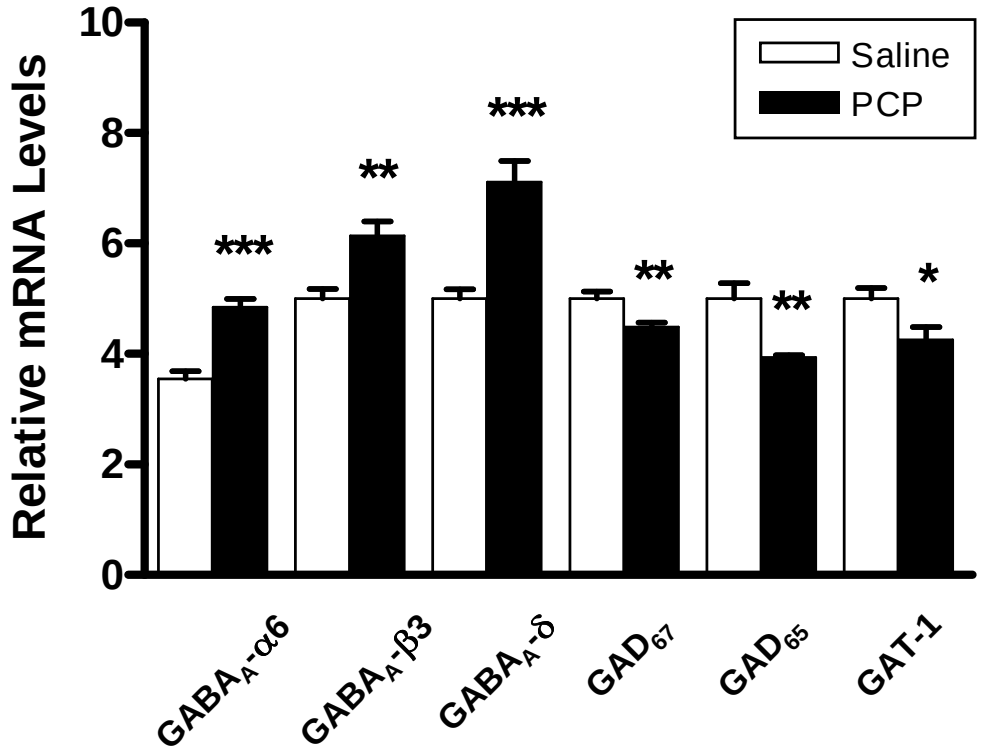


Figure 3.4 Relative expression levels of GABAergic markers in the cerebellum of PCP treated rats versus saline controls. Postsynaptic $GABA_A$ receptors α6, β3, and δ are significantly increased in PCP rats while GABA synthesizing enzymes GAD_{67} and GAD_{65}, and the reuptake transporter GAT-1, are significantly decreased. Expression levels normalized to β-actin. $*p<0.05$, $**p<0.01$, $***p<0.005$.

TABLE 3.3: Summary of Gene Expression Changes in PCP Treated Rats

Gene of Interest	*Change (%)*	*P/S ratio*	*p value*
GABAergic Markers			
$GABA_A$-α6	+55	1.55	***0.0001
$GABA_A$-β3	+28	1.28	**0.0024
$GABA_A$-δ	+55	1.55	***0.0002
GAD_{65}	-18	0.82	**0.0023
GAD_{67}	-11	0.89	**0.0060
GAT-1	-17	0.83	*0.0242
NMDA Receptor Subunits			
NR1	-5	0.95	0.1324
NR2A	0	1.00	0.0952
NR2B	-22	0.78	**0.0049
NR2C	+9	1.09	0.6822
NR2D	-36	0.74	**0.0076
Neuromodulators			
GluR6	-16	0.84	**0.0014
KA2	+39	1.39	***<0.0001
mGluR2	+37	1.37	0.0610
mGluR3	-6	0.94	0.3612
nNOS	+15	1.15	0.1862

Summary of gene expression changes seen in PCP treated rats versus saline controls. Decreases in mRNA levels of GAD_{65} and GAD_{67} are seen in the cerebellum of PCP treated rats versus saline controls as shown by both percent change and PCP/Saline (P/S) expression ratio. All other gene changes are involved in GABAergic transmission between Golgi cells and granule cells. Differences in expression determined by *t* test - **p<0.05, **p<0.01, ***p<0.0001.*

(Grunze et al., 1996; Homayoun and Moghaddam, 2007). To determine its effects on NMDA receptor expression, we evaluated expression levels of cerebellar NMDA receptor subunits. Decreased mRNA levels of two subunits, the Golgi cell specific NR2B subunit (Scherzer et al., 1997; Misra et al., 2000) (P/S=0.78; p=0.0049) and NR2D (P/S=0.74; p=0.0076) were the only significant changes seen among NMDA receptor subunits. Interestingly, NR2B and NR2D are co-localized to Golgi cells (Brickley et al., 2003), further suggesting that these cells may be preferentially affected by PCP. In contrast, the mRNA levels of NR1 (P/S=0.95; p=0.1324), NR2A (P/S=1.00; p=0.0952), and NR2C (P/S=1.09; p=0.6822) subunits were not significantly altered (Figure 3.5, Table 3.3).

3.3.3 Cerebellar Neuromodulator Expression

Considering the changes in expression of GABAergic markers and NMDA receptor subunits seen in our samples were consistent with deficits Golgi-granule cell communication, we examined major modulatory components involved in GABAergic transmission to granule cells. Here we observed a significant decrease in kainate receptor subunit GluR6 (P/S=0.84; p=0.0014), present in both Golgi and granule cells (Porter et al., 1997; Bureau et al., 2000), and a contrasting increase in the granule cell specific KA2 subunit (P/S=1.39; p<0.0001) (Porter et al., 1997; Pemberton et al., 1998). We did not find any changes in expression of metabotropic glutamate receptors mGluR2 (P/S=1.37; p=0.0610), which is present presynaptically solely in Golgi cells (Berthele et al., 1999; Watanabe and Nakanishi, 2003), nor in mGluR3 (P/S=0.94; p=0.3612), which is mainly

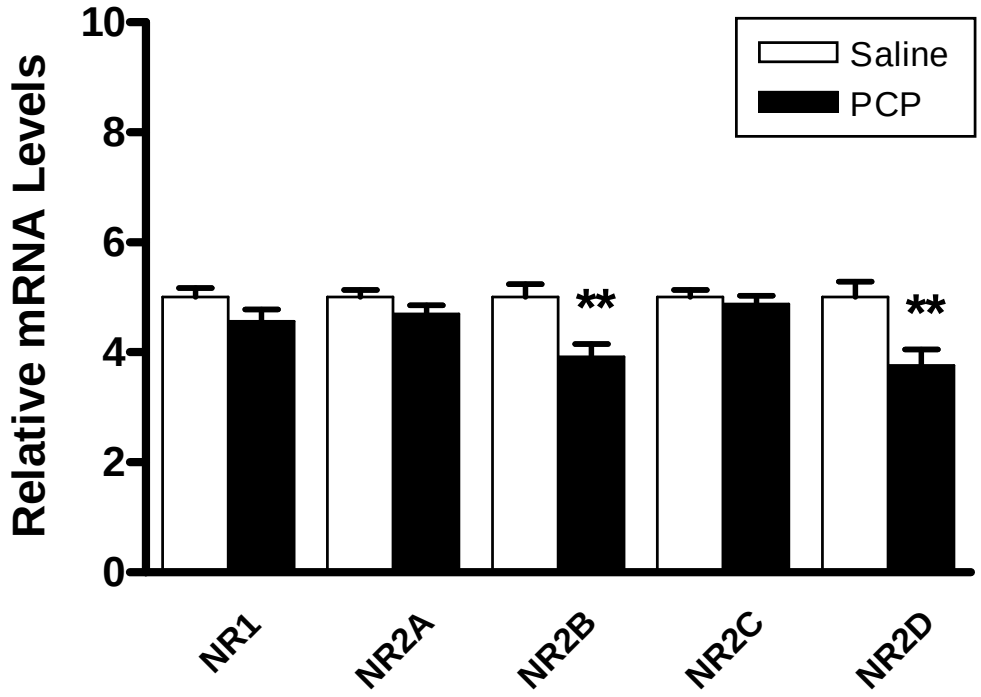


Figure 3.5 Relative expression levels of NMDA receptor subunits in the cerebellum of PCP treated rats versus saline controls. The Golgi cell specific subunit NR2B along with the interneuron marker NR2D are both significantly decreased in PCP treated rats. Expression levels normalized to β-actin. $^{**}p<0.01$.

glial (Berthele et al., 1999). Additionally, postsynaptic nNOS (P/S=1.15; p=0.1862) levels were also unchanged (Figure 3.6, Table 3.3).

3.4 Decreased GAD_{67} Expression in Golgi Cells of the Cerebellum

The GABA hypothesis of schizophrenia states that GABAergic deficits in specific subsets of interneurons are pathophysiological hallmarks of the disease (for review see Lewis et al., 2005). Chronic low dose administration of PCP preferentially inhibits GABAergic interneurons (Grunze et al., 1996; Jackson et al., 2004; Homayoun and Moghaddam, 2007) and has been proposed as an animal model of schizophrenia, mimicking many of the molecular findings seen in patients (Abe et al., 2000; Kim et al., 2000a; Cochran et al., 2003). Since many of the alterations seen in cerebellar transcript levels implicate deficient Golgi cell to granule cell inhibitory neurotransmission, we performed quantitative *in situ* hybridization to localize the changes seen in GAD_{67} expression using qRT-PCR to a particular cell type. Analysis of Golgi cells dually stained with GAD_{67} riboprobe and mGluR2 antibody, a Golgi cell specific marker (Figure 3.7a, c), showed an overall decrease in Golgi cell numbers (P/S=0.77; p=0.0014) in PCP treated rats versus controls, but not in basket/stellate cells of the molecular layer (P/S=0.96; p=0.4586) or in Purkinje cells (P/S=0.93; p=0.5262) (Figure 3.7b). Furthermore, analysis of the area covered by grains per cell type showed that PCP rats expressed significantly less GAD_{67} in Golgi cells (P/S=0.66; p=0.0058), but remained unchanged in basket/stellate cells (P/S=0.97; p=0.8319) and in Purkinje cells (P/S=0.93; p=0.5191) (Figure 3.7d). These results point to Golgi cells as the cells primarily affected by chronic intermittent exposure to a low dose of PCP.

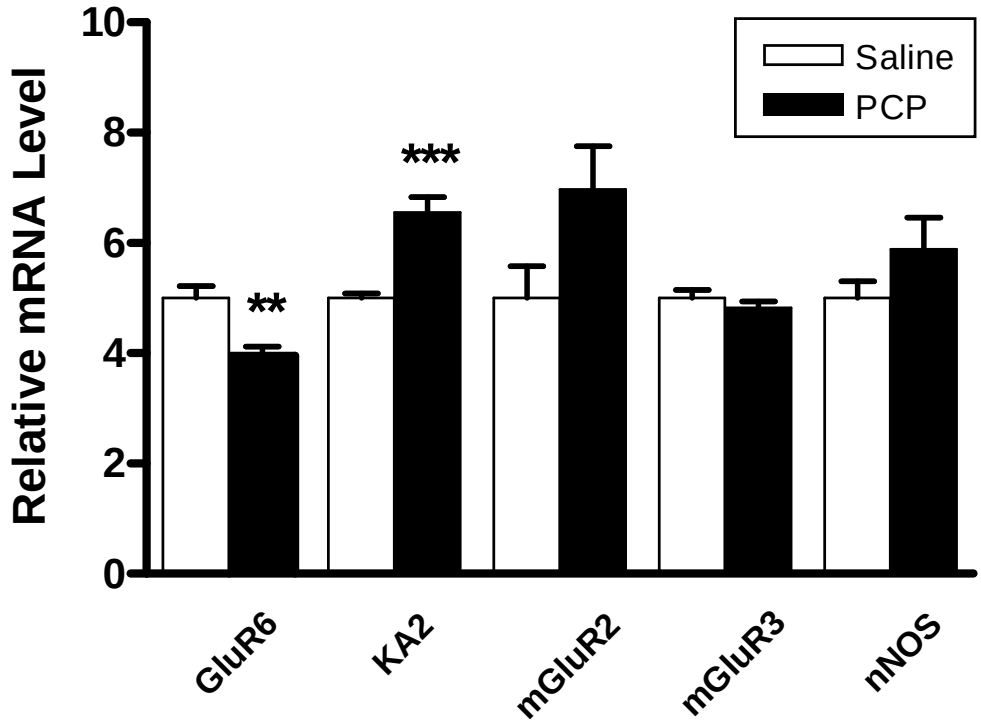


Figure 3.6 Relative expression levels of cerebellar neuromodulators of GABA release in the cerebellar glomerulus of PCP treated rats versus saline controls. Granule cell specific KA2 is significantly increased with granule and Golgi cell specific GluR6 is significantly decreased. Expression levels normalized to β-actin. $^{**}p<0.01$, $^{***}p<0.005$.

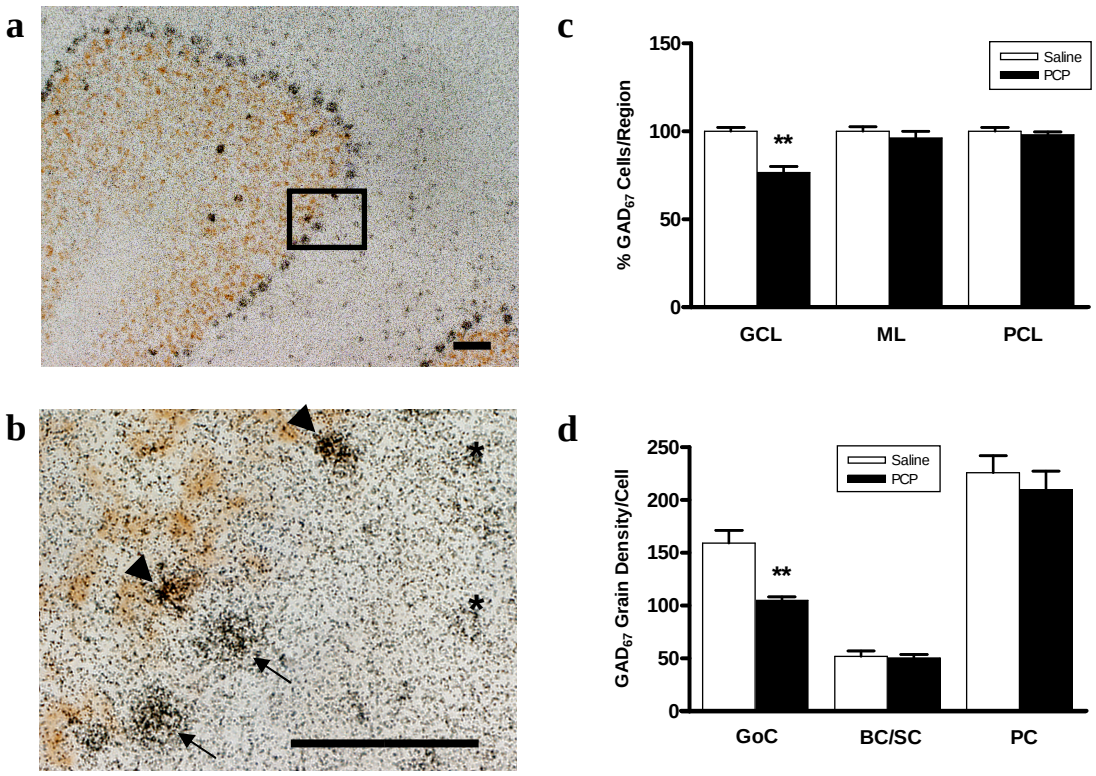


Figure 3.7 Quantitative *in situ* hybridization of GAD_{67} transcript expression levels in the cerebellar cortex of PCP treated rats. **a** 10X magnification of a cerebellar folium showing GAD_{67} ISH (grains) with mGluR2 IHC (brown). Golgi cells can be well visualized in the granule cell layer, Purkinje cells in the Purkinje cell layer, and basket and stellate cells in the molecular layer. Bar represents 50 μm. **b** 40X magnification representing inset in panel **a**. Distinction can be made between Golgi cells (arrowheads) and Purkinje cells (arrows). Basket and stellate cells are also shown (asterisks). Bar represents 50 μm. **c** Percentage of GAD_{67} expressing cells in the granule cell layer (GCL), molecular layer (ML), and Purkinje cell layer (PCL) of the cerebellum. All values normalized to saline control animal values. **d** Density of GAD_{67} silver grains in cerebellar Golgi (GoC) cells, basket (BC) and stellate (SC) cells, and Purkinje (PC) cells. $^{**}p<0.01$.

Chapter 4

Discussion

4.1 Patients with Schizophrenia

An increasing number of experimental studies support the hypothesis that GABA mediated neurotransmission is deficient in subsets of interneurons in patients with schizophrenia (for reviews see (Benes and Berretta, 2001; Lewis et al., 2005; Akbarian and Huang, 2006). While the effects of GABA mediated excitatory disinhibition have been well characterized in the PFC (Akbarian et al., 1995; Volk et al., 2000; Hashimoto et al., 2003) and limbic system (Heckers et al., 2002; Woo et al., 2004) of patients with schizophrenia, few studies have examined these deficits in the cerebellum (Guidotti et al., 2000; Fatemi et al., 2005). Analysis of post-mortem tissue from the lateral cerebellar hemisphere of patients with schizophrenia clearly demonstrates GABA deficits, which, along with altered expression of neuromodulators of granule cell activity, implicate a selective deficit in local granule cell-Golgi cell communication. A dysfunction in this local network is expected to have consequences in the function of both cerebellar micro- and macro-circuitry. Deficient tonic Golgi cell modulation may cause disinhibition of granule cells, as marked by increases in activity-dependent genes (Paz et al., 2006), and could disrupt cerebellar output by Purkinje cells. This disrupted output may, in turn, have a significant effect on other brain regions in the cortico-cerebellar-thalamic-cortical circuit, which includes the PFC (Andreasen et al., 1999).

4.1.1 GABAergic Marker Deficits in Patients with Schizophrenia

A number of discrete GABAergic interneurons reside in the cerebellum, most notably Golgi cells in the granule layer and basket and stellate cells in the molecular layer. While basket and stellate cells provide feed forward inhibition from granule cells to Purkinje cells, Golgi cells provide the sole source of feedback inhibition to granule cells (for review see (Ito, 2006). Together with Purkinje cells, these GABAergic interneurons express the GABA synthesizing enzymes GAD_{65} and GAD_{67}, which are encoded by distinct genes (Erlander et al., 1991) and exhibit different subcellular localizations (Esclapez et al., 1994). Our findings (Figure 3.1) indicate that the levels of both transcripts are decreased in patients versus control subjects, suggesting that GABA synthesis may be impaired in patients. We also found that transcript levels between the two enzymes are significantly correlated in the schizophrenic population (Figure 3.1). In agreement with our observations, Fatemi and colleagues (Fatemi et al., 2005) demonstrated decreases in the levels of both proteins in the cerebellum of a separate cohort of patients with schizophrenia, further supporting the idea of an overall deficit in GABA neurotransmission in this illness.

While GABA synthesis cannot be directly measured in post-mortem tissue, other markers can be investigated to infer whether GABAergic synaptic transmission is reduced. For example, deceased levels of transcripts encoding for the presynaptic GABA transporter, GAT-1 (Figure 3.1) may reflect a compensatory attempt to increase synaptic GABA. In adults Golgi and basket/stellate cells express GAT-1 while Purkinje cells and glia do not (Takayama and Inoue, 2005), suggesting that GAT-1 deficits may be restricted to specific

GABAergic interneurons. This is consistent with a similar decrease in GAT-1 observed in a specific subpopulation of GABAergic interneurons in association with GAD_{67} deficits in the PFC of patients with schizophrenia (Volk et al., 2001). Interestingly, the calcium binding protein parvalbumin, which has been shown to label GABAergic interneurons that are decreased in the PFC of patients with schizophrenia (Hashimoto et al., 2003), was not decreased in our patient population. Parvalbumin is expressed primarily in Purkinje and basket/stellate cells, but only in a small subpopulation of Golgi cells (Bastianelli, 2003). Conversely, the significant increases in the expression of extrasynaptic $GABA_A$ receptor subunits $\alpha 6$ and δ (Figure 3.1) suggest that a compensatory mechanism may occur to increase GABA receptivity by the granule cell in response to decreased GABA synthesis and release. These findings strongly suggest deficits selective to Golgi cells as compared to other GABAergic interneurons in the cerebellum.

4.1.2 NMDA Receptor Expression in Patients with Schizophrenia

A competing, but not mutually exclusive, hypothesis of schizophrenia implicates dysfunction of glutamatergic NMDA receptors and proposes that deficits in NMDA receptor mediated neurotransmission account for the cellular abnormalities and aberrant signaling seen in patients (Akbarian et al., 1996b). Furthermore, recent studies demonstrate that GABAergic interneurons are more vulnerable to NMDA receptor hypofunction (Grunze et al., 1996; Homayoun and Moghaddam, 2007). To get further insights into the subtypes of GABAergic interneurons that are primarily affected in the cerebellum we examined the expression of cell-selective NMDA receptor subunits

(Scherzer et al., 1997). While each cell type expresses the obligatory NR1 subunit, Purkinje cells mainly express NR2A, Golgi cells NR2B, granule cells NR2C, and basket/stellate cells NR2D. Although none of these subunits were significantly changed in the patients, the levels of NR2B subunit showed a trend towards decrease in this cohort ($p=0.0681$). Moreover, the expression of the NR2B subunit was strongly correlated with NR2D, both of which colocalize extrasynaptically in Golgi cells and might reflect altered modulation of glutamatergic tone on Golgi cells (Brickley et al., 2003). Additionally, NR2B subunit expression correlated strongly with GAD_{65} and GAT-1 expression in the patients and with GAD_{67} in both patients and controls (Figure 3.2). These findings further point to the Golgi cell as the predominately affected cerebellar interneuron in schizophrenia.

4.1.3 Neuromodulator Alterations in Patients with Schizophrenia

Given that Golgi interneurons control granule cells activity, we examined mRNA levels of proteins modulating GABA neurotransmission from Golgi to granule cells. GABA release by Golgi cells is regulated by various factors in both Golgi and granule cells including Golgi cell specific mGluR2 (Berthele et al., 1999) and nNOS located in granule cells (Wall, 2003). Activation of both systems normally decreases GABA release by Golgi cells, and both were shown to be decreased in the patient population (Figure 3.2), again presumably compensating for decreased levels of GABA in the synapse. On the other hand mGluR3, which is expressed in most cell types including glia (Berthele et al., 1999), was not changed in patients. Additionally, decreased levels of kainate receptor subunits GluR6 and KA2 mRNAs have been previously reported in the prefrontal cortex

and hippocampus of patients with schizophrenia (Porter et al., 1997; Meador-Woodruff et al., 2001). This contrasts with the increased mRNA levels of GluR6 and KA2 seen in our cohort of patients. Since these markers are expressed in granule cells and these cells show increased expression of activity dependent-genes in the same group of patients (Paz et al., 2006), it is possible that the changes in kainate receptor subunits may reflect a change in basal granule cell activity. Additionally, activation of presynaptic kainate receptors has been shown to increase GABA release (Mathew et al., 2008), further acting as compensatory processes for decreased GABA signaling. A model summarizing the changes in gene expression and putative cerebellar microcircuit alterations is shown in Figure 4.1.

An alteration in GABA neurotransmission between Golgi and granule cells is further evidenced by the positive correlations found only in patients between the Golgi specific markers mGluR2 and NR2B with the kainate receptor GluR6 subunit (Figure 3.2). Moreover, the positive correlation between nNOS with NR2B (Figure 3.2), together with the negative correlations between GAT-1 with granule cell specific NR2C and KA2, and the lack of a strong correlation between $GABA_A$-δ and mGluR2 in the patients (data not shown), suggest a defect in modulation of GABA release by Golgi cells and increased firing of granule cells.

4.1.4 Medication Effects on Gene Expression

Our gene expression findings in patients point to dysfunctional regulation of GABA release between Golgi and granule cells, but do not take into account any effects of

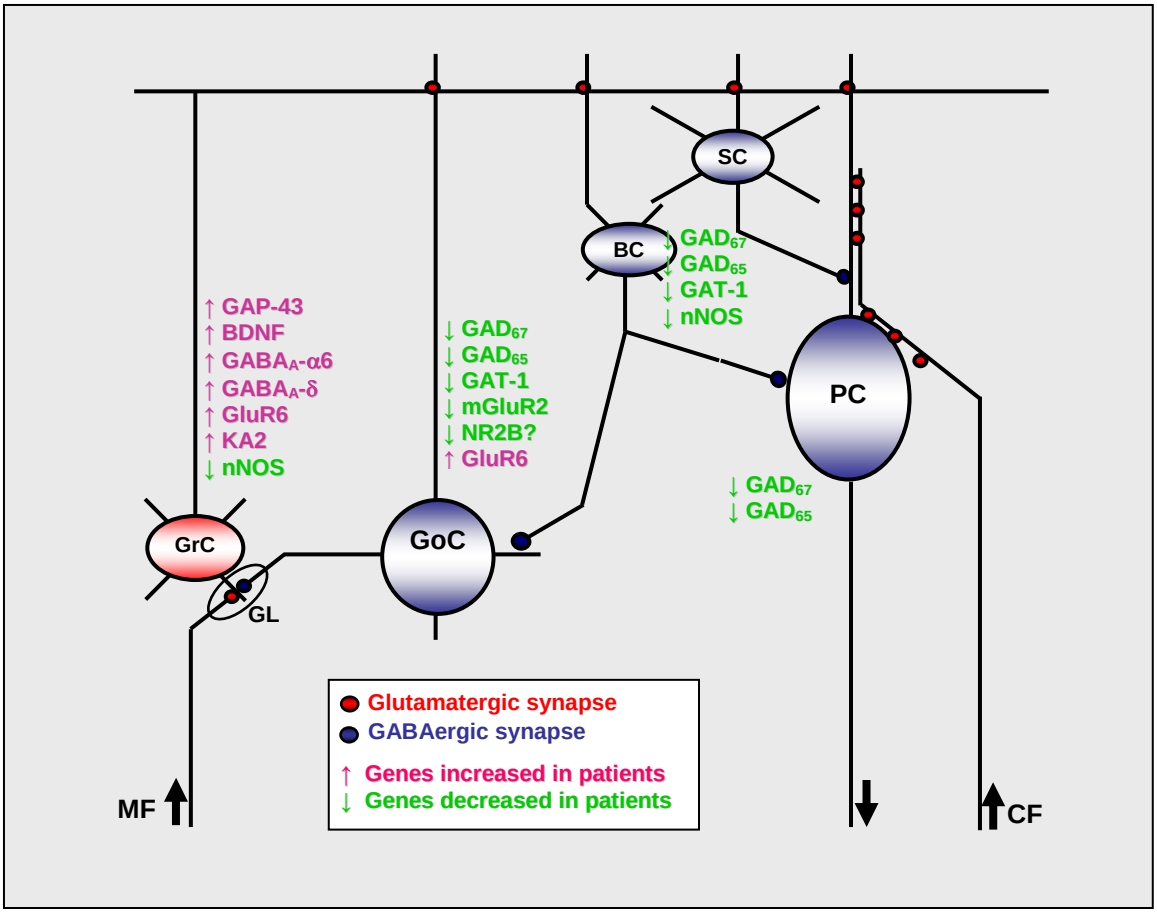


Figure 4.1: Summary of Gene Alterations in the Cerebellum of Patients with Schizophrenia. Note decreased GABA gene expression in Golgi cells and increased modulatory gene expression in Golgi and granule cells. GrC-granule cells; GoC-Golgi cells; BC-basket cells; SC-stellate cells; PC-Purkinje cells; GL-cerebellar glomerulus; MF-mossy fibers (from pontine nuclei); CF-climbing fibers (from inferior olive).

antipsychotic medication. To address this issue, we examined the levels of the same transcripts in rats treated with either haloperidol (Bustillo et al., 2006) or clozapine (Bai et al., 2004). While antipsychotic medications have been shown to affect gene expression in several brain regions, these alterations have previously not been reported for the cerebellum (Zink et al., 2004a; Zink et al., 2004b). Based on our results (Table 3.1), clozapine seems to have a more beneficial effect on gene expression of all modulatory components in the local cerebellar circuit leading to increased GABA in the synaptic cleft than does haloperidol, although both medications were effective in increasing GAD_{67}. Our results support the idea that combining different types of antipsychotics with $GABA_A$ receptor agonists and mGluR2 antagonists may have enhanced benefits for treating the underlying GABA-glutamate pathophysiology of schizophrenia.

4.1.5 Summary of GABAergic Deficits in Patients with Schizophrenia

In conclusion, we found that the expression of multiple GABAergic markers is decreased in the lateral cerebellar hemispheres of patients with schizophrenia versus pair-matched controls and that these deficits are most likely localized to Golgi cells. Additionally, both typical and atypical medications exert a level of influence on gene expression in this system. Overall, patient mRNA expression data points to GABA signaling deficits between Golgi and granule cells, possibly causing increased granule cell activity and disinhibition of glutamatergic signaling in the cerebellum leading to aberrant cerebellar output. These changes may underlie not only the alterations in cerebellar blood flow observed in the patients but also, and most importantly, the clinical symptoms associated with abnormal cerebellar function in schizophrenia (Ho et al., 2004).

4.2 PCP Treated Rats

Considering an increasing number of studies have characterized GABA expression deficits in patients with schizophrenia (for reviews see Benes and Berretta, 2001; Lewis et al., 2005; Akbarian and Huang, 2006), it is important that some of these deficits are reproduced in an animal model. One such model is a chronic low dose PCP exposure (Cochran et al., 2003) which was shown to reproduce decreases in parvalbumin mRNA in GABAergic interneurons seen in the PFC of patients with schizophrenia. Therefore, we chose this model for our studies. By chronic administration of low-dose PCP to rats, we demonstrated the decreased expression of markers of GABA mediated neurotransmission in the cerebellum of this animal model mimic the alterations found in the same markers in patients with schizophrenia . Additionally, we localized the decrease in GAD_{67} expression to a specific subtype of cerebellar GABAergic interneuron, the Golgi cell. Based upon our patient data and our findings in PCP-treated rats, we propose that Golgi cells are vulnerable to the effects of NMDA hypofunction. Since Golgi cells are responsible for negative feedback on excitatory granule cell firing, dysfunctional Golgi cell signaling may cause granule cell disinhibition and disrupted cerebellar output to other brain regions, including those implicated in schizophrenia, such as the PFC.

4.2.1 Golgi-Granule Cell Signaling – Tonic Inhibition

It has been well established that Golgi cells are responsible for tonic inhibition of granule cells (De Schutter et al., 2000; Rossi et al., 2003). This inhibitory tone is thought to be imparted by GABA release due to autorhythmic activity (Forti et al., 2006) and mediated through GABA binding to high affinity $GABA_A$-$\alpha 6/\delta$ containing receptors located

extrasynaptically (Hamann et al., 2002; Geurts et al., 2003; Fritschy and Panzanelli, 2006). This signaling occurs in a unit named the cerebellar glomerulus, comprised of the Golgi cell axon-granule cell-pontine mossy fiber axon synapses (for review see De Schutter et al., 2000). Here extracellular GABA concentrations are regulated by the neuronal presynaptic transporter GAT-1, which also effectively ends phasic GABA transmission (Morara et al., 1996). Our findings show decreased levels of GAD_{67} and GAD_{65} expression (Figure 3.4, Table 3.3, and Figure 3.7b, d), indicating deficient GABA production. These findings, along with decreased GAT-1 expression and increased levels of $GABA_A$ receptor subunits $\alpha 6$, $\beta 3$, and δ (Figure 3.4, Table 3.3), point to aberrant GABAergic neurotransmission between Golgi cells and granule cells in PCP treated rats. Insufficient GABA tone decreases the threshold of activation for granule cells, allowing for increased granule cell activity and increased expression of activity dependent genes, such as $GABA_A$-δ (Salonen et al., 2006) and the granule cell specific kainate receptor subunit KA2 (Feligioni et al., 2006).

4.2.2 Golgi-Granule Cell Signaling – Phasic Inhibition

Golgi cells also regulate granule cell activity in a phasic manner, receiving excitatory input from granule cell parallel fibers and then providing inhibitory feedback, functioning in a negative feedback system (for review see De Schutter et al., 2000). Release of glutamate by mossy fiber afferents excites granule cells while inhibiting GABA release from Golgi cells through presynaptic binding of mGluR2/3 receptors (Geurts et al., 2003), while postsynaptic mGluR2 activation by glutamate release from parallel fibers also inhibits downstream GABA release (Watanabe and Nakanishi, 2003). Further

inhibition of GABA neurotransmission takes place when granule cells release nitric oxide (NO), synthesized by the activity dependent nNOS (Wall, 2003), decreasing GABA release from Golgi cells in a retrograde manner. Hence, simultaneous granule cell activation by glutamate and inhibition of Golgi cell GABA release increases granule cell activity (Chadderton et al., 2004).

While our findings in patients with schizophrenia show perceived compensatory decreases in mGluR2 and nNOS , the PCP rat model failed to replicate these deficits. This could be due, in part, to increased NO production due to PCP administration (Wiley, 1998; Wass et al., 2006). Conversely, mGluR3 expression is not changed (Figure 3.6) as expected since it is glial (Berthele et al., 1999). However, decreased levels of GluR6 (Figure 3.6, Table 3.3), a Golgi and granule cell selective kainate receptor forming functional homomeric channels (Bureau et al., 2000) and modulating GABA release presynaptically (Mathew et al., 2008), suggest alternative methods of compensation for decreased GABA release and further implicate Golgi cells as dysfunctional. The deficits seen in Golgi cells, therefore, may lead to decreased tonic and phasic inhibition, deregulating granule cell transmission to Purkinje cells and ultimately contributing to aberrant cerebellar output.

The interaction between parallel fibers and Golgi cells is non-synaptic and has a low probability of glutamate release (Dieudonne, 1998). This indicates that Golgi cells main function is tonic, not phasic, inhibition (Rossi et al., 2003). However, glutamate is released onto Golgi cells and functions partly through NMDA receptors. Golgi cell

NMDA receptors consist of the obligatory NR1 subunit along with Golgi cell specific NR2B in synaptic channels (Misra et al., 2000) and heterotetramers containing both NR2B and NR2D at extrasynaptic sites (Brickley et al., 2003). We found decreased expression of both NR2B and NR2D subunits (Figure 3.5, Table 3.3), suggesting preferential antagonism of Golgi cell NMDA receptors. This is not surprising considering GABAergic interneurons in the hippocampus and PFC are selectively affected by PCP administration (Grunze et al., 1996; Homayoun and Moghaddam, 2007). Molecular layer basket and stellate interneurons also express NR2D (Berthele et al., 1999), but do not show deficits in GAD_{67} expression (Figure 3.7c, d) and, thus, are not affected by low dose PCP administration.

4.2.3 Purkinje Cells

Granule cells ultimately synapse on Purkinje cells, the sole source of output from the cerebellar cortex. Purkinje cells are GABAergic projection neurons that integrate signals from multiple inputs from parallel fibers, basket/stellate interneurons, and climbing fibers, and send the sole cerebellar output signal to deep cerebellar nuclei (Sastry et al., 1997). Our results suggest Purkinje cells are not directly affected by PCP administration, as seen by the unchanged levels of Purkinje cell selective NR2A (Figure 3.5, Table 3.3) and GAD_{67} (Figure 3.7d), and the decreased expression of GAT-1 (Figure 3.4, Table 3.3), which is not expressed in Purkinje cells (Takayama and Inoue, 2005). However, Purkinje cell output may be irregular due to increased granule cells activity caused by disinhibition from deficient Golgi cell GABA neurotransmission. These deficits may desynchronize Purkinje cell input, ultimately leading to aberrant cerebellar output.

4.2.4 Summary of GABAergic Deficits in PCP Treated Rats

In conclusion, we have shown that chronic low-dose administration of PCP in rats models the GABA neurotransmission deficits seen in patients with schizophrenia . Furthermore, we localized these changes to Golgi cells, a subset of cerebellar inhibitory interneurons, critical for controlling granule cell output to Purkinje cells. Our findings indicate that GABAergic neurotransmission in the cerebellum mediated by Golgi cells is defective. Aberrant cerebellar signaling, through its connections to the PFC, may be contributing to the cognitive deficits seen in the patients.

4.3 Translational Implications

Understanding the pathophysiology of schizophrenia is crucial in developing more effective treatments. Current psychopharmacotherapeutical interventions are successful in treating positive symptoms, but do not treat negative or cognitive symptoms effectively (Lewis et al., 2004; Harvey et al., 2005), which are the better prognosticators for patient outcomes (Breier et al., 1991; Milev et al., 2005). This is most likely due to the fact that typical and atypical antipsychotics were developed to treat excessive dopaminergic signaling. While it is apparent that patients exhibit problems in dopamine neurotransmission, it is unlikely that dopamine dysfunction is the underlying cause of the disease. Additionally, clinical studies have shown that typical and atypical antipsychotics show no differences in quality of life, treatment of symptoms, and cost (Jones et al., 2006). Moreover, glutamatergic agents, such as glycine or D-cycloserine, have not proven useful in reversing the negative or cognitive deficits (Buchanan et al., 2007).

Furthermore, many of the medications available have unwanted side effects that preclude patients from adhering to treatment regimens. In essence, more effective therapies with less severe side effects are the short-term goal for clinical schizophrenia. The ultimate goal is eventually finding a cure for this disease.

In light of recent data, including our own studies (Akbarian et al., 1995; Guidotti et al., 2000; Hashimoto et al., 2003; Fatemi et al., 2005; Benes et al., 2007), it is becoming more clear that GABAergic deficits are more widespread and contribute more to the underlying etiology of schizophrenia. With this in mind, more therapeutic options geared towards treating the regionally specific deficits seen in GABA signaling need to be developed and tested. Benzodiazepine-like GABA modulators, such as α2- and α5-selective $GABA_A$ receptor agonists or benzodiazepine antagonists (Lewis et al., 2004; Guidotti et al., 2005; Menzies et al., 2007), are showing promise in animal and human studies. However, truly effective treatments cannot be developed until the pathophysiological mechanisms that underlie schizophrenia are discovered. Our study provides further support to the hypothesis that specific subsets of GABAergic interneurons are affected in schizophrenia in distributed brain regions, all with a common connectivity.

4.4 Critique of Work

Any time research is preformed utilizing humans, certain problems may arise. This statement is applicable to both living human subjects and post-mortem tissue. Since humans have many complex interactions with their environment and have intricate genetic diversities, it is difficult to control for any study. However, attempts are made to lessen the confounding effects of extraneous interactions in experiments dealing with patients. This is mainly performed using statistical applications to normalize data and remove confounding factors. Our study was designed to minimize differences by pair-matching based on age and PMI. Nevertheless, factors beyond our control may contribute to our findings. To combat this, we would need to perform an extensive chart review on each patient to account for other medications, duration of treatments, other disease or conditions, etc. Since this could not be done, we controlled in the most applicable manner by pair-matching and performing more strict non-parametric tests to explain our findings.

4.4.1 Post-Mortem Tissue

qRT-PCR studies elucidating GABA deficits in patients with schizophrenia were performed using total RNA extracted from post-mortem tissue. Since it is virtually impossible to study molecular mechanisms in living humans, post-mortem tissue analysis becomes extremely important. However, this type of analysis does not allow for mechanistic studies or manipulations of the tissue. Additionally, issues with RNA quality have been raised based on PMI, but our samples showed intact quality total RNA as

shown by RIN ≥7.0. Nonetheless, this is the best way to study gene expression in humans.

4.4.2 qRT-PCR Analysis

Our results were obtained using the technique of qRT-PCR. While the experiments were all conducted in the same manner, the volume of experiments run precluded the use of only one real-time PCR machine. Time and scheduling issues caused us to run our experiments on three different Applied Biosystems thermalcyclers. Ideally, these experiments would be carried out on the same machine, but was impossible. Our data, however, was consistent and validated on each machine used.

4.4.3 Evaluation of Medication Effects in Animal Models

Since none of the patients in this study were neuroleptic naïve, the effects of antipsychotic on gene expression were evaluated using rodent models. The doses of clozapine and haloperidol that used here were based on previous studies showing high levels of D2 receptor occupancy (Bai et al., 2004). Nevertheless, one limitation is that we used a 6 month exposure paradigm for haloperidol versus a 21 day exposure paradigm for clozapine. Since the effects of these different treatments were not compared to each other, but were compared to the levels in the patients, we do not consider this to be a major shortcoming in our study.

4.4.4 Animal Model of Schizophrenia

Schizophrenia is a purely human disease involving complex perceptional and cognitive aspects. Reproducing this disease in an animal model is challenging, as many animal models of schizophrenia exist. In the chronic intermittent exposure paradigm that we used rats are sacrificed 3 days after the last PCP exposure. Thus, these animals are useful to model the residual illness in the patients rather than an active psychotic episode, which is modeled by acute PCP exposure. Furthermore, because we used a low dose of PCP, it is unlikely that the animals were experiencing any withdrawal at the time they were sacrificed. Ultimately, our model was shown to reproduce the GABAergic deficits seen in patients and is, therefore, useful in examining the GABA dysfunction seen in schizophrenia.

4.4.5 *In Situ* Hybridization Analysis

Analysis of *in situ* hybridization images was not performed blind to the experiment. This creates an inherent bias and may influence the findings and the significance of the results.

4.5 Future Perspectives

It is clear that GABAergic interneurons in distributed brain regions show deficits in markers related to signaling, such as GAD_{67} and GAT-1 (Benes and Berretta, 2001; Lewis et al., 2005). However, further research must be performed in order to determine the reason why certain subsets of GABAergic interneurons are preferentially affected over others. Polymorphisms in the promoter for GAD_{67} have been identified in patients with schizophrenia, some of which were shown to affect the rate of transcription (Straub

et al., 2007). Moreover, epigenetic mechanisms, such as increased levels of DNA methyltransferase 1 (DNMT1), which correspond to decreased levels of GAD_{67} and reelin, another marker of GABAergic cells, were found in specific cortical layers from patients (Veldic et al., 2004; Ruzicka et al., 2007). Additionally, animal models of methionine induced hypermethylation were found to affect the expression of GAD_{67} and reelin, a developmental marker (Tremolizzo et al., 2002; Dong et al., 2005). More recent epigenetic studies have implicated tri-methylation of histone H3-lysine 4 (H3K4me3) in the activity of GABAergic gene promoters, such as GAD67, GAD65, somatostatin, and reelin in patients (Huang and Akbarian, 2007; Huang et al., 2007). Epigenetic alterations linked to reelin may lead to abnormal cellular migration, as seen by increased interstitial white matter cells in the PFC (Akbarian et al., 1996a) and in the hippocampus (Eastwood and Harrison, 2003, 2006).

These genetic and epigenetic differences in patients may confer a high degree of sensitivity to GABAergic interneurons, making them more vulnerable to environmental insults. However, these findings do not explain the mechanism behind dysfunction of specific subsets of cells, placing importance on identification of new markers that can distinguish among different subtypes of GABAergic interneurons, particularly those that can predict a selective cellular dysfunction. Along these lines, it will be important to measure the levels of different $GABA_A$ receptors *in vivo* in the patients by designing specific radioligands for these receptors, whose binding could be detected using positron emission tomography (PET). Regardless of the mechanism, it is clear deficits in GABAergic interneurons contribute greatly to the pathophysiology of schizophrenia.

Patients could benefit greatly from treatment with selective GABA receptor agonists, medications targeting specific modulators of GABA release, or treatment with medications that treat epigenetic regulation in GABAergic interneurons.

4.6 Conclusion

Our study has shown that patients with schizophrenia have decreased expression of markers of GABA neurotransmission in the lateral cerebellar hemispheres, regions linked to cognition (Schmahmann and Sherman, 1998; Middleton and Strick, 2001; Ramnani, 2006; Thach, 2007). This supports the current hypothesis of GABAergic dysfunction in other brain regions of patients. Furthermore, our results from chronic intermittent low-dose PCP administration in rats reproduced many of the GABA deficits seen in our patient cohort. Furthermore, we localized deficits in one of the major GABA markers, GAD_{67}, to a specific subset of GABAergic interneurons in the cerebellar cortex, Golgi cells. This is an exciting finding which shows GABA deficits in the cerebellum are similar to those found in other brain regions, specifically those important in cognition. These findings may ultimately contribute to better therapeutic interventions to ameliorate the dysfunction experienced by patients with schizophrenia.

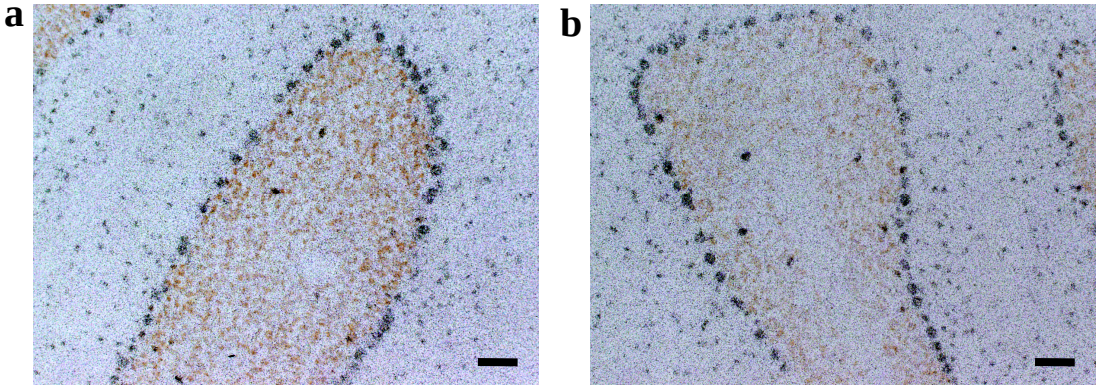
Abbreviations Used

5-HT2A	Serotonin Receptor Subunit 2A
ANCOVA	Analysis of Covariance
BC	Basket Cell
BDNF	Brain Derived Neurotrophic Factor
CCTCC	Cortico-Cerebellar-Thalamic-Cortical Circuit
CF	Climbing Fibers
COMT	Catechol-o-methyl Transferase
DA	Dopamine
DAAO	D-Amino Acid Oxidase
DAB	Diaminobenzidine
DISC1	Disrupted in Schizophrenia 1
DNMT1	DNA Methyltransferase 1
GABA	γ-aminobutyric acid
GAD_{65}	Glutamic Acid Decarboxylase 65kDa Isoform
GAD_{67}	Glutamic Acid Decarboxylase 67kDa Isoform
GAP-43	Growth Associated Protein 43
GAT-1	Presynaptic GABA Reuptake Transporter
GCL	Granule Cell Layer
GL	Cerebellar Glomerulus
GluR6	Low Affinity Ionotropic Glutamate Receptor – Kainate GluR6 Subunit
GoC	Golgi Cell
GrC	Granule Cell

H3K4me3	Histone H3-lysine 4 tri-methylation
IHC	Immunohistochemistry
KA2	High Affinity Ionotropic Glutamate Receptor – Kainate KA2 Subunit
LTP	Long Term Potentiation
MF	Mossy Fibers
MFC	Medial Frontal Cortex (Rat)
mGluR2	Metabotropic Glutamate Receptor 2
mGluR3	Metabotropic Glutamate Receptor 3
ML	Molecular Layer
MRS	Magnetic Resonance Spectroscopy
NAA	n-acetylaspartate
NAAG	n-acetylaspartylglutamate
NHS	Normal Horse Serum
NMDA	N-methyl-D-aspartate
nNOS	Neuronal Nitric Oxide Synthase
NO	Nitric Oxide
NR1	N-methyl-D-aspartate Receptor Subunit 1
NR2A-D	N-methyl-D-aspartate Receptor Subunit 2A-D
NRG1	Neuregulin 1
PC	Purkinje Cell
PCL	Purkinje Cell Layer
PCP	Phencyclidine
PF	Parallel Fibers

PFC	Prefrontal Cortex (Human)
PMI	Post-Mortem Interval
qISH	Quantitative In Situ Hybridization
qRT-PCR	Quantitative Real Time Polymerase Chain Reaction
RIN	RNA Integrity Number
RSG4	Regulator of G-Protein Signaling 4
SC	Stellate Cell
SPET	Single Positron Emission Tomography
TBS	Tris Buffered Saline

Appendix A.1

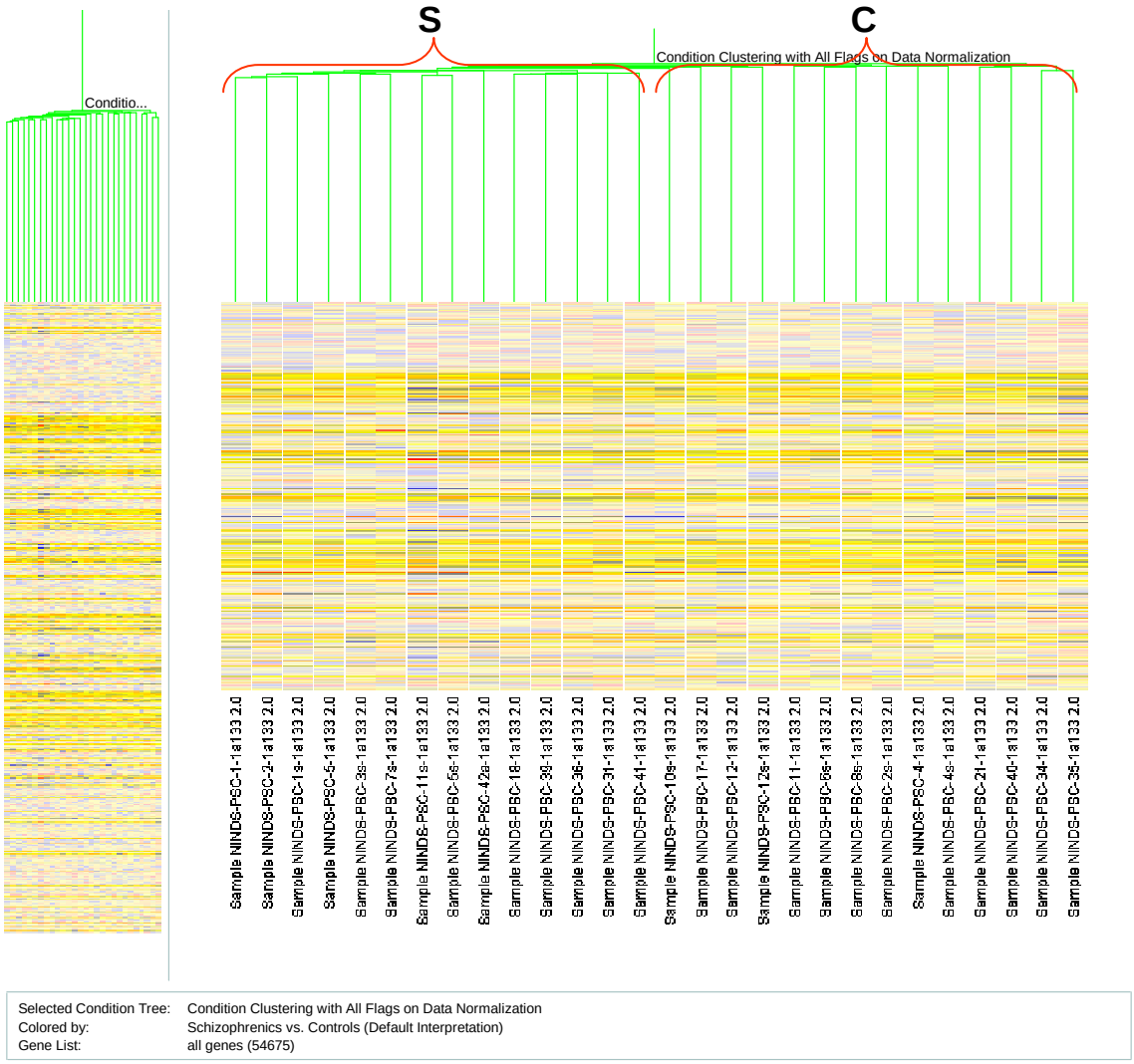


Supplementary Figure A.1.1. 10μm thick sections from rats treated with either a) saline or b) PCP. Sections were labeled with S^{35}-GAD_{67} riboprobe and counterstained with DAB following mGluR2 immunohistochemistry at 10X magnification. Decreases were seen in number of GAD_{67} expressing Golgi cells in the granule cell layer and in GAD_{67} expression of Golgi cells in PCP rats. No other changes were seen. Bar represents 50μm.

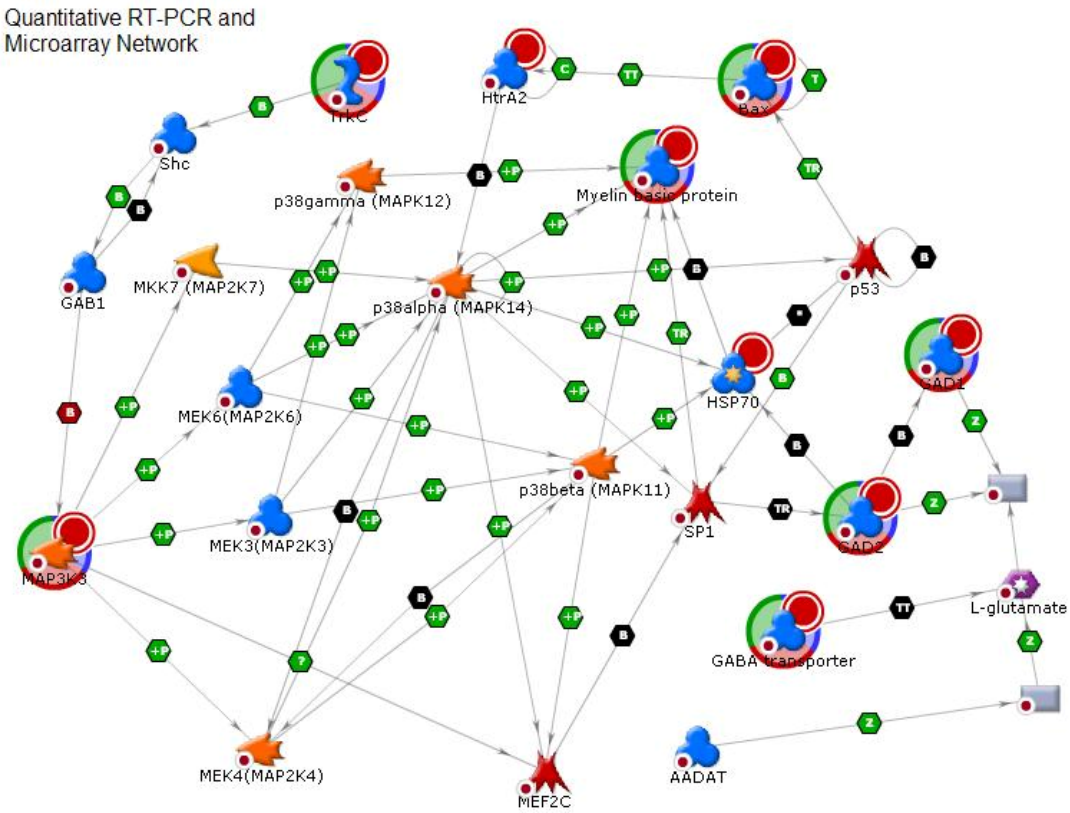
Appendix A.2

Hybridization of Affymetrix HG-U133_2.0 Plus microarrays (Affymetrix; Santa Clara, CA) from 14 patients with schizophrenia and 14 comparison subjects was performed at the Translational Genomics Research Institute (T-GEN). Raw data was analyzed using GeneSpring GX (Agilent Technologies; Santa Clara, CA). Data was normalized to the median of all chips and clustered by condition (Supplemental Figure A.2.1). Filtering on 14 of 28 flags present and fold change of 1.2 produced a gene list of 1023 genes. Certain genes from this list (BAX, MAP3K3, NTRK3, SP1,) were validated by qRT-PCR as described in the methods section (Supplementary Figures A.2.9 – A.2.17). Additionally, two genes related to GABA function (GABRB3 and NOS1) were identified and examined by qRT-PCR (Figures 3.1 and 3.3).

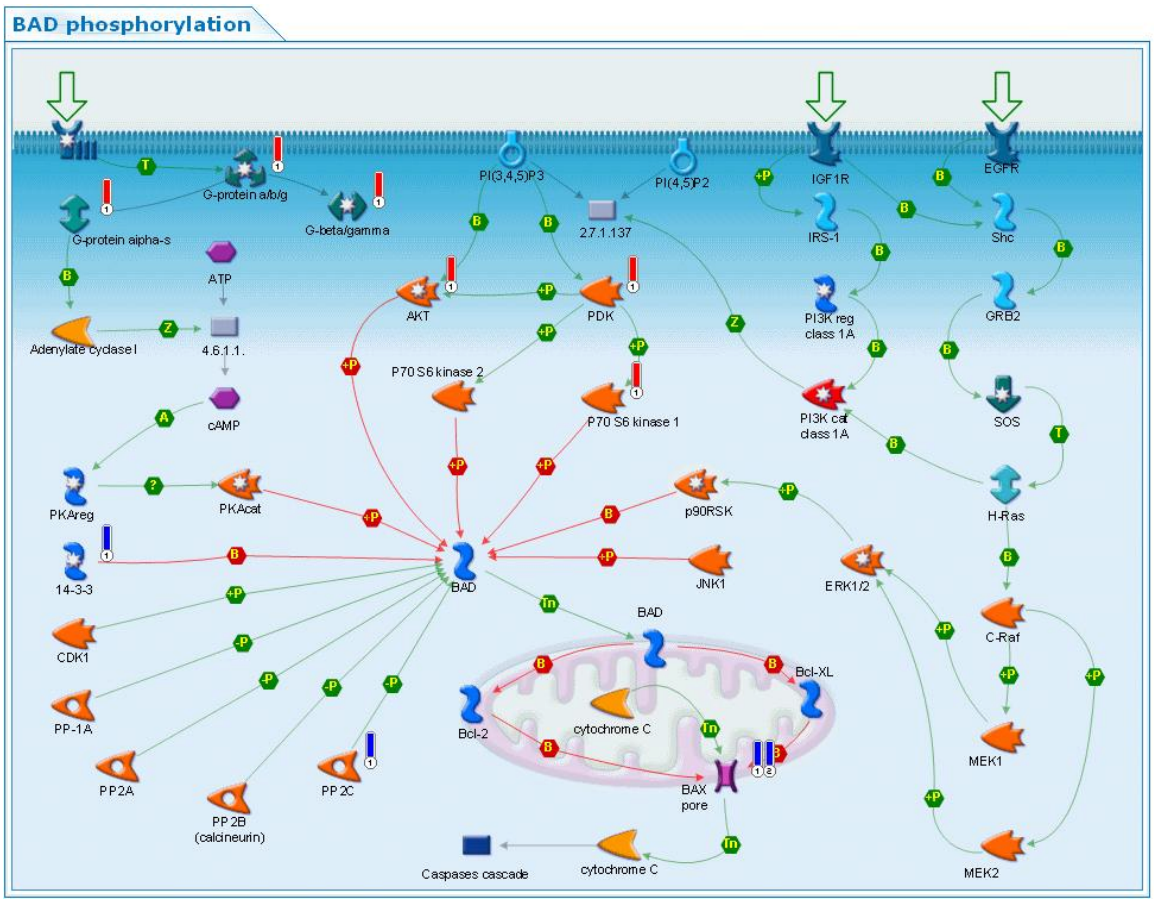
The gene list created from GeneSpring GX was entered into GeneGo MetaCore™ (Encinitas, CA) and analyzed using standard algorithms. A network showing interactions between selected genes from the gene list was created using expression data from qRT-PCR analysis (Supplementary Figure A.2.2). Additionally, maps were created from the gene list show the greatest number of hits in pathways associated with BAD phosphorylation, CDC42 pathways, G-protein mediated regulation of p38 and JNK signaling, JNK pathway, neurotrophin signaling, and p53-dependent apoptosis. These are represented below (Supplementary Figures A.2.3 – A.2.8).



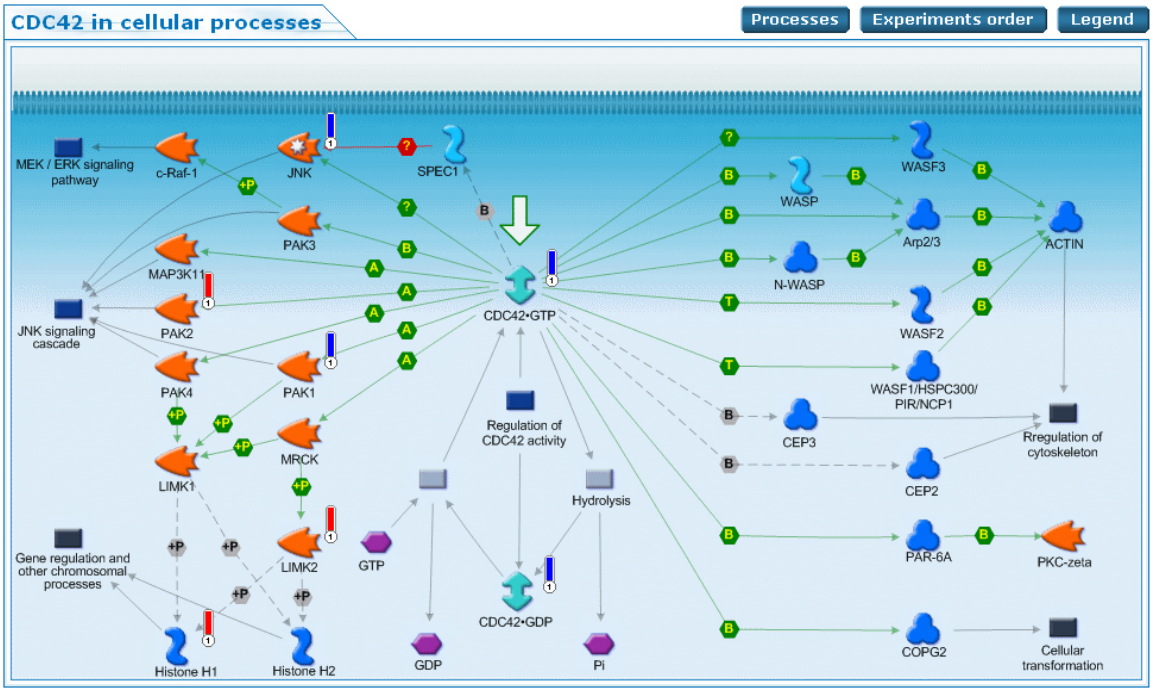
Supplementary Figure A.2.1. GeneSpring condition clustering from 14 patients with schizophrenia and 14 comparison subjects with data normalization to the median intensity of all chips. The patients with schizophrenia cluster independently from the comparison subjects without any other manipulations.



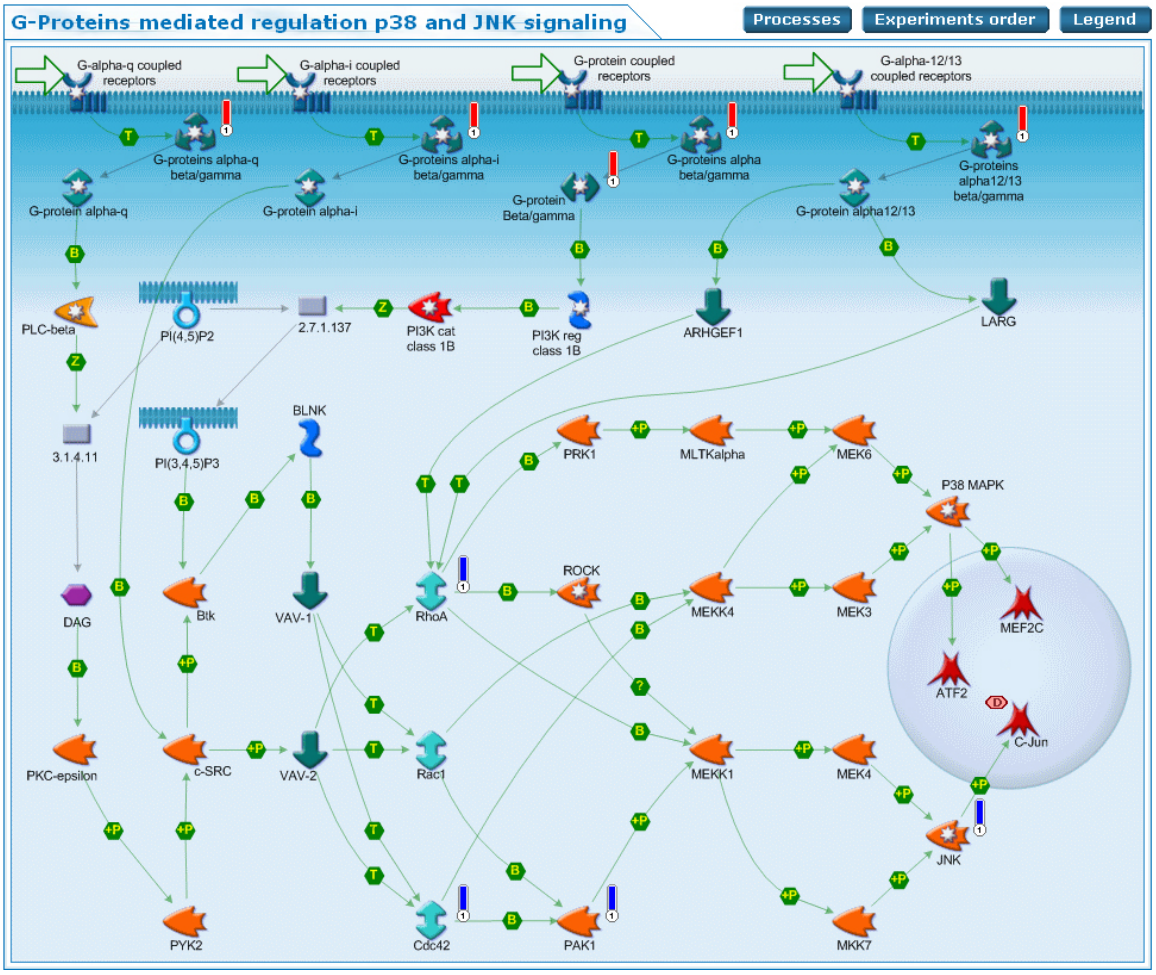
Supplementary Figure A.2.2. GeneGo MetaCore™ network showing interactions among genes validated using qRT-PCR from GeneSpring GX gene list and selected GABA markers. These genes include MAP3K3, TrkC, MBP, BAX, GAD_{67}, GAD_{65}, and GAT-1.



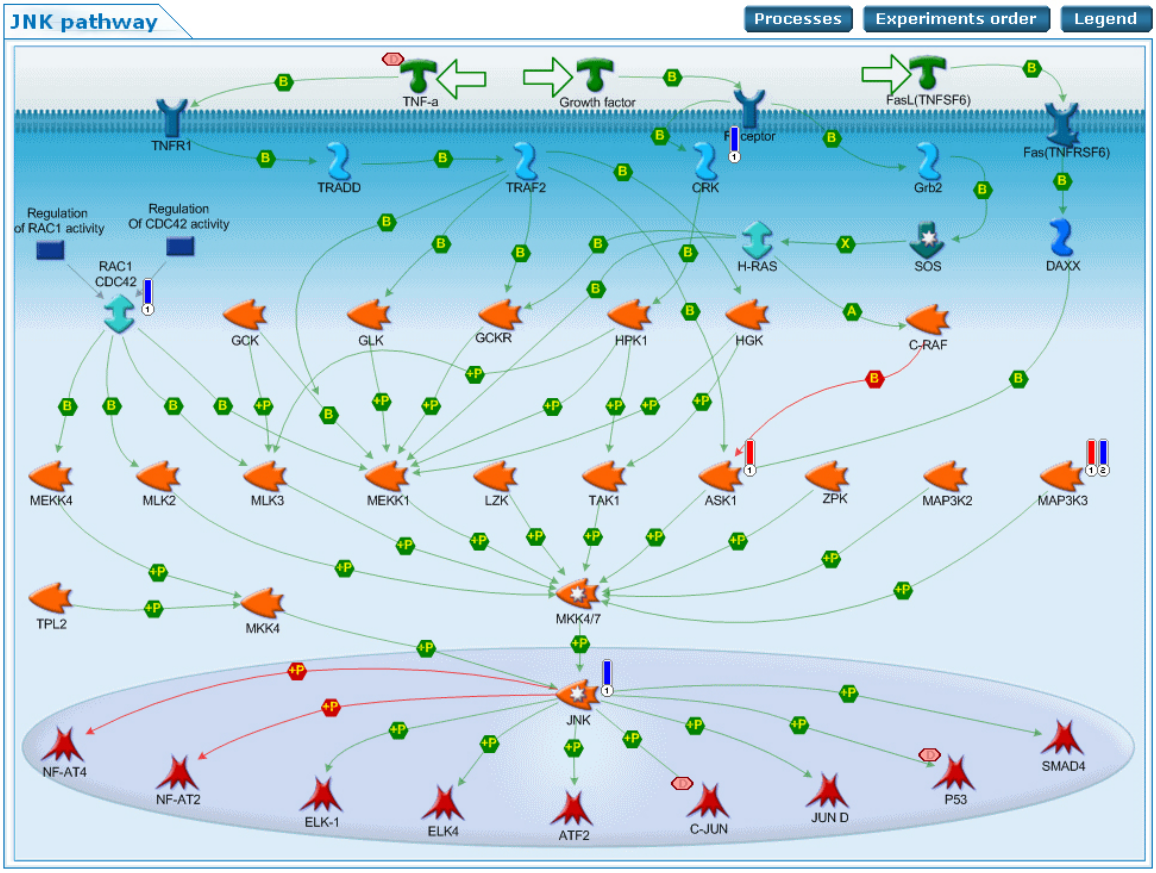
Supplementary Figure A.2.3. GeneGo MetaCore™ map showing interactions among genes from GeneSpring GX gene list involved in BAD phosphorylation.



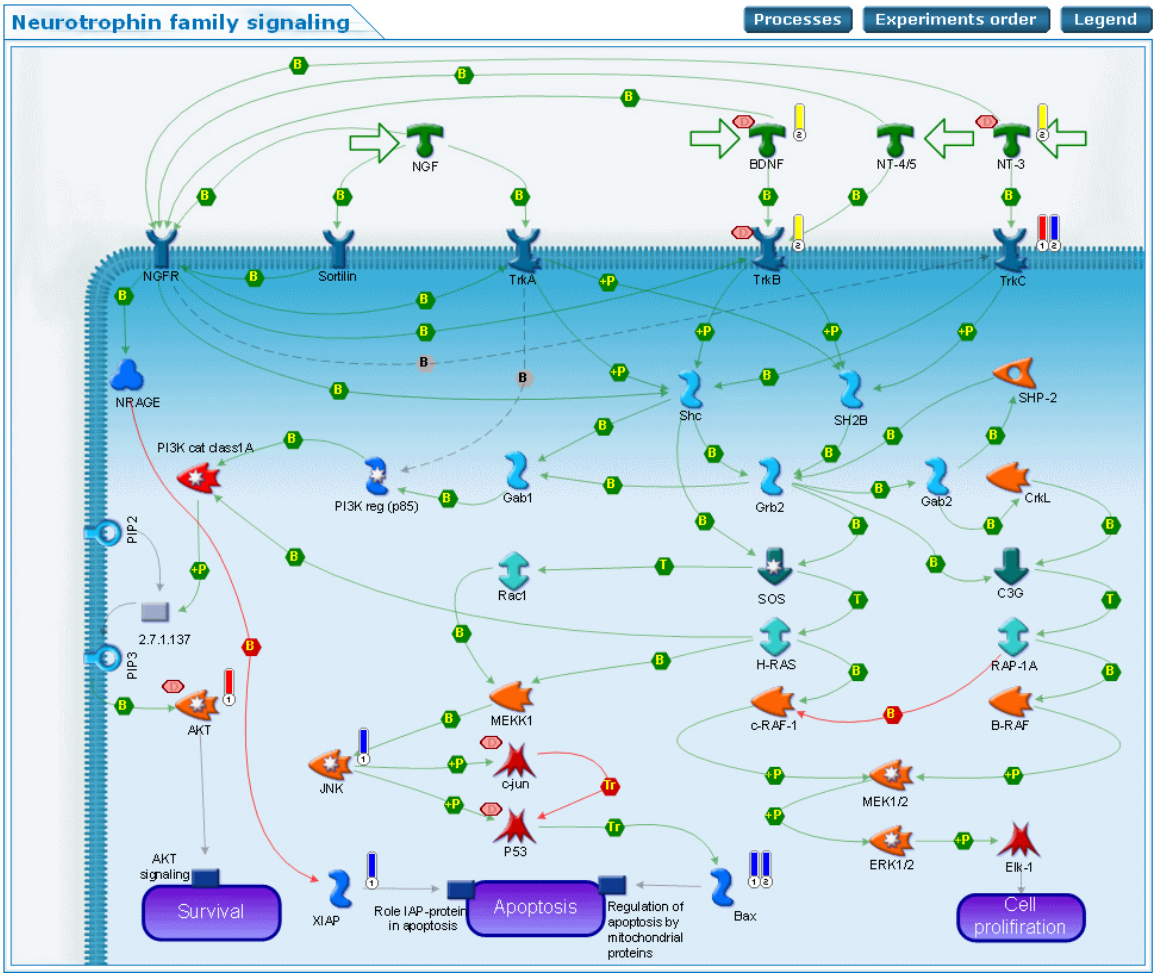
Supplementary Figure A.2.4. GeneGo MetaCore™ map showing interactions among genes from GeneSpring GX gene list involved in CDC42 in cellular processes.



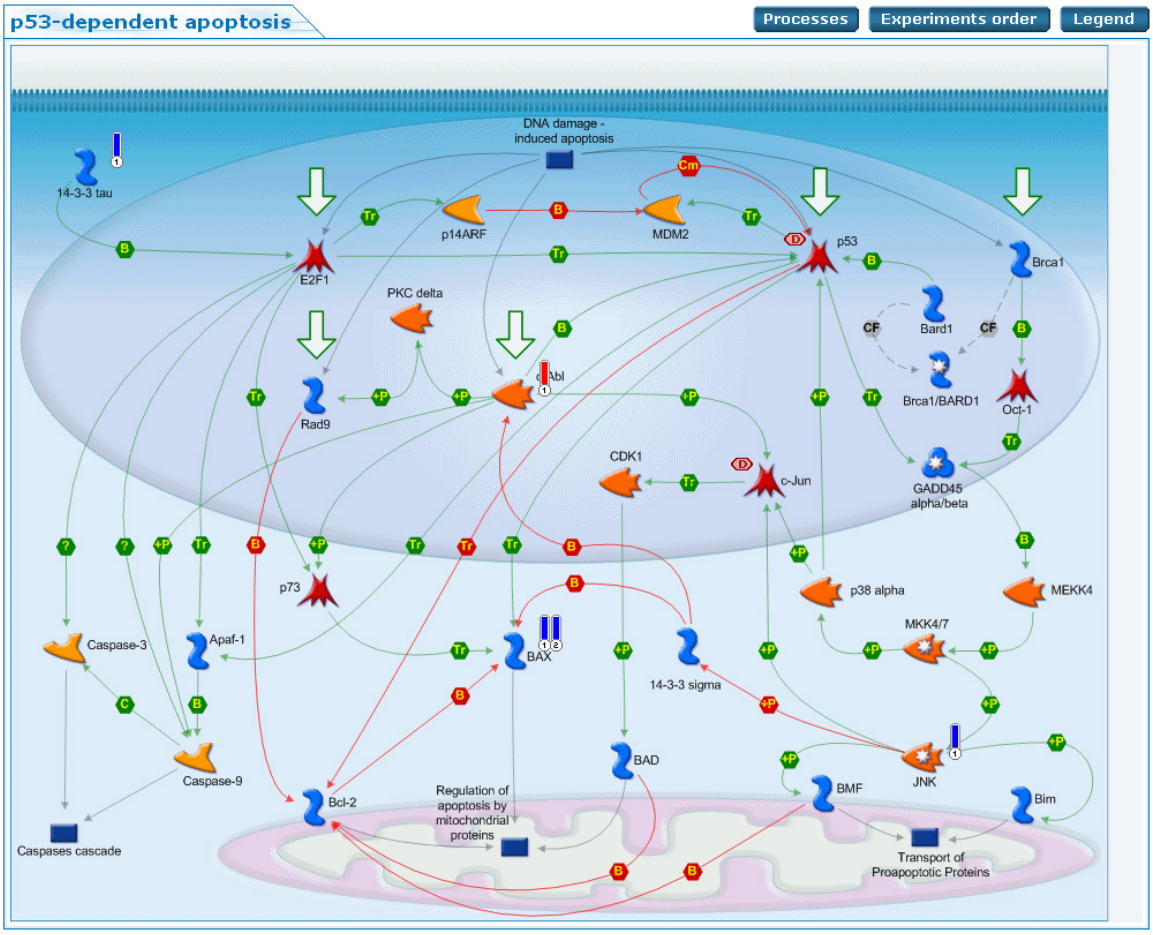
Supplementary Figure A.2.5. GeneGo MetaCore™ map showing interactions among genes from GeneSpring GX gene list involved in G-protein mediated regulation of p38 and JNK signaling.



Supplementary Figure A.2.6. GeneGo MetaCore™ map showing interactions among genes from GeneSpring GX gene list involved in the JNK pathway.

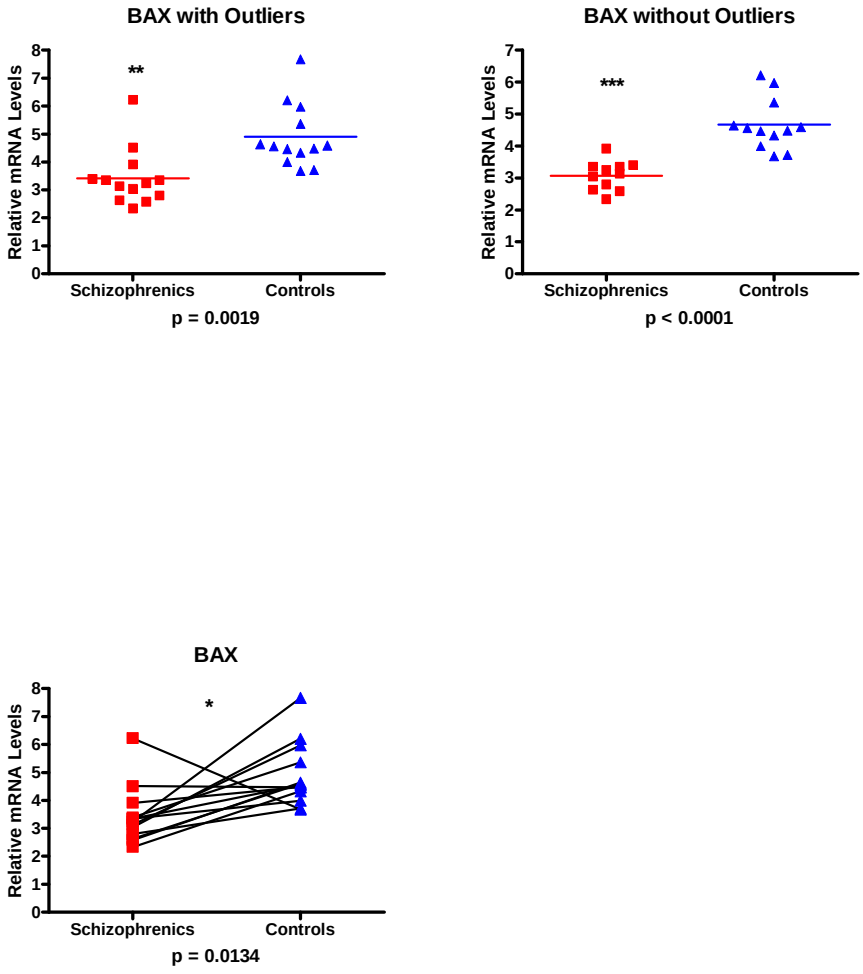


Supplementary Figure A.2.7. GeneGo MetaCore™ map showing interactions among genes from GeneSpring GX gene list involved in neurotrophin family signaling.



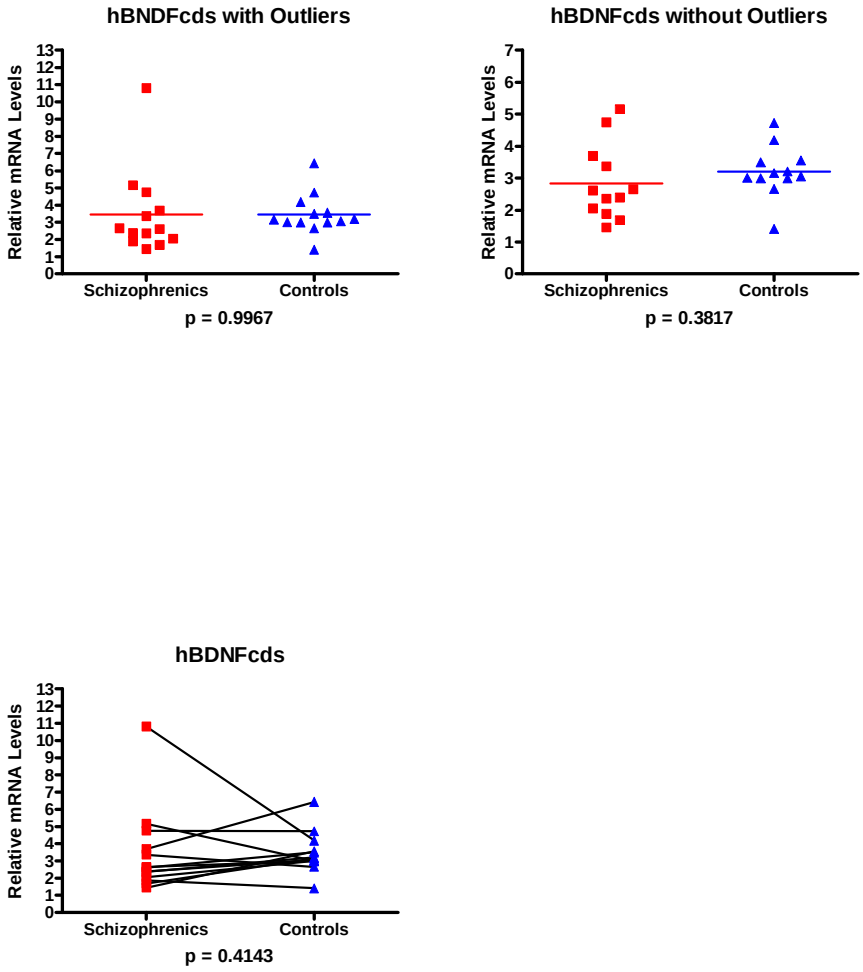
Supplementary Figure A.2.8. GeneGo MetaCore™ map showing interactions among genes from GeneSpring GX gene list involved in p53-dependent apoptosis.

BAX Graphs - Patients with Schizophrenia



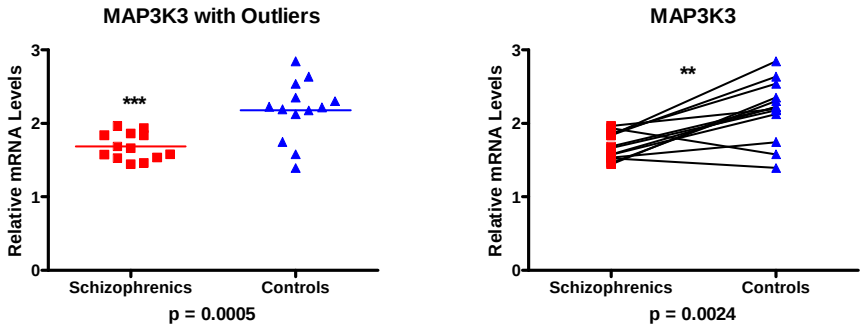
Supplementary Figure A.2.9. Analysis of BAX gene expression by SYBR® Green qRT-PCR. Data is represented as unpaired with outliers and unpaired without outliers (analysis by *t* test), and pair matched (analysis by Wilcoxon Signed Rank test). ***p<0.01, ***p<0.001*.

hBDNFcds Graphs - Patients with Schizophrenia



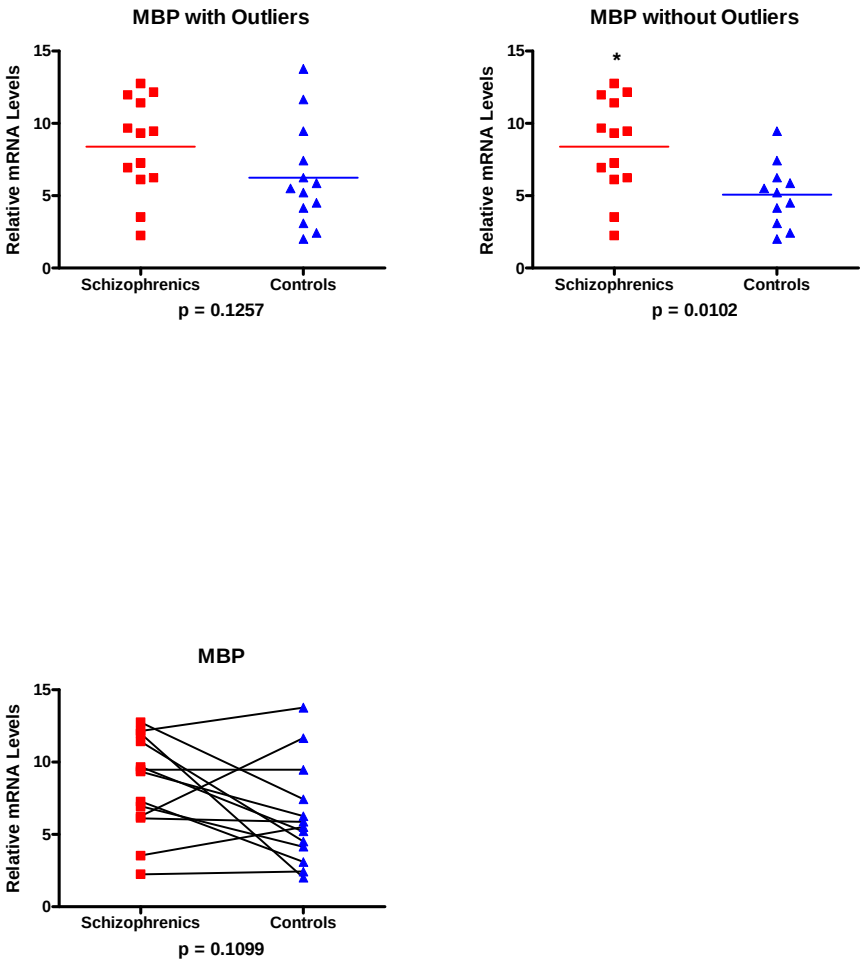
Supplementary Figure A.2.10. Analysis of hBDNFcds gene expression by SYBR® Green qRT-PCR. Data is represented as unpaired with outliers and unpaired without outliers (analysis by *t* test), and pair matched (analysis by Wilcoxon Signed Rank test).

MAP3K3 Graphs - Patients with Schizophrenia



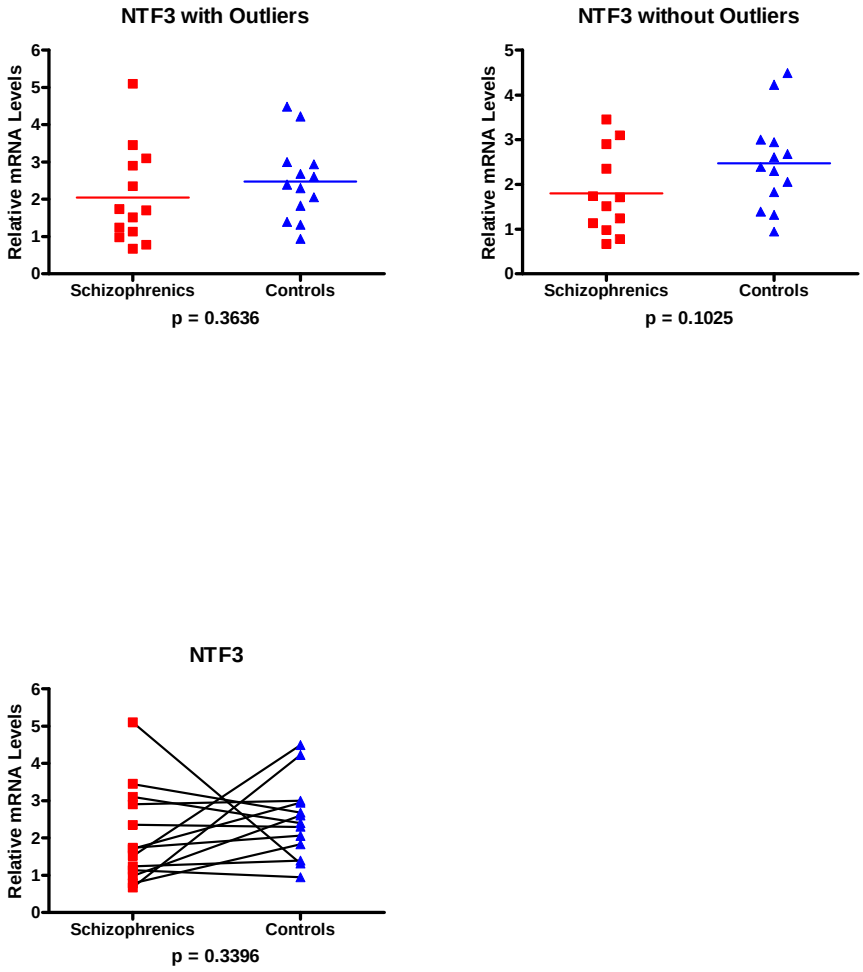
Supplementary Figure A.2.11. Analysis of MAP3K3 gene expression by SYBR® Green qRT-PCR. Data is represented as unpaired with outliers (analysis by *t* test) and pair matched (analysis by Wilcoxon Signed Rank test). ***p<0.01, ***p<0.001*.

MBP Graphs - Patients with Schizophrenia



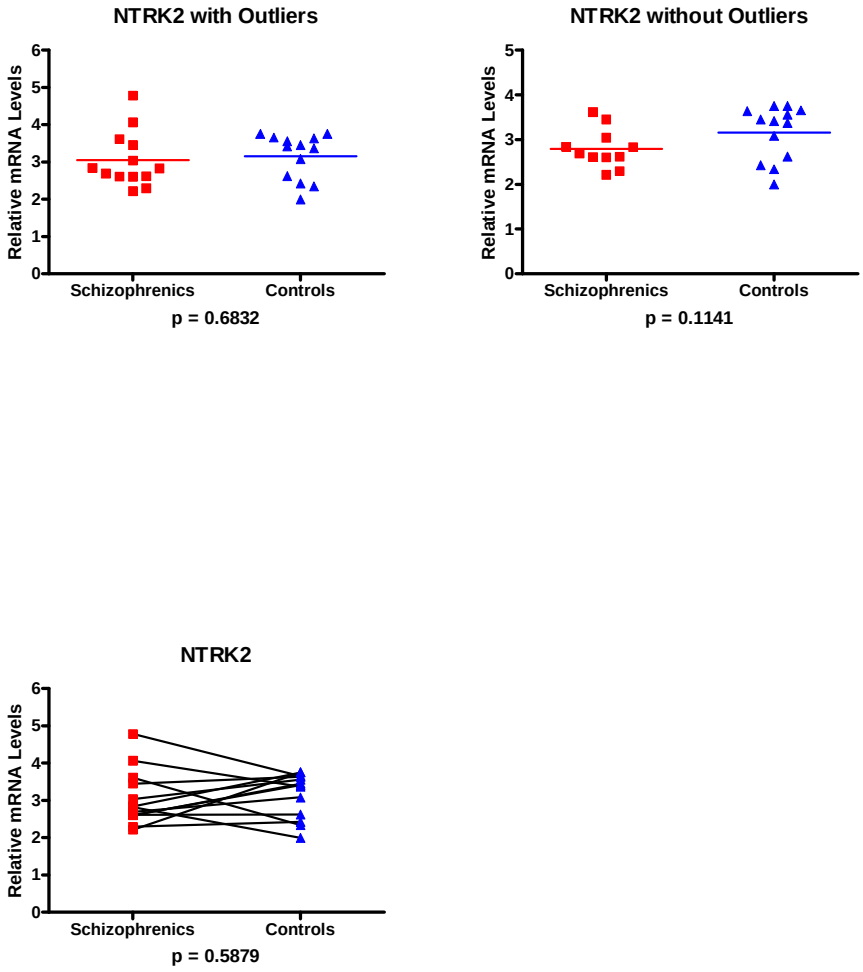
Supplementary Figure A.2.12. Analysis of MBP gene expression by SYBR® Green qRT-PCR. Data is represented as unpaired with outliers and unpaired without outliers (analysis by *t* test), and pair matched (analysis by Wilcoxon Signed Rank test). *$p<0.05$.

NF3 Graphs - Patients with Schizophrenia



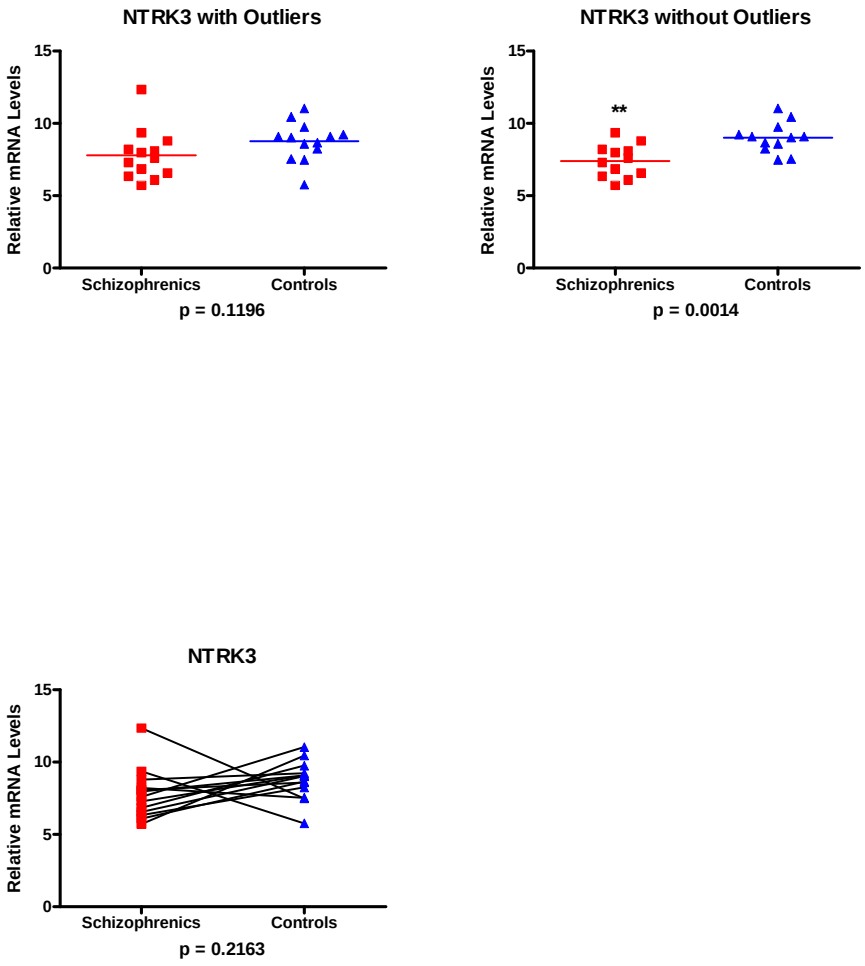
Supplementary Figure A.2.13. Analysis of NF-3 gene expression by SYBR® Green qRT-PCR. Data is represented as unpaired with outliers and unpaired without outliers (analysis by *t* test), and pair matched (analysis by Wilcoxon Signed Rank test).

TrkB Graphs - Patients with Schizophrenia



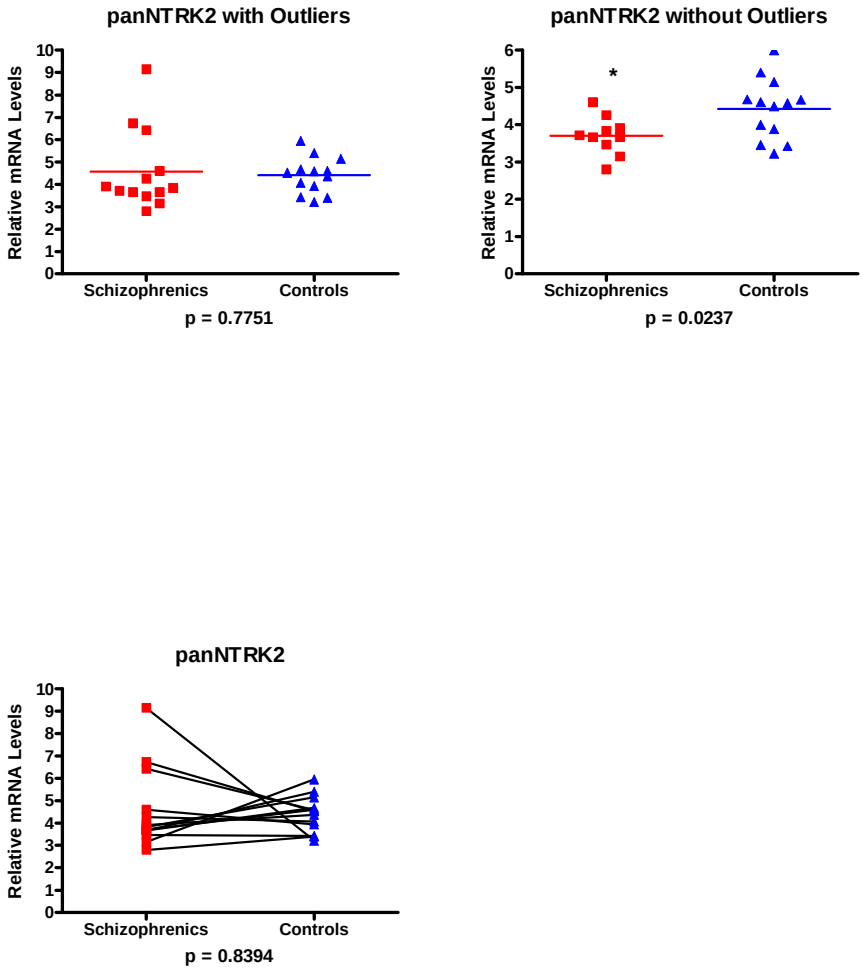
Supplementary Figure A.2.14. Analysis of TrkB gene expression by SYBR® Green qRT-PCR. Data is represented as unpaired with outliers and unpaired without outliers (analysis by *t* test), and pair matched (analysis by Wilcoxon Signed Rank test).

TrkC Graphs - Patients with Schizophrenia



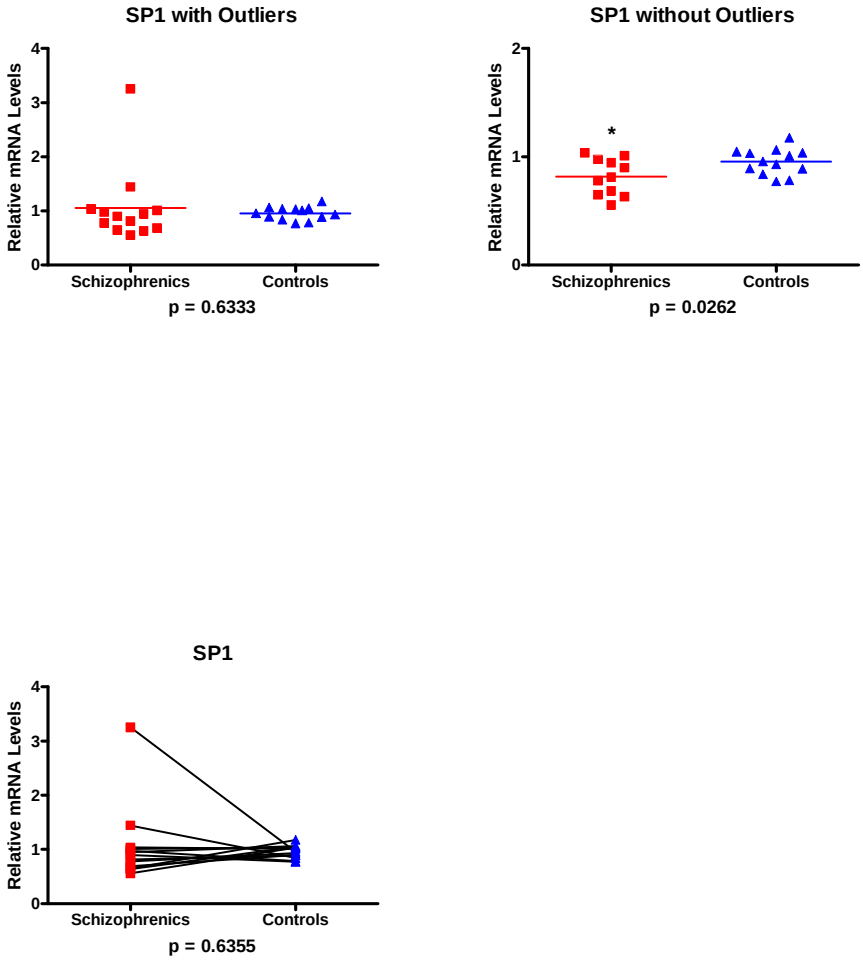
Supplementary Figure A.2.15. Analysis of TrkC gene expression by SYBR® Green qRT-PCR. Data is represented as unpaired with outliers and unpaired without outliers (analysis by *t* test), and pair matched (analysis by Wilcoxon Signed Rank test). ***p<0.01*.

panTrkB Graphs - Patients with Schizophrenia



Supplementary Figure A.2.16. Analysis of panTrkB gene expression by SYBR® Green qRT-PCR. Data is represented as unpaired with outliers and unpaired without outliers (analysis by *t* test), and pair matched (analysis by Wilcoxon Signed Rank test).

SP1 Graphs - Patients with Schizophrenia

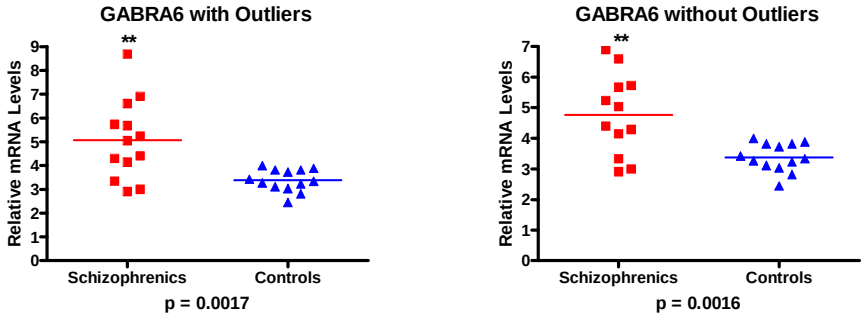


Supplementary Figure A.2.17. Analysis of SP1 gene expression by SYBR® Green qRT-PCR. Data is represented as unpaired with outliers and unpaired without outliers (analysis by *t* test), and pair matched (analysis by Wilcoxon Signed Rank test). **p<0.05.*

Appendix A.3

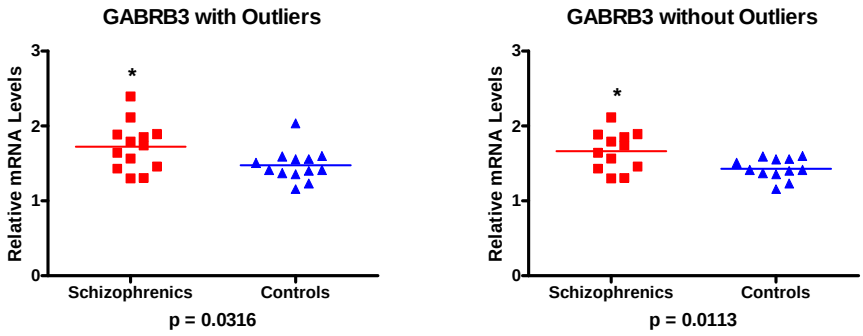
This appendix consists of unpaired qRT-PCR graphs from GABAergic markers, NMDA receptor subunits, and cerebellar neuromodulators of GABAergic release. All of these analyses were performed using t tests and show the same results as the non-parametric pair-matched data shown in the text (Figures 3.1 – 3.3) with the exception of $GABA_A$-β3, which is significantly increased using this analysis. Data is shown as average of gene expression levels of the gene of interest in patients with schizophrenia versus comparison subjects. Analyses with statistical outliers ($\pm$2 SD) and without statistical outliers are both shown.

$GABA_A$-α6 Graphs - Patients with Schizophrenia



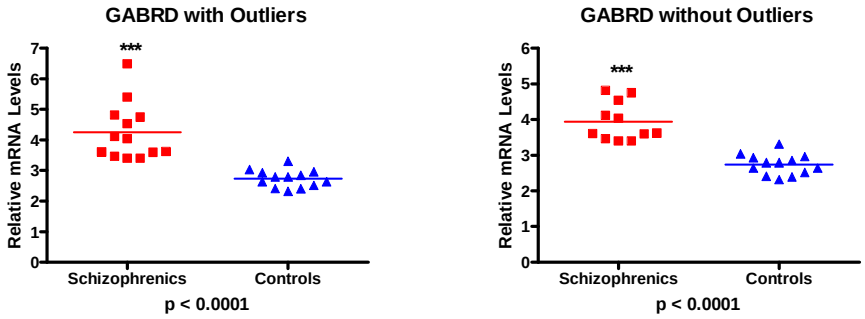
Supplementary Figure A.3.1. Analysis of $GABA_A$-α6 gene expression by TaqMan® qRT-PCR. Data is represented as unpaired with outliers and unpaired without outliers (analysis by *t* test). ***p<0.01*.

$GABA_A$-β3 Graphs - Patients with Schizophrenia



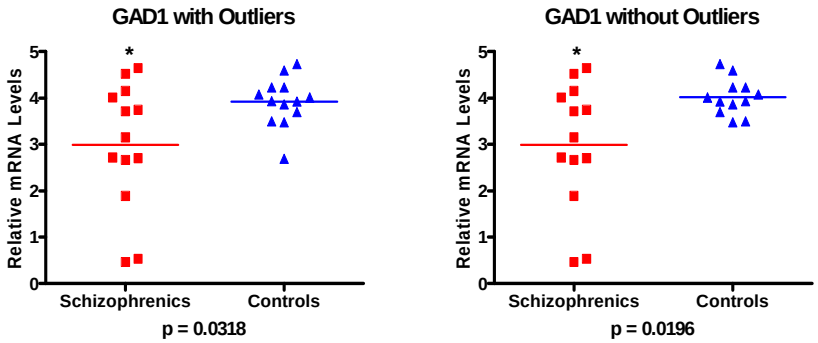
Supplementary Figure A.3.2. Analysis of $GABA_A$-β3 gene expression by SYBR® Green qRT-PCR. Data is represented as unpaired with outliers and unpaired without outliers (analysis by *t* test). **p<0.05.*

$GABA_A$-δ Graphs - Patients with Schizophrenia



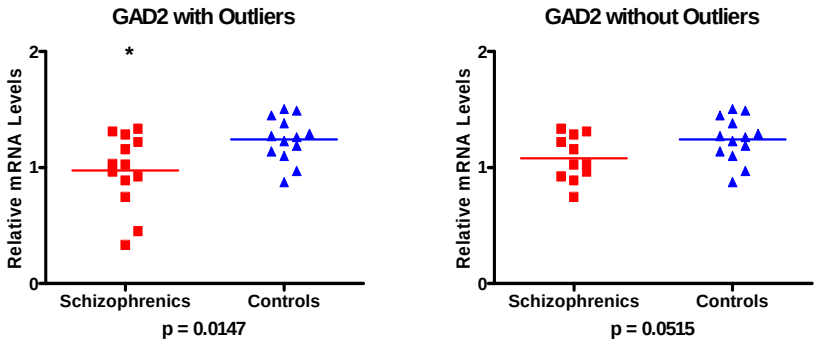
Supplementary Figure A.3.3. Analysis of $GABA_A$-δ gene expression by TaqMan® qRT-PCR. Data is represented as unpaired with outliers and unpaired without outliers (analysis by *t* test). ***p<0.01, ***p<0.001.*

GAD_{67} vs. β-actin Graphs - Patients with Schizophrenia



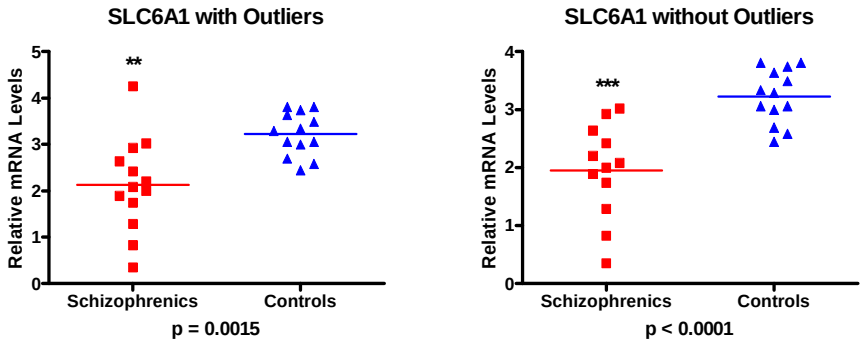
Supplementary Figure A.3.4. Analysis of GAD_{67} gene expression by SYBR® Green qRT-PCR. Data is represented as unpaired with outliers and unpaired without outliers (analysis by *t* test). **p<0.05*.

GAD_{65} vs. β-actin Graphs - Patients with Schizophrenia



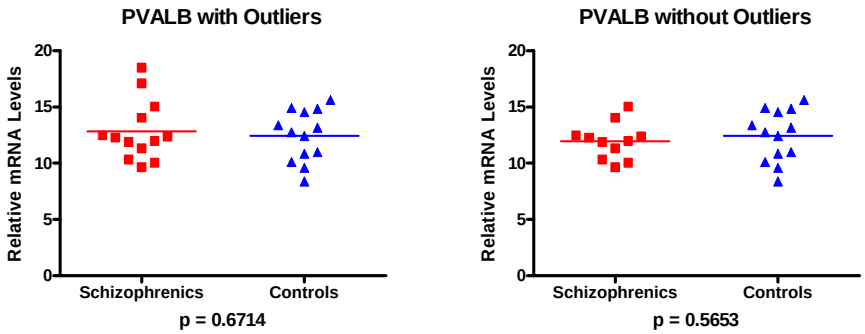
Supplementary Figure A.3.5. Analysis of GAD_{65} gene expression by SYBR® Green qRT-PCR. Data is represented as unpaired with outliers and unpaired without outliers (analysis by *t* test). **p<0.05*.

GAT-1 Graphs - Patients with Schizophrenia



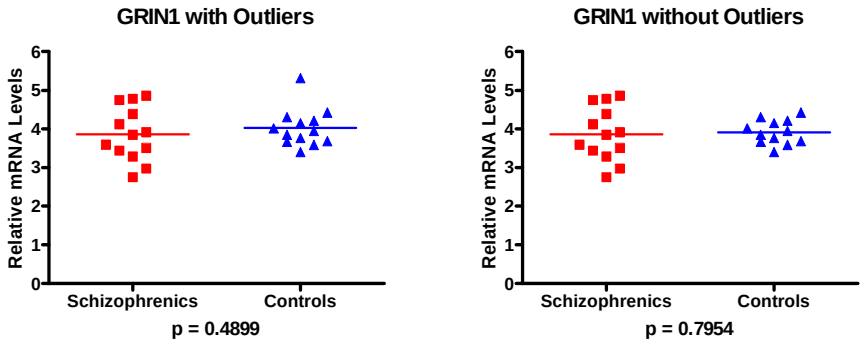
Supplementary Figure A.3.6. Analysis of GAT-1 gene expression by SYBR® Green qRT-PCR. Data is represented as unpaired with outliers and unpaired without outliers (analysis by *t* test). ***p<0.01, ***p<0.001.*

Parvalbumin Graphs - Patients with Schizophrenia



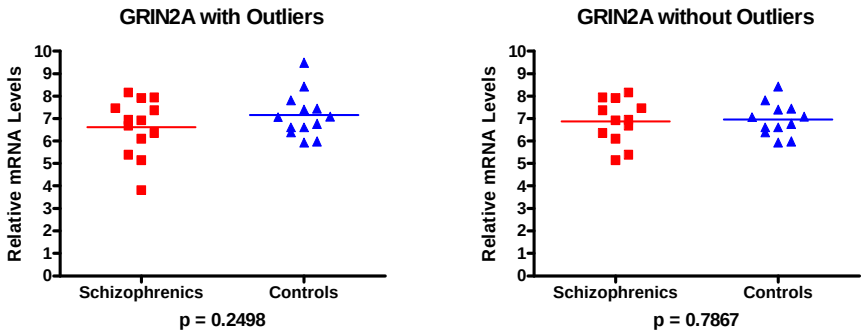
Supplementary Figure A.3.7. Analysis of Parvalbumin gene expression by SYBR® Green qRT-PCR. Data is represented as unpaired with outliers and unpaired without outliers (analysis by *t* test).

NR1 Graphs - Patients with Schizophrenia



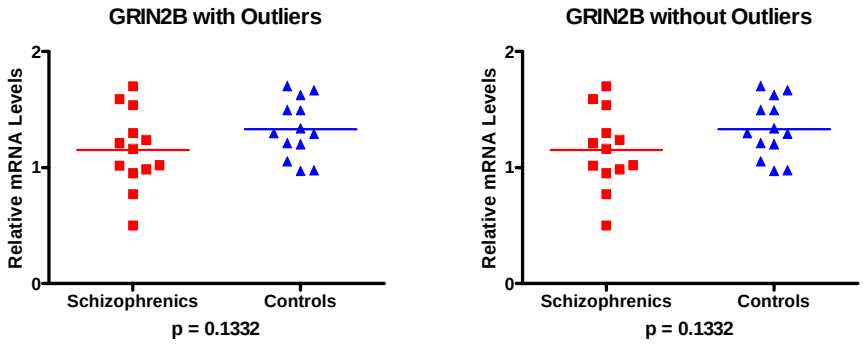
Supplementary Figure A.3.8. Analysis of NR1 gene expression by SYBR® Green qRT-PCR. Data is represented as unpaired with outliers and unpaired without outliers (analysis by *t* test).

NR2A Graphs - Patients with Schizophrenia



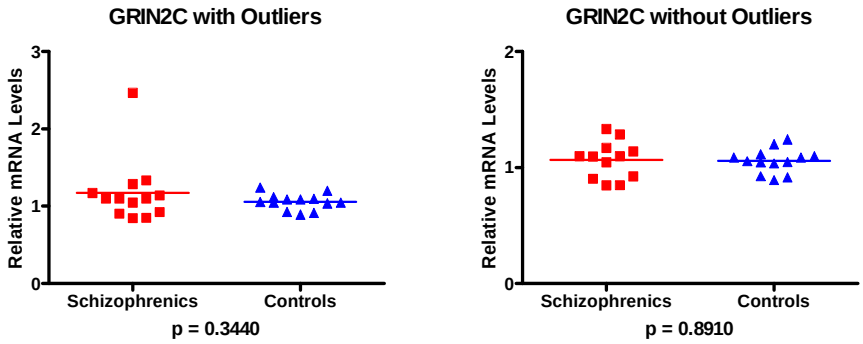
Supplementary Figure A.3.9. Analysis of NR2A gene expression by SYBR® Green qRT-PCR. Data is represented as unpaired with outliers and unpaired without outliers (analysis by *t* test).

NR2B Graphs - Patients with Schizophrenia



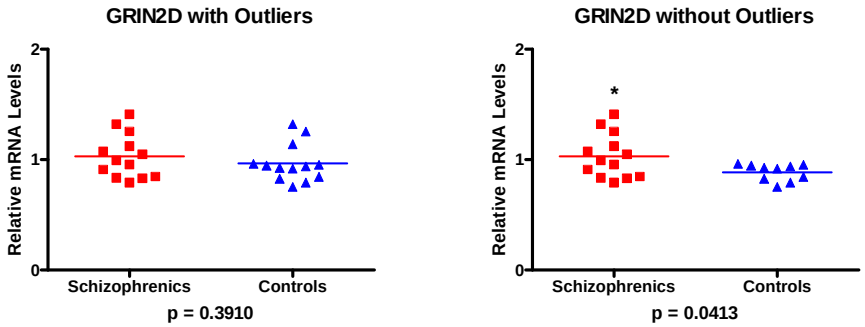
Supplementary Figure A.3.10. Analysis of NR2B gene expression by SYBR® Green qRT-PCR. Data is represented as unpaired with outliers and unpaired without outliers (analysis by *t* test).

NR2C Graphs - Patients with Schizophrenia



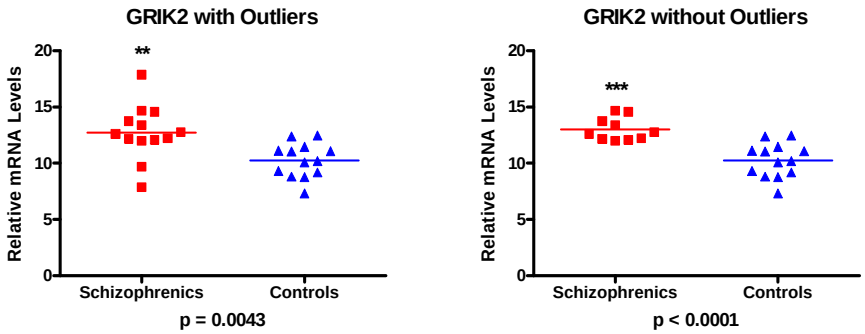
Supplementary Figure A.3.11. Analysis of NR2C gene expression by SYBR® Green qRT-PCR. Data is represented as unpaired with outliers and unpaired without outliers (analysis by *t* test).

NR2D Graphs - Patients with Schizophrenia



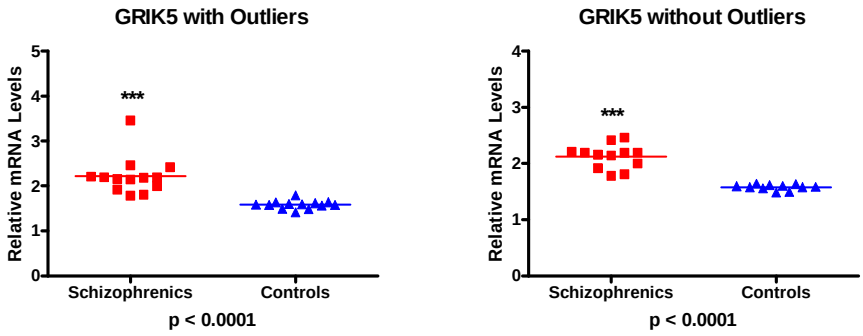
Supplementary Figure A.3.12. Analysis of NR2D gene expression by SYBR® Green qRT-PCR. Data is represented as unpaired with outliers and unpaired without outliers (analysis by *t* test). **p<0.05*.

GluR6 Graphs - Patients with Schizophrenia



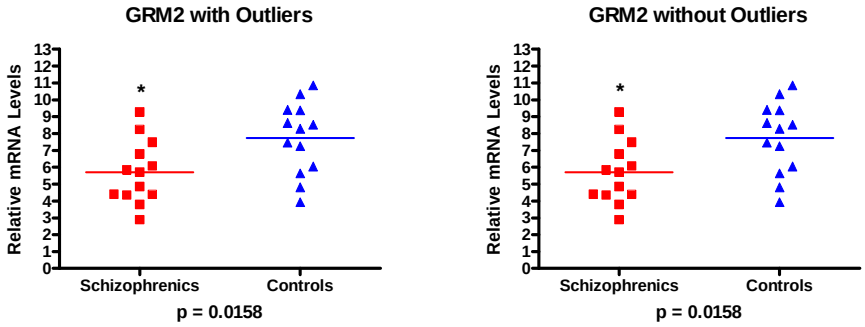
Supplementary Figure A.3.13. Analysis of GluR6 gene expression by SYBR® Green qRT-PCR. Data is represented as unpaired with outliers and unpaired without outliers (analysis by *t* test). ***p<0.01, ***p<0.001.*

KA2 Graphs - Patients withSchizophrenia



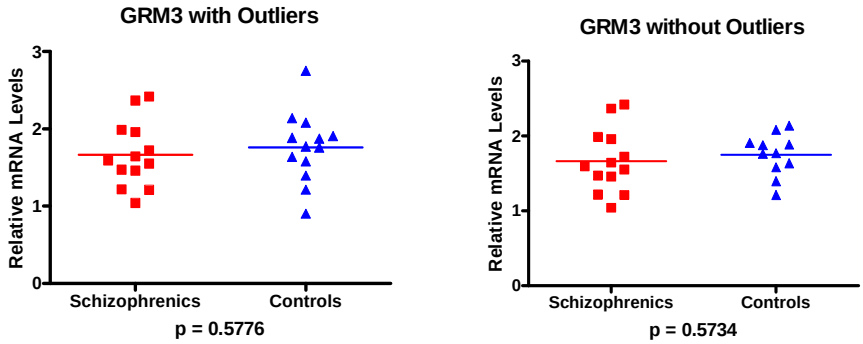
Supplementary Figure A.3.14. Analysis of KA2 gene expression by SYBR® Green qRT-PCR. Data is represented as unpaired with outliers and unpaired without outliers (analysis by *t* test). ****p<0.001*.

mGluR2 Graphs - Patients with Schizophrenia



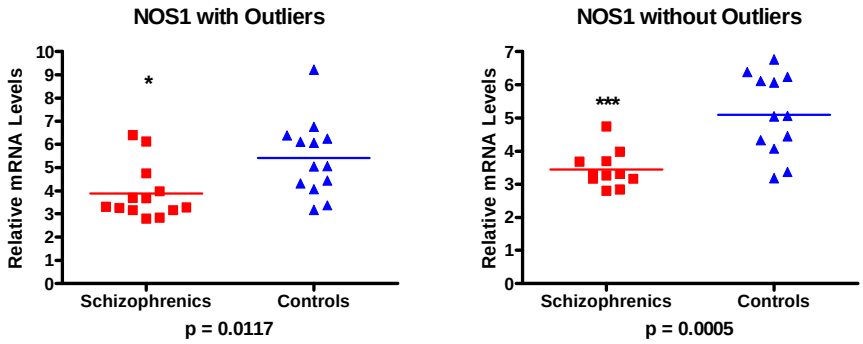
Supplementary Figure A.3.15. Analysis of mGluR2 gene expression by SYBR® Green qRT-PCR. Data is represented as unpaired with outliers and unpaired without outliers (analysis by *t* test). **p<0.05*.

mGluR3 Graphs - Patients with Schizophrenia



Supplementary Figure A.3.16. Analysis of mGluR3 gene expression by SYBR® Green qRT-PCR. Data is represented as unpaired with outliers and unpaired without outliers (analysis by *t* test).

nNOS Graphs - Patients with Schizophrenia

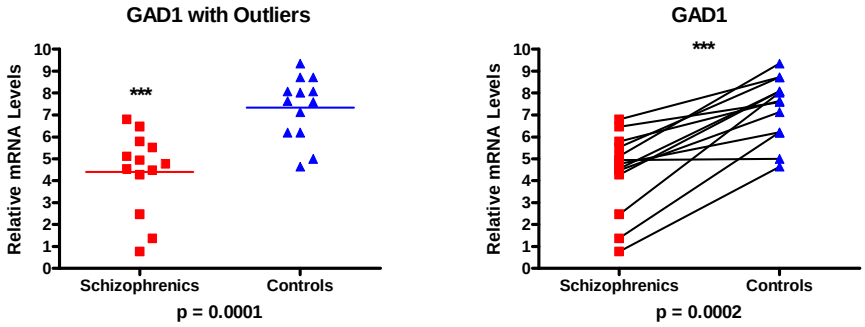


Supplementary Figure A.3.17. Analysis of nNOS gene expression by SYBR® Green qRT-PCR. Data is represented as unpaired with outliers and unpaired without outliers (analysis by *t* test). **p<0.05, ***p<0.001*.

Appendix A.4

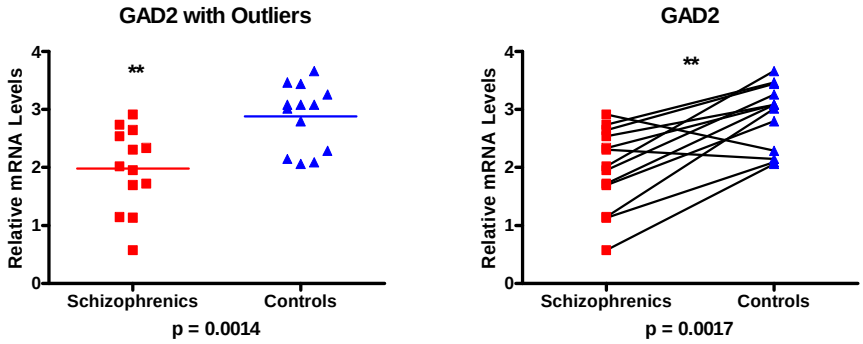
This appendix contains qRT-PCR analysis of GAD_{67}, GAD_{65}, and GAT-1 normalized to cyclophilin from patients with schizophrenia versus comparison subjects. Data was analyzed using both unpaired (analyzed by *t*-tests) and pair-matched (analyzed by non-parametric Wilcoxson Signed Rank test). Data derived from normalization to cyclophilin was similar to data derived from normalization to β-actin.

GAD_{67} vs. Cyclophilin Graphs - Patients with Schizophrenia



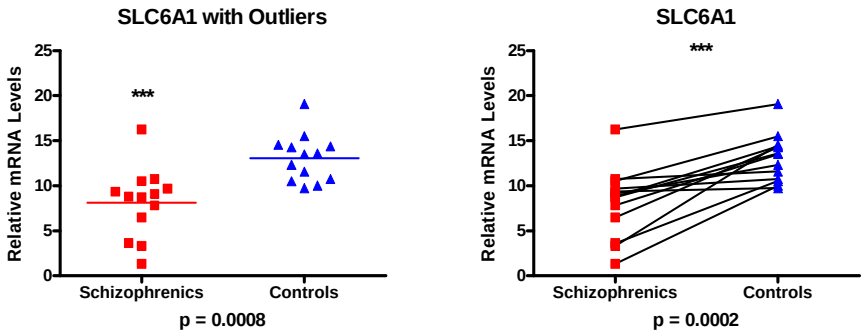
Supplementary Figure A.4.1. Analysis of GAD_{67} gene expression by SYBR® Green qRT-PCR normalized to cyclophilin. Data is represented as unpaired with outliers and unpaired without outliers (analysis by *t* test). ****p<0.001*.

GAD_{65} vs. Cyclophilin Graphs - Patients with Schizophrenia



Supplementary Figure A.4.2. Analysis of GAD_{65} gene expression by SYBR® Green qRT-PCR normalized to cyclophilin. Data is represented as unpaired with outliers and unpaired without outliers (analysis by *t* test). ***p<0.01*.

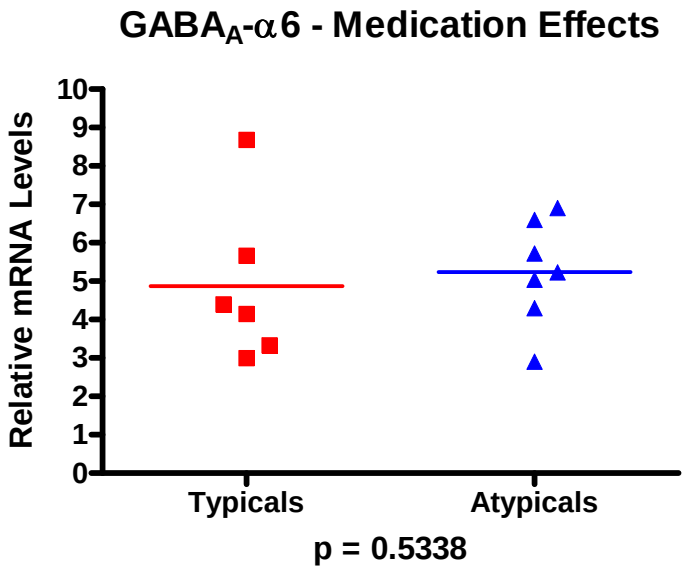
GAT-1 vs. Cyclophilin Graphs - Patients with Schizophrenia



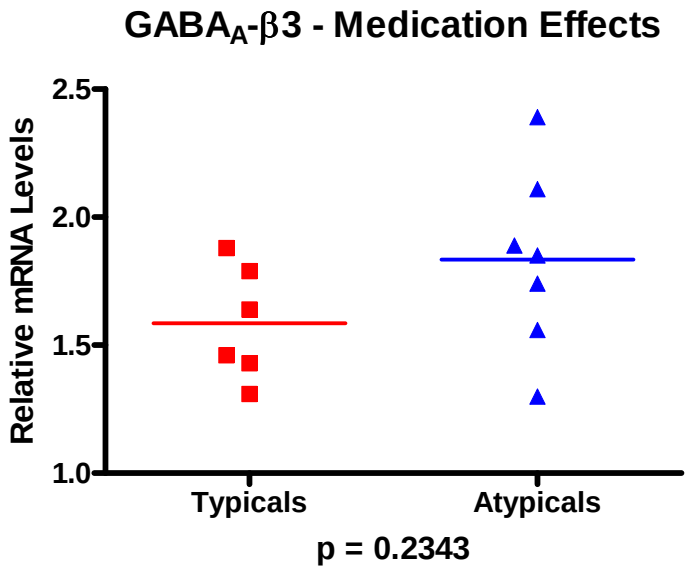
Supplementary Figure A.4.3. Analysis of GAT-1 gene expression by SYBR® Green qRT-PCR normalized to cyclophilin. Data is represented as unpaired with outliers and unpaired without outliers (analysis by *t* test). ****p<0.001*.

Appendix A.5

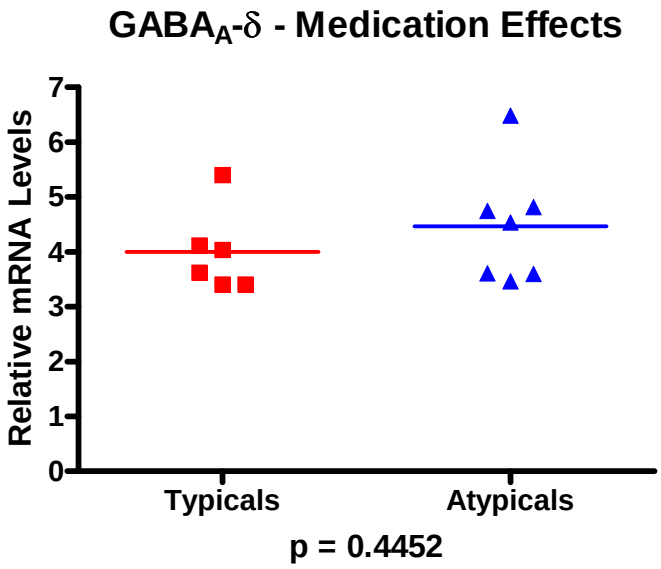
Data in this appendix is a comparison of gene expression data from patients who were receiving typical antipsychotics at the time of death versus those who were receiving atypical antipsychotics (Table 2.1). Significant changes were seen in the expression of GAD67 (Supplementary Figure A.5.4), NR2A (Supplementary Figure A.5.9), and SP1 (Supplementary Figure A.5.23). Data were analyzed using non-parametric Mann-Whitney Rank Sum test to account for non-parametric distribution of the data.



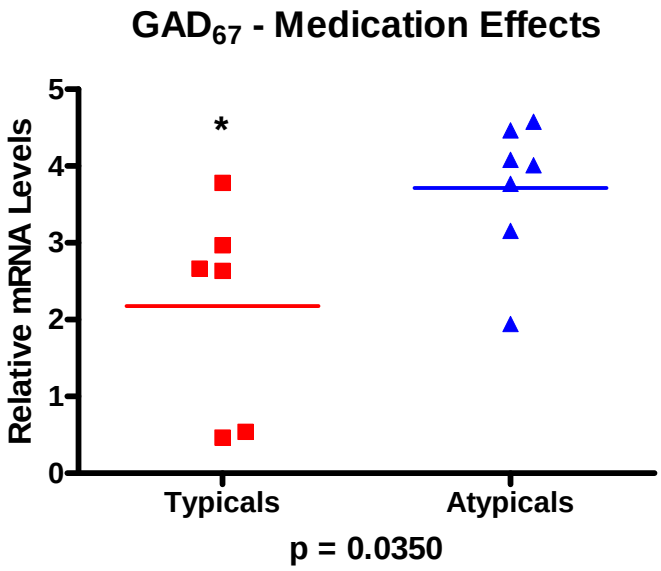
Supplementary Figure A.5.1. Medication subtype analysis of $GABA_A$-α6 gene expression by TaqMan® qRT-PCR. Data is represented as patients receiving typical antipsychotics at the time of death versus those receiving atypical antipsychotics (analysis by Mann-Whitney Rank Sum test).



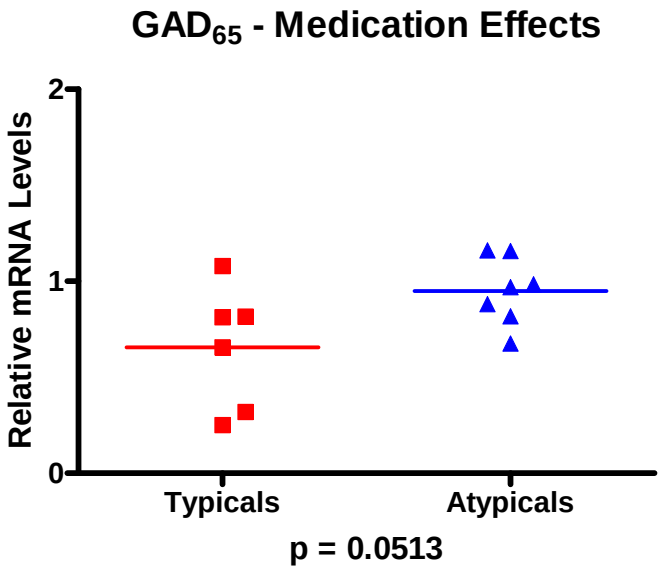
Supplementary Figure A.5.2. Medication subtype analysis of GABA$_A$-β3 gene expression by SYBR® Green qRT-PCR. Data is represented as patients receiving typical antipsychotics at the time of death versus those receiving atypical antipsychotics (analysis by Mann-Whitney Rank Sum test).



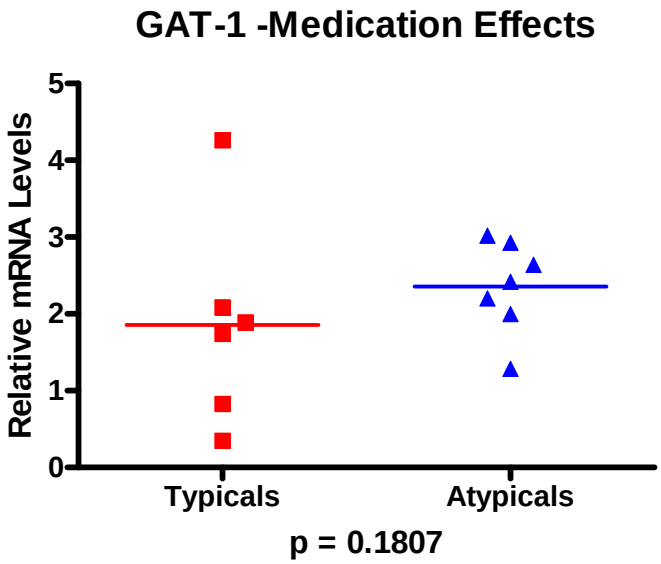
Supplementary Figure A.5.3. Medication subtype analysis of $GABA_A$-δ gene expression by TaqMan® qRT-PCR. Data is represented as patients receiving typical antipsychotics at the time of death versus those receiving atypical antipsychotics (analysis by Mann-Whitney Rank Sum test).



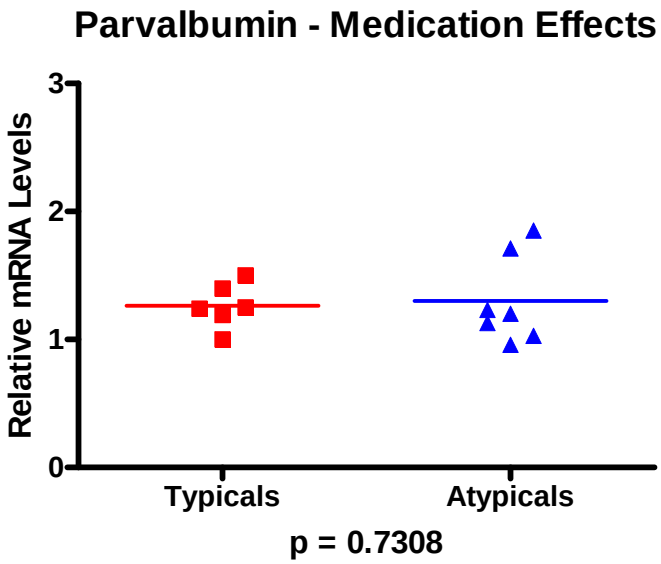
Supplementary Figure A.5.4. Medication subtype analysis of GAD_{67} gene expression by SYBR® Green qRT-PCR. Data is represented as patients receiving typical antipsychotics at the time of death versus those receiving atypical antipsychotics (analysis by Mann-Whitney Rank Sum test). *$p<0.05$.



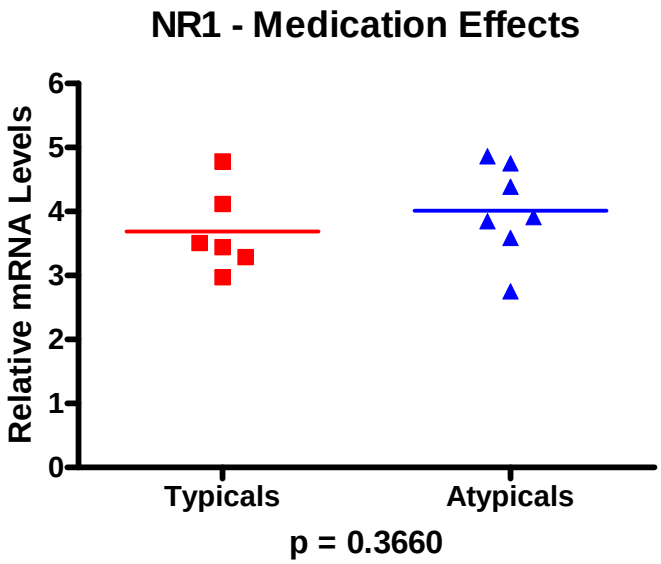
Supplementary Figure A.5.5. Medication subtype analysis of GAD_{65} gene expression by SYBR® Green qRT-PCR. Data is represented as patients receiving typical antipsychotics at the time of death versus those receiving atypical antipsychotics (analysis by Mann-Whitney Rank Sum test).



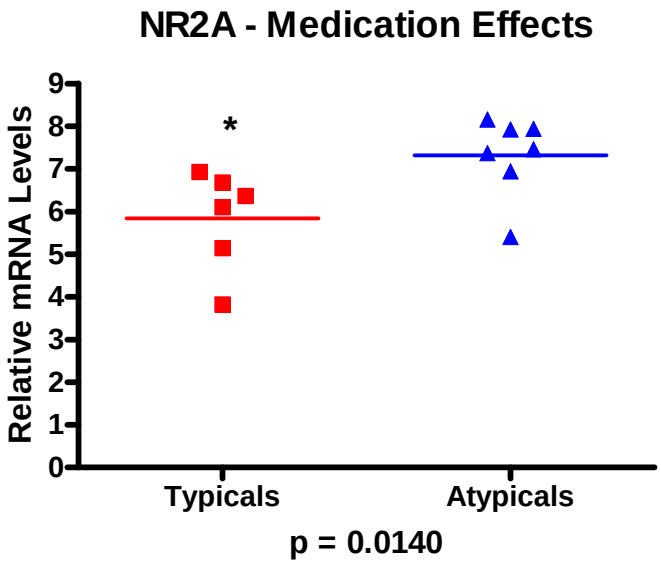
Supplementary Figure A.5.6. Medication subtype analysis of GAT-1 gene expression by SYBR® Green qRT-PCR. Data is represented as patients receiving typical antipsychotics at the time of death versus those receiving atypical antipsychotics (analysis by Mann-Whitney Rank Sum test).



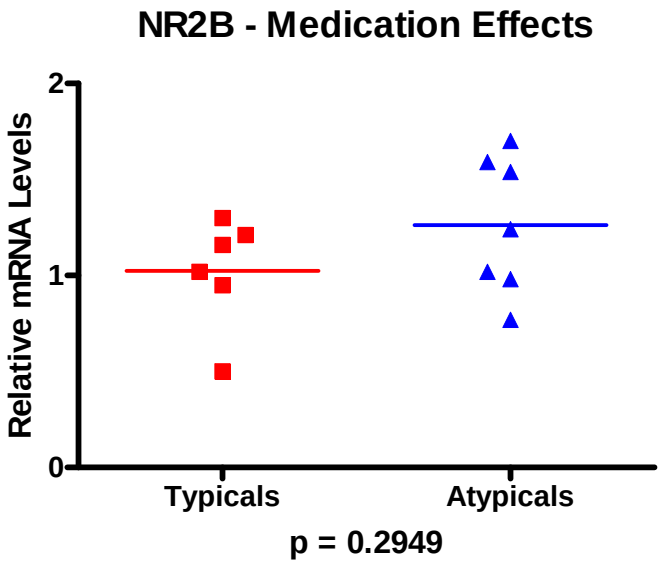
Supplementary Figure A.5.7. Medication subtype analysis of Parvalbumin gene expression by SYBR® Green qRT-PCR. Data is represented as patients receiving typical antipsychotics at the time of death versus those receiving atypical antipsychotics (analysis by Mann-Whitney Rank Sum test).



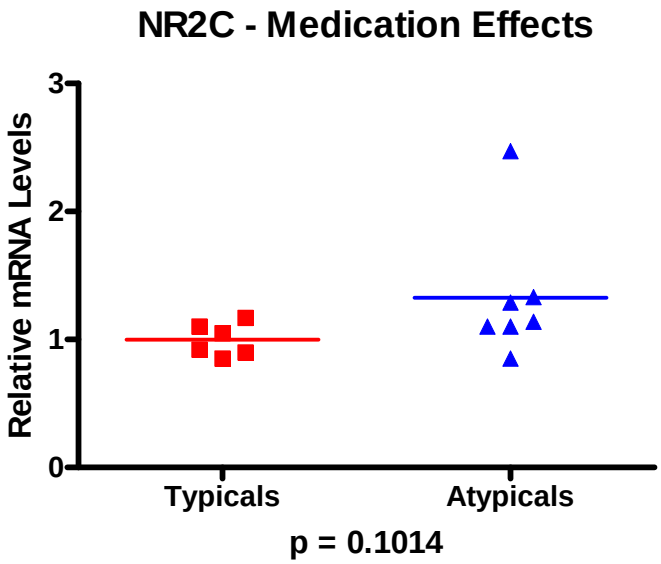
Supplementary Figure A.5.8. Medication subtype analysis of NR1 gene expression by SYBR® Green qRT-PCR. Data is represented as patients receiving typical antipsychotics at the time of death versus those receiving atypical antipsychotics (analysis by Mann-Whitney Rank Sum test).



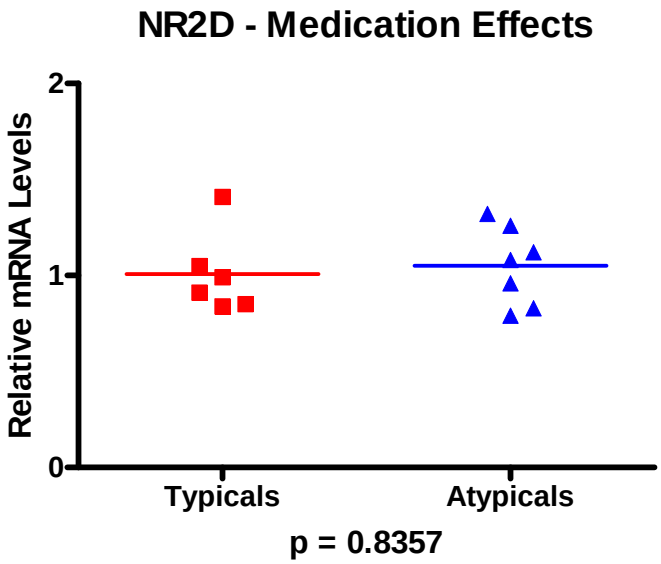
Supplementary Figure A.5.9. Medication subtype analysis of NR2A gene expression by SYBR® Green qRT-PCR. Data is represented as patients receiving typical antipsychotics at the time of death versus those receiving atypical antipsychotics (analysis by Mann-Whitney Rank Sum test). *$p<0.05$.



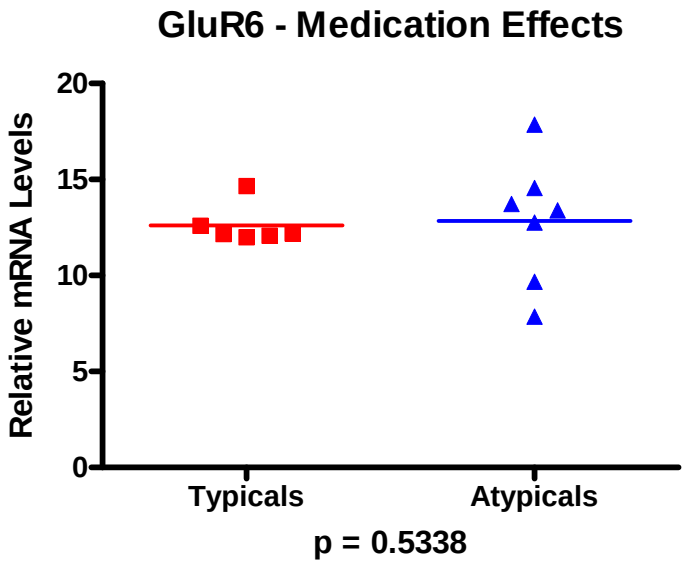
Supplementary Figure A.5.10. Medication subtype analysis of NR2B gene expression by SYBR® Green qRT-PCR. Data is represented as patients receiving typical antipsychotics at the time of death versus those receiving atypical antipsychotics (analysis by Mann-Whitney Rank Sum test).



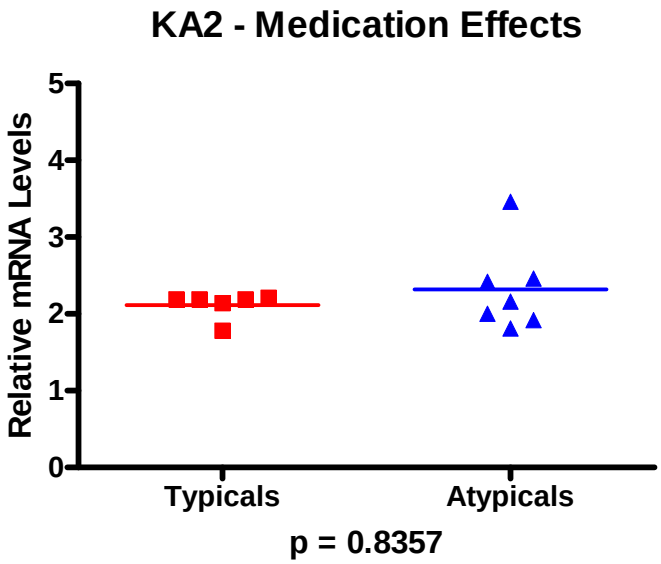
Supplementary Figure A.5.11. Medication subtype analysis of NR2C gene expression by SYBR® Green qRT-PCR. Data is represented as patients receiving typical antipsychotics at the time of death versus those receiving atypical antipsychotics (analysis by Mann-Whitney Rank Sum test).



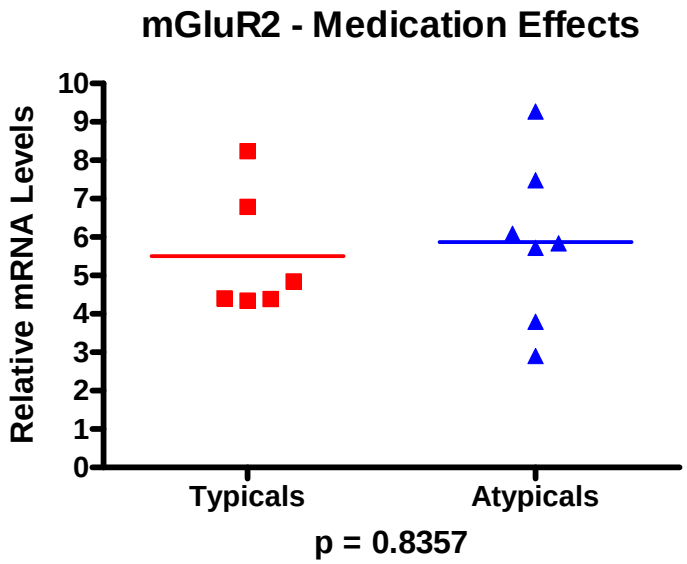
Supplementary Figure A.5.12. Medication subtype analysis of NR2D gene expression by SYBR® Green qRT-PCR. Data is represented as patients receiving typical antipsychotics at the time of death versus those receiving atypical antipsychotics (analysis by Mann-Whitney Rank Sum test).



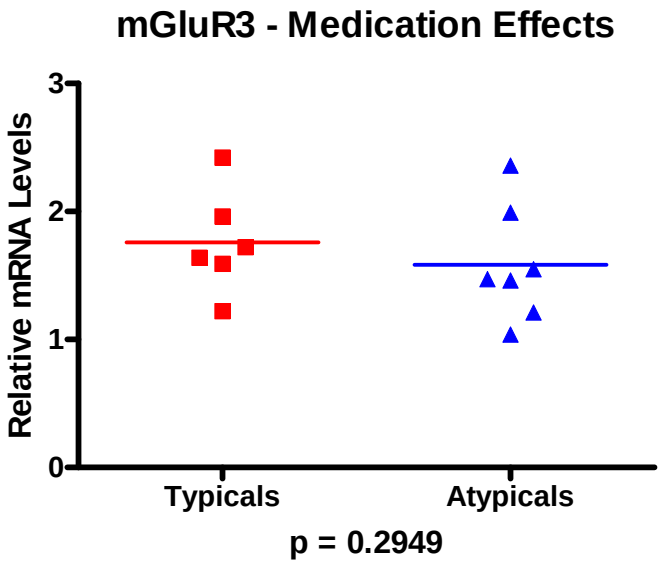
Supplementary Figure A.5.13. Medication subtype analysis of GluR6 gene expression by SYBR® Green qRT-PCR. Data is represented as patients receiving typical antipsychotics at the time of death versus those receiving atypical antipsychotics (analysis by Mann-Whitney Rank Sum test).



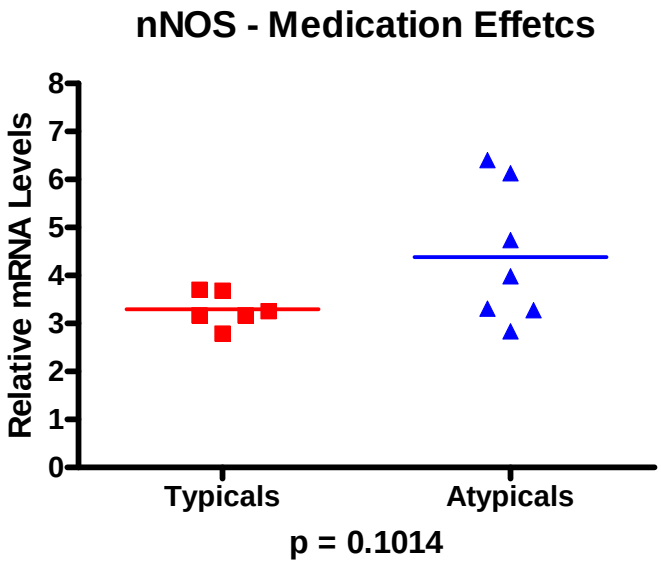
Supplementary Figure A.5.14. Medication subtype analysis of KA2 gene expression by SYBR® Green qRT-PCR. Data is represented as patients receiving typical antipsychotics at the time of death versus those receiving atypical antipsychotics (analysis by Mann-Whitney Rank Sum test).



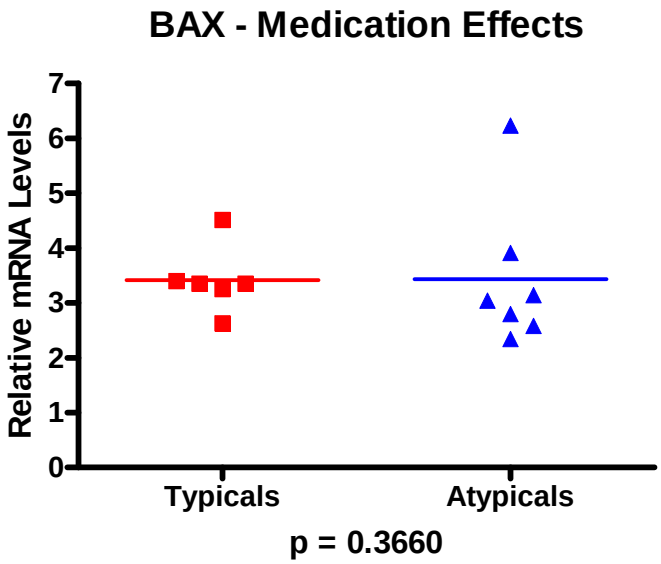
Supplementary Figure A.5.15. Medication subtype analysis of mGluR2 gene expression by SYBR® Green qRT-PCR. Data is represented as patients receiving typical antipsychotics at the time of death versus those receiving atypical antipsychotics (analysis by Mann-Whitney Rank Sum test).



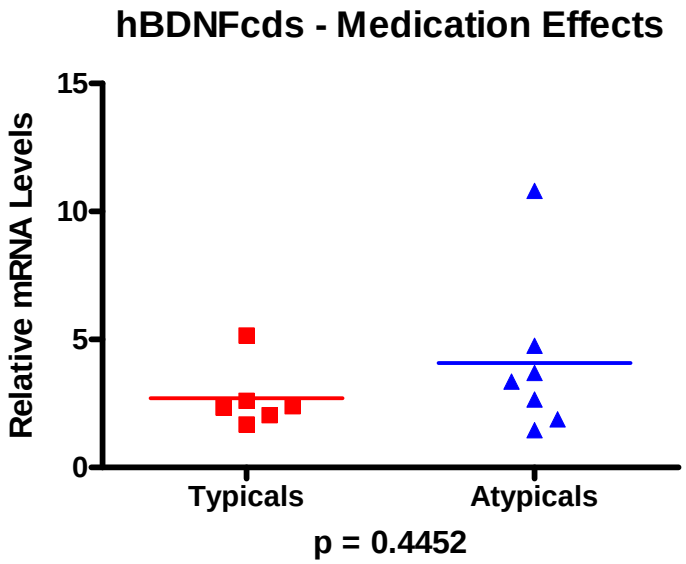
Supplementary Figure A.5.16. Medication subtype analysis of mGluR3 gene expression by SYBR® Green qRT-PCR. Data is represented as patients receiving typical antipsychotics at the time of death versus those receiving atypical antipsychotics (analysis by Mann-Whitney Rank Sum test).



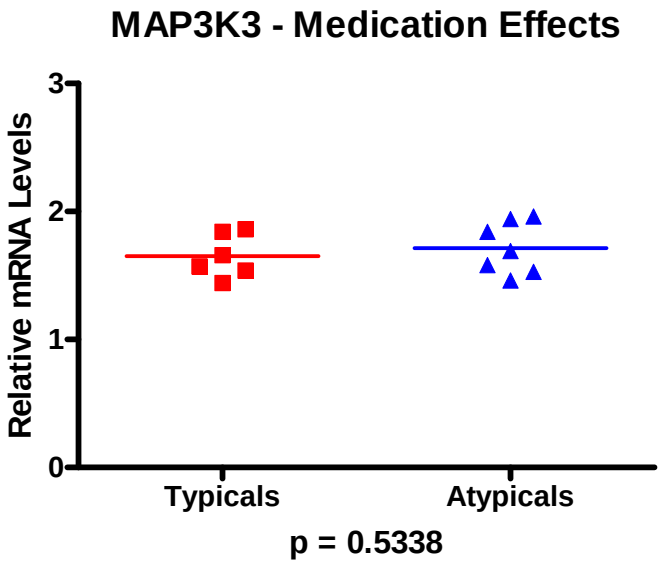
Supplementary Figure A.5.17. Medication subtype analysis of nNOS gene expression by SYBR® Green qRT-PCR. Data is represented as patients receiving typical antipsychotics at the time of death versus those receiving atypical antipsychotics (analysis by Mann-Whitney Rank Sum test).



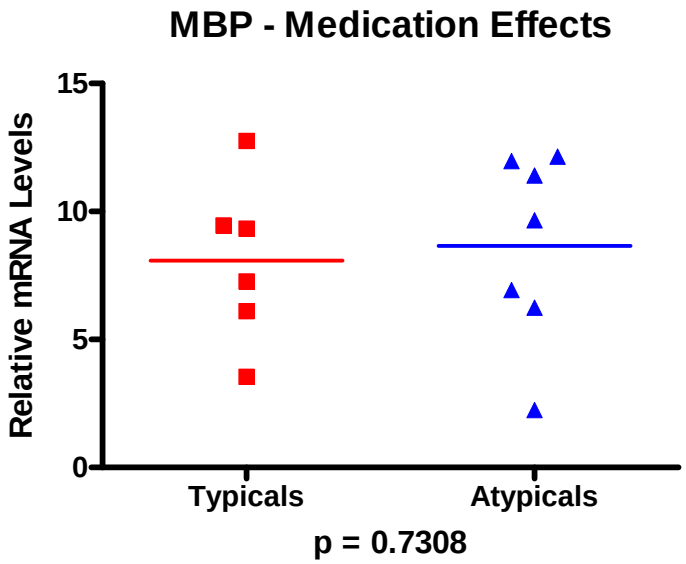
Supplementary Figure A.5.18. Medication subtype analysis of BAX gene expression by SYBR® Green qRT-PCR. Data is represented as patients receiving typical antipsychotics at the time of death versus those receiving atypical antipsychotics (analysis by Mann-Whitney Rank Sum test).



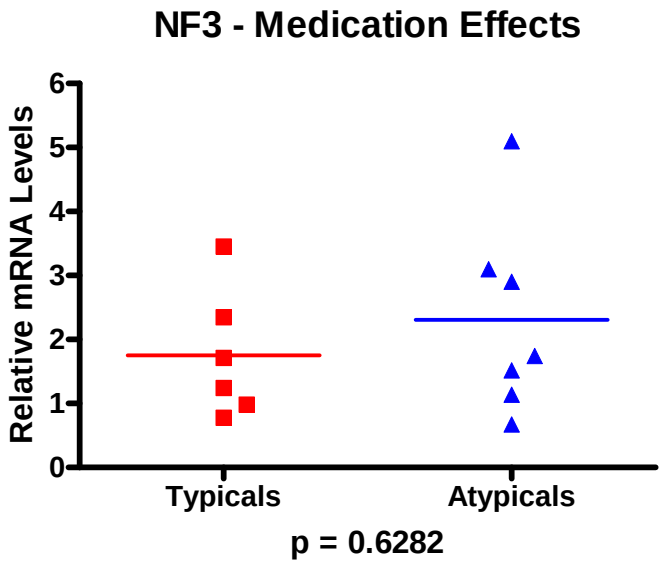
Supplementary Figure A.5.19. Medication subtype analysis of hBDNFcds gene expression by SYBR® Green qRT-PCR. Data is represented as patients receiving typical antipsychotics at the time of death versus those receiving atypical antipsychotics (analysis by Mann-Whitney Rank Sum test).



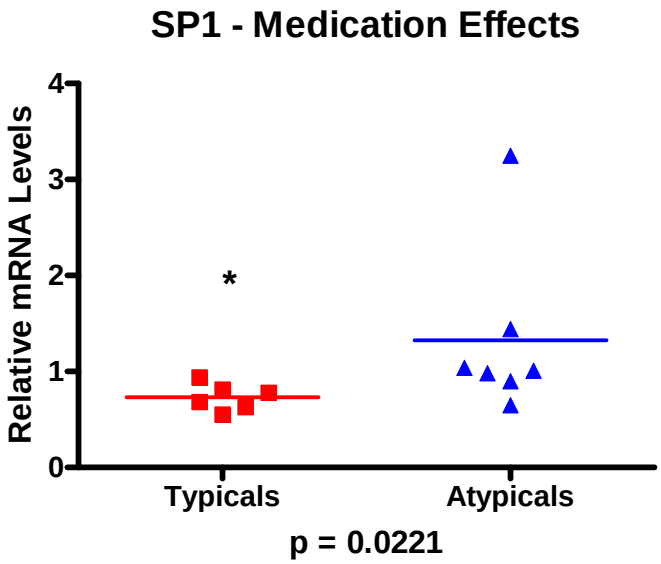
Supplementary Figure A.5.20. Medication subtype analysis of MAP3K3 gene expression by SYBR® Green qRT-PCR. Data is represented as patients receiving typical antipsychotics at the time of death versus those receiving atypical antipsychotics (analysis by Mann-Whitney Rank Sum test).



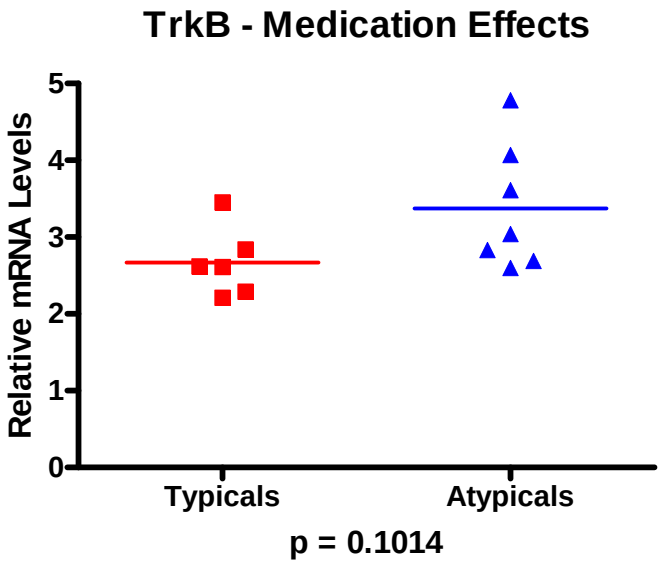
Supplementary Figure A.5.21. Medication subtype analysis of MBP gene expression by SYBR® Green qRT-PCR. Data is represented as patients receiving typical antipsychotics at the time of death versus those receiving atypical antipsychotics (analysis by Mann-Whitney Rank Sum test).



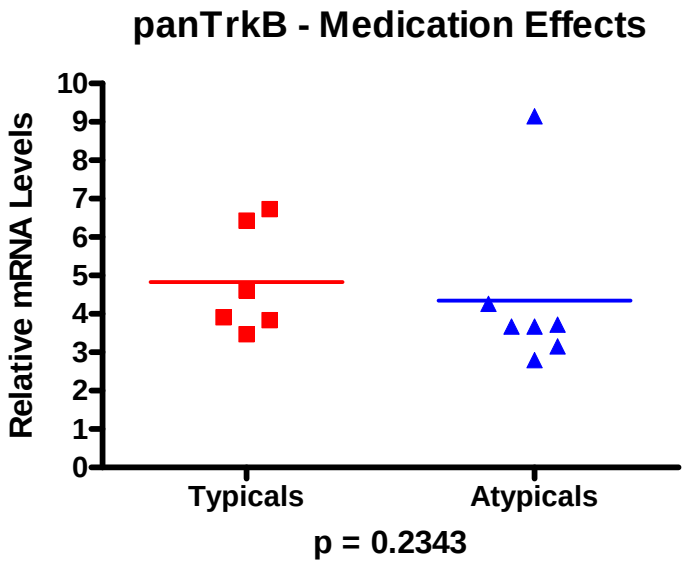
Supplementary Figure A.5.22. Medication subtype analysis of NF3 gene expression by SYBR® Green qRT-PCR. Data is represented as patients receiving typical antipsychotics at the time of death versus those receiving atypical antipsychotics (analysis by Mann-Whitney Rank Sum test).



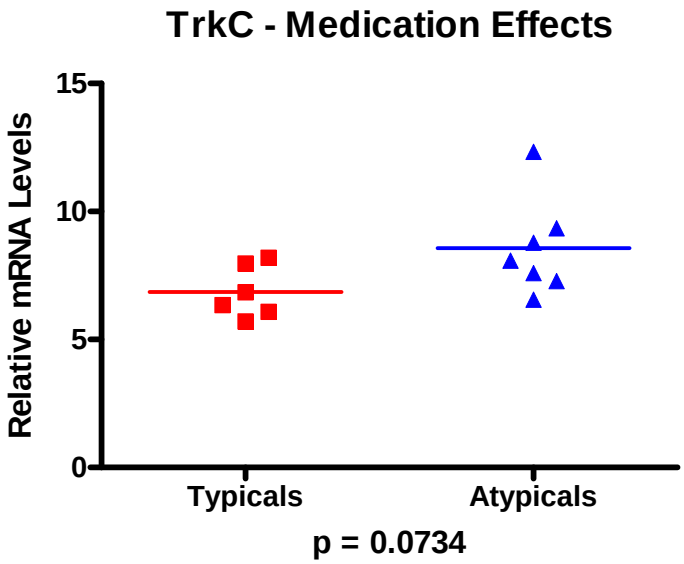
Supplementary Figure A.5.23. Medication subtype analysis of SP1 gene expression by SYBR® Green qRT-PCR. Data is represented as patients receiving typical antipsychotics at the time of death versus those receiving atypical antipsychotics (analysis by Mann-Whitney Rank Sum test). *$p<0.05$.



Supplementary Figure A.5.24. Medication subtype analysis of TrkB gene expression by SYBR® Green qRT-PCR. Data is represented as patients receiving typical antipsychotics at the time of death versus those receiving atypical antipsychotics (analysis by Mann-Whitney Rank Sum test).



Supplementary Figure A.5.25. Medication subtype analysis of panTrkB gene expression by SYBR® Green qRT-PCR. Data is represented as patients receiving typical antipsychotics at the time of death versus those receiving atypical antipsychotics (analysis by Mann-Whitney Rank Sum test).



Supplementary Figure A.5.26. Medication subtype analysis of TrkC gene expression by SYBR® Green qRT-PCR. Data is represented as patients receiving typical antipsychotics at the time of death versus those receiving atypical antipsychotics (analysis by Mann-Whitney Rank Sum test).

References

Abe S, Suzuki T, Ito T, Baba A, Hori T, Kurita H, Yamaguchi M, Shiraishi H (2000) Differential expression of GABA(A) receptor subunit mRNAs and ligand binding sites in rat brain following phencyclidine administration. Synapse 38:51-60.

Addington AM, Gornick M, Duckworth J, Sporn A, Gogtay N, Bobb A, Greenstein D, Lenane M, Gochman P, Baker N, Balkissoon R, Vakkalanka RK, Weinberger DR, Rapoport JL, Straub RE (2005) GAD1 (2q31.1), which encodes glutamic acid decarboxylase (GAD67), is associated with childhood-onset schizophrenia and cortical gray matter volume loss. Mol Psychiatry 10:581-588.

Adler CM, Malhotra AK, Elman I, Goldberg T, Egan M, Pickar D, Breier A (1999) Comparison of ketamine-induced thought disorder in healthy volunteers and thought disorder in schizophrenia. Am J Psychiatry 156:1646-1649.

Akbarian S, Huang HS (2006) Molecular and cellular mechanisms of altered GAD1/GAD67 expression in schizophrenia and related disorders. Brain Res Brain Res Rev 52:293-304.

Akbarian S, Kim JJ, Potkin SG, Hetrick WP, Bunney WE, Jr., Jones EG (1996a) Maldistribution of interstitial neurons in prefrontal white matter of the brains of schizophrenic patients. Arch Gen Psychiatry 53:425-436.

Akbarian S, Kim JJ, Potkin SG, Hagman JO, Tafazzoli A, Bunney WE, Jr., Jones EG (1995) Gene expression for glutamic acid decarboxylase is reduced without loss of neurons in prefrontal cortex of schizophrenics. Arch Gen Psychiatry 52:258-266.

Akbarian S, Sucher NJ, Bradley D, Tafazzoli A, Trinh D, Hetrick WP, Potkin SG, Sandman CA, Bunney WE, Jr., Jones EG (1996b) Selective alterations in gene expression for NMDA receptor subunits in prefrontal cortex of schizophrenics. J Neurosci 16:19-30.

Allen G, McColl R, Barnard H, Ringe WK, Fleckenstein J, Cullum CM (2005) Magnetic resonance imaging of cerebellar-prefrontal and cerebellar-parietal functional connectivity. Neuroimage 28:39-48.

American Psychiatric Association (2000) Diagnostic and statistical manual of mental disorders: DSM-IV-TR, 4th Edition. Washington, DC: American Psychiatric Association.

Andreasen NC, Nopoulos P, O'Leary DS, Miller DD, Wassink T, Flaum M (1999) Defining the phenotype of schizophrenia: cognitive dysmetria and its neural mechanisms. Biol Psychiatry 46:908-920.

Andreasen NC, O'Leary DS, Flaum M, Nopoulos P, Watkins GL, Boles Ponto LL, Hichwa RD (1997) Hypofrontality in schizophrenia: distributed dysfunctional circuits in neuroleptic-naive patients. Lancet 349:1730-1734.

Andreasen NC, Rezai K, Alliger R, Swayze VW, 2nd, Flaum M, Kirchner P, Cohen G, O'Leary DS (1992) Hypofrontality in neuroleptic-naive patients and in patients with chronic schizophrenia. Assessment with xenon 133 single-photon emission computed tomography and the Tower of London. Arch Gen Psychiatry 49:943-958.

Austin J (2005) Schizophrenia: an update and review. J Genet Couns 14:329-340.

Baader SL, Schilling K (1996) Glutamate receptors mediate dynamic regulation of nitric oxide synthase expression in cerebellar granule cells. J Neurosci 16:1440-1449.

Bai O, Zhang H, Li XM (2004) Antipsychotic drugs clozapine and olanzapine upregulate bcl-2 mRNA and protein in rat frontal cortex and hippocampus. Brain Res 1010:81-86.

Bastianelli E (2003) Distribution of calcium-binding proteins in the cerebellum. Cerebellum 2:242-262.

Benes FM, Berretta S (2001) GABAergic interneurons: implications for understanding schizophrenia and bipolar disorder. Neuropsychopharmacology 25:1-27.

Benes FM, Vincent SL, Marie A, Khan Y (1996) Up-regulation of GABAA receptor binding on neurons of the prefrontal cortex in schizophrenic subjects. Neuroscience 75:1021-1031.

Benes FM, Vincent SL, Alsterberg G, Bird ED, SanGiovanni JP (1992) Increased GABAA receptor binding in superficial layers of cingulate cortex in schizophrenics. J Neurosci 12:924-929.

Benes FM, Lim B, Matzilevich D, Walsh JP, Subburaju S, Minns M (2007) Regulation of the GABA cell phenotype in hippocampus of schizophrenics and bipolars. Proc Natl Acad Sci U S A 104:10164-10169.

Berretta S, Munno DW, Benes FM (2001) Amygdalar activation alters the hippocampal GABA system: "partial" modelling for postmortem changes in schizophrenia. J Comp Neurol 431:129-138.

Berthele A, Platzer S, Laurie DJ, Weis S, Sommer B, Zieglgansberger W, Conrad B, Tolle TR (1999) Expression of metabotropic glutamate receptor subtype mRNA (mGluR1-8) in human cerebellum. Neuroreport 10:3861-3867.

Breier A, Schreiber JL, Dyer J, Pickar D (1991) National Institute of Mental Health longitudinal study of chronic schizophrenia. Prognosis and predictors of outcome. Arch Gen Psychiatry 48:239-246.

Brickley SG, Misra C, Mok MH, Mishina M, Cull-Candy SG (2003) NR2B and NR2D subunits coassemble in cerebellar Golgi cells to form a distinct NMDA receptor subtype restricted to extrasynaptic sites. J Neurosci 23:4958-4966.

Brown SM, Kieffaber PD, Carroll CA, Vohs JL, Tracy JA, Shekhar A, O'Donnell BF, Steinmetz JE, Hetrick WP (2005) Eyeblink conditioning deficits indicate timing and cerebellar abnormalities in schizophrenia. Brain Cogn 58:94-108.

Buchanan RW, Javitt DC, Marder SR, Schooler NR, Gold JM, McMahon RP, Heresco-Levy U, Carpenter WT (2007) The Cognitive and Negative Symptoms in Schizophrenia Trial (CONSIST): the efficacy of glutamatergic agents for negative symptoms and cognitive impairments. Am J Psychiatry 164:1593-1602.

Bureau I, Dieudonne S, Coussen F, Mulle C (2000) Kainate receptor-mediated synaptic currents in cerebellar Golgi cells are not shaped by diffusion of glutamate. Proc Natl Acad Sci U S A 97:6838-6843.

Bustillo J, Barrow R, Paz R, Tang J, Seraji-Bozorgzad N, Moore GJ, Bolognani F, Lauriello J, Perrone-Bizzozero N, Galloway MP (2006) Long-term treatment of rats with haloperidol: lack of an effect on brain N-acetyl aspartate levels. Neuropsychopharmacology 31:751-756.

Chadderton P, Margrie TW, Hausser M (2004) Integration of quanta in cerebellar granule cells during sensory processing. Nature 428:856-860.

Chaperon F, Muller W, Auberson YP, Tricklebank MD, Neijt HC (2003) Substitution for PCP, disruption of prepulse inhibition and hyperactivity induced by N-methyl-D-aspartate receptor antagonists: preferential involvement of the NR2B rather than NR2A subunit. Behav Pharmacol 14:477-487.

Cochran SM, Kennedy M, McKerchar CE, Steward LJ, Pratt JA, Morris BJ (2003) Induction of metabolic hypofunction and neurochemical deficits after chronic intermittent exposure to phencyclidine: differential modulation by antipsychotic drugs. Neuropsychopharmacology 28:265-275.

Coyle JT (2006) Glutamate and Schizophrenia: Beyond the Dopamine Hypothesis. Cell Mol Neurobiol.

De Schutter E, Vos B, Maex R (2000) The function of cerebellar Golgi cells revisited. Prog Brain Res 124:81-93.

Dieudonne S (1998) Submillisecond kinetics and low efficacy of parallel fibre-Golgi cell synaptic currents in the rat cerebellum. J Physiol 510 (Pt 3):845-866.

Dong E, Agis-Balboa RC, Simonini MV, Grayson DR, Costa E, Guidotti A (2005) Reelin and glutamic acid decarboxylase67 promoter remodeling in an epigenetic methionine-induced mouse model of schizophrenia. Proc Natl Acad Sci U S A 102:12578-12583.

Dracheva S, Marras SA, Elhakem SL, Kramer FR, Davis KL, Haroutunian V (2001) N-methyl-D-aspartic acid receptor expression in the dorsolateral prefrontal cortex of elderly patients with schizophrenia. Am J Psychiatry 158:1400-1410.

Duan X, Chang JH, Ge S, Faulkner RL, Kim JY, Kitabatake Y, Liu XB, Yang CH, Jordan JD, Ma DK, Liu CY, Ganesan S, Cheng HJ, Ming GL, Lu B, Song H (2007) Disrupted-In-Schizophrenia 1 regulates integration of newly generated neurons in the adult brain. Cell 130:1146-1158.

Eastwood SL, Harrison PJ (2003) Interstitial white matter neurons express less reelin and are abnormally distributed in schizophrenia: towards an integration of molecular and morphologic aspects of the neurodevelopmental hypothesis. Mol Psychiatry 8:769, 821-731.

Eastwood SL, Harrison PJ (2006) Cellular basis of reduced cortical reelin expression in schizophrenia. Am J Psychiatry 163:540-542.

Eastwood SL, Law AJ, Everall IP, Harrison PJ (2003) The axonal chemorepellant semaphorin 3A is increased in the cerebellum in schizophrenia and may contribute to its synaptic pathology. Mol Psychiatry 8:148-155.

Erlander MG, Tillakaratne NJ, Feldblum S, Patel N, Tobin AJ (1991) Two genes encode distinct glutamate decarboxylases. Neuron 7:91-100.

Esclapez M, Tillakaratne NJ, Kaufman DL, Tobin AJ, Houser CR (1994) Comparative localization of two forms of glutamic acid decarboxylase and their mRNAs in rat brain supports the concept of functional differences between the forms. J Neurosci 14:1834-1855.

Fatemi SH, Stary JM, Earle JA, Araghi-Niknam M, Eagan E (2005) GABAergic dysfunction in schizophrenia and mood disorders as reflected by decreased levels of glutamic acid decarboxylase 65 and 67 kDa and Reelin proteins in cerebellum. Schizophr Res 72:109-122.

Feligioni M, Holman D, Haglerod C, Davanger S, Henley JM (2006) Ultrastructural localisation and differential agonist-induced regulation of AMPA and kainate receptors present at the presynaptic active zone and postsynaptic density. J Neurochem 99:549-560.

Ferrer-Montiel AV, Merino JM, Planells-Cases R, Sun W, Montal M (1998) Structural determinants of the blocker binding site in glutamate and NMDA receptor channels. Neuropharmacology 37:139-147.

Fiszman ML, Erdelyi F, Szabo G, Vicini S (2007) Presynaptic AMPA and kainate receptors increase the size of GABAergic terminals and enhance GABA release. Neuropharmacology 52:1631-1640.

Forti L, Cesana E, Mapelli J, D'Angelo E (2006) Ionic mechanisms of autorhythmic firing in rat cerebellar Golgi cells. J Physiol 574:711-729.

Freedman R (2005) The choice of antipsychotic drugs for schizophrenia. N Engl J Med 353:1286-1288.

Freichel C, Potschka H, Ebert U, Brandt C, Loscher W (2006) Acute changes in the neuronal expression of GABA and glutamate decarboxylase isoforms in the rat piriform cortex following status epilepticus. Neuroscience 141:2177-2194.

Fritschy JM, Panzanelli P (2006) Molecular and synaptic organization of GABAA receptors in the cerebellum: Effects of targeted subunit gene deletions. Cerebellum 5:275-285.

Gao XM, Hashimoto T, Tamminga CA (1998) Phencyclidine (PCP) and dizocilpine (MK801) exert time-dependent effects on the expression of immediate early genes in rat brain. Synapse 29:14-28.

Gao XM, Shirakawa O, Du F, Tamminga CA (1993) Delayed regional metabolic actions of phencyclidine. Eur J Pharmacol 241:7-15.

Geurts FJ, De Schutter E, Dieudonne S (2003) Unraveling the cerebellar cortex: cytology and cellular physiology of large-sized interneurons in the granular layer. Cerebellum 2:290-299.

Grunze HC, Rainnie DG, Hasselmo ME, Barkai E, Hearn EF, McCarley RW, Greene RW (1996) NMDA-dependent modulation of CA1 local circuit inhibition. J Neurosci 16:2034-2043.

Guidotti A, Auta J, Davis JM, Dong E, Grayson DR, Veldic M, Zhang X, Costa E (2005) GABAergic dysfunction in schizophrenia: new treatment strategies on the horizon. Psychopharmacology (Berl) 180:191-205.

Guidotti A, Auta J, Davis JM, Di-Giorgi-Gerevini V, Dwivedi Y, Grayson DR, Impagnatiello F, Pandey G, Pesold C, Sharma R, Uzunov D, Costa E (2000) Decrease in reelin and glutamic acid decarboxylase67 (GAD67) expression in schizophrenia and bipolar disorder: a postmortem brain study. Arch Gen Psychiatry 57:1061-1069.

Hajszan T, Leranth C, Roth RH (2006) Subchronic phencyclidine treatment decreases the number of dendritic spine synapses in the rat prefrontal cortex. Biol Psychiatry 60:639-644.

Hamann M, Rossi DJ, Attwell D (2002) Tonic and spillover inhibition of granule cells control information flow through cerebellar cortex. Neuron 33:625-633.

Hanaoka T, Toyoda H, Mizuno T, Kikuyama H, Morimoto K, Takahata R, Matsumura H, Yoneda H (2003) Alterations in NMDA receptor subunit levels in the brain

regions of rats chronically administered typical or atypical antipsychotic drugs. Neurochem Res 28:919-924.

Harvey PD, Rabinowitz J, Eerdekens M, Davidson M (2005) Treatment of cognitive impairment in early psychosis: a comparison of risperidone and haloperidol in a large long-term trial. Am J Psychiatry 162:1888-1895.

Hashimoto T, Volk DW, Eggan SM, Mirnics K, Pierri JN, Sun Z, Sampson AR, Lewis DA (2003) Gene expression deficits in a subclass of GABA neurons in the prefrontal cortex of subjects with schizophrenia. J Neurosci 23:6315-6326.

Heckers S, Stone D, Walsh J, Shick J, Koul P, Benes FM (2002) Differential hippocampal expression of glutamic acid decarboxylase 65 and 67 messenger RNA in bipolar disorder and schizophrenia. Arch Gen Psychiatry 59:521-529.

Hirano T, Watanabe D, Kawaguchi SY, Pastan I, Nakanishi S (2002) Roles of inhibitory interneurons in the cerebellar cortex. Ann N Y Acad Sci 978:405-412.

Ho BC, Mola C, Andreasen NC (2004) Cerebellar dysfunction in neuroleptic naive schizophrenia patients: clinical, cognitive, and neuroanatomic correlates of cerebellar neurologic signs. Biol Psychiatry 55:1146-1153.

Homayoun H, Moghaddam B (2007) NMDA receptor hypofunction produces opposite effects on prefrontal cortex interneurons and pyramidal neurons. J Neurosci 27:11496-11500.

Huang HS, Akbarian S (2007) GAD1 mRNA expression and DNA methylation in prefrontal cortex of subjects with schizophrenia. PLoS ONE 2:e809.

Huang HS, Matevossian A, Whittle C, Kim SY, Schumacher A, Baker SP, Akbarian S (2007) Prefrontal dysfunction in schizophrenia involves mixed-lineage leukemia

1-regulated histone methylation at GABAergic gene promoters. J Neurosci 27:11254-11262.

Ito M (2006) Cerebellar circuitry as a neuronal machine. Prog Neurobiol 78:272-303.

Jackson ME, Homayoun H, Moghaddam B (2004) NMDA receptor hypofunction produces concomitant firing rate potentiation and burst activity reduction in the prefrontal cortex. Proc Natl Acad Sci U S A 101:8467-8472.

Jentsch JD, Roth RH (1999) The neuropsychopharmacology of phencyclidine: from NMDA receptor hypofunction to the dopamine hypothesis of schizophrenia. Neuropsychopharmacology 20:201-225.

Jentsch JD, Tran A, Le D, Youngren KD, Roth RH (1997a) Subchronic phencyclidine administration reduces mesoprefrontal dopamine utilization and impairs prefrontal cortical-dependent cognition in the rat. Neuropsychopharmacology 17:92-99.

Jentsch JD, Redmond DE, Jr., Elsworth JD, Taylor JR, Youngren KD, Roth RH (1997b) Enduring cognitive deficits and cortical dopamine dysfunction in monkeys after long-term administration of phencyclidine. Science 277:953-955.

Jiang X, Zhu D, Okagaki P, Lipsky R, Wu X, Banaudha K, Mearow K, Strauss KI, Marini AM (2003) N-methyl-D-aspartate and TrkB receptor activation in cerebellar granule cells: an in vitro model of preconditioning to stimulate intrinsic survival pathways in neurons. Ann N Y Acad Sci 993:134-145; discussion 159-160.

Jones PB, Barnes TR, Davies L, Dunn G, Lloyd H, Hayhurst KP, Murray RM, Markwick A, Lewis SW (2006) Randomized controlled trial of the effect on Quality of Life

of second- vs first-generation antipsychotic drugs in schizophrenia: Cost Utility of the Latest Antipsychotic Drugs in Schizophrenia Study (CUtLASS 1). Arch Gen Psychiatry 63:1079-1087.

Kapur S, Remington G (2001) Atypical antipsychotics: new directions and new challenges in the treatment of schizophrenia. Annu Rev Med 52:503-517.

Kelly RM, Strick PL (2003) Cerebellar loops with motor cortex and prefrontal cortex of a nonhuman primate. J Neurosci 23:8432-8444.

Kim HS, Choi HS, Lee SY, Oh S (2000a) Changes of GABA(A) receptor binding and subunit mRNA level in rat brain by infusion of subtoxic dose of MK-801. Brain Res 880:28-37.

Kim JJ, Mohamed S, Andreasen NC, O'Leary DS, Watkins GL, Boles Ponto LL, Hichwa RD (2000b) Regional neural dysfunctions in chronic schizophrenia studied with positron emission tomography. Am J Psychiatry 157:542-548.

Kristiansen LV, Beneyto M, Haroutunian V, Meador-Woodruff JH (2006) Changes in NMDA receptor subunits and interacting PSD proteins in dorsolateral prefrontal and anterior cingulate cortex indicate abnormal regional expression in schizophrenia. Mol Psychiatry 11:737-747, 705.

Kristiansen LV, Huerta I, Beneyto M, Meador-Woodruff JH (2007) NMDA receptors and schizophrenia. Curr Opin Pharmacol 7:48-55.

Laprade N, Soghomonian JJ (1995) Differential regulation of mRNA levels encoding for the two isoforms of glutamate decarboxylase (GAD65 and GAD67) by dopamine receptors in the rat striatum. Brain Res Mol Brain Res 34:65-74.

Lewis DA, Gonzalez-Burgos G (2000) Intrinsic excitatory connections in the prefrontal cortex and the pathophysiology of schizophrenia. Brain Res Bull 52:309-317.

Lewis DA, Lieberman JA (2000) Catching up on schizophrenia: natural history and neurobiology. Neuron 28:325-334.

Lewis DA, Volk DW, Hashimoto T (2004) Selective alterations in prefrontal cortical GABA neurotransmission in schizophrenia: a novel target for the treatment of working memory dysfunction. Psychopharmacology (Berl) 174:143-150.

Lewis DA, Hashimoto T, Volk DW (2005) Cortical inhibitory neurons and schizophrenia. Nat Rev Neurosci 6:312-324.

Lewis DA, Cruz DA, Melchitzky DS, Pierri JN (2001) Lamina-specific deficits in parvalbumin-immunoreactive varicosities in the prefrontal cortex of subjects with schizophrenia: evidence for fewer projections from the thalamus. Am J Psychiatry 158:1411-1422.

Lieberman JA, Stroup TS, McEvoy JP, Swartz MS, Rosenheck RA, Perkins DO, Keefe RS, Davis SM, Davis CE, Lebowitz BD, Severe J, Hsiao JK (2005) Effectiveness of antipsychotic drugs in patients with chronic schizophrenia. N Engl J Med 353:1209-1223.

Lindahl JS, Keifer J (2004) Glutamate receptor subunits are altered in forebrain and cerebellum in rats chronically exposed to the NMDA receptor antagonist phencyclidine. Neuropsychopharmacology 29:2065-2073.

Lipska BK, Lerman DN, Khaing ZZ, Weickert CS, Weinberger DR (2003) Gene expression in dopamine and GABA systems in an animal model of schizophrenia: effects of antipsychotic drugs. Eur J Neurosci 18:391-402.

Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 25:402-408.

Llansola M, Sanchez-Perez A, Cauli O, Felipo V (2005) Modulation of NMDA receptors in the cerebellum. 1. Properties of the NMDA receptor that modulate its function. Cerebellum 4:154-161.

Malaspina D, Harkavy-Friedman J, Corcoran C, Mujica-Parodi L, Printz D, Gorman JM, Van Heertum R (2004) Resting neural activity distinguishes subgroups of schizophrenia patients. Biol Psychiatry 56:931-937.

Mansbach RS, Geyer MA (1989) Effects of phencyclidine and phencyclidine biologs on sensorimotor gating in the rat. Neuropsychopharmacology 2:299-308.

Mathew SS, Pozzo-Miller L, Hablitz JJ (2008) Kainate modulates presynaptic GABA release from two vesicle pools. J Neurosci 28:725-731.

McCullumsmith RE, Kristiansen LV, Beneyto M, Scarr E, Dean B, Meador-Woodruff JH (2007) Decreased NR1, NR2A, and SAP102 transcript expression in the hippocampus in bipolar disorder. Brain Res 1127:108-118.

Meador-Woodruff JH, Davis KL, Haroutunian V (2001) Abnormal kainate receptor expression in prefrontal cortex in schizophrenia. Neuropsychopharmacology 24:545-552.

Menzies L, Ooi C, Kamath S, Suckling J, McKenna P, Fletcher P, Bullmore E, Stephenson C (2007) Effects of gamma-aminobutyric acid-modulating drugs on working memory and brain function in patients with schizophrenia. Arch Gen Psychiatry 64:156-167.

Middleton FA, Strick PL (2001) Cerebellar projections to the prefrontal cortex of the primate. J Neurosci 21:700-712.

Midtgaard J, Lasser-Ross N, Ross WN (1993) Spatial distribution of Ca2+ influx in turtle Purkinje cell dendrites in vitro: role of a transient outward current. J Neurophysiol 70:2455-2469.

Milev P, Ho BC, Arndt S, Andreasen NC (2005) Predictive values of neurocognition and negative symptoms on functional outcome in schizophrenia: a longitudinal first-episode study with 7-year follow-up. Am J Psychiatry 162:495-506.

Misra C, Brickley SG, Farrant M, Cull-Candy SG (2000) Identification of subunits contributing to synaptic and extrasynaptic NMDA receptors in Golgi cells of the rat cerebellum. J Physiol 524 Pt 1:147-162.

Mohn AR, Gainetdinov RR, Caron MG, Koller BH (1999) Mice with reduced NMDA receptor expression display behaviors related to schizophrenia. Cell 98:427-436.

Monyer H, Burnashev N, Laurie DJ, Sakmann B, Seeburg PH (1994) Developmental and regional expression in the rat brain and functional properties of four NMDA receptors. Neuron 12:529-540.

Morara S, Brecha NC, Marcotti W, Provini L, Rosina A (1996) Neuronal and glial localization of the GABA transporter GAT-1 in the cerebellar cortex. Neuroreport 7:2993-2996.

Morris BJ, Cochran SM, Pratt JA (2005) PCP: from pharmacology to modelling schizophrenia. Curr Opin Pharmacol 5:101-106.

Ninan I, Jardemark KE, Wang RY (2003) Olanzapine and clozapine but not haloperidol reverse subchronic phencyclidine-induced functional hyperactivity of N-methyl-

D-aspartate receptors in pyramidal cells of the rat medial prefrontal cortex. Neuropharmacology 44:462-472.

Paz RD, Andreasen NC, Daoud SZ, Conley R, Roberts R, Bustillo J, Perrone-Bizzozero NI (2006) Increased expression of activity-dependent genes in cerebellar glutamatergic neurons of patients with schizophrenia. Am J Psychiatry 163:1829-1831.

Pemberton KE, Belcher SM, Ripellino JA, Howe JR (1998) High-affinity kainate-type ion channels in rat cerebellar granule cells. J Physiol 510 (Pt 2):401-420.

Pilowsky LS, Bressan RA, Stone JM, Erlandsson K, Mulligan RS, Krystal JH, Ell PJ (2006) First in vivo evidence of an NMDA receptor deficit in medication-free schizophrenic patients. Mol Psychiatry 11:118-119.

Porter RH, Eastwood SL, Harrison PJ (1997) Distribution of kainate receptor subunit mRNAs in human hippocampus, neocortex and cerebellum, and bilateral reduction of hippocampal GluR6 and KA2 transcripts in schizophrenia. Brain Res 751:217-231.

Potkin SG, Alva G, Fleming K, Anand R, Keator D, Carreon D, Doo M, Jin Y, Wu JC, Fallon JH (2002) A PET study of the pathophysiology of negative symptoms in schizophrenia. Positron emission tomography. Am J Psychiatry 159:227-237.

Ramnani N (2006) The primate cortico-cerebellar system: anatomy and function. Nat Rev Neurosci 7:511-522.

Rapoport JL, Addington AM, Frangou S, Psych MR (2005) The neurodevelopmental model of schizophrenia: update 2005. Mol Psychiatry 10:434-449.

Reynolds LM, Cochran SM, Morris BJ, Pratt JA, Reynolds GP (2005) Chronic phencyclidine administration induces schizophrenia-like changes in N-acetylaspartate and N-acetylaspartylglutamate in rat brain. Schizophr Res 73:147-152.

Ross CA, Margolis RL, Reading SA, Pletnikov M, Coyle JT (2006) Neurobiology of schizophrenia. Neuron 52:139-153.

Rossi DJ, Hamann M, Attwell D (2003) Multiple modes of GABAergic inhibition of rat cerebellar granule cells. J Physiol 548:97-110.

Rujescu D, Bender A, Keck M, Hartmann AM, Ohl F, Raeder H, Giegling I, Genius J, McCarley RW, Moller HJ, Grunze H (2006) A pharmacological model for psychosis based on N-methyl-D-aspartate receptor hypofunction: molecular, cellular, functional and behavioral abnormalities. Biol Psychiatry 59:721-729.

Ruzicka WB, Zhubi A, Veldic M, Grayson DR, Costa E, Guidotti A (2007) Selective epigenetic alteration of layer I GABAergic neurons isolated from prefrontal cortex of schizophrenia patients using laser-assisted microdissection. Mol Psychiatry 12:385-397.

Salonen V, Kallinen S, Lopez-Picon FR, Korpi ER, Holopainen IE, Uusi-Oukari M (2006) AMPA/kainate receptor-mediated up-regulation of GABAA receptor delta subunit mRNA expression in cultured rat cerebellar granule cells is dependent on NMDA receptor activation. Brain Res 1087:33-40.

Sams-Dodd F (1997) Effect of novel antipsychotic drugs on phencyclidine-induced stereotyped behaviour and social isolation in the rat social interaction test. Behav Pharmacol 8:196-215.

Sastry BR, Morishita W, Yip S, Shew T (1997) GABA-ergic transmission in deep cerebellar nuclei. Prog Neurobiol 53:259-271.

Scherzer CR, Landwehrmeyer GB, Kerner JA, Standaert DG, Hollingsworth ZR, Daggett LP, Velicelebi G, Penney JB, Jr., Young AB (1997) Cellular distribution of NMDA glutamate receptor subunit mRNAs in the human cerebellum. Neurobiol Dis 4:35-46.

Schmahmann JD, Sherman JC (1998) The cerebellar cognitive affective syndrome. Brain 121 (Pt 4):561-579.

Schweighofer N, Doya K (2003) Meta-learning in reinforcement learning. Neural Netw 16:5-9.

Sears LL, Andreasen NC, O'Leary DS (2000) Cerebellar functional abnormalities in schizophrenia are suggested by classical eyeblink conditioning. Biol Psychiatry 48:204-209.

Simmons DM, Arriza JL, Swanson LW (1989) A complete protocol for in situ hybridization of messenger RNAs in brain and other tissues with radio-labeled single-stranded RNA probes. J Histotechnology 12:169-181.

Sloviter RS, Dichter MA, Rachinsky TL, Dean E, Goodman JH, Sollas AL, Martin DL (1996) Basal expression and induction of glutamate decarboxylase and GABA in excitatory granule cells of the rat and monkey hippocampal dentate gyrus. J Comp Neurol 373:593-618.

Snyder SH (2006) Dopamine receptor excess and mouse madness. Neuron 49:484-485.

Spencer KM, Nestor PG, Perlmutter R, Niznikiewicz MA, Klump MC, Frumin M, Shenton ME, McCarley RW (2004) Neural synchrony indexes disordered

perception and cognition in schizophrenia. Proc Natl Acad Sci U S A 101:17288-17293.

Straub RE, Lipska BK, Egan MF, Goldberg TE, Callicott JH, Mayhew MB, Vakkalanka RK, Kolachana BS, Kleinman JE, Weinberger DR (2007) Allelic variation in GAD1 (GAD67) is associated with schizophrenia and influences cortical function and gene expression. Mol Psychiatry 12:854-869.

Takayama C, Inoue Y (2005) Developmental expression of GABA transporter-1 and 3 during formation of the GABAergic synapses in the mouse cerebellar cortex. Brain Res Dev Brain Res 158:41-49.

Thach WT (2007) On the mechanism of cerebellar contributions to cognition. Cerebellum 6:163-167.

Torrey EF, Barci BM, Webster MJ, Bartko JJ, Meador-Woodruff JH, Knable MB (2005) Neurochemical markers for schizophrenia, bipolar disorder, and major depression in postmortem brains. Biol Psychiatry 57:252-260.

Traub RD, Whittington MA, Stanford IM, Jefferys JG (1996) A mechanism for generation of long-range synchronous fast oscillations in the cortex. Nature 383:621-624.

Tremolizzo L, Carboni G, Ruzicka WB, Mitchell CP, Sugaya I, Tueting P, Sharma R, Grayson DR, Costa E, Guidotti A (2002) An epigenetic mouse model for molecular and behavioral neuropathologies related to schizophrenia vulnerability. Proc Natl Acad Sci U S A 99:17095-17100.

Uhl GR, Grow RW (2004) The burden of complex genetics in brain disorders. Arch Gen Psychiatry 61:223-229.

Veldic M, Caruncho HJ, Liu WS, Davis J, Satta R, Grayson DR, Guidotti A, Costa E (2004) DNA-methyltransferase 1 mRNA is selectively overexpressed in telencephalic GABAergic interneurons of schizophrenia brains. Proc Natl Acad Sci U S A 101:348-353.

Volk D, Austin M, Pierri J, Sampson A, Lewis D (2001) GABA transporter-1 mRNA in the prefrontal cortex in schizophrenia: decreased expression in a subset of neurons. Am J Psychiatry 158:256-265.

Volk DW, Austin MC, Pierri JN, Sampson AR, Lewis DA (2000) Decreased glutamic acid decarboxylase67 messenger RNA expression in a subset of prefrontal cortical gamma-aminobutyric acid neurons in subjects with schizophrenia. Arch Gen Psychiatry 57:237-245.

Volk DW, Pierri JN, Fritschy JM, Auh S, Sampson AR, Lewis DA (2002) Reciprocal alterations in pre- and postsynaptic inhibitory markers at chandelier cell inputs to pyramidal neurons in schizophrenia. Cereb Cortex 12:1063-1070.

Wall MJ (2003) Endogenous nitric oxide modulates GABAergic transmission to granule cells in adult rat cerebellum. Eur J Neurosci 18:869-878.

Wass C, Archer T, Palsson E, Fejgin K, Klamer D, Engel JA, Svensson L (2006) Effects of phencyclidine on spatial learning and memory: nitric oxide-dependent mechanisms. Behav Brain Res 171:147-153.

Watanabe D, Nakanishi S (2003) mGluR2 postsynaptically senses granule cell inputs at Golgi cell synapses. Neuron 39:821-829.

Watanabe D, Inokawa H, Hashimoto K, Suzuki N, Kano M, Shigemoto R, Hirano T, Toyama K, Kaneko S, Yokoi M, Moriyoshi K, Suzuki M, Kobayashi K, Nagatsu

T, Kreitman RJ, Pastan I, Nakanishi S (1998) Ablation of cerebellar Golgi cells disrupts synaptic integration involving GABA inhibition and NMDA receptor activation in motor coordination. Cell 95:17-27.

Wiley JL (1998) Nitric oxide synthase inhibitors attenuate phencyclidine-induced disruption of prepulse inhibition. Neuropsychopharmacology 19:86-94.

Wolkin A, Sanfilipo M, Wolf AP, Angrist B, Brodie JD, Rotrosen J (1992) Negative symptoms and hypofrontality in chronic schizophrenia. Arch Gen Psychiatry 49:959-965.

Woo TU, Walsh JP, Benes FM (2004) Density of glutamic acid decarboxylase 67 messenger RNA-containing neurons that express the N-methyl-D-aspartate receptor subunit NR2A in the anterior cingulate cortex in schizophrenia and bipolar disorder. Arch Gen Psychiatry 61:649-657.

Wu X, Jiang X, Marini AM, Lipsky RH (2005) Delineating and understanding cerebellar neuroprotective pathways: potential implication for protecting the cortex. Ann N Y Acad Sci 1053:39-47.

Xifro X, Malagelada C, Minano A, Rodriguez-Alvarez J (2005) Brief exposure to NMDA produces long-term protection of cerebellar granule cells from apoptosis. Eur J Neurosci 21:827-840.

Yan XX, Ribak CE (1998) Developmental expression of gamma-aminobutyric acid transporters (GAT-1 and GAT-3) in the rat cerebellum: evidence for a transient presence of GAT-1 in Purkinje cells. Brain Res Dev Brain Res 111:253-269.

Zink M, Schmitt A, May B, Muller B, Braus DF, Henn FA (2004a) Differential effects of long-term treatment with clozapine or haloperidol on GABA transporter expression. Pharmacopsychiatry 37:171-174.

Zink M, Schmitt A, May B, Muller B, Demirakca T, Braus DF, Henn FA (2004b) Differential effects of long-term treatment with clozapine or haloperidol on GABAA receptor binding and GAD67 expression. Schizophr Res 66:151-157.