Glutamate-mediated excitotoxicity in schizophrenia: A review

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Contributors

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Conflict of interest

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Abstract

Findings from neuroimaging studies in patients with schizophrenia suggest widespread structural changes although the mechanisms through which these changes occur are currently unknown. Glutamatergic activity appears to be increased in the early phases of schizophrenia and may contribute to these structural alterations through an excitotoxic effect. The primary aim of this review was to describe the possible role of glutamate-mediated excitotoxicity in explaining the presence of neuroanatomical changes within schizophrenia. A Medline® literature search was conducted, identifying English language studies on the topic of glutamate-mediated excitotoxicity in schizophrenia, using the terms “schizophreni*” and “glutam*” and (“MRS” or “MRI” or “magnetic resonance”) or (“computed tomography” or “CT”). Studies concomitantly investigating glutamatergic activity and brain structure in patients with schizophrenia were included. Results are discussed in the context of findings from preclinical studies. Seven studies were identified that met the inclusion criteria. These studies provide inconclusive support for the role of glutamate-mediated excitotoxicity in the occurrence of structural changes within schizophrenia, with the caveat that there is a paucity of human studies investigating this topic. Preclinical data suggest that an excitotoxic effect may occur as a result of a paradoxical increase in glutamatergic activity following N-methyl-D-aspartate receptor hypofunction. Based on animal literature, glutamate-mediated excitotoxicity may account for certain structural changes present in schizophrenia, but additional human studies are required to substantiate these findings. Future studies should adopt a longitudinal design and employ magnetic resonance imaging techniques to investigate whether an association between glutamatergic activity and structural changes exists in patients with schizophrenia.

Keywords

Schizophrenia; Glutamate; Glutamine; MRS; Excitotoxicity; Psychosis

1. Introduction

This section provides a comprehensive explanation of topics relevant to the study of glutamate-mediated excitotoxicity in schizophrenia, beginning with a background of the illness and its dopaminergic hypothesis. The limitations of the dopaminergic hypothesis are important in bringing forth the glutamatergic hypothesis of schizophrenia. Subsequently, N-methyl-D-aspartate (NMDA) receptor hypofunction is introduced as a model for schizophrenia, which is followed by an explanation of glutamatergic dysfunction in...
1.1. Schizophrenia

Schizophrenia is a debilitating illness, present in approximately 1% of the global population and characterized by positive, negative and cognitive symptoms (Sullivan et al., 2003; Weiser et al., 2005). The primary treatment for schizophrenia is dopamine receptor antagonism with anti-psychotic medication (Frangou, 2008). The clinical effects of dopamine receptor antagonists have provided the basis for the dopamine hypothesis of schizophrenia (Creese et al., 1976; Seeman and Lee, 1975), which posits that patients with the illness have aberrant functioning of the dopaminergic system (Abi-Dargham et al., 1998; Breier et al., 1997; Hietala et al., 1995; Laruelle et al., 1996). The dopamine hypothesis is limited in that it only addresses positive symptoms (Javitt et al., 2012); antipsychotics have minimal efficacy in the treatment of negative and cognitive symptoms (George et al., 2013; Miyamoto et al., 2012). Another limitation of the dopamine hypothesis is that 20–35% of patients show partial or no response to antipsychotic treatments (Lindenmayer, 2000; Suzuki et al., 2011). In addition, this hypothesis does not appear to adequately explain the neuroanatomical changes in patients with schizophrenia (Stone et al., 2007). Thus, the dopaminergic system does not describe the illness in its entirety (Moghaddam and Javitt, 2012). The glutamatergic hypothesis provides an alternate mechanism to explain the pathophysiology of schizophrenia.

1.2. Glutamatergic hypothesis of schizophrenia

Glutamate antagonists, such as phencyclidine (PCP) and ketamine, are well known to transiently induce symptoms similar to those observed in patients with schizophrenia (Coyle et al., 2003). Glutamate antagonists are unique in that they not only produce psychotomimetic effects, but also elicit negative and cognitive symptoms (Javitt and Zukin, 1991; Vollenweider and Geyer, 2001). Such effects have been reported following the acute administration of glutamate antagonists to healthy volunteers (Adler et al., 1999; Krystal et al., 1994, 2000, 2005; Malhotra et al., 1996), while administration of these agents to patients with schizophrenia exacerbates symptoms (Lahti et al., 1995a, 1995b). The observed symptomatic effects of glutamate antagonists provide the basis for the glutamatergic hypothesis of schizophrenia (Kantrowitz and Javitt, 2012).

1.3. NMDA receptor hypofunction

Glutamate antagonists induce schizophrenia-like symptoms through modulation of the NMDA receptor. PCP and ketamine are both non-competitive antagonists that exert their physiological effects by binding to the PCP receptor, a specific hydrophobic binding site coupled to the NMDA receptor (Javitt, 2007). Through this binding, PCP and ketamine inhibit the action of glutamate at the NMDA receptor, suggesting that the pathophysiology of schizophrenia may similarly result from dysregulation of the NMDA receptor (Javitt et al., 2012). Current proponents of the glutamatergic hypothesis postulate that hypofunctional NMDA receptors located on gamma-aminobutyric acid (GABA)–ergic inhibitory
interneurons disinhibit pyramidal neurons, leading to a paradoxical increase in glutamatergic activity (Moghaddam and Krystal, 2012; Nakazawa et al., 2012; Stone et al., 2007).

### 1.4. Glutamatergic dysfunction in schizophrenia

The role of the NMDA receptor in increasing glutamate is supported by both preclinical and human studies using NMDA receptor antagonists. Acute treatment of rodents with NMDA receptor antagonists results in increased extracellular glutamate in the striatum and prefrontal cortex (Bustos et al., 1992; Moghaddam et al., 1997), and increased glutamine (the main metabolite of glutamate) in the pre-frontal cortex (Iltis et al., 2009). Studies in healthy human participants employing proton magnetic resonance spectroscopy ($^{1}{H}$-MRS) report increased glutamate and glutamine in the anterior cingulate after the acute administration of a sub-anaesthetic dose of ketamine (Rowland et al., 2005; Stone et al., 2012). In addition, agents that inhibit glutamate release reverse behavioural, cognitive, and cerebral blood flow changes induced by NMDA receptor antagonists in healthy human volunteers (Anand et al., 2000; Deakin et al., 2008; Doyle et al., 2013).

The aforementioned findings in rodents and healthy humans following acute treatment with NMDA receptor antagonists are comparable to $^{1}{H}$-MRS studies in patients with schizophrenia, which report increased glutamate levels in antipsychotic-free and naïve subjects during their first episodes of psychosis, as well as in subjects at ultra-high risk for psychosis (de la Fuente-Sandoval et al., 2011, 2013a; Kegeles et al., 2012; Kraguljac et al., 2013; Purdon et al., 2008). $^{1}{H}$-MRS studies have also demonstrated higher glutamine levels in antipsychotic-naïve patients with schizophrenia (Bartha et al., 1997; Theberge et al., 2002). While there is strong evidence to support increased glutamatergic activity in patients with untreated schizophrenia, it should be noted that studies investigating medicated patients with schizophrenia have reported glutamatergic marker decreases or levels similar to healthy controls (Bustillo et al., 2011; de la Fuente-Sandoval et al., 2013b; Goto et al., 2012; Kegeles et al., 2012; Ohrmann et al., 2005; Rowland et al., 2013; Theberge et al., 2003). Thus far, two studies have made direct comparisons between unmedicated and medicated patients, both showing elevated glutamate levels in the unmedicated state and normal glutamate levels in the medicated state. Using a longitudinal within-subject comparison, one study in particular administered clinically effective antipsychotic treatment (reduction of at least 30% on the total score of the Positive and Negative Syndrome Scale after 4 weeks) to antipsychotic-naïve patients with first-episode psychosis, significantly decreasing elevated baseline glutamate in the associative striatum, such that levels following treatment did not differ from controls (de la Fuente-Sandoval et al., 2013b). Notably, this study specifically included patients who responded to treatment. Another study utilized a cross-sectional approach to compare unmedicated patients, medicated patients and healthy controls, reporting increased Glx in the medial prefrontal cortex region of unmedicated patients, in comparison to controls, whereas no such difference existed between medicated patients and the control group (Kegeles et al., 2012). To further elucidate the role of treatment in changing glutamatergic activity, a recent review noted that glutamatergic levels are elevated in anti-psychotic naïve patients but are similar to those of healthy controls in medicated patients with schizophrenia, independent of stage of illness (Poels et al., 2014a). This is contrasted by a meta-analysis that demonstrated that glutamate and glutamine concentrations...
decrease at a faster rate with age in patients with schizophrenia, as compared to healthy controls (Marsman et al., 2013).

However, it should be noted that recent research observed higher glutamate levels in the anterior cingulate cortex of antipsychotic-treated first episode patients with unremitting psychotic symptoms and in treatment-resistant patients than in medication responders (Demjaha et al., 2014; Egerton et al., 2012). These findings suggest that an alternative underlying pathophysiology may exist in patients with treatment-resistant schizophrenia than in patients who respond well to antipsychotics – one that similarly involves the glutamatergic system, yet is not modulated by dopaminergic regulation.

1.5. Glutamate as an excitotoxic factor

Glutamate has the potential to induce neuronal dysfunction and degeneration when present in abnormally high extracellular concentrations (Lahti and Reid, 2011; Lau and Tymianski, 2010; Mehta et al., 2013). This process is referred to as excitotoxicity, a term coined by John Olney (Olney, 1969; Olney and Sharpe, 1969), who posited that excessive stimulation by glutamate has the capacity to vastly increase intracellular calcium, affecting calcium homeostatic mechanisms and triggering a cascade of events that ultimately result in cell death (Lau and Tymianski, 2010). Though the exact mechanisms of this phenomenon are only partially known, calcium influx is highly implicated (Belousov, 2012; Choi, 1988; Hardingham and Bading, 2010). In schizophrenia, the disruption in glutamatergic signalling may result in an excitotoxic effect secondary to excess stimulation of non-NMDA glutamate receptors (i.e AMPA and Kainate), leading to the structural findings associated with the illness (Abbott and Bustillo, 2006; Deutsch et al., 2001).

1.6. Structural changes in schizophrenia

Neuroanatomical changes are often reported in patients with schizophrenia; for example, progressive loss of grey matter volume is common in both early and chronic stages of the illness (Arango et al., 2012; Hulshoff Pol and Kahn, 2008; Meyer-Lindenberg, 2011; van Haren et al., 2008). Recent meta-analyses investigating grey matter losses in schizophrenia most commonly identify volumetric reduction within superior temporal, medial temporal, superior prefrontal, medial prefrontal and insular regions, along with the thalamus and basal ganglia (Bora et al., 2011; Chan et al., 2011; Ellison-Wright et al., 2008; Glahn et al., 2008; Honea et al., 2005; Shenton et al., 2001; Steen et al., 2006). Whole brain volume reductions, ventricular enlargement and white-matter alterations are also frequently reported (Colibazzi et al., 2013; Connor et al., 2011; Lawrie and Abukmeil, 1998; Nazeri et al., 2013; Shenton et al., 2001; Voneskos et al., 2013).

In addition, reductions in cortical thickness are common in patients with schizophrenia. Various studies have observed cortical thinning in schizophrenia, particularly within frontal, temporal, parietal and cingulate regions, though insular and occipital areas are also affected (Goldman et al., 2009; Kuperberg et al., 2003; Narr et al., 2005; Rais et al., 2010; Rimol et al., 2010; van Haren et al., 2011; White et al., 2003).

The occurrence of these neuroanatomical changes is largely unexplained. Though the changes may conceivably result from medication intake and prolonged illness progression...
(Moncrieff and Leo, 2010; Navari and Dazzan, 2009; Torres et al., 2013; van Haren et al., 2012; Vita et al., 2012), studies utilizing first episode schizophrenia patients have provided evidence that structural changes occur irrespective of continuous antipsychotic treatment and years of illness duration. First episode schizophrenia patients with little or no exposure to antipsychotics exhibit neuroanatomical alterations within a number of brain regions in comparison with healthy controls (Chen et al., 2014; Fornito et al., 2008; Narr et al., 2005; Ren et al., 2013; Schultz et al., 2010; Sprooten et al., 2013; Steen et al., 2006; Venkatasubramanian et al., 2008; Vita et al., 2012). Glutamate-mediated excitotoxicity may contribute to these structural changes present in patients with schizophrenia (Abbott and Bustillo, 2006; Goff and Coyle, 2001; Stone et al., 2007).

1.7. Aim of this review

The glutamatergic hypothesis offers a mechanism through which neuroanatomical changes may occur: glutamate-mediated excitotoxicity. In short, elevated glutamatergic neurotransmission, which is highly implicated in the pathology of schizophrenia, may have neurotoxic effects. The primary aim of this review was to describe the potential role of glutamate-mediated excitotoxicity as an explanatory mechanism for the neuroanatomical changes observed in patients with schizophrenia. To do so, findings from human studies were reviewed and discussed, followed by a presentation of the evidence from preclinical literature. Limitations of both human and preclinical studies were considered in drawing conclusions and providing future research directions.

2. Experimental procedures

A Medline literature search (1946-April Week 3 2014) was performed to identify studies, reviews or case reports relevant to glutamate-mediated excitotoxicity in patients with schizophrenia. The search was conducted using the terms “schizophreni*” (Subheadings: schizophrenia, antipsychotic agents and psychotic disorders) and “glutam*” (Subheading: magnetic resonance spectroscopy) and (“MRS” or “MRI” or “magnetic resonance”) or (“computed tomography or “CT”)). Only English language human publications were included. Reference sections of major review articles (Deutsch et al., 2001; Javitt, 2007; Marsman et al., 2013; Poels et al., 2014a; Stone et al., 2007; Tsai and Coyle, 2002) were examined for additional, relevant articles that were overlooked by the search strategy. Articles were included if they concomitantly measured markers of glutamatergic activity and brain structure using magnetic resonance imaging (MRI) or computed tomography (CT), and investigated the relationship between the two measurements. Studies utilizing participants deemed to be at risk for schizophrenia were included. The last search was conducted on April 10th 2014. Findings resulting from this search are discussed in the context of established preclinical data.

3. Results

The Medline search yielded 622 publications. All titles and abstracts were read by two of the authors (E.P. and S.N.). Thirteen papers concurrently investigated glutamatergic activity and brain structure, and were thus selected and reviewed. Six articles were excluded because they failed to include statistics regarding the relationship between glutamatergic markers and
neuroanatomical measures (Bartha et al., 1999; Duncan et al., 2013; Gruber et al., 2012; Kegeles et al., 2000; Rusch et al., 2008; Wood et al., 2010). The supplementary search through the reference sections of the specified review articles resulted in no additional articles that concurrently measured glutamatergic activity and brain structure. Thus, all remaining studies (n=7) (Aoyama et al., 2011; Klar et al., 2010; Kraguljac et al., 2013; Stone et al., 2009; Tandon et al., 2013; Theberge et al., 2007; Tsai et al., 1998) that reported on the relationship between glutamatergic activity and brain structure in patients with schizophrenia were retained. These studies are summarized in Table 1. Six of the studies utilized 1H-MRS to assess glutamatergic markers, while one measured cerebrospinal fluid (CSF) glutamate. Six of the studies used volumetric measurements to assess structure – four of which were specific to grey matter – and one study assessed structure through ventricle to brain ratio (VBR) and pre-frontal atrophy.

Studies that met the inclusion criteria indicate that glutamate, along with its metabolite glutamine, may have a relationship with neuroanatomical measurements. One study identified a negative correlation between two clusters of grey matter volume in the hippocampus and hippocampal Glx, a combined measure of glutamate and glutamine concentrations (Kraguljac et al., 2013). By contrast, another study that measured glutamate within the hippocampus and hippocampal volume failed to find such a relationship (Klar et al., 2010).

Two other publications report on a study that employed a longitudinal design and found a relationship between thalamic glutamine and grey matter volume (Aoyama et al., 2011; Theberge et al., 2007). Theberge et al. (2007) noted an association between decreased thalamic glutamine levels and parietal and temporal grey matter volume loss over the course of 30 months, beginning with a never-treated state. Aoyama et al. (2011) extended these findings to an 80-month follow-up, and noted a positive relationship between change in both thalamic glutamine and grey matter volume within frontal, parietal, temporal and limbic regions; thalamic glutamine levels and grey matter volume both decreased over the course of 80 months. Notably, no relationships between changes in grey matter volume and anterior cingulate glutamine or tGL, a summed measure of glutamate and glutamine levels, were reported within this study. This longitudinal design included the introduction of antipsychotic medication over the duration of the study.

A study involving individuals with an at-risk mental state (ARMS) investigated the relationship between grey matter volume and glutamate in the thalamus and glutamine in the anterior cingulate (Stone et al., 2009). This study identified positive associations in participants with an ARMS between thalamic glutamate and grey matter volume in the left prefrontal cortex, insula, cingulate, superior temporal gyrus and temporal pole, and bilaterally in the cerebellum and lingual gyrus. In the same study, negative correlations were found between thalamic glutamate and grey matter volume in the dorsal anterior cingulate extending to the posterior cingulate gyrus. Anterior cingulate glutamine was positively correlated with grey matter volume in the posterior cingulate gyrus and negatively correlated with grey matter volume in the left cerebellum. In another study that included individuals at familial high risk for schizophrenia, non-significant negative correlations were observed in...
the at-risk group between thalamic and caudate Glx, and thalamic and caudate volumes, respectively (Tandon et al., 2013).

Lastly, a CT investigation assessing CSF glutamate and VBR in antipsychotic-free chronic schizophrenia patients reported an inverse relationship between CSF glutamate and VBR (Tsai et al., 1998). Overall, the identified studies suggest that an association between markers of glutamatergic activity and structural measures may exist, although conflicting findings are reported within available literature.

4. Discussion

The overall aim of this review was to explore the evidence in humans for the relationship between glutamate related compounds (glutamate, glutamine and Glx) and structural brain measurements in patients with schizophrenia. A review of existing literature was conducted to elucidate the role of glutamate-mediated excitotoxicity in the structural brain changes associated with schizophrenia. Unexpectedly, the search yielded only seven studies that met inclusion criteria, reflecting the paucity of literature available to effectively address this topic in humans.

4.1. Analysis of reviewed studies

Of the seven studies identified from the search, Kraguljac et al. (2013) offered the most direct evidence for a glutamate-mediated excitotoxic effect. The authors attributed structural changes observed in the hippocampus to increases in glutamatergic activity by reporting a negative correlation between two clusters of grey matter volume and Glx within the hippocampus in the schizophrenia group, whereas no such relationship existed in the healthy control group (Kraguljac et al., 2013). In this study, the patient group was unmedicated. In contrast, Klar et al. (2010), which included medicated patients, failed to find an association between hippocampal glutamate and volume; however, the study may have been underpowered to find a significant relationship ($r= -0.356, p=0.074$).

Interestingly, in other studies that resulted from the search, measures of thalamic glutamatergic activity were associated with volumetric loss in a number of different brain regions. Decreases in thalamic glutamine paralleled grey matter volume decreases within frontal, temporal, parietal and limbic areas (Aoyama et al., 2011; Theberge et al., 2007). Based on the regions in which volume loss occurred and the involvement of the thalamus, excitotoxic damage was considered in the explanation of these findings. Results suggest that neurodegeneration secondary to glutamate-mediated excitotoxicity may result from decreased levels of thalamic glutamatergic activity. Consistent with this notion, a decrease in thalamic glutamatergic markers was observed in both studies, which may have resulted in diminished stimulation of NMDA receptors on GABAergic interneurons in the thalamus. This hypostimulation could subsequently result in toxicity induced by paradoxically high levels of glutamate in cortical regions through the disinhibition of thalamocortical circuits that use glutamate as a neurotransmitter.

Two additional studies offer evidence that the relationship between glutamatergic markers and volumetric measures is present in the early stages of schizophrenia. In one study of
participants with an ARMS, thalamic glutamate and anterior cingulate glutamine levels were associated with grey matter volume, demonstrating both positive and negative correlations depending on the brain region (Stone et al., 2009). Another study, which included individuals with familial high-risk for schizophrenia, reported that Glx in both the thalamus and the caudate was negatively associated with regional brain volume, though both correlations were not significant (Tandon et al., 2013).

Finally, one study measured CSF glutamate and VBR using CT in antipsychotic-free chronic schizophrenia patients (Tsai et al., 1998). An inverse relationship was reported between CSF glutamate and VBR, suggesting that glutamatergic cells may have degenerated over the duration of the illness as a result of toxic levels of glutamate, resulting in a lower glutamatergic measure at the time of assessment, consistent with an excitotoxic effect.

4.2. Limitations of reviewed studies

Overall, the studies listed in Table 1 have several limitations, and as such, do not individually offer sufficient evidence to make conclusive claims about the role of glutamatergic markers in the structural alterations observed in schizophrenia. The publications by Theberge et al. (2007) and Aoyama et al. (2011) were limited largely by the introduction of medication during the study. Antipsychotic treatment very likely confounded the relationship between markers of glutamatergic activity and volumetric losses; although patients were monitored throughout the study, it is expected that the introduction of medications influenced the serial neurochemical and structural measurements. This said, we acknowledge the practical challenges associated with performing a longitudinal study in medication-free patients. The studies conducted by Stone et al. (2009) and Tandon et al. (2013) were limited by the nature of the participant population. The authors included at-risk individuals, and although useful, this sample is not necessarily generalizable to patients with schizophrenia, as many at-risk participants may not progress to the illness (McGorry et al., 2009); one study reported a 35% transition rate to a psychotic disorder over a 10-year follow-up (Nelson et al., 2013). The studies by Kraguljac et al. (2013) and Klar et al. (2010) were limited by their targeted focus on glutamatergic levels and grey matter volume in the hippocampus. A common limitation shared among the aforementioned studies was the use of 1H-MRS, which quantifies the concentration of glutamate or glutamate-related compounds but cannot distinguish between intracellular and extracellular glutamate pools, therefore failing to precisely measure glutamate neurotransmission (Poels et al., 2014b).

Finally, the study performed by Tsai et al. (1998) was limited by the use of VBR, as this index of brain structure lacks sensitivity to minor neuroanatomical alterations, though this measurement was necessitated by the usage of CT. Further, the same study included participants with chronic schizophrenia; in this population, ongoing medication exposure and/or illness progression very likely confounded the relationship between glutamate levels and structural irregularities. Due to the limitations present within the reviewed studies, and the general dearth of literature exploring this topic, it remains unclear whether glutamate-mediated excitotoxicity is responsible for the structural changes observed in schizophrenia.
4.3. Evidence from preclinical literature

In contrast to currently available human studies, preclinical studies offer more conclusive evidence for the role of glutamate-mediated excitotoxicity in schizophrenia. Pharmacological NMDA receptor antagonism is believed to reflect a similar state to schizophrenia, as evidenced by changes in symptomology, blood flow and cognition (Anticevic et al., 2012; Krystal et al., 2005; Lahti et al., 1995a; Nagels et al., 2011). Established and replicated preclinical studies in rodents have reported increased extracellular measures of glutamatergic activity within cortical and subcortical brain regions following NMDA receptor antagonism (Adams and Moghaddam, 1998, 2001; Bustos et al., 1992; Moghaddam et al., 1997). The administration of NMDA receptor antagonists also consistently leads to neurotoxic injury, characterized by neuronal vacuolization, neurodegeneration and the appearance of heat-shock protein in affected cells (Farber et al., 2002; Ikonomidou et al., 1999; Olney and Farber, 1995; Olney et al., 1999; Sharp et al., 1991, 1994). Moreover, preventing glutamate release in these preclinical models, using agonists of metabotropic glutamate receptors types 2 and 3, has been shown to block the neurotoxic effect of NMDA receptor antagonists (Carter et al., 2004; Moghaddam and Adams, 1998; Okamura et al., 2003; Schobel et al., 2013). A study by Schobel et al. (2013) utilized a preclinical rodent model to test the hypothesis that excess glutamate serves as a common upstream mechanism for hippocampal hypermetabolism and atrophy, after these phenomena were identified in participants who fulfilled “clinical high-risk” criteria. Acute ketamine administration led to hippocampal hypermetabolism, whereas chronic ketamine administration additionally resulted in hippocampal atrophy and parvalbumin-expressing interneuron downregulation; pre-treatment with an agonist of metabotropic glutamate receptors types 2 and 3 prevented the effects of ketamine. Thus, this study provided translational evidence to implicate increased glutamate in certain metabolic and structural abnormalities present in schizophrenia, notably concluding that excess glutamate may drive hippocampal atrophy.

Furthermore, the injury induced by NMDA receptor antagonists in preclinical models is comparable to the damage seen in schizophrenia in that both are age-dependent and affect similar locations within the brain. In schizophrenia, symptoms and certain structural changes usually appear after puberty, in late adolescence (Kessler et al., 2007; Lieberman et al., 2001; van Os and Kapur, 2009). The psychotomimetic properties of NMDA receptor antagonists are also age-dependent (Farber, 2003; Reich and Silvay, 1989). NMDA receptor antagonists begin to cause injury to rodent brain cells around the time of puberty (approximately 45 days of age), an effect that becomes more severe as rodents progress into adulthood (Auer, 1996; Farber et al., 1995). Moreover, NMDA receptor antagonism in preclinical models damages regions similar to those affected in patients with schizophrenia, including limbic and neocortical brain areas (Ellison, 1994; Hargreaves et al., 1993; Olney and Farber, 1995; Olney et al., 1989; Sharp et al., 2001). These findings offer support for the role of glutamate-mediated excitotoxicity in causing structural changes, and are consistent with the NMDA receptor hypofunction theory of schizophrenia.

The use of this preclinical model of schizophrenia also offers an opportunity to explore the mechanism by which glutamate-mediated excitotoxicity causes neuroanatomical changes.
Rodent studies have suggested that the thalamus may be a primary site of NMDA receptor hypofunction. NMDA receptor hypofunction on thalamic GABAergic inter-neurons may lead to decreased GABA production and subsequent disinhibition of thalamocortical circuits, which utilize glutamate as a neurotransmitter (Sharp et al., 2001). As the thalamus has a multitude of projection neurons, NMDA receptor hypofunction on thalamic GABAergic inter-neurons provides one explanation for the increase in glutamatergic markers across multiple brain regions in schizophrenia. This is supported by studies where a GABA_A agonist is injected into the thalamus, preventing NMDA receptor antagonist-induced neurotoxicity (Sharp et al., 2001; Tomitaka et al., 2000). Also, NMDA receptor antagonist administration into the anterior nucleus of the thalamus results in cortical degeneration, while injection into cortical regions has no effect (Sharp et al., 2001; Tomitaka et al., 2000). Notably, unlike systemic treatment with NMDA antagonists, cortical injection of ketamine also does not induce glutamate release (Lorrain et al., 2003).

The surge in glutamate following NMDA receptor hypofunction is believed to act on AMPA/kainate receptors, possibly resulting in calcium influx that leads to an excitotoxic effect. In support of this theory, administration of AMPA/kainate receptor antagonists and calcium channel blockers individually protects against neuronal injury by NMDA receptor antagonism (Deutsch et al., 2001; Ikonomidou et al., 1999; Sharp et al., 1995).

The ability to generalize these findings from preclinical studies to patients with schizophrenia is dependent on both the validity of the NMDA receptor hypothesis of schizophrenia and on the comparability of acute NMDA receptor deficits to the chronic NMDA receptor hypofunction presumed to exist in the illness. Thus, though findings from preclinical studies are not wholly generalizable to patients with schizophrenia, they certainly suggest that an excitotoxic effect may occur as a result of a paradoxical increase in glutamatergic activity following NMDA receptor hypofunction.

### 4.4. Future directions

Results from these preclinical studies provide sufficient evidence for future research to investigate the role of glutamate-mediated excitotoxicity in patients with schizophrenia. Several prior studies have assessed the relationship between functional imaging measures and glutamatergic markers (Hutcheson et al., 2012; Reid et al., 2010; Valli et al., 2011). The challenge in transferring this strategy to analysing structural imaging data relates to the fact that structural changes likely do not evolve acutely. As such, future studies should adopt a longitudinal design, serially measuring glutamatergic markers and brain structure in individuals at high risk for psychosis as they progress to their first episode of schizophrenia. In addition, the use of carbon magnetic resonance spectroscopy (^{13}C-MRS) along with ^1H-MRS would provide a complementary approach to understanding glutamatergic activity and excitototoxicity in schizophrenia. ^13C-MRS has the capacity to assess neuronal and astrocyte metabolic activity, and has been previously employed to study the glutamatergic system in rodent models of schizophrenia and human participants (Eyjolfsson et al., 2011; Kondziella et al., 2006; Rothman et al., 2011). Lastly, it is for a future review to investigate the relationship between markers of glutamatergic activity and N-acetyl-aspartate measures, as the latter is believed to reflect neuronal viability (Steen et al., 2005). Further, N-acetyl-
aspartate is altered in schizophrenia and has been shown to decrease following PCP administration in rodents (Brugger et al., 2011; Bustillo et al., 2012; Kraguljac et al., 2012; Natsubori et al., 2013; Reynolds et al., 2005).

4.5. Limitations of present review

This review is not without limitations. The principal aim may have been too narrow, yielding few studies specifically examining glutamate-mediated excitotoxicity in schizophrenia, thus rendering it challenging to make meaningful conclusions. Similarly, relevant articles may have been omitted from the Medline® search due to selection of the search terms.

Further, this review is biased to studies that used 1H-MRS. It may have benefited from including additional imaging techniques other than MRI and CT, such as PET and SPECT. However, the few studies that have employed these methods to investigate the glutamatergic system in schizophrenia have not addressed the relationship between structure and glutamatergic activity (Pilowsky et al., 2006; Stone et al., 2006). Moreover, MRI is currently regarded as the most sensitive, non-invasive imaging technique and does not utilize ionizing radiation. Thus, it is likely that the majority of subsequent research will be performed using this modality.

5. Conclusion

The glutamatergic hypothesis of schizophrenia provides an alternate or complementary mechanism to the dopaminergic hypothesis of schizophrenia. Excitotoxic levels of glutamate secondary to NMDA receptor hypofunction on GABAergic inhibitory interneurons may contribute to the structural abnormalities observed in schizophrenia. However, currently available literature from human studies fails to adequately address this topic.

The present review performed a Medline® search to investigate whether glutamate-mediated excitotoxicity is supported by findings from human schizophrenia studies. This search resulted in a small number of studies that met inclusion criteria: MRI or CT studies that concomitantly measured glutamatergic activity and brain structure. From the articles that met these criteria, it remains inconclusive whether glutamate-mediated excitotoxicity adequately explains the neuroanatomical changes observed in patients with schizophrenia. It is possible that publication bias exists within the literature, such that negative findings on this topic are less likely to be published.

In contrast, a subsequent discussion of preclinical studies does provide sufficient evidence of increased glutamatergic activity and associated structural alterations in response to the administration of NMDA receptor antagonists. Based on this body of literature, further studies in humans are warranted to determine the degree to which glutamate-mediated excitotoxicity could explain the structural deficits present in schizophrenia. Determining whether glutamate-mediated excitotoxicity contributes to the structural changes in schizophrenia may aid in the understanding of illness progression, along with the development of adjunctive therapies. Studies investigating the use of glutamatergic modulators to this point have provided useful, yet inconclusive evidence and as such, a
better understanding of glutamatergic dysfunction within schizophrenia is required (Heresco-Levy, 2005; Javitt et al., 2012; Large et al., 2005; Weiser et al., 2012).

Future studies investigating the relationship between glutamatergic function and brain structure should employ MRI techniques and adopt a longitudinal study design, including individuals at a high risk for schizophrenia as they transition into the illness. Future research would also benefit from performing MRS studies to assess glutamatergic activity in unmedicated patients. Glutamatergic activity is commonly, reliably and non-invasively evaluated through MRS, and structure can be assessed through analyses of T1-weighted images.

Acknowledgments

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### Table 1

Summary of studies that met inclusion criteria (n=7).

<table>
<thead>
<tr>
<th>Authors, year, journal</th>
<th>n</th>
<th>Mean age, (SD)</th>
<th>Study design</th>
<th>Phase of illness</th>
<th>Antipsychotic status</th>
<th>Glutamatergic marker(s)</th>
<th>Structural measure(s)</th>
<th>Key findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kraguljac et al. (2013), JAMA Psychiatry</td>
<td>27 SCZ; 27 HC</td>
<td>SCZ: 32.63 (9.28); HC: 32.85 (9.39)</td>
<td>CS</td>
<td>Mixed</td>
<td>Unmedicated at assessment</td>
<td>Glx in hippocampus. Assessed by 1H-MRS</td>
<td>Hippocampal GM volume. Assessed by MRI</td>
<td>In SCZ: Negative correlation between hippocampal Glx and GM volume in two hippocampal clusters</td>
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<td>In HC: No significant correlation between hippocampal Glx and GM volume in hippocampus</td>
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<tr>
<td>Tandon et al. (2013), Schizophr. Res.</td>
<td>23 HR; 24 HC</td>
<td>HR: 15.92 (4.21); HC: 15.57 (3.99)</td>
<td>CS</td>
<td>At risk</td>
<td>Unmedicated at assessment</td>
<td>Gls in thalamus and caudate. Assessed by 1H-MRS</td>
<td>Thalamic and caudate volume. Assessed by MRI</td>
<td>In HR: Non-significant negative correlation between thalamic Gls and thalamic volume</td>
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<td>Non-significant negative correlation between caudate Gls and caudate volume</td>
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<tr>
<td>Aoyama et al. (2011), Br. J. Psychiatry</td>
<td>17 SCZ, 17 HC</td>
<td>SCZ: 25 (7); HC: 29 (10)</td>
<td>L; 80 months</td>
<td>FES</td>
<td>Unmedicated at initial assessment; medicated at 30- and 80-month assessments</td>
<td>Glutamine in thalamus and anterior cingulate; tGL in thalamus and anterior cingulate. Assessed by 1H-MRS</td>
<td>Whole brain GM volume. Assessed by MRI</td>
<td>In SCZ: Positive correlation between thalamic glutamine loss and GM volume reduction in frontal, temporal, parietal and limbic lobes</td>
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<td>No significant correlation between anterior cingulate glutamine and GM volume loss</td>
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<td>No significant correlation between thalamic or anterior cingulate tGL and GM volume loss</td>
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<td>Study involved introduction of medication</td>
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<td>In HC: No significant correlation between loss of thalamic glutamine and GM volume reduction</td>
</tr>
<tr>
<td>Klar et al. (2010), NeuroImage</td>
<td>29 SCZ; 44 HC</td>
<td>SCZ: 27.6 (6.8); HC: 30.9 (7.9)</td>
<td>CS</td>
<td>Mixed</td>
<td>Medicated at assessment</td>
<td>Glutamate in hippocampus. Assessed by 1H-MRS</td>
<td>Hippocampal volume. Assessed by MRI</td>
<td>In SCZ: Non-significant negative correlation between hippocampal glutamate and volume in hippocampus</td>
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<td>In HC: Non-significant negative correlation between hippocampal glutamate and volume in hippocampus</td>
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<tr>
<td>Stone et al. (2009), Biol. Psychiatry</td>
<td>27 ARMS; 27 HC</td>
<td>ARMS: 25 (5); HC: 25 (4)</td>
<td>CS</td>
<td>At risk</td>
<td>Unmedicated at assessment</td>
<td>Glutamate in thalamus; glutamine in anterior</td>
<td>Whole brain GM volume. Assessed by MRI</td>
<td>In ARMS:</td>
</tr>
</tbody>
</table>

- Eur Neuropsychopharmacol. Author manuscript; available in PMC 2015 June 17.
<table>
<thead>
<tr>
<th>Authors, year, journal</th>
<th>n</th>
<th>Study design</th>
<th>Phase of illness</th>
<th>Antipsychotic status</th>
<th>Glutamatergic marker(s)</th>
<th>Structural measure(s)</th>
<th>Key findings</th>
</tr>
</thead>
</table>
| Theberge et al. (2007), Br. J. Psychiatry | 16 SCZ, 16 HC | L; 30 months | FES | Unmedicated at initial assessment; medicated at 10- and 30-month assessments | Glutamate in thalamus and anterior cingulate. Assessed by 1H-MRS | Whole brain GM volume and 1H-MRS voxel GM volume. Assessed by MRI | • Positive correlation between thalamic glutamate and GM volume in left cingulate. Assessed by 1H-MRS.  
• Negative correlation between thalamic glutamate and GM volume in dorsal anterior cingulate extending to posterior cingulate gyrus.  
• Positive correlation between anterior cingulate glutamine and GM volume in posterior cingulate gyrus.  
• Negative correlation between anterior cingulate glutamine and GM volume in left cerebellum. In HC:  
• No significant correlation between thalamic glutamate and GM volume.  
• Positive correlation between anterior cingulate glutamine and GM volume in right temporal cortex.  
• Negative correlation between anterior cingulate glutamine and GM volume in medial frontal and orbitofrontal cortex. 

| Tsai et al. (1998), Biol. Psychiatry | 61 SCZ, 23 HC | CS | Chronic | Unmedicated at assessment | CSF glutamate | VBR; prefrontal atrophy. Assessed by CT | • Negative correlation between CSF glutamate and VBR.  
• Non-significant correlations between CSF glutamate and VBR. 

| Plitman et al. (2019), Biol. Psychiatry | 6 SCZ, 6 HC | CS | Chronic | Unmedicated at assessment | Glutamate+Citrulline in striatum. Assessed by 1H-MRS | VBR; prefrontal atrophy. Assessed by CT | • Non-significant correlations between CSF glutamine loss and VBR.  
• Non-significant correlations between CSF glutamine loss and CSF glutamate loss.  
• Non-significant correlations between CSF glutamine loss and CSF glutamate loss.  

| ARMS: at risk mental state; CS: cross-sectional; CT: computed tomography; FES: first-episode schizophrenia; Glx: Glutamate+Glutamine; GM: grey matter; HC: healthy controls; HR: high-risk; L: longitudinal; MRI: magnetic resonance imaging; ARMS; at risk mental state; CS: cross-sectional; CT: computed tomography; FES: first-episode schizophrenia; Glx: Glutamate+Glutamine; GM: grey matter; HC: healthy controls; HR: high-risk; L: longitudinal; MRI: magnetic resonance imaging.  
| a Only measures that were utilized for the investigation of the relationship between glutamatergic markers and brain structure, and were subsequently reported upon, are included in this table.  
| b Included schizoaffective patients.