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Dysregulations of Synaptic Vesicle Trafficking in Schizophrenia

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Abstract

Schizophrenia is a serious psychiatric illness which is experienced by about 1% of individuals worldwide and has a debilitating impact on perception, cognition and social function. Over the years, several models/hypotheses have been developed which link schizophrenia to dysregulations of the dopamine, glutamate and serotonin receptor pathways. An important segment of these pathways that have been extensively studied for the pathophysiology of schizophrenia is the presynaptic neurotransmitter release mechanism. This set of molecular events is an evolutionarily well-conserved process that involves vesicle recruitment, docking, membrane fusion and recycling, leading to efficient neurotransmitter delivery at the synapse. Accumulated evidence indicate dysregulation of this mechanism impacting post-synaptic signal transduction via different neurotransmitters in key brain regions implicated in schizophrenia. In recent years, after ground-breaking work that elucidated the operations of this mechanism, research efforts have focused on the alterations in the mRNA and protein expression of pre-synaptic neurotransmitter release molecules in schizophrenia and other neuropsychiatric conditions. In this review article, we present recent evidence from schizophrenia human post-mortem studies that key proteins involved in the pre-synaptic release mechanism are dysregulated in the disorder. We also discuss the potential impact of dysfunctional pre-synaptic neurotransmitter release on the various neurotransmitter systems implicated in schizophrenia.

Keywords

Schizophrenia; Presynaptic; Neurotransmitter; SNARE (soluble N-ethylmaleimide-sensitive factor attachment protein receptor); SNAP-25 (synaptosomal-associated protein 25); Synaptobrevin; Synaptophysin; Syntaxin; Synapsin; Munc18; Complexin

Introduction/Background

Schizophrenia is a chronic and disabling psychiatric disorder that affects about 1% of the population worldwide (NIMH). Schizophrenia affects multiple brain regions, including

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Compliance with Ethics Guidelines

Conflict of Interest

Chijioke N. Egbujo, Duncan Sinclair, and Chang-Gyu Hahn declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent

This article does not contain any studies with human or animal subjects performed by any of the authors.

regions responsible for perception, movement, cognition, and affective function. The etiology of schizophrenia is complex and is thought to involve interplay between genetic and environmental factors. Different hypotheses have been postulated to address the pathophysiology of schizophrenia including the dopamine and NMDAR models of schizophrenia. The dopamine hypothesis attributes schizophrenic psychosis to hyperactive dopaminergic transmission in the basal ganglia, and the cognitive decline is attributed to underactive dopaminergic transmission in prefrontal areas [1]. The glutamate pathway dysregulation hypothesis posits that dysfunction of glutamatergic neurotransmission, N-methyl-D-aspartate (NMDAR) signaling in particular, perturb the function of synaptic strengths and plasticity leading to circuit dysregulations underlying various manifestations of the illness [2]. These two pathways involve distinct molecular machinery; yet emerging evidence indicate alterations in neurotransmitter release mechanisms in both in schizophrenia and as such suggest an interesting possibility that alterations in these two pathways may interact and converge in the presynaptic segment.

This dysregulation at the pre-synaptic terminals could result in significant impact on the post-synaptic terminals of different neurons including pyramidal, medium spiny and interneurons and could underlie the both the dopamine and glutamate deficits reported in the illness.[3, 4] [5, 6]. It is feasible that such synaptic changes may underlie the aberrant functional connectivity widely purported to occur in the disorder. [7–9]. For these reasons, much research has focused on determining whether synaptic alterations are a feature of the brain in schizophrenia. To that end, presynaptic proteins have been widely utilized as proxy markers of synapses [10, 11], and their altered expression is mostly interpreted as indicative of changes in synaptic density or function, thereby denoting synaptic pathology.

Although data have sometimes been inconsistent, some studies have found molecular evidence in support of a synaptic pathology of schizophrenia. Several reviews and articles have dealt with the post synaptic pathology of schizophrenia [12, 13] and a significant portion of the data implicate the PSD (post synaptic density) and its associated proteins [14–16] in the pathophysiology of schizophrenia. There are limited reviews that have looked at the available data on the involvement of presynaptic signaling proteins in schizophrenia.

Hence this review is focused on key proteins responsible for the mechanism of membrane fusion and neurotransmitter release. This primarily involves the SNARE complex and its associated interacting proteins. SNAP-25, syntaxin-1A and synaptobrevin form the SNARE (soluble N-ethylmaleimide-sensitive factor attachment protein receptors). Interactions of the SNARE proteins and other key interacting proteins like Munc18-1 influence all of the steps leading to exocytosis, including vesicle recruitment, docking and membrane fusion[17, 18] [19–21]. It has been proposed that abnormalities of this vesicular transport mechanism might participate in the pathogenesis of schizophrenia by triggering dysfunctional neuroexocytosis.

The Pre-synaptic release mechanism

The release of neurotransmitter at the presynaptic terminal generally involves intracellular membrane fusion which is triggered by the electrical currents and mediated by the SNARE proteins and synaptic membrane (SM) proteins that undergo cyclical interactions leading to membrane fusion and neurotransmitter exocytosis. At the presynaptic terminal, a change the

conformation of syntaxin-1 mediated by its binding to munc18 leads to the SNARE complex assembly in which the synaptobrevin on the vesicular membrane forms a complex with syntaxin-1 and SNAP-25 on the plasma membrane [17]. Prior to SNARE complex formation, syntaxin-1 is present in a closed conformation that cannot engage in SNARE complex formation; syntaxin-1 has to open for SNARE complex assembly to proceed [22, 23]. This SNARE complex formation between the vesicular SNARE and membranous SNARE leads fusion and neurotransmitter release [19–22]. After release the neurotransmitter enters the synaptic cleft and binds to its receptors at the post-synaptic terminal end of the synapse which is specialized to receive the neurotransmitter signal released from the presynaptic terminal and transduce it into electrical and biochemical changes in the postsynaptic cell. This signaling mechanism is tightly regulated in and dysregulations in this mechanism is thought to result in schizophrenia.

Efficient functioning of the mechanism involves proper – Neurotransmitter recruitment and concentration in the vesicles-Targeting of the pools of vesicles to the presynaptic terminal-Vesicle exocytosis-Vesicle endocytosis and recycling. In each of these steps there are key proteins that play crucial roles in its regulations and control.

Synaptophysin

Synaptophysin has been studied extensively since it was first identified in 1985 [24]. It is abundant in neurons and neuroendocrine cells where it is integral to synaptic vesicle formation and function. It is a 313 AA protein found in presynaptic terminals and is encoded by the SYP Gene which is located in Xp11.23-p11.22.

Synaptophysin is one of the most abundant synaptic vesicle proteins, accounting for 7% of the protein in this organelle. It forms multimeric complexes that have been reported to form channels in lipid bilayers and is also involved in organizing other membrane components and targeting the vesicles to the plasma membrane as shown in Fig 1. It also interacts with synaptobrevin which is a key SNARE protein and is involved in the regulation synaptic plasticity and neurotransmitter release [25], a process thought to be disrupted in schizophrenia.

Multiple groups have studied synaptophysin expression at the protein and mRNA levels in brain regions implicated for schizophrenia. While not always consistent, these findings strongly suggest alterations in the expression levels of synaptophysin in some key brain regions. Deficits in the expression of synaptophysin were reported in the lateral and basolateral amygdaloid nucleus of individuals with schizophrenia in a study using immunohistochemical methods conducted by [26•]. The subjects in the study had been appropriately matched for age and other key parameters and had no evidence of neurodegenerative disease. The amygdala have been reported to play a critical role in the recognition and the response to emotional stimuli, including fear and anxiety [27, 28] which are abnormal in subjects with schizophrenia. In addition, the amygdala is intimately connected to the frontal cortex, which is involved in executive function and emotional regulation [29–31].

Other studies have looked at the cingulate gyrus, which is part of limbic system and have been implicated in schizophrenia. In a study that used western blot for quantification, synaptophysin was significantly decreased in the schizophrenic group when compared with the control group [32]. Another study made similar observations in the cingulate cortex using western blot and also showed that such alterations are not likely from antipsychotic use[33]. Reductions in synaptophysin expression in schizophrenia have also been reported in different brain regions by various groups including, in the left thalamus using radioimmunoassay by [34] and in the medial temporal lobe using immunoradiography [35]. Furthermore synaptophysin immunoreactivity was significantly reduced in both the inner and outer molecular layers of the dentate gyrus [36] and also in the glomeruli of the olfactory bulb [37••]

Several groups have also looked at mRNA expression levels including[38••] who examined both the mRNA and the protein expression levels of synaptophysin in DLPFC using the same cohort and reported significantly decreased levels of both protein and mRNA in schizophrenia. Similar observations were made by [39] who reported significant decreases in levels of synaptophysin RNA transcripts in the entorhinal cortex of schizophrenia subjects compared to controls. In another study using in situ hybridization to assess the expression of synaptophysin mRNA and protein respectively in medial temporal lobe, [35] reported that synaptophysin mRNA was reduced bilaterally in CA4, CA3, subiculum and parahippocampal gyrus, with a similar trend in dentate gyrus but no change in CA1 region in the individuals with schizophrenia,

Overall a preponderance of these studies have shown a decrease in the expression levels of synaptophysin protein and mRNA in key brain areas affected by schizophrenia. Though it is not yet clear what role if any this plays in the etiopathogenesis and symptomatology of the illness, it is conceivable that it could impair the targeting of neurotransmitter rich vesicles to the synaptic terminal and hence contributes to dysfunctional neuroexocytosis. The defective neurotransmitter release could affect signal transmission across the synapses and also the function and structure of the post-synaptic terminals which have been reported in the illness.

Synapsins

They are a group of proteins that play a critical role in the regulation of neurotransmitter release at synapses. They are thought to be involved in regulating the number of synaptic vesicles available for release via exocytosis at any given time as shown in Fig 1. Evidence suggest that synapsins bind synaptic vesicles to components of the cytoskeleton which prevents them from migrating to the presynaptic membrane and releasing transmitter [40]. During an action potential, synapsins are phosphorylated by PKA releasing the synaptic vesicles and allowing them to move to the membrane and release their neurotransmitter.

Few groups have studied the expression of multiple synapsin isoforms at the protein and mRNA levels in brain regions implicated for schizophrenia. Although findings vary between studies, synapsins were reported to be altered in some of the studies. In a study by [41] they reported a decrease in the expression of synapsin-2a and 3a in the hippocampus of individuals with schizophrenia compared to matched controls. While another group that looked at DLPFC reports significantly decreased synapsin-3 levels in individuals with

schizophrenia compared to controls.[42]. Another study that used immunohistochemistry to study protein expression with the glomeruli of olfactory bulbs also reported significantly decreased synapsin-2a in schizophrenia compare to matched controls.[37••]

Similarly mRNA expression using real-time reverse transcriptase-PCR showed decreased synapsin-2a and 2b mRNA in DLPFC of patients with schizophrenia when compared with healthy subjects [43••]. A different group however reported that there were no differences in either the mRNA or protein levels of synapsin-2 in the prefrontal cortex of schizophrenic and control samples [44].

SNARE proteins

The SNARE (Soluble NSF Attachment protein receptors) is composed of the proteins synaptobrevin, also called VAMP, (vesicle-associated membrane protein), Syntaxin, and SNAP-25 (Soluble NSF Attachment protein of 25 kDa). They acting in concert play a crucial role in vesicle fusion for neurotransmitter release. Two of the SNARE proteins termed t-SNAREs are located at the cell plasma membrane (SNAP-25 and Syntaxin), and synaptobrevin or v-SNARE are located on the vesicles. SNAREs are ubiquitous membrane proteins necessary for membrane fusion and thus are involved in vesicle trafficking and exocytosis [21, 45]. At the presynaptic active zone, the t-SNARE interacts with the v-SNARE to form a heterotrimer known as the SNARE complex as shown in Figure 2, which is necessary for synaptic vesicle exocytosis [46]. The exact mechanism by which this vesicle fusion occurs is still poorly understood. However the critical importance of this protein in exocytosis and neurotransmission is demonstrated by the finding that proteolytic cleavage of the SNARE proteins by neurotoxins results in strong inhibition of neurotransmitter release in neurons.[47]

SNAP-25

It is a SNARE protein that is involved in the molecular regulation of neurotransmitter release. It plays an important role in the synaptic function of specific neuronal systems and associates with proteins involved in vesicle docking and membrane fusion.[48] It also regulates plasma membrane recycling through its interaction with other proteins. Expression levels of SNAP-25 been studied in schizophrenia given its key role in neurotransmitter release. Immunocytochemical studies demonstrated reduced SNAP-25 immunoreactivity in schizophrenia compared to controls, particularly in the terminal fields of entorhinal cortex projections [49]. The results also showed some reduction in hippocampal SNAP-25 in schizophrenia, but with relatively greater loss of SNAP-25 in the terminal zones of perforant pathway afferents. This might indicate that intrinsic connections of the hippocampus may also be affected in schizophrenia. Which is corroborated by other studies that showed significantly decreased SNAP-25 in the hippocampus of the schizophrenia patients using Western blotting [50] and immunohistochemistry [51].

Others have made similar findings and reported decreased levels of SNAP-25 in both the inferior temporal cortex and prefrontal association cortex in postmortem brains of patients with schizophrenia relative to non-schizophrenic controls using Western blotting while normal levels were noted in the primary visual cortex(Brodmann's area 17) [52]. These

findings suggest that the altered level of SNAP-25 are region specific, and affects regions that are known to be affected in schizophrenia.

Similar significant reductions in SNAP25 expression in schizophrenia measured with quantitative IHC have also been reported in other brain regions including ventromedial caudate (VMC) and Nucleus accumbens (NAc), [53] and glomeruli of olfactory bulb [37••]. However one study looking at the hippocampus using Western blotting found no significant changes in SNAP-25 between schizophrenia and controls. [54].

SNAP25 occurs on chromosome 20p12.2, which has been linked to schizophrenia in some samples, [55–59]. No identified studies have examined the levels of SNAP25 mRNA transcripts in Schizophrenia, but a preponderance of the studies that looked at the protein levels have shown a decrease in the expression levels of SNAP25 protein in key brain areas affected by schizophrenia.

Syntaxin-1 and syntaxin binding protein-1/munc18

Along with SNAP-25 is a t-SNARE protein involved in the molecular regulation of neurotransmitter release. Syntaxin-1 is present in synaptic terminals in a closed conformation that cannot engage in SNARE complex formation; syntaxin-1 has to open for SNARE complex assembly to proceed in a process initiated by the binding of syntaxin binding protein-1 to the closed conformation of syntaxin-1 [22]. This binding opens up syntaxin-1, enabling SNARE complex formation and exocytosis. Syntaxin is a large family of proteins that are found in variety of tissues and cellular compartments but have two neuron-specific isoforms (syntaxin 1A and 1B) [60]

Multiple groups have studied syntaxin expression at the protein and mRNA levels in brain regions implicated for schizophrenia. While not always consistent, these findings strongly suggest alterations in the expression levels of syntaxin in some key brain regions. Increased expression levels of syntaxin in the cingulate cortex of subjects with schizophrenia using Western blotting, however this contrasted with three other cortical regions, superior frontal gyrus, temporal gyrus and the inferior parietal lobule in which no difference was found. In a different study [61•], reported an increase in the expression of syntaxin-1 in the dorsolateral prefrontal cortex of schizophrenic subjects. The study also showed a positive correlation between munc18-1 and syntaxin-1a protein expression in controls but showed a marked increase in the ratio of syntaxin 1a to munc18-1. This showed that the increase in syntaxin was not associated with a corresponding increase in its binding protein. A study demonstrated an increase in syntaxin immunoreactivity in the SNARE heterotrimeric complex in the prefrontal cortex of schizophrenics [62]. This indicates a dysregulation in the protein-protein interactions of key SNARE proteins. Interestingly a study that looked at the phosphorylated syntaxin-1 reported decreased levels of the phosphorylated protein [63] in contrast to most of the other studies that had reported a total increase in Syntaxin levels.

Other studies found no significant difference between schizophrenics and controls in the expression levels of syntaxin in the prefrontal cortex.[64] and in the cerebellum.[65]. In contrast to other SNARE proteins most studies showed either no change or an increase in the expression levels of syntaxin-1 protein in key brain areas affected by schizophrenia. Given

that a study showed decreased levels of phosphorylated syntaxin-1, it is conceivable that a defect in the phosphorylation of Syntaxin-1 could lead to dysregulation in the protein-protein interactions of key vesicular proteins could underlie the pathophysiology synaptic abnormalities in schizophrenia.

Genetic studies also have found a statistically significant genetic association between the STX1A intron 7 polymorphism and schizophrenia [66] but no identified studies have looked at the syntaxin-1 mRNA.

Few studies have looked at syntaxin binding protein which binds to syntaxin in order to allow for the conformational change necessary for exocytosis to occur. In a study by [67] reported a significant increase in the expression levels of syntaxin-1 binding protein. Interestingly in another study a transgenic mouse strain that overexpresses the protein isoform munc18-1a in the brain was characterized and it displayed several schizophrenia-related behaviors like super sensitivity to hallucinogenic drugs and deficits in prepulse inhibition that reverse after antipsychotic treatment.[68]. In a different study however they found no changes in the expression levels of munc18-1a/b isoforms though the presence of antipsychotic drugs reduced the basal content of munc18-1a isoform [61]. Thus the evidence though very limited suggests a relative increase in syntaxin with no corresponding increase in its binding protein in schizophrenia.

Synaptobrevin (VAMP)—Is involved in the targeting and/or fusion of transport vesicles to their target membrane. Synaptobrevin binds with a high affinity to the t-SNARE complex formed by SNAP-25 and syntaxin. This forms a coiled coil structure, with binding initiating at the N-termini and zippering along the remainder of the binding domain. This serves to pull the two membranes into a closer proximity, enhancing the potential for fusion to occur.

Few studies have looked at the expression levels of synaptobrevin in schizophrenia and the results have largely been varied. Synaptobrevin was significantly decreased in the PFC of individuals with schizophrenia when compared with the matched controls using Western blot.[64] (Halim, Weickert e While another study that also used Western blot to look at protein levels found no significant difference in the levels of synaptobrevin in DLPFC between schizophrenia and controls. Though the levels of synaptobrevin showed a trend towards decrease in schizophrenia, it however was not statistically significant [69]. Another group made a similar observation when they looked at three different brain regions affected in schizophrenia-Brodman's areas 10, 40 and 46. They found no differences in the expression levels of synaptobrevin between schizophrenia and controls [54]

A study that looked at snaptobrevin-1 mRNA levels in the DLPFC found no significant differences between schizophrenia and healthy controls or bipolar-1 patients [70] while another found no genetic association between schizophrenia and synaptobrevin-2 gene [71]. Though the results are varied but the few available show that there is no significant association on the genetic and protein level between synaptobrevin and schizophrenia

Complexins—Positively regulates a late step in synaptic vesicle exocytosis. Negatively regulates the formation of synaptic vesicle clustering at active zone to the presynaptic

membrane in post-mitotic neurons. Organizes the SNAREs into a cross-linked zigzag topology that, when interposed between the vesicle and plasma membranes, is incompatible with fusion, thereby preventing SNAREs from releasing neurotransmitters until an action potential arrives at the synapse. There are two common isoforms, complexin 1&2. Complexin-2 are expressed primarily by excitatory neurons, whereas complexin-1 is mainly expressed by inhibitory neurons. Post-mortem and other studies have provided evidence for alterations in complexin expression in schizophrenia. Studies on complexin expression at the protein and mRNA levels in brain regions implicated for schizophrenia, while not always consistent, strongly suggest significant decrease in the expression levels of complexin in some key brain regions. A study of post mortem prefrontal cortex using ELISA [72] showed significant decrease in complexin1 immunoreactivity levels in schizophrenia and also significantly increased complexin-2/I mean ratios. In the hippocampus reported decreased complexin-1&2 expressions and lower complexin-2/complexin-1 ratio in subjects with schizophrenia which was inversely correlated with the severity of ante mortem cognitive impairment. This suggests that alterations in complexins' function play a crucial role in the cognitive impairment in schizophrenia which might be related to other negative symptoms. Another group using post mortem tissue reported that the total tissues homogenates of complexin-1&2 were unchanged in the orbitofrontal and the anterior cingulate cortexes of schizophrenia [73•], however after immunoprecipitation with SNAP-25, complexin-1 but not complexin-2 was found to be significantly enriched in the immunoprecipitate. This indicates that though the expression levels were unaltered however the relative amounts of complexin-1 interacting with SNAP-25 was increased in schizophrenia.

Genetic studies have shown single-nucleotide polymorphisms, distributed over the CPLX2 gene, were found to be highly associated with current cognition of schizophrenic subjects compared with matched controls [74, 75].

In studies looking at mRNA expression complexin-2 mRNA was reduced in dorsolateral prefrontal cortex (DPFC), superior temporal cortex (STC), and complexin-1 mRNA decreased in STC.[76]

Other interacting proteins

There are multiple other protein molecules that interact with the SNARE and its interacting proteins to smooth and efficient presynaptic neurotransmitter release, for details see [20] [19]. These include the synaptotagmin family, which is localized in cells either in synaptic vesicles or the cellular membrane, and is known to act as a calcium sensor and plays a crucial role in neurotransmitter release. There are also the chaperone systems that involve HSC 70/alpha-CSP/SGT and synuclein that are crucial in maintaining the SNARE in a functional state [19]. It is critically important to study these proteins and their interactions to better appreciate their roles in schizophrenia and other psychiatric illnesses. However at this point there are no published studies on the database about the expression and interactions of these proteins in schizophrenia. The key findings from the different studies are shown in Table 1.

Conclusions

The mechanism involved in the release of neurotransmitters at pre-synaptic terminals in the brain is complex and involves several intricate steps from vesicular recruitment/formation to membrane fusion/neurotransmitter release and endocytosis. Dysregulations at any of these intricate steps would adversely affect efficient neurotransmission across the synapses with significant neurobiologic and behavioral effects.

There is strong evidence for a link between key presynaptic proteins and schizophrenia, although some studies looking at these proteins in schizophrenia have been inconclusive. This evidence points to alterations in the SNARE complex and its interacting proteins expression in the post mortem brains of patients with schizophrenia as table –1 illustrates. This makes it plausible as the common pathway for the development of schizophrenia that fits most of the theories of schizophrenia available to date. Significantly most of the studies point towards a significant decrease in the expression levels of synaptophysin and synapsins which are reliable markers for presynaptic terminal density. Of the SNARE proteins SNAP25 and synaptobrevin were decreased in a number of studies and none of the studies reported an increase in the expression level while in the case of syntaxin none of the studies reported of an increase while one noted significantly decreased expression levels of syntaxin-1. This indicates that it is very plausible that the alterations are region specific in the brain and might also reflect altered interactions between the different proteins involved in the presynaptic neurotransmitter release mechanism. These studies have however relied on postmortem tissues for protein and mRNA estimation and there are significant problems with the use of postmortem tissues as there are several confounding factors that are difficult to control for which include variables in medications, smoking, alcohol and other drug use. In addition there are also significant variables in postmortem tissue including but not limited to the postmortem interval (PMI), tissue PH, age and handling/method of storage. Given all these variables, the greatest challenges lie with the difficulty in studying synaptic activities at the molecular level in humans given that the processes taking place at the presynaptic terminals are very dynamic with multiple interactions in very small micro domains. A good way to overcome some of these obstacles will involve real time imaging study of the synapses in live subjects and at this time there is no technology to appropriately do that. The next best option is to use a neural circuit that is affected in schizophrenia and is easily accessible such as the olfactory circuit. Given the relatively easy accessibility to the olfactory neurons, this might represent the best approach to the real time study of the dynamics of presynaptic activity in live subjects.

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Figure 1. Presynaptic Terminal

Schematic diagram of a presynaptic terminal with synaptic vesicles (SV), showing steps involved vesicle formation, fusion, neurotransmitter release and vesicle recycling via endocytosis at the active zone. Ca^{2+} -channels and other pre and post-synaptic receptors along with the postsynaptic density (PSD) are also shown.

Figure 2. Synaptic Vesicle

Schematic diagram of a synaptic vesicle showing the SNARE and other key proteins involved in vesicular formation, targeting and membrane fusion and recycling at the presynaptic terminals. The arrows indicate the relative expression levels of each protein in schizophrenia when compared to matched controls.

Table 1

Study	Summary table of studies.				Assay	Finding
	Gene	Brain region	Molecule			
Halim et al, 2003	Synaptophysin	PFC	Protein	WB	No change	
Weickert et al, 2004	Synaptophysin	Dorsolateral PFC	Protein	ISH	No change	
Scarr et al, 2006	Synaptophysin	Dorsolateral PFC	Protein	WB	No change	
Gray et al, 2010	Synaptophysin	Anterior PFC	Protein	WB	No change	
		Dorsolateral PFC				
Egbujo et al, 2015	Synaptophysin	Olfactory Bulb	Protein	IHC	Decreased	
Varea et al, 2012	Synaptophysin	Amygdala	Protein	IHC	Decreased	
Landen et al, 2002	Synaptophysin	Cingulate gyrus	Protein	WB	Decreased	
Eastwood et al, 2001	Synaptophysin	Cingulate gyrus	Protein	WB	Decreased	
Rao et al, 2013	Synaptophysin	Dorsolateral PFC	Protein	WB	Decreased	
Rao et al, 2013	Synaptophysin	Dorsolateral PFC	mRNA	RT-PCR	Decreased	
Chambers et al, 2005	Synaptophysin	Dentate gyrus	Protein	IHC	Decreased	
Vavter et al, 2002	Synapsin-2a & 3a	Hippocampus	Protein	WB	Decreased	
Porton et al, 2007	Synapsin-3	Dorsolateral PFC	Protein	WB	Decreased	
Tan et al, 2014	Synapsin-2a & 2b	Dorsolateral PFC	mRNA	RT-PCR	Decreased	
Egbujo et al, 2015	Synapsin-2a	Olfactory bulb	Protein	IHC	Decreased	
Imai et al, 2001	Synapsin-2	Dorsolateral PFC	Protein	WB	No change	
Imai et al, 2002	Synapsin-2	Dorsolateral PFC	mRNA	RT-PCR	No change	
Barakauskas et al, 2010	SNAP-25	Venteromedial cortex	Protein	IHC	Decreased	
		Nucleus accumbens				
Young et al, 1998	SNAP-25	Entorhinal cortex	Protein	IHC	Decreased	
Thompson et al, 1998	SNAP-25	Temporal cortex	Protein	WB	Decreased	
		Dorsolateral PFC				
		Ant PFC				
Thompson et al, 1999	SNAP-25	Visual cortex	Protein	WB	No change	
Fatemi et al, 2001	SNAP-25	Hippocampus	Protein	IHC	Decreased	
Thompson et al, 2003	SNAP-25	Hippocampus	Protein	WB	Decreased	

	Summary table of studies.					
Study	Gene	Brain region	Molecule	Assay	Finding	
Halim et al, 2003	SNAP-25	PFC	Protein	WB	No change	
Scarr et al, 2006	SNAP-25	Dorsolateral PFC	Protein	WB	No change	
Scarr et al, 2006	SNAP-25	Dorsolateral PFC	mRNA	RT-PCR	No change	
Gray et al, 2010	SNAP-25	Ant PFC	Protein	WB	No change	
		Dorsolateral PFC				
Barakauskas et al, 2010	SNAP-25	Venteromedial cortex	Protein	WB	Decreased	
		Nac				
Egbujo et al, 2015	SNAP25	Olfactory Bulb	Protein	IHC	Decreased	
Gabriel et al, 1997	Syntaxin-1	Cingulate gyrus	Protein	WB	Increased	
Gabriel et al, 1997	Syntaxin-1	Superior frontal gyrus	Protein	WB	No change	
		Temporal gyrus				
		Inferior parietal lobule				
Honer et al, 2002	Syntaxin-1	PFC	Protein	WB	Increased	
Halim et al, 2003	Syntaxin-1	PFC	Protein	WB	No change	
Scarr et al, 2006	Syntaxin-1	Dorsolateral PFC	Protein	WB	No change	
Gray et al, 2010	Syntaxin-1	Anterior PFC	Protein	WB	No change	
		Dorsolateral PFC				
Gil-Pisa et al, 2012	Syntaxin-1	Dorsolateral PFC	Protein	WB	Increased	
Ramos-Miguel et al, 2015	Synataxin	Orbitofrontal	Protein	WB	No change/Enriched in SNAP-25 IP	
Halim et al, 2003	Synaptobrevin	PFC	Protein	WB	Decreased	
Scarr et al, 2006	Synaptobrevin	Dorsolateral PFC	Protein	WB	No change	
Gray et al, 2010	Synaptobrevin	Ant PFC	Protein	WB	No change	
		Dorsolateral PFC				
Sawada et al, 2002	Complexin-1	Dorsolateral PFC	Protein	ELISA	Decreased	
Eastwood et al, 2005	Complexin-1	Superior temporal cortex	mRNA	ISH	Decreased	
		Orbitofrontal AC				
Ramos-Miguel et al, 2015	Complexin-1		Protein	WB	No change/Enriched in SNAP-25 IP	

Summary table of studies.					
Study	Gene	Brain region	Molecule	Assay	Finding
Sawada et al, 2002	Complexin-2	Dorsolateral PFC	Protein	ELISA	No change
		Ant PFC			
Ramos-Miguel et al, 2015	Complexin-2	Orbitofrontal	Protein	WB	No change
		Anterior Cingulate			
Eastwood et al, 2005	Complexin-2	DLPFC	mRNA	ISH	Decreased
		Superior temporal cortex			