Pharmacological interventions in clozapine-refractory schizophrenia
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This thesis focuses on treatment challenges such as severe cognitive impairments, prominent persistent negative symptoms and treatment-resistant positive symptoms. New treatment approaches to overcome glutamatergic deficits in schizophrenia seem promising in treatment-resistant schizophrenia. The glutamate hypothesis proposes that the specific combination of the glutamate agonist clozapine and the voltage-dependent N-methyl-D-aspartate (NMDA) receptor antagonist memantine results in upregulation of the NMDA receptor. The encouraging results of a first proof-of-concept study with large effect size for all symptom domains of schizophrenia impelled me to further investigate this combination therapy. My research team and I have provided additional encouraging clinical results for memantine as an adjunctive to clozapine in refractory schizophrenia. Memantine slightly improved memory and negative symptoms after twelve weeks of treatment in a double-blind, placebo-controlled study. In an open-label, one-year extension study the small improvement of memory was sustained and a large improvement in negative and positive symptoms and impaired psychosocial functioning was found in the first 26 weeks and second 26 weeks. In conclusion, further research on memantine as clozapine augmentation strategy in patients suffering from treatment-resistant schizophrenia is justified.
Pharmacological Interventions in Clozapine-Refractory Schizophrenia

Selene Roxane Tamasine Veerman
Pharmacological Interventions in Clozapine-Refractory Schizophrenia

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Chapter 1

Introduction
Introduction

While the lifetime prevalence estimate for psychotic experiences is 5.8% (McGrath et al. 2016), schizophrenia is a relatively rare severe mental illness with a lifetime prevalence estimate of 0.4% (McGrath et al. 2008). Symptoms of schizophrenia can be clustered into three symptom domains: cognitive dysfunction, negative symptoms, and positive symptoms (Tamminga, 2008). While symptomatic heterogeneity results in a high diversity of presentations of schizophrenia, the severity and course also vary significantly. In 20% to 30% of patients suffering from schizophrenia functional remission is achieved, but the majority of patients experience a more debilitating course with recurrent or persistent symptoms and disabling impairments (Walker et al. 2004). However, a partial recovery is often achieved after intensive treatment. Treatment resistance affects approximately 20% to 30% of schizophrenia patients (Conley & Kelly, 2001) and is a major health concern (Porcelli et al. 2012). A substantial proportion of patients suffering from treatment-resistant schizophrenia (TRS) shows a partial response with estimates between 40% and 70% (Buckley et al. 2001; Kontaxakis et al. 2005; Agid et al. 2010). In approximately 30% of TRS patients residual symptoms persist, even after controlling for clozapine plasma level (Schulte et al. 2003). At the moment evidence-based treatments for clozapine-resistant symptoms are scant.

The main aim of this thesis is to examine whether memantine, which specifically targets the N-methyl-D-aspartate (NMDA) receptor, is efficacious in patients with clozapine-resistant schizophrenia. The NMDA receptor, an ionotropic glutamate receptor, plays a pivotal role in learning and memory through synaptogenesis and the pruning of unwanted synapses (Jarskog, 2006; Kornmeier & Sosic-Vasic, 2012). The NMDA receptor also plays a key role in several hypotheses regarding the underlying pathogenic mechanism of schizophrenia (Stone et al. 2007; Kantrowitz et al. 2010; Lin et al. 2012). The combination of clozapine, a glutamate agonist, and the voltage-dependent NMDA receptor antagonist memantine may cause upregulation of the NMDA receptor (Joshi et al. 2007).

We tested the hypothesis that the combination of clozapine and memantine has beneficial effects on symptom domains in refractory schizophrenia, inspired by the encouraging results of the first proof-of-concept study by de Lucena et al. (2009). This small 12-week double-blind, placebo-controlled, randomized clinical trial (RCT) on the addition of memantine in clozapine-resistant schizophrenia showed remarkably high effect sizes (ESs) for global clinical impression, global cognitive function, positive symptoms and overall clinical symptoms, and in particular for negative symptoms.

In our attempt to replicate these favourable effects of adjunctive memantine to
clozapine in treatment-resistant schizophrenia, we conducted a larger and more elaborate study than the first proof-of-concept study. Because the combination of clozapine and memantine may have a synergistic, neuroprotective effect (Joshi et al. 2007), memantine is hypothesized to improve cognitive impairments in clozapine-refractory schizophrenia. We therefore assessed cognitive function as the primary outcome in our second proof-of-concept study, with a comprehensive cognitive test battery.

We analyzed the effects of twelve weeks of adjunctive memantine treatment in patients suffering from clozapine-resistant schizophrenia on memory, executive function, negative symptoms, positive symptoms, overall symptoms of schizophrenia and global severity of psychopathology in a 26-week placebo-controlled, double-blind crossover trial (Veerman et al. 2016). The safety and tolerability of memantine addition to clozapine were also assessed. We studied long-term effects and tolerability of memantine in completers of the placebo-controlled trial who experienced beneficial effects during 12 weeks of memantine treatment in an open-label one-year extension study (Veerman et al. 2017a).

**Neurobiological hypotheses for the pathogenesis of schizophrenia**

There are six main neurobiological hypotheses about the pathogenesis of primary negative symptoms. These hypotheses are not mutually exclusive (in fact they are mostly interconnected) and they are thought to explain primary negative symptoms, positive symptoms and cognitive impairments.

*Dopamine hypothesis*

According to the dopamine hypothesis a hypodopaminergic state in the prefrontal cortex (PFC) causes anhedonia. Hypofunction in mesolimbic and mesocortical systems results in apathy and social withdrawal (Segarra et al. 2001). Disturbances in anticipatory pleasure, reward learning, effort-based decision-making and social motivation contribute to primary negative symptoms (Reddy et al. 2016). Impaired reward processing appears to be related to working memory abilities, implicating dopaminergic dysregulation in the orbital and dorsal prefrontal structures and functional changes within the frontostriatal circuits (Gold et al. 2008). Attention deficits stem from dysregulation of the mesocortical pathway. Due
to a reciprocal relationship between cortical and subcortical dopamine systems, reduced dopaminergic prefrontal activity leads to disinhibition of subcortical dopaminergic systems (Abi-Dargham et al. 2003). It has been suggested that positive symptoms may arise from hyperdopaminergic activity with elevated striatal dopamine synthesis and release in subcortical structures (Howes et al. 2011; Bonoldi & Howes, 2014), causing aberrant attribution of salience to irrelevant or otherwise neutral stimuli (Howes & Kapur, 2009; Boehme et al. 2015).

**Glutamate hypothesis**

The glutamate hypothesis posits that in schizophrenia the ionotropic \(N\)-Methyl-D-Aspartate (NMDA) receptor is hypofunctional (Kantrowitz & Javitt, 2010). This receptor plays a pivotal role in neuroplasticity through synaptogenesis and pruning of unwanted synapses (Jarskog, 2006). NMDA receptor hypofunction decreases glutamate uptake and leads to glutamate spill-over in the synaptic cleft, which triggers cell death (Lin et al. 2012; Stone et al. 2007; Veerman et al. 2014a). Increased apoptotic activity in the early stages of schizophrenia may account for neurodevelopmental abnormalities, which contribute to cognitive and primary negative symptoms. Negative symptoms are believed to arise from dysregulated glutamate, gamma-aminobutyric acid (GABA) and dopamine neurotransmission in the PFC (Stone et al. 2007; Veerman et al. 2014a; Homayoun & Moghaddam, 2007), while positive symptoms may be caused by dysregulation of prefrontal cortical GABA interneurons, which are responsible for recurrent inhibition of pyramidal neurons (Veerman et al. 2014a).

**Dysconnection hypothesis**

A related hypothesis postulates that both primary negative symptoms and cognitive disturbances such as impaired executive functions and memory are caused by widespread loss of cortical synaptic connectivity of the pyramidal neurons, leading to cortical atrophy and abnormalities in brain circuits (Coyle et al. 2016). These abnormalities are thought to be related to regulation of the NMDA receptor by neurotransmitters such as dopamine, serotonin or acetylcholine (Stephan et al. 2009). Dysregulation of the NMDA receptor results in aberrant synaptic, cellular and cortical plasticity. Disconnection of cortico-cortical prefron-
to-parietal and prefronto-temporal pathways and cortico-striato-thalamic pathways causes abnormal functional integration of brain processes leading to primary negative symptoms (among other symptoms) (Lesh et al. 2011). Recent evidence suggests that grey matter reduction in brain regions in the reward network, especially the left caudate nucleus may be specifically related to persistent primary negative symptoms (Li et al. 2017).

**Muscarinic hypothesis**

Apart from dopamine, acetylcholine seems to play an important role in the pathogenesis of schizophrenia (Raedler et al. 2007). Ionotropic nicotine receptors and metabotropic muscarinic receptors have been linked to cognition and schizophrenia (Gray & Roth, 2007). The dopaminergic system and cholinergic system interact with each other closely at different levels in the brain (Raedler et al. 2007). In schizophrenia a hypercholinergic state with an abnormal increase in the reactivity of the cholinergic neurotransmission may result in impaired regulation of the mesolimbic dopaminergic neurotransmission. However, changes in activity of the muscarinic cholinergic system in schizophrenia may also be of a secondary nature, caused by primary changes in the dopaminergic system.

**Immune hypothesis**

The immune hypothesis proposes that in the first years of the development of schizophrenia an inflammatory process activates microglial cells, releasing glutamate and neurotoxic substances, such as proinflammatory cytokines and free radicals (Muller & Schwarz, 2006; Chew et al. 2013). Synaptic NMDA receptor activity boosts antioxidant defences through increased expression of antioxidant enzymes (Papadia et al. 2008). However, NMDA receptor activity is inhibited by kynurenic acid, an endogenous NMDA receptor antagonist, which is a product of altered tryptophan catabolism. Selective blockade of the NMDA receptor and the release of glutamate through the activation of microglial cells cause glutamatergic-dopaminergic dysregulation and brain volume loss through an excitotoxic cascade (Kahn & Sommer, 2015). This mechanism may explain the development of cognitive disturbances and primary negative symptoms in the years preceding positive symptoms.
Oestrogen and testosterone hypothesis

Another hypothesis is that sex hormones modulate the sensitivity of the D2 receptor (Gogos et al. 2015) and influence not only dopaminergic and glutamatergic neurotransmission but also GABA neurotransmission (Godar & Bortalota, 2014). Based on sex differences, oestrogens seem to protect against the development of schizophrenia (Gogos et al. 2015). Testosterone is also thought to influence vulnerability to developing primary negative symptoms. Low levels of testosterone in men are associated with more severe negative symptoms (da Silva & Ravindran, 2015).

In the following part of the introduction, I will address several aspects of the symptom domains of schizophrenia: the characteristics, the diagnosis and an evaluation of the effect of interventions targeting these three domains of dysfunction in schizophrenia. Cognitive dysfunction and positive symptoms are discussed in the introduction and negative symptoms are described in a separate chapter.

Cognitive dysfunction

Cognitive functioning and negative symptoms are the best predictors of functional outcome such as work skills, community activities and interpersonal functioning (Bowie & Harvey, 2006; Lepage et al. 2014, Ventura et al. 2015). Cognitive impairments associated with schizophrenia are impaired speed of processing, attention, working memory, verbal learning and memory, visual learning and memory, reasoning and problem solving (executive function), and social cognition. These seven independent cognitive domains are included in the Measurement and Treatment Response to Improve Cognition in Schizophrenia (MATRICS) Consensus Cognitive Battery (MCCB), recommended by the National Institute of Mental Health (NIMH) (Nuechterlein et al. 2008).

Unfortunately, there is no known pharmacological treatment which ameliorates cognitive symptoms such that clinical improvement is achieved (Javitt, 2015). Comparisons of the effects of second-generation antipsychotics (SGAs) versus placebo on neurocognition are limited to two negative RCTs (Takeuchi et al. 2017). Evidence from clinical studies indicates that clozapine has beneficial effects on verbal fluency, executive function (Meltzer & McGurk, 1999), verbal and working memory (Molina et al. 2014), and the orienting function of attention (Spagna et al. 2015). However, a recent meta-analysis found no overall differences in neurocognitive effects between SGAs and first-generation antipsychotics (FGAs) and no single antipsychotic stood out with a uniform positive cognitive profile (Nielsen et al. 2015).
Neurocognitive and social cognitive interventions may benefit neurocognition and social cognition (Roder et al. 2011; Kurtz & Richardson, 2012; Lindenmayer et al. 2013; Pinkham & Harvey, 2013; Mueller et al. 2015). In a meta-analysis integrated psychological therapy (IPT) combining neurocognitive and social cognitive interventions with social skills and problem-solving approaches was found to be superior over placebo-attention conditions and standard care for cognition (ES=0.53, p<0.01), neurocognition (ES=0.52, p<0.01) and social cognition (ES=0.70, p<0.01) (Roder et al. 2011). A meta-analysis of controlled studies of social cognitive training found a moderate to large effect for facial affect recognition (identification, $d=0.71$ and discrimination, $d=1.01$) and a small effect for theory of mind ($d=0.46$) (Kurtz & Richardson, 2012). Integrated neurocognitive therapy (INT) is a novel, more extensive form of cognitive remediation which shows more promise, with long-term favourable effects. In a recent RCT ($n=156$) small beneficial effects were found for attention ($d=0.09$, $p=0.02$), verbal memory ($d=0.26$, $p=0.03$) and social cognition ($d=0.24$, $p=0.008$) after fifteen weeks of INT at 9-month follow-up (Mueller et al. 2015). Cognitive adaptation training is a home-based therapy incorporating environmental supports such as signs, alarms, checklists and organization of belongings in order to bypass the cognitive deficits of schizophrenia. In a 24-month randomized study ($n=120$) cognitive adaptation training significantly improved functional outcome ($\beta=5.65$, $p<0.0001$) compared to generic environmental supports and treatment as usual. Greater engagement in functional behaviour led to better performance on cognitive testing ($d=0.30$), suggesting that compensatory interventions have restorative effects in the long term (Fredrick et al. 2015).

A meta-analysis of seven RCTs ($n=297$) evaluating the efficacy of physical exercise – mainly aerobic exercise – on cognitive functioning in schizophrenia showed a small beneficial effect on global cognition (Hedges’ $g=0.41$, $p<0.001$) (Firth et al. 2016). Exercise improved several cognitive domains: social cognition ($n=81$, Hedges’ $g=0.71$, $p=0.002$), attention/vigilance ($n=104$, Hedges’ $g=0.66$, $p=0.005$) and working memory ($n=282$, Hedges’ $g=0.39$, $p=0.024$). Furthermore, higher levels of weekly exercise showed a trend towards greater improvement in cognition ($p=0.065$). Another meta-analysis of six RCTs ($n=354$) including aerobic exercise (endurance training, cardiovascular exercises, treadmill walking), anaerobic exercise (muscle strength training) and yoga did not find a significant effect of physical exercise on cognition compared to treatment as usual (Dauwan et al. 2016). A subanalysis including two RCTs ($n=184$) revealed that yoga was more efficacious for long-term memory in schizophrenia (Hedges’ $g=0.32$, $p<0.05$) than treatment as usual. However, effects of yoga on other cognitive domains were not significant.
Negative symptoms

Debilitating negative symptoms are a major health concern, because these symptoms hamper personal relationships, social participation, education and work, and diminish the likelihood of living independently (Fenton & McGlashan, 1994; Hofer et al. 2005; Ventura et al. 2015).

Primary negative symptoms are thought to be intrinsic to schizophrenia. Negative symptoms date back to the earliest descriptions of Kraepelin and Bleuler and are one of the five A criteria for the diagnosis of schizophrenia in the Diagnostic and Statistical Manual of Mental Disorders, 5th edition (DSM-5) (Carpenter et al. 1985; American Psychiatric Association, 2013). The negative syndrome or deficit syndrome entails the absence or diminished expression of emotion and normally present behaviour. Expressive deficits and social amotivation are two subdomains of the negative syndrome (Millan et al. 2014).

There are various causes for secondary negative symptoms (Murphy et al. 2006). An excessively high dosage of antipsychotic medication causes negative subjective experience and more severe negative symptoms (de Haan et al. 2000, 2003, 2004; Mizrahi et al. 2007). Behavioural inhibition may be caused by psychosis, disorganization, anxiety and depression (Goekoop & Goekoop, 2014). Chronic abuse of illicit drugs and alcohol may lead to loss of motivation or emotional attachment, detachment from reality, social withdrawal and reduced attention and memory (Rovai et al. 2013). This amotivational syndrome shows a close resemblance to the negative syndrome in psychosis. In addiction sensitivity to natural reward is diminished (Goldstein & Volkow, 2002), while in schizophrenia motivational drive to experience reward may be impaired (Gold et al. 2008; Reddy et al. 2016). Psychosocial factors which may contribute significantly to negative symptoms are demoralization, social deprivation, and residence in an institution. Stigma and self-stigma may cause low self-esteem and hopelessness, which have a negative impact on recovery (Vass et al. 2015; Kao et al. 2016).

It may be difficult to distinguish between primary and secondary negative symptoms, but it is essential to do so in order to treat symptoms adequately. In the second chapter of this thesis I address the evidence of the past decade concerning specific pharmacological and non-pharmacological interventions for unspecified negative symptoms, and possible explanations of their mode of action.
Positive symptoms

Positive symptoms are hallucinations, delusions, bizarre behaviour and formal thought disorder.

Antipsychotic medication has a moderate effect on positive symptoms (Leucht et al. 2009a, Leucht et al. 2009b, Zhang et al. 2013, Leucht et al. 2009c). Below is a summary of the outcomes for positive symptom improvement in different meta-analytic comparisons of antipsychotic medication.

Comparisons between SGAs versus FGAs demonstrate no consistent superiority of SGAs over FGAs (Leucht et al. 2009b, Zhang et al. 2013). Furthermore, when superiority was found, the differences were small and non-equivalent dosing of SGAs versus FGAs as well as industry sponsoring may confound these findings. A meta-analysis of 150 RCTs (n=21,533) comparing nine SGAs (amisulpride, aripiprazole, clozapine, olanzapine, quetiapine, risperidone, sertindole, ziprasidone and zotepine) with FGAs in acutely ill schizophrenia patients found that clozapine (ten RCTs, n=1,080, Hedges’ g=0.36, p<0.0001), amisulpride (four RCTs, n=703, Hedges’ g=0.22, p=0.005), olanzapine (24 RCTs, n=4,189, Hedges’ g=0.15, p<0.0001), and risperidone (28 RCTs, n=3,286, Hedges’ g=.13, p=0.001) were marginally more efficacious than FGAs in treating positive symptoms (Leucht et al. 2009b). In a meta-analysis of eleven RCTs (n=1,932) no superiority for positive symptom improvement was found for SGAs (olanzapine, risperidone, clozapine, amisulpride, quetiapine, and ziprasidone) over FGAs (haloperidol, molindone, zuclopenthixol, chlorpromazine) (Zhang et al. 2013). Subanalysis of antipsychotics showed that amisulpride was superior to haloperidol (ES=0.54, p<0.05).

Comparisons between different antipsychotics do not reveal consistent superiority of any single antipsychotic for the treatment of positive symptoms (Leucht et al. 2009c; Samara et al. 2016; Harvey et al. 2016). A meta-analysis of 78 RCTs (n=13,558) on the efficacy of the same nine SGAs mentioned previously in a selection of patients with predominantly positive symptoms found olanzapine to be superior to quetiapine (six RCTs, n=646, Weighted Mean Difference (WMD)=1.9, p<0.001) and ziprasidone (two RCTs, n=730, WMD=3.1, p<0.001) (Leucht et al. 2009c). Risperidone was more efficacious for positive symptoms than quetiapine (seven RCTs, n=1,264, WMD=1.8, p<0.001) and ziprasidone (one RCT, n=204, WMD=2.5, p=0.021). For clozapine a lack of superior efficacy for positive symptoms was found, which may be due to low mean dosages (below 400 mg) and poor study quality.

A recent network meta-analysis of 40 RCTs (n=5,172) compared the efficacy of nine
antipsychotics in treatment-resistant schizophrenia (Samara et al. 2016). Superiority for positive symptoms was found for risperidone (Standard Mean Difference (SMD)=0.43), clozapine (SMD=0.40) and olanzapine (SMD=0.33) over quetiapine. Risperidone (SMD=0.29) and clozapine (SMD=0.27) were more effective for positive symptoms than haloperidol. However, caution should be observed in interpreting these results since the data on ziprasidone, fluphenazine, quetiapine, chlorpromazine, and sertindole were limited. Another recent network meta-analysis of eleven RCTs (n=1,714) on the efficacy of eight antipsychotics (aripiprazole, haloperidol, molindone, olanzapine, paliperidone, quetiapine, risperidone and ziprasidone) in patients with early-onset schizophrenia (age of onset before 18 years) showed a statistically significant reduction in positive symptoms for haloperidol, olanzapine and risperidone compared with placebo (Harvey et al. 2016). Comparisons between these antipsychotics showed a trend for haloperidol, followed by olanzapine and risperidone as the most efficacious antipsychotics for reducing positive symptoms. A possible explanation for the lack of consistent evidence for the superiority of a certain antipsychotic could be the small difference in efficacy, but also non-equivalent dosing, industry sponsoring and poor study quality.

Clozapine is the treatment of choice for patients with TRS (Hasan et al. 2012). However, clozapine has not consistently been shown to be superior to other antipsychotics for positive symptoms in patients suffering from TRS. A meta-analysis of seven RCTs (n=648, duration 8–28 weeks) showed superior efficacy of clozapine over olanzapine (SMD=0.51) for positive symptoms in TRS patients (Souza et al. 2013). A recent meta-analysis comparing clozapine with olanzapine, haloperidol and chlorpromazine in TRS found a superior short-term effect of clozapine for positive symptoms (Siskind et al. 2016). However, clozapine was not superior to olanzapine in the long term. No differential efficacy for positive symptoms was found between clozapine and risperidone in the short or long term. Possible confounders were funding by the pharmaceutical industry and non-equivalent dosing of clozapine. An important finding was the association of higher mean psychosis scores at baseline and a greater response for clozapine in the long term (p=0.003). Taken together, the most convincing evidence for positive symptom improvement in patients with TRS is for clozapine.

Below is an overview of the main findings published from 2014 until now regarding augmentation strategies and non-pharmacological interventions for positive symptoms and my recommendations for clinical care. Studies from before 2014 are discussed in the fourth and fifth chapters. Two recent meta-analyses demonstrated that antipsychotic polyphar-
macy in clozapine and non-clozapine augmentation studies is not efficacious for positive symptoms (Galling et al. 2017, Zheng et al. 2016a). In a recent meta-analysis of eight RCTs (n=436, mean dose 165 mg) topiramate was found to be superior to placebo as regards positive symptom improvement (SMD=0.37, p=0.002) (Zheng et al. 2016b). In a subgroup analysis of four RCTs on topiramate augmentation to non-clozapine antipsychotics (n=223) a small improvement of positive symptoms was found compared to placebo (SMD=0.32, p=0.02). Topiramate augmentation to clozapine (four RCTs, n=213) was significantly superior to that of placebo with a moderate ES (SMD=0.49, p=0.04) (Zheng et al. 2016b). Another recent meta-analysis of eight RCTs in patients with schizophrenia and related psychosis (n=423, two RCTs on clozapine) also revealed a superior efficacy of antipsychotic augmentation with topiramate (SMD=0.4, p=0.001) compared to antipsychotic alone or placebo plus antipsychotic (Okuyama et al. 2016).

A meta-analysis of ten RCTs of sex hormone treatment showed significant improvement in positive symptoms (Hedges’ g=0.42, p<0.001) (Heringa et al. 2015). Subgroup analysis revealed a small significant effect for oestrogen (0.05 mg patch–0.2 mg patch/0.625 mg–2 mg) in 462 premenopausal women (Hedges’ g=0.48, p<0.001). The selective oestrogen receptor modulator (SERM) raloxifene was not found to improve positive symptoms in a subanalysis of 114 postmenopausal women (Hedges’ g=0.41, p=0.120) or in two recent short-term RCTs (n=79 and n=46) in premenopausal women and men (Weickert et al. 2015; Khodaie-Ardakani et al. 2015). Other hormones such as testosterone and oxytocin also failed to demonstrate benefits for treating positive symptoms (Ko et al. 2008; Williams et al. 2017).

Psychological interventions are slightly beneficial for positive symptoms. A meta-analysis showed that cognitive behavioural therapy has proven efficacy with a small ES for positive symptoms (33 RCTs, ES=0.25, p<0.001) and for hallucinations in particular (15 RCTs, ES=0.34, p=0.01) (Jauhar et al. 2014). For mindfulness interventions a small beneficial effect for positive symptoms (Hedges’ g=0.32, p<0.0081) was found in a meta-analysis of seven RCTs (Khoury et al. 2013). Cognitive adaptation training significantly reduced positive symptoms in three single studies (Velligan et al. 2000, 2002, 2015).

Moderate to vigorous physical exercise improved positive symptoms in schizophrenia patients with a moderate ES (SMD=0.54, p=0.009) in a meta-analysis of four RCTs (Firth et al. 2015). In a more recent meta-analysis of fifteen RCTs (n=715) physical exercise improved positive symptoms with a small ES (Hedges’ g=0.32, p<0.01). It seems that aerobic exercise (Hedges’ g=0.43, p<0.05) and yoga (Hedges’ g=0.31, p<0.01) are equally
Electroconvulsive therapy was tested in one single RCT \( n=39 \) with at least a 40% reduction of positive symptoms in approximately 50% of patients (Petrides \etal 2015).

**Aims of the thesis**

1. This thesis focuses on efficacious interventions for important treatment challenges in schizophrenia:
   a. severe cognitive impairments;
   b. prominent and persistent negative symptoms;
   c. treatment-resistant positive symptoms.

2. We investigate the efficacy and tolerability of memantine in addition to clozapine in patients suffering from TRS.

**Outline of the thesis**

In the *second chapter* I describe interventions to prevent or treat negative symptoms associated with schizophrenia. Primary negative symptoms are distinguished from secondary negative symptoms. I explain the importance of early treatment and low dosing of antipsychotics (Veerman \etal 2017b). Furthermore, I address the efficacy and supposed mechanism of action of both pharmacological and non-pharmacological treatments for unspecified negative symptoms, supported by evidence from meta-analytic research and reviews. I analyse whether there is superior efficacy of a particular treatment over other interventions in patients with negative symptoms of schizophrenia (without discrimination between primary and secondary negative symptoms). In addition, I explore whether pharmacological interventions for negative symptoms (without primary/secondary distinction) are different in schizophrenia patients receiving non-clozapine antipsychotics compared to patients with TRS receiving clozapine. Subsequently, I make recommendations that are relevant to clinical practice. Finally, I describe limitations and confounding variables regarding research on primary negative symptoms of schizophrenia and I present recommendations for future studies on this important health problem.

In the *third chapter* I focus on the glutamate system and explain the mechanism of action of the NMDA receptor (Veerman \etal 2014a). I then present four lines of evidence
supporting the glutamate hypothesis. I subsequently explore this pathogenic pathway of schizophrenia in order to explain cognitive disturbances, negative symptoms and positive symptoms. Furthermore, I identify ionotropic and metabotropic glutamate receptors as potential new treatment targets. Finally, I review randomized, double-blind studies on glutamate modulators in schizophrenia and glutamatergic augmentation strategies in patients with clozapine-resistant schizophrenia. I analyse whether glutamate agonists are efficacious in combination with non-clozapine antipsychotics or with clozapine, which is itself a glutamate agonist. In addition, I analyse whether glutamate antagonists have clinical benefits as add-on therapy to both non-clozapine antipsychotics and clozapine. I explain how the specific combination of memantine and clozapine may improve cognitive, negative and positive symptoms in TRS.

In the fourth chapter I review double-blind RCTs on the efficacy of non-glutamatergic clozapine augmentation strategies for negative symptoms, positive symptoms, and overall symptoms of schizophrenia and affective symptoms (Veerman et al. 2014b). I assess the potential clinical utility of a second antipsychotic, an antidepressant, ethyl-eicosapentaenoic acid (E-EPA), lithium and extract of ginkgo biloba.

In the fifth chapter I review double-blind RCTs on the efficacy of adjunctive glutamatergic agents in clozapine-resistant schizophrenia for negative, positive, and overall symptoms of schizophrenia (Veerman et al. 2014c). I assess the clinical benefits of clozapine augmentation with NMDA receptor agonists, a positive modulator of the amino-3-hydroxy-5-methyl-isoxazole-4-proprionic acid (AMPA) and glutamate antagonists.

In the sixth chapter I describe the results of twelve weeks of adjunctive memantine treatment in patients suffering from clozapine-resistant schizophrenia in a 26-week placebo-controlled, double-blind crossover trial (Veerman et al. 2016). Memory, executive function, negative symptoms, positive symptoms, overall symptoms of schizophrenia and global severity of psychopathology were the primary endpoints. The safety and tolerability of memantine addition to clozapine were secondary endpoints. I compare our results with the results of the first proof-of-concept study by De Lucena et al. (2009) and discuss the differences in efficacy of adjunctive memantine to clozapine.

In the seventh chapter I present the long-term effects and tolerability of memantine, which I studied in an open-label, one-year extension study in completers of the placebo-controlled trial who experienced beneficial effects from memantine treatment (Veerman et al. 2017a). Finally, I present recommendations for future research on memantine augmentation to clozapine.

The eighth and final chapter of this thesis contains the summary and discussion.
Chapter 2

Treatment for negative symptoms in schizophrenia: a comprehensive review

Selene R.T. Veerman, Peter F.J. Schulte, Lieuwe de Haan

What pharmacological and non-pharmacological interventions are efficacious for negative symptoms of schizophrenia?

Drugs 2017; Accepted
Abstract

Negative symptoms (such as amotivation and diminished expression) associated with schizophrenia are a major health concern. Adequate treatment would mean important progress with respect to quality of life and participation in society. Distinguishing primary from secondary negative symptoms may inform treatment options. Primary negative symptoms are part of schizophrenia. Well-known sources of secondary negative symptoms are psychotic symptoms, disorganisation, anxiety, depression, chronic abuse of illicit drugs and alcohol, an overly high dosage of antipsychotic medication, social deprivation, lack of stimulation and hospitalisation. We present an overview of reviews and meta-analyses of double-blind, controlled randomised trials, in which the efficacy of pharmacological and non-pharmacological interventions for negative symptoms was assessed. Unfortunately there have been very few clinical trials focusing on primary negative symptoms and selecting chronically ill patients with predominant persistent negative symptoms. An important limitation in many of these studies is the failure to adequately control for potential sources of secondary negative symptoms. At present there is no convincing evidence regarding efficacy for any treatment of predominant persistent primary negative symptoms. However, for several interventions there is short-term evidence of efficacy for negative symptoms. This evidence has mainly been obtained from studies in chronically ill patients with residual symptoms and studies with a heterogeneous study population of patients in both the acute and chronic phase. Unfortunately, reliable information regarding the distinction between primary and secondary negative symptoms is lacking. Currently, early treatment of psychosis, add-on therapy with aripiprazole, antidepressants or topiramate, music therapy and exercise have been found to be useful for unspecified negative symptoms. These interventions can be considered carefully in a shared decision-making process with patients and are promising enough to be examined in large, well-designed long-term studies focusing on primary negative symptoms. Future research should be aimed at potential therapeutic interventions for primary negative symptoms since there is a lack of research in this field.
Key Points

- Available research data on treatments for primary negative symptoms are scarce and limited by the failure to control for sources of secondary negative symptoms.

- Interventions for negative symptoms have been investigated mainly in chronically ill patients with residual symptoms and heterogeneous study populations (acute and chronic phase) without distinction between primary and secondary negative symptoms, and to a lesser extent in acutely ill patients with specific reference to secondary negative symptoms.

- There is no standard treatment for negative symptoms in schizophrenia.

- Meta-analyses and reviews provide no consistent evidence of the superior efficacy of any particular intervention in patients with negative symptoms of schizophrenia.

- Early treatment of psychosis, add-on therapy with aripiprazole, antidepressants or topiramate, music therapy and exercise seem useful and viable interventions to reduce the severity of unspecified negative symptoms, and can be considered. The beneficial effect of these interventions may be limited to secondary negative symptoms.
1. Introduction

The lifetime prevalence estimate for schizophrenia is 0.4% [1]. Schizophrenia is a heterogeneous disorder presenting with positive and negative symptoms, emotional dysregulation and cognitive disturbances. Antipsychotic drugs have a moderate beneficial effect on positive symptoms [2,3]. Unfortunately, negative symptoms respond poorly to medication [4], while these symptoms mainly account for functional and social outcome in schizophrenia [5].

Primary negative symptoms are thought to be intrinsic to schizophrenia. By definition, negative symptoms mean the absence of normal behaviour. Two subdomains can be distinguished: 1. avolition, apathy, lack of energy, anhedonia and social withdrawal, and 2. expressive deficits which include blunted affect and poverty of speech [6]. Negative symptoms are associated with neurocognitive impairments [7] involving olfaction, social cognition, global cognition and language [8].

Patients with prominent negative symptoms fail to respond to both internal and external stimuli. Apathy does not seem to be associated with reduced attention to novel stimuli, but with slowed information processing [9]. Avolition may result from aberrant reinforcement learning. Patients with prominent negative symptoms fail to recognize the relative value of different rewards [10]. Impaired reward processing results in a limited behavioural repertoire and failure to activate behaviour to accomplish goals. In addition to disruptions in anticipatory pleasure and reward learning, impaired effort-based decision-making and social motivation also contribute to negative symptoms [11].

Psychomotor poverty with a flattened affect and decrease in spontaneous movements contribute to the loss of expression of emotions [12]. It is possible that psychomotor slowing with a decreased processing speed underlies verbal working memory impairment, which may mediate the association between working memory span deficit and negative symptoms [13]. A correlation has been found between working memory and awareness of illness, which explains the association between negative symptoms and poorer insight [14].

In the first five years of schizophrenia negative symptoms decrease or remain stable [15]. Evidence indicating that negative symptoms persist in the long term is inconsistent [15,16], which may suggest that more improvement of negative symptoms can be achieved than previously assumed [16].

Secondary negative symptoms are caused by other symptoms or circumstances. Psychosis, disorganisation, anxiety and depression sometimes lead to behavioural
inhibition for which adequate treatment of the underlying psychopathology is recommended with psychotherapy, antipsychotics or antidepressants [17]. Chronic abuse of illicit drugs and alcohol may cause an amotivational syndrome which shows a close resemblance to the negative syndrome in psychosis [18]. In such cases, motivational interviewing may help to reduce substance abuse. However, at present there is no compelling evidence to support this intervention [19–22]. There is some indication that training family members in motivational interviewing results in greater reduction of cannabis use in their relatives [23,24]. Lack of stimulation, demoralisation with reduced self-esteem, social deprivation, and residence in an institution are psychosocial factors which may contribute significantly to secondary negative symptoms for which rehabilitation [25], social skills training, cognitive remediation, family interventions [26] and physical activity [27] are appropriate interventions. Higher dosage of antipsychotics, and higher dopamine D2 receptor blockade have been hypothesised to cause iatrogenic negative symptoms. The impact of excessively high dopamine D2 receptor antagonism on subjective wellbeing has been found repeatedly [28–31]. In particular, patients complain of feeling numb, having diminished thoughts, amotivation and loss of hedonia. Therefore, too high a dosage of antipsychotic medication is thought to contribute to secondary negative symptoms. Unfortunately, despite increased use of lower dosage and/or second-generation antipsychotics (SGAs) the prevalence of neuroleptic-induced parkinsonism with resting tremor, rigidity, bradykinesia, loss of postural reflexes, flexed posture and motor blocking (freezing) is still substantial [32,33]. Finally, premorbid personality dimensions, such as the schizoid dimension and, to a lesser extent, schizotypic and passive-dependent dimensions are non-specific risk factors for the expression of higher levels of negative symptomatology at the beginning of psychosis [34].

Estimates of healthcare resources utilisation and the costs associated with negative symptoms are high [35]. Although reduction of prominent negative symptoms may significantly improve functional and social outcome, relatively little research has been conducted in patients suffering predominantly from persistent primary negative symptoms. Primary negative symptoms which are residual, chronic and relatively stable are seldom the primary outcome in studies. In general double-blind, randomised, placebo-controlled trials (RCTs) include acutely ill schizophrenia patients with primarily positive symptoms. However, study populations with predominantly positive symptoms are not appropriate for examining primary negative symptoms. Given this, and the fact that a better understanding of the pathogenesis of negative symptoms may help to intervene more effectively, research into the causes and treatment of negative symptoms is a high priority [36].
We examined the recent evidence supporting interventions for negative symptoms in schizophrenia spectrum disorders. Our aim was to provide a comprehensive overview of treatments for primary negative symptoms, but unfortunately it proved impossible to achieve this aim (see section 2.1 for further information). We briefly addressed the supposed explanation of action of these pharmacological and non-pharmacological interventions for negative symptoms in schizophrenia. We also aimed to explore whether pharmacological treatment options for negative symptoms are different in schizophrenia patients receiving non-clozapine antipsychotics compared to patients with treatment-resistant schizophrenia (TRS) receiving clozapine.

In this review we will address the following topics: early intervention; low dosage of antipsychotics; pharmacological monotherapy; add-on therapy with dopaminergic medication; add-on therapy with serotonergic or noradrenergic medication; add-on therapy with glutamatergic medication; add-on therapy with cholinesterase inhibitors; add-on therapy with anti-inflammatory agents; add-on therapy with antioxidants; add-on therapy with hormone treatment; non-pharmacological treatments.

2. Methodological considerations

2.1 Search strategy and selection criteria
We conducted a literature search of the PubMed database and the Cochrane Library (latest issue), limited to publications in the past decade up to 12/05/2017, concerning negative symptoms in schizophrenia, evidence supporting specific pharmacological and non-pharmacological interventions for these symptoms and the mechanism of action of the interventions. We limited our search to meta-analyses and reviews in order to increase the statistical power for group comparisons and to overcome the limitation of the small sample sizes used in several RCTs.

In an initial explorative search we focused on interventions for primary negative symptoms, using the following terms: ‘primary negative symptoms', 'schizophrenia' and 'meta-analysis.' When this first search for effective interventions for primary negative symptoms failed to reveal sufficient hits that were relevant to clinical practice, we imposed minimum constraints on our eligibility criteria. We selected meta-analytic comparisons between first-generation antipsychotics (FGAs) and SGAs, and between clozapine and FGAs and SGAs, and reviews and meta-analyses of double-blind, randomised controlled trials (RCTs)
which targeted either primary negative symptoms in patients with predominant persistent negative symptoms or negative symptoms as primary or secondary outcome in acutely ill patients or chronically ill patients stable on medication with a schizophrenia spectrum disorder. Keywords used in the search process of the PubMed database included ‘negative symptoms’, ‘schizophrenia’, ‘meta-analysis’ and ‘review’. A total of 147 hits were returned from PubMed. In addition, an equivalent search of the Cochrane Library was made, using the keywords ‘negative symptoms’ and ‘schizophrenia’. A total of 47 hits were returned from the Cochrane database. Relevant cross-references and related articles displayed in PubMed website pages were also screened and added to the common pool if found to be appropriate. After examining titles, abstracts and related articles, we selected relevant reviews and meta-analyses.

Reviews and meta-analyses on single medications were excluded, except when a specific medication was considered relevant and would not otherwise have been covered. Research on the efficacy of clozapine and amisulpride was specifically included, because of the unique receptor-binding profile of these antipsychotic medications. Clozapine has a distinct receptor-binding profile with a wide range of receptor affinities, including affinity to D4, D2, 5-HT1A, 5-HT2A, 5-HT2C, α1, α2, acetylcholine, histamine, gamma-aminobutyric acid (GABA) and glutamate receptors [37]. The superior efficacy of clozapine is hypothesised to be caused by inverse 5-HT2A agonism and D4 receptor antagonism.

![Flow diagram of the study selection process in the PubMed and Cochrane database](image)
Through antagonism of D4 receptors clozapine acts as a glutamate agonist and through GABA the signal-to-noise ratio also improves in the prefrontal cortex [39]. However, the exact mechanism of clozapine is still unknown. Amisulpride may exert its efficacy for negative symptoms through a unique binding profile with a preferable affinity for D3 receptors, which are concentrated in limbic and cortical areas [40]. Moreover, at low doses amisulpride preferentially blocks presynaptic dopaminergic autoreceptors, increasing dopaminergic transmission in the mesolimbic system and decreasing subcortical synthesis and the release of dopamine.

Articles without randomisation or control group and single-blind pharmacological studies were not included in the current review. Studies comparing two drugs in the absence of a placebo group were not included, because at present there is no evidence to assure that the active-control would have shown superior efficacy for negative symptoms over placebo, had a placebo group been included in the study. Moreover, if one drug is not found to be superior to a second, no conclusion can be drawn regarding the efficacy of either. Unpublished articles were also not included. There were no language restrictions. The diagram illustrates the process of review and exclusion of studies (see Figure 1).

2.2 Outcome measures
The articles included were heterogeneous, covering a wide range of interventions. The most important characteristics and findings of individual reviews and meta-analyses are described, including study design, inclusion criteria, description of the included population, comparisons and main results (see Tables 1–16). There are few studies focusing on primary negative symptoms which included chronically ill patients with predominant and persistent negative symptoms. Studies of chronically ill patients failed to distinguish between primary negative symptoms and unresponsive secondary negative symptoms. In studies with a heterogeneous population of patients in both the acute phase and residual phase of their illness reliable information regarding the distinction between primary and secondary negative symptoms was again not available. In acutely ill patients negative symptoms were characterized as secondary negative symptoms.

The limitations are assessed by reporting the between-study heterogeneity and whether included reviews and meta-analyses conducted sensitivity analyses to assess potential influences of any one single study on the pooled effect size. Bold results indicate statistical significance. Funding of included reviews and meta-analyses was reported
as none, independent or pharmaceutical industry. The methodological quality of included reviews and meta-analyses was assessed using the validated AMSTAR (Assessment of Multiple Systematic Reviews) tool [41,42], which characterises quality at three levels: 0 to 3 is low-quality, 4 to 7 is medium-quality and 8 to 11 is high-quality [43].

3. Early intervention

Early detection and treatment of psychosis may improve primary negative symptoms. An early age of onset in children and adolescents, and an insidious symptom onset predict a more serious negative syndrome [44–46]. However, negative symptoms are more difficult to detect in patients with a non-acute mode onset. Primary negative symptoms contribute to a delay in diagnosis, resulting in a longer duration of untreated psychosis (DUP) compared with patients with an acute mode onset [45]. A meta-analysis of 28 studies (N=3,339) showed that a shorter DUP is associated with less severe negative symptoms (without distinguishing between primary and secondary negative symptoms) at short-term (1–2 years) and long-term (5–8 years) follow-up [47]. Improvement of negative symptoms was substantially greater in patients with a DUP shorter than nine months. Early intervention is important, because a short DUP appears to be a predictor of short-term symptom and functional outcomes, based on the results of a meta-analysis of 43 RCTs of first-episode patients [48]. A meta-analysis of 33 RCTs revealed that a short DUP also predicts long-term recovery and functional remission [49]. The role of DUP in functional outcome appears to be mediated largely by primary negative symptoms [50]. Screening and ultra high risk (UHR) assessment of help-seeking individuals using the Comprehensive Assessment of at Risk Mental States (CAARMS) may enhance early detection of psychosis and shorten the DUP [51]. However, a long DUP may be not causally related to outcome in severely ill patients with poor insight [14], who often do not seek help. It may be that long DUP is a marker of a more severe form of schizophrenia with more substantial and persistent primary negative symptoms.

Furthermore, more severe negative symptoms are associated with less perceived social support, less friend support and higher levels of loneliness in young adults at UHR for psychosis [52]. Therefore, early interventions should also focus on increasing social networks in the prodromal phase, which may act as a protective factor against further development of secondary negative symptoms.
4. Low dosage of antipsychotics

The striatal, temporal and insular regions are involved in the control of motivation and reward. Occupancy of D2 receptors in these specific regions by antipsychotic medication appears to influence subjective experiences [31]. D2 occupancy by antipsychotics of above 70% is related to negative subjective experience, more severe negative symptoms and depression [28–30].

However, antipsychotics in a relatively low dose do not seem to cause or worsen amotivation, which is an important feature of primary negative symptoms. In a recent prospective RCT (N=520) motivational deficits and social amotivation were not found to be related to chronic antipsychotic treatment (olanzapine, perphenazine, quetiapine, risperidone, or ziprasidone) in a dose-dependent manner [53]. Moreover, in 121 patients who were antipsychotic-free at baseline no significant changes in motivational deficits and social amotivation were found following six months of antipsychotic treatment. It is important to realise that the doses used in this study were in the relatively low range.

In conclusion, early intervention with a relatively low dosage of antipsychotic medication and dose reduction in patients with stable chronic schizophrenia may prevent or reduce secondary negative symptoms.

5. Pharmacological monotherapy

5.1 Antipsychotic medication

While FGAs mainly block the D2 receptor and improve – probably indirectly – only those negative symptoms that are secondary to positive and depressive symptoms, the receptor-binding profile of most SGAs extends beyond D2 receptor antagonism. Except for amisulpride, which is a pure D2 and D3 receptor antagonist [40], most SGAs also bind to serotonin, glutamate, histamine, alpha (α)-adrenergic and muscarinic receptors [54]. The combination of D2 blockade and inhibition of serotonin 5-HT2 receptors increases dopamine release in the frontal lobe, which may explain why SGAs could be superior to FGAs as regards improvement in primary negative symptoms [55].

However, evidence on the efficacy of SGAs for negative symptoms is disappointing, based on medium to high-quality meta-analyses with significant heterogeneity across the included studies (see Tables 1A–B). A meta-analysis of – mainly short-term – RCTs eva-
Table 1A. Meta-analytic comparisons evaluating the efficacy of FGAs and SGAs versus placebo for negative symptom improvement.

<table>
<thead>
<tr>
<th>Study</th>
<th>Inclusion criteria</th>
<th>Specification study population and NSS</th>
<th>Medication</th>
<th>k</th>
<th>n</th>
<th>SMD</th>
<th>( P )</th>
<th>( I^2(%) )</th>
<th>SA</th>
<th>Funding</th>
<th>AMSTAR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leucht et al. 2009a [2]</td>
<td>schizophrenia</td>
<td>acute episode with predominant positive symptoms (^{\text{a}}) II NSS</td>
<td>amisulpride, aripiprazole, clozapine, olanzapine, quetiapine, risperidone, sertindole, ziprasidone, zotepine</td>
<td>36</td>
<td>5403</td>
<td>0.39</td>
<td>(&lt;0.0001)</td>
<td>+</td>
<td>+</td>
<td>none</td>
<td>8</td>
</tr>
<tr>
<td>Fusar-Poli et al. 2015 [4]</td>
<td>schizophrenia</td>
<td>aged ( \geq 18 ) heterogeneous I/II NSS</td>
<td>SGAs FGAs</td>
<td>38</td>
<td></td>
<td>0.58</td>
<td>(&lt;0.001)</td>
<td>85</td>
<td>+</td>
<td>independent</td>
<td>10</td>
</tr>
<tr>
<td>Harvey et al. 2016 [56]</td>
<td>EOS</td>
<td>mean age 15 heterogeneous I/II NSS</td>
<td>antipsychotics</td>
<td>11</td>
<td>1417</td>
<td>0.08</td>
<td>( P )</td>
<td>0.069</td>
<td>90</td>
<td>+</td>
<td>none</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>aripiprazole, clozapine, haloperidol, molindone, olanzapine, paliperidone, quetiapine, risperidone, ziprasidone</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>mild to moderate</td>
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<td></td>
<td></td>
<td>1.37</td>
<td>0.007</td>
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<td>+</td>
<td>+</td>
<td>none</td>
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<td></td>
<td>3.42</td>
<td>0.002</td>
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<td>+</td>
<td>+</td>
<td>none</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td>3.42</td>
<td>0.002</td>
<td></td>
<td>+</td>
<td>+</td>
<td>none</td>
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<td></td>
<td></td>
<td></td>
<td>2.91</td>
<td>0.007</td>
<td></td>
<td>+</td>
<td>+</td>
<td>none</td>
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<td></td>
<td>1.01</td>
<td>0.002</td>
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<td>+</td>
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<td>none</td>
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<td></td>
<td>1.20</td>
<td>0.001</td>
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<td>+</td>
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<td>none</td>
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<td></td>
<td>2.87</td>
<td>0.006</td>
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<td>+</td>
<td>+</td>
<td>none</td>
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<td></td>
<td></td>
<td>0.38</td>
<td>0.004</td>
<td></td>
<td>+</td>
<td>+</td>
<td>none</td>
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<tr>
<td>Pagsberg et al. 2017 [57]</td>
<td>EOS</td>
<td>age 8–19 acute episodes II NSS</td>
<td>antipsychotics</td>
<td>12</td>
<td>2158</td>
<td>1.00</td>
<td>( P )</td>
<td>0.001</td>
<td>+</td>
<td>+</td>
<td>independent</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>aripiprazole, asenapine, paliperidone, risperidone, quetiapine, olanzapine, molindone, ziprasidone</td>
<td></td>
<td></td>
<td>1.00</td>
<td>( P )</td>
<td>0.001</td>
<td>+</td>
<td>+</td>
<td>independent</td>
</tr>
</tbody>
</table>

NSS, negative symptoms of schizophrenia; SA, sensitivity analysis; II, secondary; I/II, no primary/secondary distinction; EOS, early-onset schizophrenia; NS, non-significant.

\(^{\text{a}}\)4 amisulpride studies, 1 olanzapine and amisulpride study and 1 zotepine study examined patients with predominantly negative symptoms.

\(\bullet\)positive trend.

\(\dagger\)significantly superior to placebo except paliperidone, quetiapine and ziprasidone.
<table>
<thead>
<tr>
<th>Comparison</th>
<th>FGAs</th>
<th>SGAs</th>
</tr>
</thead>
<tbody>
<tr>
<td>FGAs</td>
<td>haloperidol</td>
<td>olanzapine</td>
</tr>
<tr>
<td>FGAs</td>
<td>molindone</td>
<td>amisulpride</td>
</tr>
<tr>
<td>FGAs</td>
<td>zuclopenthixol</td>
<td>clozapine</td>
</tr>
<tr>
<td>FGAs</td>
<td>chlorpromazine</td>
<td>risperidone</td>
</tr>
<tr>
<td>FGAs</td>
<td></td>
<td>sertindole</td>
</tr>
<tr>
<td>FGAs</td>
<td></td>
<td>quetiapine</td>
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<tr>
<td>SGAs</td>
<td>olanzapine</td>
<td></td>
</tr>
<tr>
<td>SGAs</td>
<td></td>
<td>risperidone</td>
</tr>
<tr>
<td>SGAs</td>
<td></td>
<td>clozapine</td>
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<tr>
<td>SGAs</td>
<td></td>
<td>amisulpride</td>
</tr>
<tr>
<td>SGAs</td>
<td></td>
<td>quetiapine</td>
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<tr>
<td>SGAs</td>
<td></td>
<td>ziprasidone</td>
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<tr>
<td>SGAs</td>
<td></td>
<td>zotepine</td>
</tr>
</tbody>
</table>

Table IB. Meta-analytic comparisons evaluating the efficacy of SGAs versus FGAs for negative symptoms improvement.
Table 1C. Meta-analytic comparisons evaluating the efficacy of antipsychotics within a class for negative symptom improvement.

<table>
<thead>
<tr>
<th>Study</th>
<th>Inclusion criteria</th>
<th>Specification study population and NSS</th>
<th>Medication</th>
<th>Comparison</th>
<th>k</th>
<th>n</th>
<th>WMD</th>
<th>SMD</th>
<th>P</th>
<th>Heterogeneity</th>
<th>SA</th>
<th>Funding</th>
<th>AMSTAR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leucht et al. 2009c [59]</td>
<td>schizophrenia acute episodes with predominant positive symptoms II NSS</td>
<td>quetiapine clozapine</td>
<td>2</td>
<td>142</td>
<td>2.2●</td>
<td>&lt;0.001</td>
<td>+</td>
<td>+</td>
<td>independent</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Samara et al. 2016 [60]</td>
<td>schizophrenia spectrum disorder TRS I/II NSS</td>
<td>olanzapine clozapine risperidone haloperidol chlorpromazine sertindole quetiapine fluphenazine ziprasidone</td>
<td>0.14●</td>
<td>0.24●</td>
<td>0.24●</td>
<td>0.26●</td>
<td>0.44●</td>
<td>0.26●</td>
<td>0.44●</td>
<td>0.03●</td>
<td>2 = 0.08</td>
<td>+</td>
<td>independent</td>
</tr>
<tr>
<td></td>
<td></td>
<td>chlorpromazine sertindole</td>
<td>-0.02●</td>
<td>0.00●</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

NSS, negative symptoms of schizophrenia; SA, sensitivity analysis; II, secondary; I/II, no primary/secondary distinction; NS, non-significant.

*WMD, weighted mean difference; ●SMD, standard mean difference.

* significant, but not stated specifically.
luating the efficacy of nine SGAs compared to placebo found a small effect size (ES) for improvement of secondary negative symptoms in schizophrenia patients during an acute episode with predominant positive symptoms [2]. Although SGAs were found to be efficacious for negative symptoms (without primary/secondary distinction) in a meta-analysis of 38 RCTs, no evidence was found that SGAs are superior to FGAs, since a trend towards superior efficacy of FGAs was based on an analysis of a small number (ten) of RCTs [4]. A network meta-analysis on the efficacy of two FGAs and six SGAs in patients with early-onset schizophrenia (EOS, age of onset before 18 years) showed a positive trend towards reducing negative symptoms (without primary/secondary distinction) compared with placebo [56]. Comparisons between these antipsychotics did not yield significant differences. A more recent network meta-analysis on the efficacy of one FGA and seven SGAs used for the acute treatment of EOS found a superior efficacy of aripiprazole, asenapine, risperidone, olanzapine and molindone for secondary negative symptoms over placebo [57].

The results of medium-quality meta-analytic comparisons between SGAs versus FGAs without distinction between primary and secondary negative symptoms demonstrate no consistent superiority of SGAs over FGAs. Heterogeneity between primary studies proved large and when superiority was found, the differences were small (see Table 1B). A meta-analysis comparing nine SGAs with FGAs in acutely ill schizophrenia patients found that olanzapine, amisulpride, clozapine and risperidone were marginally more efficacious than FGAs in treating negative symptoms [3]. Non-equivalent dosing of SGAs versus FGAs confounds these findings. In another meta-analysis in first-episode schizophrenia disorders SGAs showed superior efficacy over FGAs [58]. However, this small ES disappeared and no significant difference was found between SGAs and FGAs after exclusion of industry-sponsored studies on SGAs (seven RCTs, N=1,430, \( p=0.001 \)) and analysis of independently funded studies (four RCTs, N=501, \( p=0.72 \)).

Comparisons between different antipsychotics within a class revealed significant heterogeneity levels across the included studies and did not show that any single antipsychotic was superior for the treatment of negative symptoms (see Table 1C). A medium-quality meta-analysis on the efficacy of the nine SGAs mentioned previously in a selection of patients with predominantly positive symptoms found insufficient evidence to prove any single SGA superior to any other for the treatment of secondary negative symptoms, with the exception of two small studies favouring quetiapine over clozapine [59]. A recent high-quality network meta-analysis compared the efficacy of nine antipsychotics in TRS [60]. For negative symptoms (without primary/secondary distinction), olanzapine was found to be
Table 2A. Cochrane reviews evaluating the efficacy of clozapine versus other antipsychotic medication for negative symptom improvement.

<table>
<thead>
<tr>
<th>Study</th>
<th>Inclusion criteria</th>
<th>Specification study population and NSS</th>
<th>Medication</th>
<th>Comparison</th>
<th>k</th>
<th>n</th>
<th>WMD</th>
<th>SMD</th>
<th>P</th>
<th>Heterogeneity</th>
<th>SA</th>
<th>Funding</th>
<th>AMSTAR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Essali et al. 2009 [64]</td>
<td>schizophrenia spectrum disorder</td>
<td>mostly TRS I/II NSS</td>
<td>clozapine</td>
<td>FGA</td>
<td>6</td>
<td>196</td>
<td>7.21●</td>
<td>*</td>
<td>I²=69%</td>
<td>+</td>
<td>none</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Aenjo et al. 2010 [65]</td>
<td>schizophrenia spectrum disorder</td>
<td>mostly TRS I/II NSS</td>
<td>clozapine</td>
<td>olanzapine</td>
<td>6</td>
<td>592</td>
<td>0.78●</td>
<td>NS</td>
<td>I²=0%</td>
<td>+</td>
<td>none</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Barber et al. 2017 [66]</td>
<td>schizophrenia spectrum disorder</td>
<td>age ≥18</td>
<td>clozapine</td>
<td>olanzapine + ziprasidone</td>
<td>1</td>
<td>60</td>
<td>0.80●</td>
<td>NS</td>
<td>NA</td>
<td>+</td>
<td>none</td>
<td>11</td>
<td></td>
</tr>
</tbody>
</table>

NSS, negative symptoms of schizophrenia; SA, sensitivity analysis; I/II, no primary/secondary distinction; NS, non-significant; NA, not applicable.

* statistically significant effect.

Table 2B. Meta-analytic comparisons evaluating the efficacy of clozapine versus other antipsychotic medication for negative symptom improvement.

<table>
<thead>
<tr>
<th>Study</th>
<th>Inclusion criteria</th>
<th>Specification study population and NSS</th>
<th>Medication</th>
<th>Comparison</th>
<th>k</th>
<th>n</th>
<th>SMD</th>
<th>P</th>
<th>Heterogeneity</th>
<th>SA</th>
<th>Funding</th>
<th>AMSTAR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Souza et al. 2013 [67]</td>
<td>TRS age ≥18</td>
<td>96% schizophrenia FES I/II NSS</td>
<td>clozapine</td>
<td>olanzapine</td>
<td>7</td>
<td>677</td>
<td>0.50</td>
<td>*</td>
<td>-</td>
<td>+</td>
<td>independent</td>
<td>5</td>
</tr>
<tr>
<td>Siskind et al. 2016 [68]</td>
<td>TRS</td>
<td>not specified I/II NSS</td>
<td>clozapine</td>
<td>FGAs and SGAs*</td>
<td>11</td>
<td>5</td>
<td>0.25</td>
<td>*</td>
<td>I²=6%</td>
<td>+</td>
<td>independent</td>
<td>5</td>
</tr>
<tr>
<td>Samara et al. 2016 [60]</td>
<td>TRS</td>
<td>not specified I/II NSS</td>
<td>clozapine</td>
<td>olanzapine, risperidone, haloperidol, ziprasidone, fluphenazine, quetiapine, chlorpromazine, sertindole</td>
<td>-0.14</td>
<td>*</td>
<td>τ²=0.08</td>
<td>+</td>
<td>independent</td>
<td>8</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NSS, negative symptoms of schizophrenia; SA, sensitivity analysis; I/II, no primary/secondary distinction; NS, non-significant; FES, first-episode schizophrenia spectrum disorder.

* statistically significant effect.

* chlorpromazine, haloperidol, olanzapine, risperidone, quetiapine, ziprasidone.
superior to clozapine, risperidone, haloperidol, chlorpromazine and sertindole. Ziprasidone was more effective for negative symptoms than chlorpromazine and sertindole. These results should be interpreted with caution since the data on ziprasidone, fluphenazine, quetiapine, chlorpromazine and sertindole are limited.

While clozapine is the standard treatment for patients with TRS [61,62], the superior efficacy of clozapine relative to other antipsychotic medication seems not to apply to negative symptoms [63]. Three Cochrane reviews compared the efficacy of clozapine with that of other antipsychotics in improving negative symptoms (without distinguishing between primary and secondary negative symptoms) in patients suffering from a schizophrenia spectrum disorder, most of whom were treatment-resistant (see Table 2A). An early Cochrane review comparing short-term treatment with clozapine and FGAs in schizophrenia spectrum disorders found a significantly greater reduction of negative symptoms in clozapine-treated patients, but there was a substantial degree of heterogeneity between studies [64]. A Cochrane review comparing clozapine and SGAs found that clozapine was not more efficacious for negative symptoms than olanzapine or risperidone [65]. In a recent Cochrane review comparing combinations of clozapine with various other antipsychotics in TRS, negative symptoms were assessed in only one RCT, which found they did not improve significantly [66].

Findings of meta-analyses comparing clozapine with individual antipsychotics in TRS are inconsistent and primary negative symptoms were not distinguished from secondary negative symptoms (see Table 2B). A medium-quality meta-analysis showed superior efficacy of clozapine over olanzapine for negative symptoms (trial duration 8–28 weeks) [67]. A more recent medium-quality meta-analysis comparing clozapine with two FGAs and four SGAs in TRS found a superior short-term effect of clozapine for negative symptoms. The effect was no longer superior after a treatment duration of three months or more [68]. This discrepancy may be confounded by pharmaceutical industry funding of included studies and by the significantly lower doses of clozapine compared with those of control group medication, which may have biased the data against clozapine. In a recent high-quality network meta-analysis comparing the efficacy of FGAs and SGAs olanzapine was found to be significantly more efficacious for negative symptoms than clozapine in TRS [60]. Clozapine did not substantially differ from risperidone, haloperidol, ziprasidone, fluphenazine, quetiapine, chlorpromazine or sertindole. However, evidence on these last five antipsychotics is scarce and blinded RCTs for the comparison of clozapine with other
Table 3. Meta-analysis of double-blind, randomised trials evaluating the efficacy of amisulpride for negative symptom improvement.

<table>
<thead>
<tr>
<th>Study</th>
<th>Inclusion criteria</th>
<th>Specification study population and NSS</th>
<th>Medication</th>
<th>Comparison</th>
<th>k</th>
<th>n</th>
<th>r</th>
<th>P</th>
<th>Heterogeneity</th>
<th>SA</th>
<th>Funding</th>
<th>AMSTAR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leucht et al. 2002 [69]</td>
<td>schizophrenia spectrum disorder</td>
<td>acute episode with predominant positive symptoms II NSS</td>
<td>amisulpride (200–1200 mg)</td>
<td>conventional antipsychotics*</td>
<td>5</td>
<td>1563</td>
<td>0.14</td>
<td>&lt;0.0001</td>
<td>−</td>
<td>+</td>
<td>independent</td>
<td>5</td>
</tr>
<tr>
<td>Leucht et al. 2002 [69]</td>
<td>schizophrenia spectrum disorder</td>
<td>predominant and persistent negative symptoms I NSS</td>
<td>amisulpride (50–300 mg)</td>
<td>conventional antipsychotics* placebo</td>
<td>3</td>
<td>130</td>
<td>0.08</td>
<td>0.44</td>
<td>−</td>
<td>+</td>
<td>independent</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4</td>
<td>624</td>
<td>0.26</td>
<td>&lt;0.0001</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NSS, negative symptoms of schizophrenia; SA, sensitivity analysis; II, secondary; I, primary.
*olanzapine, quetiapine, risperidone and sertindole.
SGAs are limited. Moreover, clozapine plasma concentrations were not examined in most studies included in this meta-analysis and the clozapine titration speed was high. The sedative effects of clozapine may therefore explain why clozapine showed no superior efficacy over other antipsychotics.

There are not many studies involving schizophrenia patients with prominent and persistent negative symptoms. Most of these studies have focused on the efficacy of low doses of amisulpride. A medium-quality meta-analysis comparing amisulpride with SGAs in acutely ill patients with predominant positive symptoms revealed a slight superior efficacy of amisulpride for secondary negative symptom improvement (see Table 3) [69]. In the same meta-analysis superior efficacy of low-dose amisulpride (treatment duration 6–24 week) was found for primary negative symptoms over placebo in patients suffering from schizophrenia spectrum disorders with predominant and persistent negative symptoms [69]. However, potential sources of secondary negative symptoms were not assessed which may have confounded the effect on primary negative symptoms.

5.2 Metabotropic glutamate 2/3 receptor (mGluR 2/3) agonists

Pomaglumetad methionil is a potent and highly selective mGluR 2/3 agonist [70]. A beneficial effect on negative symptoms may arise from activation of these mGlu2 and mGlu3 receptors, which are found in high levels in the limbic system. Due to the inhibition of presynaptic glutamate release and modulation of synaptic plasticity, mGluR 2/3 agonists may influence negative symptoms. Although three RCTs did not show superiority over placebo in patients with acutely exacerbated schizophrenia, a post hoc exploratory study of five RCTs found a beneficial effect of pomaglumetad methionil (40 mg twice daily) on secondary negative symptoms in specific subgroups: patients with a duration of illness shorter than three years and patients who had previously received antipsychotic medication with predominant D2 receptor antagonism and without 5-HT2A receptor antagonist activity [71].

MGluR positive allosteric modulators (PAMs) are efficacious in animal studies and are currently being tested in phase II studies [72]. Although functional selectivity of third-generation antipsychotics was considered a breakthrough in the treatment of schizophrenia, clinical expectations of success of mGluR agonists have not been met and the development of these ligands for schizophrenia has been discontinued.
Table 4A. Meta-analysis evaluating the efficacy of antipsychotic augmentation versus monotherapy for negative symptom improvement.

<table>
<thead>
<tr>
<th>Study</th>
<th>Inclusion criteria</th>
<th>Specification study population and NSS</th>
<th>Medication</th>
<th>k</th>
<th>n</th>
<th>SMD</th>
<th>P</th>
<th>I^2 (%)</th>
<th>SA</th>
<th>Funding</th>
<th>AMSTAR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Galling et al. 2017 [73]</td>
<td>schizophrenia</td>
<td>mostly inpatients I/II NSS</td>
<td>antipsychotic augmentation</td>
<td>18</td>
<td>931</td>
<td>0.38</td>
<td>0.003</td>
<td>+</td>
<td>+</td>
<td>independent</td>
<td>7</td>
</tr>
<tr>
<td>Galling et al. 2017 [73] Subanalysis</td>
<td>schizophrenia</td>
<td>not specified I/II NSS</td>
<td>two D2 antagonists</td>
<td>10</td>
<td>399</td>
<td>0.36</td>
<td>0.055</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Galling et al. 2017 [73] Subanalysis</td>
<td>schizophrenia</td>
<td>not specified I/II NSS</td>
<td>aripiprazole</td>
<td>8</td>
<td>532</td>
<td>0.41</td>
<td>0.036</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Galling et al. 2017 [73] Subanalysis</td>
<td>schizophrenia</td>
<td>not specified I/II NSS</td>
<td>aripiprazole</td>
<td>4</td>
<td>355</td>
<td>0.28</td>
<td>0.043</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NSS, negative symptoms of schizophrenia; SA, sensitivity analysis; I/II, no primary/secondary distinction.

*1 RCT with clozapine and 7 RCTs combined with other SGAs.

*high-quality studies.
Table 4B. Meta-analyses of double-blind, randomized trials evaluating the efficacy of add-on therapy with dopamine antagonists for negative symptom improvement.

<table>
<thead>
<tr>
<th>Study</th>
<th>Medication</th>
<th>Comparator</th>
<th>Inclusion criteria</th>
<th>NSS and SS</th>
<th>Publication</th>
<th>AMSTAR</th>
<th>Funding</th>
<th>F (%), p</th>
<th>n</th>
<th>SMD</th>
<th>k</th>
<th>u</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>non-Clozapine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Add-on clozapine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Add-on haloperidol</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Add-on sulpiride</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Add-on sertindole</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Note:** NSS, negative symptoms of schizophrenia; SS, secondary symptoms; I/II, no primary/secondary distinction.
6. Add-on therapy with dopaminergic medication

6.1 Dopamine antagonists

On theoretical grounds it is unlikely that the addition of a second dopamine antagonist to a non-clozapine antipsychotic would improve negative symptoms. Nevertheless, a recent medium-quality meta-analysis comparing antipsychotic augmentation versus monotherapy, mainly in inpatients with schizophrenia, favoured antipsychotic augmentation treatment over monotherapy for negative symptom improvement (see Table 4A) [73]. However, a significant degree of heterogeneity was found between the included studies, which did not distinguish primary from secondary negative symptoms and this favourable effect was restricted to augmenting with the partial dopamine agonist aripiprazole.

An earlier medium-quality meta-analysis showed that a second antipsychotic as adjunctive treatment to clozapine significantly but weakly improved negative symptoms (without primary/secondary distinction) in TRS after exclusion of one outlier study on pimozide (see Table 4B) [74]. Risperidone and aripiprazole are the two antipsychotics which have been studied the most in combination with clozapine. While the results on risperidone showed no significant improvement of negative symptoms compared to placebo, two separate meta-analyses of three RCTs of aripiprazole (duration 8–24 weeks) showed a similar trend towards reducing negative symptoms [74,75] (see Table 4B). A recent high-quality meta-analysis of placebo-controlled and open-label RCTs on the efficacy of aripiprazole augmentation showed a significant effect on negative symptoms in patients with a schizophrenia spectrum disorder who were receiving clozapine or non-clozapine antipsychotics (see Table 4B) [76]. Important limitations were the inclusion of low-quality RCTs, a high level of heterogeneity across the included studies and the failure to discriminate primary from secondary negative symptoms.

6.2 Dopamine agonists

Psychostimulants such as modafinil and armodafinil block the dopamine transporter, resulting in increased dopamine levels in the synaptic cleft [77]. Although theoretically a dopamine agonist could worsen positive symptoms, a meta-analysis of the effects of modafinil or armodafinil as add-on therapy to antipsychotics (both clozapine and non-clozapine antipsychotics) revealed no significant change in positive symptoms compared to placebo [78]. A beneficial effect on negative symptoms was hypothesised based on the
### Table 5

**A. Meta-analysis of double-blind, placebo-controlled, randomised trials evaluating the efficacy of dopamine agonists added to non-clozapine antipsychotics for negative symptom improvement.**

<table>
<thead>
<tr>
<th>Study Population</th>
<th>NSS</th>
<th>Medication</th>
<th>SMD</th>
<th>n</th>
<th>Funding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Andrade et al. 2015 [78]</td>
<td>none</td>
<td>dopamine agonists</td>
<td>0.26</td>
<td>322</td>
<td>non-significant</td>
</tr>
<tr>
<td>Andrade et al. 2015 [78]</td>
<td>none</td>
<td>dopamine agonists</td>
<td>0.17</td>
<td>276</td>
<td>non-significant</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Specification</th>
<th>study population and NSS combination of acute episode and stable on medication</th>
</tr>
</thead>
<tbody>
<tr>
<td>I/II NSS</td>
<td>stable dose of antipsychotic medication for ≥4 weeks</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>I/II</th>
<th>NSS</th>
<th>AMSTAR</th>
</tr>
</thead>
<tbody>
<tr>
<td>I/II</td>
<td>NSS</td>
<td>9</td>
</tr>
</tbody>
</table>

**Funding**

None

### Table 5

**B. Meta-analysis and Cochrane review of double-blind, placebo-controlled, randomised trials evaluating the efficacy of add-on therapy with psychostimulants for negative symptom improvement.**

<table>
<thead>
<tr>
<th>Study Population</th>
<th>NSS</th>
<th>Medication</th>
<th>SMD</th>
<th>n</th>
<th>Funding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nolte et al. 2004 [82]</td>
<td>none</td>
<td>amphetamine</td>
<td>0.26</td>
<td>6</td>
<td>non-significant</td>
</tr>
<tr>
<td>Lindenmayer et al. 2013 [83]</td>
<td>none</td>
<td>methylphenidate</td>
<td>0.17</td>
<td>8</td>
<td>significant effect</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Specification</th>
<th>study population and NSS</th>
</tr>
</thead>
<tbody>
<tr>
<td>I/II NSS</td>
<td>chronic inpatients</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>I/II</th>
<th>NSS</th>
<th>AMSTAR</th>
</tr>
</thead>
<tbody>
<tr>
<td>I/II</td>
<td>NSS</td>
<td>11</td>
</tr>
</tbody>
</table>

**Funding**

None

### Table 5

**Medication**

<table>
<thead>
<tr>
<th>Specification</th>
<th>study population and NSS</th>
</tr>
</thead>
<tbody>
<tr>
<td>I/II NSS</td>
<td>predominant negative symptoms</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>I/II</th>
<th>NSS</th>
<th>AMSTAR</th>
</tr>
</thead>
<tbody>
<tr>
<td>I/II</td>
<td>NSS</td>
<td>2</td>
</tr>
</tbody>
</table>
### Table 6A. Meta-analyses of double-blind, randomised trials evaluating the efficacy of add-on therapy with serotonergic medication compared with placebo or no treatment for negative symptom improvement.

<table>
<thead>
<tr>
<th>Study</th>
<th>Inclusion criteria</th>
<th>Specification study population and NSS</th>
<th>Medication</th>
<th>Comparison</th>
<th>k</th>
<th>n</th>
<th>SMD</th>
<th>P</th>
<th>Heterogeneity</th>
<th>funding</th>
<th>AMSTAR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Singh et al. 2010a [84]</td>
<td>chronic schizophrenia</td>
<td>severely negative symptoms similar to baseline</td>
<td>antidepressants</td>
<td>placebo</td>
<td>23</td>
<td>819</td>
<td>0.48</td>
<td>*</td>
<td>+</td>
<td>independent</td>
<td>5</td>
</tr>
<tr>
<td>Subanalysis</td>
<td></td>
<td></td>
<td>SSRIs</td>
<td>placebo</td>
<td>2</td>
<td>73</td>
<td>0.83</td>
<td>*</td>
<td>+</td>
<td>independent</td>
<td>5</td>
</tr>
<tr>
<td>Singh et al. 2010a [84]</td>
<td>chronic schizophrenia</td>
<td>severely negative symptoms similar to baseline</td>
<td>trazodone</td>
<td>placebo</td>
<td>2</td>
<td>72</td>
<td>0.70</td>
<td>*</td>
<td>+</td>
<td>independent</td>
<td>5</td>
</tr>
<tr>
<td>Subanalysis</td>
<td></td>
<td></td>
<td>fluoxetine</td>
<td>placebo</td>
<td>4</td>
<td>136</td>
<td>0.42</td>
<td>*</td>
<td>+</td>
<td>independent</td>
<td>5</td>
</tr>
<tr>
<td>Singh et al. 2010a [84]</td>
<td>chronic schizophrenia</td>
<td>severity positive symptoms similar to baseline</td>
<td>antidepressants</td>
<td>placebo</td>
<td>26</td>
<td>650</td>
<td>0.35</td>
<td>0.001</td>
<td>F = 56.3%</td>
<td>+</td>
<td>independent</td>
</tr>
<tr>
<td>Subanalysis</td>
<td></td>
<td></td>
<td>SSRIs</td>
<td>placebo</td>
<td>26</td>
<td>406</td>
<td>0.58</td>
<td>0.002</td>
<td>F = 67.6%</td>
<td>+</td>
<td>independent</td>
</tr>
<tr>
<td>Fussar Poli et al. 2015 [4]</td>
<td>schizophrenia spectrum disorder</td>
<td>aged ≥ 18</td>
<td>antidepressants</td>
<td>placebo</td>
<td>48</td>
<td>1905</td>
<td>0.30</td>
<td>&lt;0.0001</td>
<td>+</td>
<td>independent</td>
<td>10</td>
</tr>
<tr>
<td>Helfer et al. 2016 [85]</td>
<td>schizophrenia spectrum disorder</td>
<td>heterogeneous</td>
<td>antidepressants</td>
<td>placebo</td>
<td>48</td>
<td>1905</td>
<td>0.30</td>
<td>&lt;0.0001</td>
<td>+</td>
<td>independent</td>
<td>10</td>
</tr>
<tr>
<td>Fussar Poli et al. 2015 [4]</td>
<td>schizophrenia spectrum disorder</td>
<td>aged ≥ 18</td>
<td>antidepressants</td>
<td>no treatment</td>
<td>48</td>
<td>1905</td>
<td>0.30</td>
<td>&lt;0.0001</td>
<td>+</td>
<td>independent</td>
<td>10</td>
</tr>
<tr>
<td>Subanalysis</td>
<td></td>
<td></td>
<td>SSRIs</td>
<td>placebo</td>
<td>26</td>
<td>406</td>
<td>0.58</td>
<td>0.002</td>
<td>F = 67.6%</td>
<td>+</td>
<td>independent</td>
</tr>
<tr>
<td>Singh et al. 2014a [87]</td>
<td>schizophrenia spectrum disorder</td>
<td>patients with clinical instability</td>
<td>5-HT3R-ANTs</td>
<td>placebo</td>
<td>5</td>
<td>261</td>
<td>1.10</td>
<td>0.002</td>
<td>+</td>
<td>none</td>
<td>6</td>
</tr>
</tbody>
</table>

NSS, negative symptoms of schizophrenia; SA, sensitivity analysis; I/II, no primary/secondary distinction; 1, primary, 2, secondary, 3, tertiary, 4, quaternary, 5, quintary, 6, quinty, 7, sevengy, 8, eyegy, 9, nineny, 10, tenten, 11, eleven, 12, twelven.
increase of dopamine in the prefrontal cortex [79] and the nucleus accumbens [80], and a
dose-dependent regional increase of glutamate and decrease of GABA [77]. In a medium-
quality meta-analysis on adjunctive modafinil or armodafinil a small significant improvement
of negative symptoms (without discrimination between primary and secondary symptoms)
was found compared with placebo in schizophrenia patients after eight weeks of treatment
(see Table 5A) [78]. In all but one RCT patients were on a stable dose of antipsychotic
medication for at least four weeks. However, after exclusion of one RCT in which
participants were inpatients in the active phase of the illness [81], the beneficial effect on
negative symptoms was no longer significant.

In a Cochrane review evidence for efficacy of amphetamine for negative symptoms
(without primary/secondary distinction) was limited to a single small study in chronic inpa-
tients (see Table 5B) [82]. In a low-quality review of psychostimulant treatment of negative
symptoms in schizophrenia no reduction of primary negative symptoms was found in a
single study after treatment with methylphenidate in patients suffering from schizophrenia
spectrum disorders with predominant negative symptoms (see Table 5B) [83].

7. Add-on therapy with serotonergic or noradrenergic medication

7.1 Serotonergic medication
A synergistic effect of a selective serotonin re-uptake inhibitor (SSRI) and an antipsychotic
through modulation of the GABA-A receptor and its regulating system is hypothesised to
improve negative symptoms [55].

Medium to high-quality meta-analyses of RCTs in mostly chronic schizophrenia
patients revealed superior efficacy of serotonergic medication compared to placebo for
negative symptom improvement (without discrimination between primary and secondary
negative symptoms) (see Table 6A). A medium-quality meta-analysis of add-on treatment
with antidepressants included RCTs examining SSRIs, the 5-HT2 receptor antagonist
ritanserin, the serotonin antagonist and reuptake inhibitor (SARI) trazodone, alpha-2
receptor antagonists (mirtazapine and mianserin) and the noradrenalin reuptake inhibitor
(NRI) reboxetine in chronic schizophrenia [84]. Antidepressants were found to moderately
improve negative symptoms with a NNT (number needed to treat) of ten to fifteen. A
subanalysis showed that ritanserin (NNT=5), trazodone (NNT=6) and fluoxetine (NNT=11)
were the most efficacious. However, due to the relatively small sample sizes of separate
Table 6B. Meta-analyses of double-blind, placebo-controlled, randomised trials evaluating the efficacy of alpha-2 receptor antagonists and noradrenalin reuptake inhibitors as add-on therapy for negative symptom improvement.

<table>
<thead>
<tr>
<th>Study</th>
<th>Inclusion criteria</th>
<th>Specification study population and NSS</th>
<th>Medication</th>
<th>k</th>
<th>n</th>
<th>SMD</th>
<th>P</th>
<th>Heterogeneity</th>
<th>SA</th>
<th>Funding</th>
<th>AMSTAR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hecht &amp; Landy, 2012 [89]</td>
<td>schizophrenia</td>
<td>not specified I/II NSS</td>
<td>mirtazapine (30 mg) mianserin (15–30 mg)</td>
<td>8</td>
<td>244</td>
<td>0.84 *</td>
<td>Q=37.4</td>
<td>–</td>
<td>none</td>
<td>none</td>
<td>2</td>
</tr>
<tr>
<td>Kishi &amp; Iwata, 2014b [90]</td>
<td>schizophrenia</td>
<td>mostly inpatients II NSS</td>
<td>alpha-2 receptor antagonists</td>
<td>9</td>
<td>240</td>
<td>0.88 0.001</td>
<td>37%</td>
<td>+</td>
<td>none</td>
<td>none</td>
<td>8</td>
</tr>
<tr>
<td>Subanalysis</td>
<td></td>
<td></td>
<td></td>
<td>6</td>
<td>172</td>
<td>1.25 0.0001</td>
<td>37%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kishi &amp; Iwata, 2014b [90]</td>
<td>schizophrenia</td>
<td>mostly inpatients II NSS</td>
<td>mirtazapine (15–30 mg)</td>
<td>3</td>
<td>68</td>
<td>0.15 0.54</td>
<td>0%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subanalysis</td>
<td></td>
<td></td>
<td></td>
<td>3</td>
<td>218</td>
<td>0.02 0.89</td>
<td>0%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kishi et al. 2013a [93]</td>
<td>schizophrenia</td>
<td>mostly outpatients II NSS</td>
<td>NRIs</td>
<td>7</td>
<td>218</td>
<td>0.02 0.89</td>
<td>0%</td>
<td></td>
<td>+</td>
<td>none</td>
<td>8</td>
</tr>
<tr>
<td>Subanalysis</td>
<td></td>
<td></td>
<td></td>
<td>3</td>
<td>73</td>
<td>0.01 0.97</td>
<td>0%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kishi et al. 2013a [93]</td>
<td>schizophrenia</td>
<td>mostly outpatients II NSS</td>
<td>atomoxetine</td>
<td>3</td>
<td>73</td>
<td>0.01 0.97</td>
<td>0%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subanalysis</td>
<td></td>
<td></td>
<td></td>
<td>3</td>
<td>145</td>
<td>0.02 0.89</td>
<td>0%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NSS, negative symptoms of schizophrenia; SA, sensitivity analysis; I/II, no primary/secondary distinction; II, secondary.

* significant, but not stated specifically.
RCTs it was not possible to determine the superiority of a single antidepressant. A smaller ES was found for antidepressants in a high-quality meta-analysis of a range of different treatments for negative symptoms in patients with a schizophrenia spectrum disorder [4]. A more recent medium-quality meta-analysis on the efficacy of adjunctive antidepressants for negative symptoms also found a small beneficial effect on negative symptoms compared to placebo in patients with a schizophrenia spectrum disorder (NNT=9) [85]. SSRIs stood out as a class with fluvoxamine and citalopram showing consistent efficacy for negative symptoms. A subanalysis of patients with predominant negative symptoms revealed a higher ES for primary negative symptoms, but secondary negative symptoms were not eliminated from this analysis.

While selective serotonin 3 receptor antagonists (5-HT3R-ANTs) are generally used for the treatment of patients with postoperative or chemotherapy-induced nausea and vomiting, 5-HT3R-ANTs may exert efficacy for negative symptoms in schizophrenia through modulation of mesolimbic and mesocortical dopaminergic activity [86]. In a medium-quality meta-analysis on the short-term efficacy of 5-HT3R-ANTs (tropisetron, ondansetron and granisetron) (duration 10 days–12 weeks, median 8 weeks) this add-on therapy to haloperidol or risperidone was found to be especially beneficial for negative symptoms in schizophrenia with clinical stability [87]. However, a significant degree of heterogeneity was found between the included studies. Further research of 5-HT3R-ANTs add-on therapy is necessary to confirm these positive findings and investigate long-term effectiveness.

7.2 Alpha-2 receptor antagonists
A presynaptic alpha-2 receptor antagonist combined with a D2 antagonist may cause an efflux of dopamine in the frontal cortex [88]. Two meta-analyses with a significant degree of heterogeneity show evidence of the efficacy of alpha-2 receptor antagonists for negative symptoms in mostly acutely ill schizophrenia patients (see Table 6B). A low quality meta-analysis showed a large significant improvement of negative symptoms (without discrimination between primary and secondary symptoms) compared to placebo after four to eight weeks adjunctive treatment with mirtazapine or mianserin [89].

A high-quality meta-analysis evaluating the efficacy of mirtazapine and mianserin as augmentation therapy found a similar ES for secondary negative symptoms in mostly inpatients with schizophrenia [90]. Subanalysis revealed that adjunctive mirtazapine was superior to placebo, while mianserin did not show significant change compared to placebo.
for negative symptoms.

Mirtazapine (6 of 7 studies) and mianserin (1 of 3 studies) showed efficacy for negative symptoms (without primary/secondary distinction) in a low-quality review (no funding, AMSTAR=1) of several different add-on antidepressants (selective serotonin reuptake inhibitors, duloxetine, imipramine, mianserin, mirtazapine, nefazodone, reboxetin, trazodone and bupropion) in schizophrenia [91]. A beneficial effect for negative symptoms was also found for SSRIs: fluvoxamine (2 of 2 studies), paroxetin (1 study) and fluoxetine (2 of 6 studies). Trazodone (2 of 3 studies) and duloxetine (1 study) also improved negative symptoms.

7.3 Noradrenalin reuptake inhibitors (NRIs)
NRIs increase dopaminergic activity in the PFC and influence the noradrenergic reward pathway, which could theoretically result in improvement of negative symptoms [92]. However, a high-quality meta-analysis on the efficacy of NRI augmentation to antipsychotic medication demonstrated no superiority of NRI add-on therapy to placebo for negative symptoms (without discrimination between primary and secondary negative symptoms) in chronic schizophrenia (see Table 6B) [93]. Subanalyses of atomoxetine and reboxetine showed no effect on negative symptoms.

7.4 Augmentation to clozapine
While considerable research has been conducted concerning antidepressant medication as add-on therapy to non-clozapine antipsychotics, only one meta-analysis of four RCTs (N=111) examined the effect of the specific combination of an antidepressant and clozapine (no funding, AMSTAR=5) [74]. Antidepressants (fluoxetine, mirtazapine and duloxetine) added to clozapine treatment compared to placebo addition showed a positive trend with a large ES (Hedges’ g=0.87, p=0.062) for reduction of negative symptoms (without primary/secondary distinction). The heterogeneity of the included studies was high (I²=80%).
Table 7A. Meta-analyses of double-blind, placebo-controlled, randomised trials evaluating the efficacy of adjunctive topiramate for negative symptom improvement.

<table>
<thead>
<tr>
<th>Study</th>
<th>Concomitant medication</th>
<th>Specification</th>
<th>Conclusion</th>
<th>Meta-analysis type</th>
<th>NSS, negative symptoms; SA, sensitivity analysis; II, secondary; I/II, no primary/second ary distinction; NS, non-significant.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>clozapine</td>
<td>mostly inpatients</td>
<td>statistically significant</td>
<td>II NSS</td>
<td>mostly inpatients</td>
</tr>
<tr>
<td>2</td>
<td>olanzapine</td>
<td>mostly inpatients</td>
<td>non-significant</td>
<td>II NSS</td>
<td>mostly inpatients</td>
</tr>
<tr>
<td>3</td>
<td>clozapine</td>
<td>mostly inpatients</td>
<td>non-significant</td>
<td>II NSS</td>
<td>mostly inpatients</td>
</tr>
<tr>
<td>4</td>
<td>clozapine</td>
<td>mostly inpatients</td>
<td>non-significant</td>
<td>II NSS</td>
<td>mostly inpatients</td>
</tr>
<tr>
<td>5</td>
<td>clozapine</td>
<td>mostly inpatients</td>
<td>non-significant</td>
<td>II NSS</td>
<td>mostly inpatients</td>
</tr>
<tr>
<td>6</td>
<td>non-clozapine antipsychotics</td>
<td>mostly inpatients</td>
<td>non-significant</td>
<td>II NSS</td>
<td>mostly inpatients</td>
</tr>
</tbody>
</table>

**Notes:**
- AMSTAR: Assessment of Methodological Quality score.
- SMD: Standardised Mean Difference.
- Hedges'g: Hedges' unbiased estimate of effect size.
- Other abbreviations and symbols are explained within the table.

**References:**
- Sommer et al. 2012 [98]
- Veerman et al. 2014 [99]
- Correll et al. 2016 [100]
- Okuyama et al. 2016 [101]
- Zheng et al. 2016 [102]
8. Add-on therapy with glutamatergic medication

8.1 Glutamate antagonists

Pharmacological treatment which targets the NMDA receptor is supposed to improve the balance of glutamate [94–96]. We have hypothesised that the negative symptoms and cognitive impairment associated with schizophrenia could be prevented if dysregulation of the NMDA receptor is addressed shortly after the onset of symptoms [97]. Novel pharmacological strategies which are expected to boost the NMDA receptor function have been thoroughly investigated over the past decade.

Topiramate potentiates GABA-ergic neurotransmission and decreases the presynaptic release of glutamate through antagonism of postsynaptic kainate receptors and amino-3-hydroxy-5-methyl-isoxazole-4-proprionic acid (AMPA) receptors [97]. Research on topiramate augmentation to antipsychotic medication in schizophrenia spectrum disorders is extensive with six meta-analyses, but heterogeneity across primary studies is substantial (see Table 7A). Whereas topiramate augmentation to clozapine did not show significant efficacy for secondary negative symptoms in two early, medium-quality meta-analyses of short-term RCTs (duration 8–24 weeks) in acutely ill patients with TRS [98,99], three more recent medium to high-quality meta-analyses consistently demonstrated the efficacy of adjunctive topiramate in mostly inpatients suffering from schizophrenia spectrum disorders [100–102]. However, no distinction is made between primary and secondary negative symptoms.

Subgroup analyses in the most recent high-quality meta-analysis revealed that topiramate augmentation to clozapine may be more efficacious than topiramate augmentation to non-clozapine antipsychotics.

Lamotrigine is an antagonist of postsynaptic voltage-sensitive sodium channels [36]. Three medium-quality meta-analyses show inconsistent evidence of the efficacy of lamotrigine augmentation to clozapine for negative symptoms in TRS (without no distinction between primary and secondary negative symptoms) [103,98,99] (see Table 7B). While an early meta-analysis of short-term RCTs (10–24 weeks) found superior efficacy of lamotrigine augmentation to clozapine compared with placebo [103], another meta-analysis of the same five RCTs found no significant difference in negative symptom improvement between lamotrigine and placebo after exclusion of an outlier study [98]. In a more recent meta-analysis of six RCTs (duration 10–24 weeks) on adjunctive lamotrigine to clozapine the effect of lamotrigine on negative symptoms was on a trend level after excluding two...
Table 7B. Meta-analyses of double-blind, placebo-controlled, randomised trials evaluating the efficacy of adjunctive lamotrigine, amantadine and memantine for negative symptom improvement.

<table>
<thead>
<tr>
<th>Study</th>
<th>Medication</th>
<th>Concomitant medication</th>
<th>Specification of study population and NSS</th>
<th>Meta-analytic approach</th>
<th>Funding</th>
<th>AMSTAR 4</th>
<th>I2 (%)</th>
<th>n (SMD)</th>
<th>p (SA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tiihonen et al. 2009</td>
<td>Lamotrigine (100–400 mg)</td>
<td>Clozapine</td>
<td>TRS</td>
<td>Meta-regression of double-blind, placebo-controlled, randomised trials evaluating the efficacy of adjunctive lamotrigine, amantadine and memantine for negative symptom improvement</td>
<td>Independent</td>
<td>7</td>
<td>87</td>
<td>0.05</td>
<td>0.87</td>
</tr>
<tr>
<td>Sommer et al. 2012</td>
<td>Lamotrigine (100–400 mg)</td>
<td>Clozapine</td>
<td>TRS</td>
<td>Meta-regression of double-blind, placebo-controlled, randomised trials evaluating the efficacy of adjunctive lamotrigine, amantadine and memantine for negative symptom improvement</td>
<td>Independent</td>
<td>5</td>
<td>69</td>
<td>0.41</td>
<td>NS</td>
</tr>
<tr>
<td>Veerman et al. 2013</td>
<td>Lamotrigine (100–400 mg)</td>
<td>Clozapine</td>
<td>TRS</td>
<td>Meta-regression of double-blind, placebo-controlled, randomised trials evaluating the efficacy of adjunctive lamotrigine, amantadine and memantine for negative symptom improvement</td>
<td>Independent</td>
<td>5</td>
<td>64</td>
<td>0.41</td>
<td>NS</td>
</tr>
<tr>
<td>Matsuda et al. 2013</td>
<td>Lamotrigine (100–400 mg)</td>
<td>Clozapine</td>
<td>TRS</td>
<td>Meta-regression of double-blind, placebo-controlled, randomised trials evaluating the efficacy of adjunctive lamotrigine, amantadine and memantine for negative symptom improvement</td>
<td>Independent</td>
<td>4</td>
<td>8</td>
<td>0.008</td>
<td>0.43</td>
</tr>
</tbody>
</table>

NSS, negative symptoms of schizophrenia; SA, sensitivity analysis; I/II, no primary/secondary distinction; NS, non-significant.
Table 7C. Meta-analyses of double-blind, placebo-controlled, randomised trials evaluating the efficacy of adjunctive NMDA-receptor agonists for negative symptom improvement.

<table>
<thead>
<tr>
<th>Study</th>
<th>Inclusion criteria</th>
<th>Specification study population and NSS</th>
<th>Regular medication</th>
<th>Medication</th>
<th>k</th>
<th>n</th>
<th>SMD</th>
<th>P</th>
<th>Heterogeneity</th>
<th>SA</th>
<th>Funding</th>
<th>AMSTAR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Singh &amp; Singh, 2011 [111]</td>
<td>schizophrenia</td>
<td>TRS I/II NSS</td>
<td>antipsychotics</td>
<td>NMDA receptor agonists</td>
<td>32</td>
<td>1413</td>
<td>0.27♠</td>
<td>0.01</td>
<td>QE=97.70</td>
<td>+</td>
<td>none</td>
<td>6</td>
</tr>
<tr>
<td>Singh &amp; Singh, 2011 [111]</td>
<td>schizophrenia</td>
<td>TRS I/II NSS</td>
<td>non-clozapine antipsychotics</td>
<td>D-serine</td>
<td>4</td>
<td>183</td>
<td>0.54♠</td>
<td>0.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Singh &amp; Singh, 2011 [111]</td>
<td>schizophrenia</td>
<td>TRS I/II NSS</td>
<td>non-clozapine antipsychotics</td>
<td>N-acetylcysteine</td>
<td>1</td>
<td>140</td>
<td>0.45♠</td>
<td>0.01</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Singh &amp; Singh, 2011 [111]</td>
<td>schizophrenia</td>
<td>TRS I/II NSS</td>
<td>non-clozapine antipsychotics</td>
<td>sarcosine</td>
<td>3</td>
<td>112</td>
<td>0.39♠</td>
<td>0.04</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Singh &amp; Singh, 2011 [111]</td>
<td>schizophrenia</td>
<td>TRS I/II NSS</td>
<td>non-clozapine antipsychotics</td>
<td>D-alanine</td>
<td>1</td>
<td>31</td>
<td>0.51♠</td>
<td>0.03</td>
<td></td>
<td></td>
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<tr>
<td>Singh &amp; Singh, 2011 [111]</td>
<td>schizophrenia</td>
<td>TRS I/II NSS</td>
<td>non-clozapine antipsychotics</td>
<td>glycine</td>
<td>5</td>
<td>219</td>
<td>0.60♠</td>
<td>0.05</td>
<td></td>
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<tr>
<td>Singh &amp; Singh, 2011 [111]</td>
<td>schizophrenia</td>
<td>TRS I/II NSS</td>
<td>non-clozapine antipsychotics</td>
<td>ampakine CX516</td>
<td>1</td>
<td>105</td>
<td>0.17♠</td>
<td>0.38</td>
<td></td>
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</tr>
<tr>
<td>Singh &amp; Singh, 2011 [111]</td>
<td>schizophrenia</td>
<td>TRS I/II NSS</td>
<td>non-clozapine antipsychotics</td>
<td>D-cycloserine</td>
<td>9</td>
<td>326</td>
<td>0.04♠</td>
<td>0.72</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Singh &amp; Singh, 2011 [111]</td>
<td>schizophrenia</td>
<td>TRS I/II NSS</td>
<td>non-clozapine antipsychotics</td>
<td>memantine</td>
<td>1</td>
<td>135</td>
<td>0.05♠</td>
<td>0.76</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Singh &amp; Singh, 2011 [111]</td>
<td>schizophrenia</td>
<td>TRS I/II NSS</td>
<td>doxapine</td>
<td>D-cycloserine ampakine CX516</td>
<td>1</td>
<td>34</td>
<td>1.83♠</td>
<td>0.00</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Singh &amp; Singh, 2011 [111]</td>
<td>schizophrenia</td>
<td>TRS I/II NSS</td>
<td>doxapine</td>
<td>D-serine ampakine CX516</td>
<td>1</td>
<td>18</td>
<td>0.47♠</td>
<td>0.36</td>
<td></td>
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<tr>
<td>Singh &amp; Singh, 2011 [111]</td>
<td>schizophrenia</td>
<td>TRS I/II NSS</td>
<td>doxapine</td>
<td>glycine</td>
<td>2</td>
<td>49</td>
<td>0.11♠</td>
<td>0.43</td>
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<tr>
<td>Singh &amp; Singh, 2011 [111]</td>
<td>schizophrenia</td>
<td>TRS I/II NSS</td>
<td>doxapine</td>
<td>sarcosine</td>
<td>1</td>
<td>20</td>
<td>0.07♠</td>
<td>0.70</td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Veerman et al. 2014c [99]</td>
<td>schizophrenia</td>
<td>TRS I/II NSS</td>
<td>doxapine</td>
<td>glycine</td>
<td>3</td>
<td>57</td>
<td>0.07♠</td>
<td>0.77</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NSS, negative symptoms of schizophrenia; SA, sensitivity analysis; I/II, no primary/secondary distinction.

♠SMD, standard mean difference; ♠Hedges’ g.
outlier studies [99].

Memantine is a voltage-dependent, non-competitive NMDA receptor antagonist that binds with a higher affinity than magnesium [97]. The neuroprotective properties of memantine are based upon this higher affinity, which blocks the effects of excessive glutamate. Thus, memantine may increase the signal-to-noise ratio, improve neurotransmission and reduce neuronal oxidative stress and degenerative changes in patients with increased levels of glutamate in the brain, such as patients with schizophrenia or Alzheimer’s disease. A medium-quality meta-analysis on the augmentation of NMDA receptor antagonists to ongoing antipsychotic treatment in schizophrenia showed superior efficacy to placebo for negative symptoms (without primary/secondary distinction) [104,105] (see Table 7B). Evidence for memantine addition to non-clozapine antipsychotics is limited to three RCTs. If the single positive study (ES=1.5, \( p<0.001 \)) by Rezaei et al. (2013) is regarded as an outlier [106], the trend towards a superior effect of memantine (20 mg) compared with placebo in non-clozapine antipsychotics disappears [97]. The combination of clozapine and memantine is assumed to cause up-regulation of the NMDA receptor, resulting in an improvement of plasticity and glutamatergic tone in the prefrontal cortex. When the signal-to-noise ratio improves, negative symptoms will be reduced. In a small 12-week proof-of-concept study in patients with predominant persistent negative symptoms an exceptionally large ES of 3.33 (\( p=0.001 \)) was found in the memantine group (N=10) compared with the placebo group (N=11) [107]. However, this beneficial effect on primary negative symptoms was confounded by a large improvement in positive symptoms (ES=1.38, \( p=0.007 \)), while the confounding factor of depressive symptoms was not assessed. A more recent crossover RCT (N=52) detected no significant difference in positive, depressive and extrapyramidal symptoms between the memantine and placebo group and showed a small significant improvement in negative symptoms compared to placebo after twelve weeks of add-on therapy with memantine (ES=0.29, \( p=0.043 \)) [108].

Amantadine is a weak, non-competitive NMDA receptor antagonist [109]. Neuroprotective properties are related to both stimulation of the release of neurotrophic factors by astrocytes and inhibition of the release of inflammatory factors by activated microglial cells. The effect of amantadine (200 mg) on negative symptoms (without primary/secondary distinction) was studied in only a single 6-week crossover study (N=23), which showed no superiority to placebo for negative symptoms (SMD=0.17, \( p=0.68 \)) [110].
Table 8. Meta-analysis and Cochrane review of double-blind, placebo-controlled randomised trials evaluating the efficacy of cholinesterase inhibitors as add-on therapy for negative symptom improvement.

<table>
<thead>
<tr>
<th>Study</th>
<th>Medication</th>
<th>Specification</th>
<th>N</th>
<th>SMD</th>
<th>MD</th>
<th>%I2</th>
<th>P</th>
<th>AMSTAR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ribeiz et al. 2010</td>
<td>donepezil</td>
<td>heterogeneous</td>
<td>8</td>
<td>0.17</td>
<td>0.17</td>
<td>0</td>
<td>0.499</td>
<td>4</td>
</tr>
<tr>
<td>Singh et al. 2012</td>
<td>rivastigmine</td>
<td>inpatients</td>
<td>2</td>
<td>31</td>
<td>1.69</td>
<td>0</td>
<td>+</td>
<td>11</td>
</tr>
</tbody>
</table>

NSS, negative symptoms of schizophrenia; SA, sensitivity analysis; I/II, no primary/secondary distinction.

*SMD, standard mean difference; ΔMD, mean difference; *, significant, but not stated specifically.
8.2 Glutamate agonists

In a medium-quality meta-analysis on the efficacy of NMDA receptor agonists as add-on therapy to antipsychotics (both non-clozapine and clozapine) in treating negative, positive and overall symptoms in TRS a small significant improvement of negative symptoms (without primary/secondary distinction) was found compared to placebo (see Table 7C) [111]. Subanalysis of specific NMDA receptor agonists showed significant beneficial effects of D-serine, N-acetylcysteine (NAC) and sarcosine in combination with non-clozapine antipsychotics. Beneficial effects on negative symptoms of D-alanine combined with non-clozapine antipsychotics and D-cycloserine combined with clozapine in single RCTs should be interpreted with caution.

In a more recent medium-quality review and meta-analysis of short-term RCTs (duration 6–14 weeks) the combination of clozapine and an NMDA receptor agonist (glycine, D-serine, D-cycloserine or sarcosine) was found not to differ from placebo treatment as regards negative symptom improvement in TRS (without discrimination between primary and secondary negative symptoms) (see Table 7C) [99].

Results of phase III trials of bitopertin, a non-competitive glycine transporter I (GlyT1) inhibitor, were disappointing, and adverse drug reactions and complex dose titration in individual patients hampered further clinical development [112]. Although the development and evaluation of new Food and Drug Administration (FDA)-approved antipsychotics, inspired by the NMDA receptor hypofunction hypothesis, have cost a tremendous amount of research funding over the past two decades, this line of research has not produced clinically relevant results.

9. Add-on therapy with cholinesterase inhibitors

Cholinesterase inhibitors (donepezil, rivastigmine or galantamine) increase the intrasynaptic concentration of acetylcholine through inhibition of the enzyme acetylcholinesterase and therefore act as indirect cholinergic agonists at muscarinic and nicotinic receptors [113]. Galantamine also acts as a PAM at the α7 nicotinic receptor, which does not cause α7 receptor desensitisation [114]. Negative symptoms may be alleviated due to direct muscarinic effects (independent of dopamine) or through a modulatory effect on the dopaminergic system [113]. The findings of a medium-quality meta-analysis and a Cochrane review on the efficacy of cholinesterase inhibitors for negative symptoms (without discrimination between primary
Table 9. Meta-analyses of double-blind, placebo-controlled, randomised trials evaluating the efficacy of anti-inflammatory drugs as add-on therapy for negative symptom improvement.

<table>
<thead>
<tr>
<th>Study</th>
<th>Inclusion criteria</th>
<th>Study population and NSS</th>
<th>Concomitant medication</th>
<th>Study medication</th>
<th>k</th>
<th>n</th>
<th>SMD</th>
<th>P</th>
<th>I² (%)</th>
<th>SA</th>
<th>Funding</th>
<th>AMSTAR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sommer et al. 2012b [119]</td>
<td>schizophrenia spectrum disorder</td>
<td>not specified I/II NSS</td>
<td>SGA</td>
<td>group acetylsalicylic acid (1000 mg) celecoxib (400 mg)</td>
<td>5</td>
<td>264</td>
<td>0.26</td>
<td>0.03</td>
<td>0</td>
<td>-</td>
<td>none</td>
<td>4</td>
</tr>
<tr>
<td>Nitta et al. 2013 [120]</td>
<td>schizophrenia spectrum disorder</td>
<td>mostly acute episode with moderate negative symptoms I/II NSS</td>
<td>SGA</td>
<td>group acetylsalicylic acid (1000 mg) celecoxib (400 mg)</td>
<td>8</td>
<td>774</td>
<td>0.026</td>
<td>0.72</td>
<td>2.4</td>
<td>+</td>
<td>independent</td>
<td>8</td>
</tr>
<tr>
<td>Zheng et al. 2017 [121]</td>
<td>schizophrenia</td>
<td>chronic with acute exacerbation FEP II NSS</td>
<td>SGA</td>
<td>celecoxib (400 mg)</td>
<td>6</td>
<td>513</td>
<td>0.12</td>
<td>0.35</td>
<td>36</td>
<td>+</td>
<td>independent</td>
<td>8</td>
</tr>
<tr>
<td>Zheng et al. 2017 [121]</td>
<td>schizophrenia</td>
<td>FEP II NSS</td>
<td>SGA</td>
<td>celecoxib (400 mg)</td>
<td>3</td>
<td>180</td>
<td>0.32</td>
<td>0.02</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oya et al. 2014 [129]</td>
<td>schizophrenia spectrum disorder</td>
<td>heterogeneous I/II NSS</td>
<td>antipsychotic minocycline (50–200 mg)</td>
<td>5</td>
<td>267</td>
<td>0.86</td>
<td>0.0002</td>
<td>66</td>
<td>+</td>
<td>none</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Oya et al. 2014 [129]</td>
<td>schizophrenia spectrum disorder</td>
<td>heterogeneous I/II NSS</td>
<td>risperidone minocycline</td>
<td>2</td>
<td>119</td>
<td>1.36</td>
<td>-0.00001</td>
<td>49</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oya et al. 2014 [129]</td>
<td>schizophrenia spectrum disorder</td>
<td>heterogeneous I/II NSS</td>
<td>other antipsychotics than risperidone minocycline</td>
<td>3</td>
<td>148</td>
<td>0.49</td>
<td>0.004</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

NSS, negative symptoms of schizophrenia; SA, sensitivity analysis; FEP, first-episode psychosis; I/II, no primary/secondary distinction; II, secondary.
and secondary negative symptoms) are disappointing (see Table 8). In the meta-analysis on the efficacy of cholinesterase inhibitors (donepezil, rivastigmine and galantamine) as adjunctive therapy in patients with schizophrenia the effect on negative symptoms with a treatment duration varying from 8 to 24 was not significant [115]. A Cochrane review analysis of two RCTs on the efficacy of donepezil and rivastigmine for negative symptoms in schizophrenia patients favoured an acetylcholinesterase inhibitor over placebo [116]. However, the quality of these studies was poor, with few participants (N=31) and a short study duration. The lack of efficacy found for pure cholinesterase inhibitors (donepezil and rivastigmine) may have been due to cigarette smoking, which causes desensitisation of α7 nicotinic receptors. A combination of an acetylcholinesterase inhibitor and a PAM of the α7 receptor would therefore deserve further study [117].

10. Add-on therapy with anti-inflammatory agents

10.1 Nonsteroidal Anti-inflammatory Drugs (NSAIDs)
The immune hypothesis predicts that anti-inflammatory agents such as acetylsalicylic acid and celecoxib may have beneficial effects on the severity of overall symptoms of schizophrenia [118]. The combination of NSAIDs and SGAs was investigated in three meta-analyses in chronic schizophrenia patients with an acute exacerbation and first-episode psychosis (FEP) patients (see Table 9) [119–121]. In an early, medium-quality meta-analysis on NSAID augmentation small but significant effect for negative symptoms (without primary/secondary distinction) was found compared to placebo [119]. Adjunctive NSAIDs seem to hold little promise, since no significant differences were found compared to placebo for improvement of secondary negative symptoms in two more recent high-quality meta-analyses concerning NSAIDs (trial duration 5–16 weeks) [120,121]. A subgroup analysis revealed selective superiority of celecoxib in first-episode schizophrenia for secondary negative symptom improvement [121]. Maybe the use of NSAIDs in schizophrenia should only be considered when inflammatory markers are identified in early stages of schizophrenia [122]. However, more research is needed.

10.2 N-acetylcysteine (NAC)
Apart from being an anti-inflammatory agent and antioxidant [123], NAC provides cysteine for glutathione synthesis and is a NMDA receptor modulator [97]. A medium-quality review
performed on NAC (no funding, AMSTAR=5) revealed that evidence for NAC in schizophrenia is limited to two RCTs [124]. Addition of NAC (2,000 mg) (N=69) to antipsychotics (45% on clozapine) in patients with chronic schizophrenia showed no significant differences compared to placebo (N=71) in the Positive and Negative Syndrome Scale (PANSS) negative subscale after eight weeks of treatment, but after 24 weeks negative symptoms were significantly improved (SMD=0.8, \( p=0.018 \)) in the adjunctive NAC group [125]. The study design failed to discriminate between primary and secondary negative symptoms. A post hoc analysis of clozapine-treated patients showed a different pattern with a small significant reduction of negative symptoms (\( d=0.30 \)) after eight weeks of NAC treatment (N=28) compared to placebo (N=27) and no significant change after 24 weeks [126].

In an 8-week RCT (N=46) on NAC (2,000 mg) in addition to risperidone NAC-treated patients with chronic schizophrenia and prominent negative symptoms showed significantly greater improvement of primary negative symptoms than the placebo-group (\( p<0.001 \)) [127]. No significant changes in confounding factors such as positive, depressive and extrapyramidal symptoms were detected during the course of this trial.

10.3 Minocycline
The antibiotic minocycline has neuroprotective properties due to a NMDA receptor antagonistic and an anti-inflammatory effect (see Table 9) [128]. A high-quality meta-analysis with a mean study duration of 25 weeks (range 8–52) found superior efficacy of adjunctive minocycline compared with placebo, especially for negative symptoms [129]. However, primary negative symptoms were not distinguished from secondary negative symptoms in the heterogeneous study population of patients in both the acute phase and residual phase of their illness. Moreover, heterogeneity across included studies was substantial. Post hoc sensitivity analysis as regards antipsychotic class revealed that larger ESs were found for minocycline as an add-on to risperidone compared to other antipsychotics.

11. Add-on therapy with antioxidants
Oxygen free radicals may contribute to the pathogenesis of negative symptoms, including increased lipid peroxidation, fatty acids and alterations in blood levels of anti-oxidant enzymes [130]. Antioxidants which neutralise these free radicals and reduce oxidative stress may therefore have potential to reduce negative symptoms.
Table 10. Meta-analyses of double-blind, placebo-controlled, randomised trials evaluating the efficacy of extract of ginkgo biloba as add-on therapy for negative symptom improvement.

<table>
<thead>
<tr>
<th>Study</th>
<th>AMSTAR</th>
<th>Funding</th>
<th>SA</th>
<th>Heterogeneity</th>
<th>p</th>
<th>n</th>
<th>SMD</th>
<th>Meta-analysis</th>
<th>Specification study</th>
<th>NSS</th>
<th>Induction criteria</th>
<th>Population</th>
</tr>
</thead>
<tbody>
<tr>
<td>Singh et al. 2010</td>
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<tr>
<td>Brondino et al. 2013</td>
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<tr>
<td>Chen et al. 2013</td>
<td></td>
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</tr>
</tbody>
</table>

- 6 double-blind RCTs and 1 single-blind RCT.
- 2 double-blind RCTs, 2 single-blind RCTs and 1 open-label study.
- NSS: negative symptoms of schizophrenia, SA: sensitivity analysis, I/II: no primary/secondary distinction, I: primary.

- 2 double-blind RCTs and 2 single-blind RCTs.
- 1 double-blind RCT and 1 single-blind RCT.

<table>
<thead>
<tr>
<th>AMSTAR</th>
<th>Funding</th>
<th>SA</th>
<th>Heterogeneity</th>
<th>p</th>
<th>n</th>
<th>SMD</th>
<th>Meta-analysis</th>
<th>Specification study</th>
<th>NSS</th>
<th>Induction criteria</th>
<th>Population</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td></td>
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<td></td>
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<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>6</td>
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<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
11.1 Ethyl eicosapentaenoic acid (E-EPA)
E-EPA increases glutathione availability, modulates the glutamate/glutamine cycle and promotes antioxidative defence mechanisms [131]. E-EPA add-on therapy (4 RCTs in the chronic phase of schizophrenia and 1 RCT in FEP patients) [132–136] and E-EPA monotherapy in FEP patients [132] did not differ from placebo as regards negative symptom improvement (without discrimination between primary and secondary negative symptoms) after 12 to 16 weeks of treatment.

11.2 Extract of ginkgo biloba
Extract of ginkgo biloba has antioxidant [137] and vasoactive properties [138]. Early research findings on ginkgo biloba concerning efficacy for negative symptoms were inconsistent [139,140] (see Table 10). A medium-quality meta-analysis of three double-blind RCTs, two single-blind RCTs and one open-label study (trial duration 8–16 weeks) found a moderate beneficial effect of extract of ginkgo biloba on negative symptoms in chronic schizophrenia and TRS [139]. A high-quality meta-analysis of three RCTs (two double-blind and one single-blind) found a non-significant improvement of negative symptoms in chronic schizophrenia after a treatment duration of 8 to 12 weeks [140]. However, a more recent large, high-quality meta-analysis of eight Chinese RCTs (duration 8–16 weeks) showed moderate superior efficacy of ginkgo biloba over placebo for primary negative symptoms of chronic schizophrenia [141]. Further study of ginkgo biloba is warranted, since this positive result may be limited to the Chinese population and confounding factors including positive, depressive and extrapyramidal symptoms were not assessed.

12. Add-on therapy with hormone treatment

12.1 Sex hormone treatment
The action of oestrogen receptors in the brain may be blunted in women and men with schizophrenia [142]. Evidence of the efficacy of sex hormone treatment for negative symptoms is limited to a medium-quality meta-analysis, a Cochrane review (see Table 11) and two single RCTs. A medium-quality meta-analysis of sex hormone treatment and oxytocin showed significant improvement in negative symptoms [143]. However, primary negative symptoms were not distinguished from secondary negative symptoms.
<table>
<thead>
<tr>
<th>Study</th>
<th>Medication</th>
<th>Specified symptom improvement</th>
<th>n</th>
<th>SMD</th>
<th>Hedges' g</th>
<th>ΔES</th>
<th>WMD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heringa et al. 2015 [143] &amp; Subanalysis</td>
<td>Oxytocin (24–80 IU)</td>
<td>mostly prominent positive symptoms</td>
<td>114</td>
<td>0.14</td>
<td>0.38</td>
<td>0.47</td>
<td>0.34</td>
</tr>
<tr>
<td>Heringa et al. 2015 [143] &amp; Subanalysis</td>
<td>Oxytocin (40–46 IU)</td>
<td>mostly prominent negative symptoms</td>
<td>6</td>
<td>0.11</td>
<td>0.39</td>
<td>0.47</td>
<td>0.34</td>
</tr>
<tr>
<td>Heringa et al. 2015 [143] &amp; Subanalysis</td>
<td>Oxytocin (60–120 mg)</td>
<td>mostly prominent negative symptoms</td>
<td>3</td>
<td>0.11</td>
<td>0.39</td>
<td>0.47</td>
<td>0.34</td>
</tr>
<tr>
<td>Heringa et al. 2015 [143] &amp; Subanalysis</td>
<td>Oxytocin (60–170 mg)</td>
<td>mostly prominent negative symptoms</td>
<td>6</td>
<td>0.11</td>
<td>0.39</td>
<td>0.47</td>
<td>0.34</td>
</tr>
</tbody>
</table>

**Notes:**
- Meta-analysis and Cochrane review of double-blind, placebo-controlled, randomized trials evaluating the efficacy of sex hormones and oxytocin as add-on therapy for negative symptoms of schizophrenia.
- NSS, negative symptoms of schizophrenia; SA, sensitivity analysis; I/II, no primary/secondary distinction; I, primary; II, secondary; SERM, selective estrogen receptor modulator; DHEA, dehydroepiandrosterone; NS, non-significant; NA, not applicable.

**Table 11: Meta-analyses and a Cochrane review of double-blind, placebo-controlled, randomized trials evaluating the efficacy of sex hormones and oxytocin as add-on therapy for negative symptoms of schizophrenia**

<table>
<thead>
<tr>
<th>Study</th>
<th>Medication</th>
<th>Inclusion criteria</th>
<th>n</th>
<th>SMD</th>
<th>Hedges' g</th>
<th>ΔES</th>
<th>WMD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heringa et al. 2015 [143] &amp; Subanalysis</td>
<td>Oxytocin (24–80 IU)</td>
<td>mostly prominent positive symptoms</td>
<td>114</td>
<td>0.14</td>
<td>0.38</td>
<td>0.47</td>
<td>0.34</td>
</tr>
<tr>
<td>Heringa et al. 2015 [143] &amp; Subanalysis</td>
<td>Oxytocin (40–46 IU)</td>
<td>mostly prominent negative symptoms</td>
<td>6</td>
<td>0.11</td>
<td>0.39</td>
<td>0.47</td>
<td>0.34</td>
</tr>
<tr>
<td>Heringa et al. 2015 [143] &amp; Subanalysis</td>
<td>Oxytocin (60–120 mg)</td>
<td>mostly prominent negative symptoms</td>
<td>3</td>
<td>0.11</td>
<td>0.39</td>
<td>0.47</td>
<td>0.34</td>
</tr>
<tr>
<td>Heringa et al. 2015 [143] &amp; Subanalysis</td>
<td>Oxytocin (60–170 mg)</td>
<td>mostly prominent negative symptoms</td>
<td>6</td>
<td>0.11</td>
<td>0.39</td>
<td>0.47</td>
<td>0.34</td>
</tr>
</tbody>
</table>

**Notes:**
- Meta-analysis and Cochrane review of double-blind, placebo-controlled, randomized trials evaluating the efficacy of sex hormones and oxytocin as add-on therapy for negative symptoms of schizophrenia.
- NSS, negative symptoms of schizophrenia; SA, sensitivity analysis; I/II, no primary/secondary distinction; I, primary; II, secondary; SERM, selective estrogen receptor modulator; DHEA, dehydroepiandrosterone; NS, non-significant; NA, not applicable.

**Table 11: Meta-analyses and a Cochrane review of double-blind, placebo-controlled, randomized trials evaluating the efficacy of sex hormones and oxytocin as add-on therapy for negative symptoms of schizophrenia**
A moderate degree of heterogeneity was found between the included studies. A small significant effect was demonstrated in both treatment with oestrogen in premenopausal women and treatment with the selective oestrogen receptor modulator (SERM) raloxifene in postmenopausal women. No significant effects were found for negative symptoms in subanalyses of pregnenolone, dehydroepiandrosterone (DHEA), testosterone or oxytocin.

A crossover RCT comparing six weeks of treatment with raloxifene (120 mg, N=40) with placebo (N=39) in premenopausal women and men suffering from chronic schizophrenia showed significant improvement of attention/processing speed and attention [144]. However, because negative symptoms (without primary/secondary distinction) were mild at baseline, there was not much room for improvement of negative symptoms in this study. In an 8-week RCT on the effect of raloxifene (120 mg) add-on therapy to risperidone in male chronic schizophrenia patients with residual negative symptoms (N=46) minimal changes in positive, depressive and extrapyramidal symptoms were detected and a large reduction of primary negative symptoms was found compared to placebo (d=1.3, p<0.001) [145].

At present, the evidence for raloxifene in both male and female schizophrenia patients is encouraging, but limited [146]. Further research is needed to examine efficacy and potential long-term side effects. Raloxifene seems a safer hormone treatment than oestrogens, which are associated with an increased risk of cardiovascular disease, thromboembolic disease, breast cancer, and endometrial cancer in long-term use [147]. A potential side effect of raloxifene is a small increase of the risk of venous thromboembolism, but there is no evidence that this SERM affects the uterus [148].

A Cochrane review revealed that evidence regarding DHEA was limited to one single study of chronic schizophrenia patients with prominent negative symptoms, which demonstrated no beneficial effect on primary negative symptoms treated with DHEA compared to placebo [149]. Although no significant difference in positive and depressive symptoms was found between DHEA and placebo groups, extrapyramidal symptoms were not assessed as a possible confounding factor.

A single placebo-controlled RCT showed moderate significant improvement in primary negative symptoms after four weeks of administration of testosterone (5 g of 1% gel) in 30 male chronic schizophrenics with residual negative symptoms (ES=0.64, p=0.001), while confounding factors including positive, depressive and extrapyramidal symptoms did not change significantly [150]. Total and free testosterone were the only serum hormone levels that significantly increased. Therefore conversion of testosterone into oestradiol [151] cannot explain this beneficial effect.
Table 12. A Cochrane review and meta-analyses of double-blind, randomised trials evaluating the efficacy of physical exercise and dance therapy for negative symptom improvement.

<table>
<thead>
<tr>
<th>Study</th>
<th>Treatment</th>
<th>Comparison</th>
<th>AMSTAR</th>
<th>Funding</th>
<th>Hegges, SD</th>
<th>Hegges, MD</th>
<th>Hegges, 9</th>
<th>Gender</th>
<th>p</th>
<th>n</th>
<th>k</th>
<th>Treatmen</th>
<th>Inclusion criteria</th>
<th>Exclusion criteria</th>
<th>Study and NSS</th>
<th>NSS population</th>
<th>Induction criteria</th>
<th>Specification</th>
<th>Study population</th>
</tr>
</thead>
</table>
12.2 Oxytocin

Oxytocin is a neurohormone which also acts as a neurotransmitter regulating social cognition, stress, learning and memory [152]. While a medium-quality and a high-quality meta-analysis found inconsistent evidence of improvement in negative symptoms (without primary/secondary distinction) with intranasal oxytocin as add-on therapy to SGAs [153,154], an updated high-quality multivariate Bayesian meta-analysis of eight RCTs in patients suffering from schizophrenia spectrum disorders, mostly with prominent negative symptoms, revealed that oxytocin was not beneficial for treating primary negative symptoms (see Table 11) [155]. While no significant difference in positive symptoms between the oxytocin and placebo groups was found, other potential sources of secondary negative symptoms (depressive and extrapyramidal symptoms) were not assessed.

13. Non-pharmacological treatments

13.1 Physical activity

While researchers have not unravelled the exact underlying mechanism of exercise, there is substantial evidence of the efficacy of physical activity for negative symptoms (see Table 12). Unfortunately, no distinction was made between primary negative symptoms and secondary negative symptoms. Early medium to high-quality meta-analyses revealed no beneficial effect on negative symptoms in inpatients or outpatients with schizophrenia spectrum disorders treated with yoga compared with usual care [156], yoga compared with exercise (two RCTs, N=102) [156] or exercise compared with usual care [157]. A substantial degree of heterogeneity was found across the included studies. In a Cochrane review on the efficacy of yoga compared to standard care a beneficial effect of yoga was based on a single small study, which included inpatients with schizophrenia [158]. However, a more recent high-quality meta-analysis on the effect of interventions involving approximately 90 minutes of moderate-to-vigorous exercise per week (range 75–120 minutes) found a small-to-moderate significant reduction of negative symptoms in patients with a schizophrenia spectrum disorder [159]. Another high-quality meta-analysis including aerobic exercise (endurance training, cardiovascular exercises, treadmill walking), anaerobic exercise (muscle strength training) and yoga found that exercise was more efficacious for negative symptoms in schizophrenia spectrum disorder than treatment as usual (TAU) [27]. Subanalyses revealed superiority of aerobic exercise and yoga above
Table 13. Meta-analyses of double-blind, randomised trials evaluating the efficacy of cognitive behavioural therapy for negative symptom improvement.

<table>
<thead>
<tr>
<th>Study</th>
<th>Inclusion criteria</th>
<th>Specification study</th>
<th>Treatment population NSS</th>
<th>Negative symptoms of schizophrenia</th>
<th>Treatment comparison</th>
<th>AMSTAR</th>
<th>Funding</th>
<th>Heterogeneity</th>
<th>Hedges’ g</th>
<th>n</th>
<th>MWD</th>
<th>Hedges’ g</th>
<th>MD</th>
<th>SMD</th>
<th>Comparison</th>
<th>Inclusion criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Velthorst et al. 2015</td>
<td>mostly chronic phase</td>
<td>NSS secondary outcome</td>
<td>acute phase inpatients</td>
<td>heterogeneous</td>
<td>I/II NSS</td>
<td>post-treatment</td>
<td>none</td>
<td>+</td>
<td>%</td>
<td>1.72%</td>
<td>16</td>
<td>0.44</td>
<td>0.02</td>
<td>0.22</td>
<td>none</td>
<td>independent</td>
</tr>
<tr>
<td>Velthorst et al. 2015</td>
<td>mostly chronic phase</td>
<td>NSS secondary outcome</td>
<td>chronic outpatients</td>
<td>heterogeneous</td>
<td>I/II NSS</td>
<td>post-treatment</td>
<td>none</td>
<td>+</td>
<td>%</td>
<td>1.29%</td>
<td>6</td>
<td>0.39</td>
<td>0.02</td>
<td>0.22</td>
<td>none</td>
<td>independent</td>
</tr>
<tr>
<td>Velthorst et al. 2015</td>
<td>mostly chronic phase</td>
<td>NSS secondary outcome</td>
<td>chronic outpatients</td>
<td>heterogeneous</td>
<td>I/II NSS</td>
<td>post-treatment</td>
<td>none</td>
<td>+</td>
<td>%</td>
<td>1.29%</td>
<td>6</td>
<td>0.39</td>
<td>0.02</td>
<td>0.22</td>
<td>none</td>
<td>independent</td>
</tr>
<tr>
<td>Velthorst et al. 2015</td>
<td>mostly chronic phase</td>
<td>NSS secondary outcome</td>
<td>chronic outpatients</td>
<td>heterogeneous</td>
<td>I/II NSS</td>
<td>post-treatment</td>
<td>none</td>
<td>+</td>
<td>%</td>
<td>1.29%</td>
<td>6</td>
<td>0.39</td>
<td>0.02</td>
<td>0.22</td>
<td>none</td>
<td>independent</td>
</tr>
<tr>
<td>Velthorst et al. 2015</td>
<td>mostly chronic phase</td>
<td>NSS secondary outcome</td>
<td>chronic outpatients</td>
<td>heterogeneous</td>
<td>I/II NSS</td>
<td>post-treatment</td>
<td>none</td>
<td>+</td>
<td>%</td>
<td>1.29%</td>
<td>6</td>
<td>0.39</td>
<td>0.02</td>
<td>0.22</td>
<td>none</td>
<td>independent</td>
</tr>
</tbody>
</table>
### Table 14. Meta-analyses of double-blind, randomised trials evaluating the efficacy of cognitive remediation and neurocognitive therapy for negative symptom improvement.

<table>
<thead>
<tr>
<th>Study</th>
<th>Inclusion criteria</th>
<th>Specification study population, NSS and analysis</th>
<th>Treatment</th>
<th>Comparison</th>
<th>k</th>
<th>n</th>
<th>Hedges’ g SMD</th>
<th>P</th>
<th>Heterogeneity</th>
<th>SA</th>
<th>Funding</th>
<th>AMSTAR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cella et al. 2016 [168]</td>
<td>schizophrenia spectrum disorder</td>
<td>heterogeneous I/II NSS post-treatment</td>
<td>cognitive remediation</td>
<td>TAU active treatment</td>
<td>42</td>
<td>2318</td>
<td>0.30 ▲</td>
<td>&lt;0.01</td>
<td>$I^2 = 0%$</td>
<td>+</td>
<td>none</td>
<td>9</td>
</tr>
<tr>
<td>Cella et al. 2016 [168]</td>
<td>schizophrenia spectrum disorder</td>
<td>heterogeneous I/II NSS follow-up</td>
<td>cognitive remediation</td>
<td>TAU active treatment</td>
<td>14</td>
<td>737</td>
<td>0.36 ▲</td>
<td>&lt;0.01</td>
<td>$I^2 = 0%$</td>
<td>+</td>
<td></td>
<td>9</td>
</tr>
<tr>
<td>Cella et al. 2016 [168] Subanalysis</td>
<td>schizophrenia spectrum disorder</td>
<td>CTAM&lt;65 CTAM≥65 I/II NSS</td>
<td>cognitive remediation</td>
<td>TAU active treatment</td>
<td>21</td>
<td>24</td>
<td>0.27 ▲</td>
<td>*</td>
<td>*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>24</td>
<td></td>
<td>0.40 ▲</td>
<td>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lutgens et al. 2017 [160]</td>
<td>schizophrenia spectrum disorder</td>
<td>heterogeneous I/II NSS post-treatment</td>
<td>neurocognitive therapy</td>
<td>TAU active treatment</td>
<td>14</td>
<td></td>
<td>0.15 ▲</td>
<td>*</td>
<td>$I^2 = 74%$</td>
<td>+</td>
<td>none</td>
<td>7</td>
</tr>
</tbody>
</table>

NSS, negative symptoms of schizophrenia; SA, sensitivity analysis; I/II, no primary/secondary distinction; TAU, treatment as usual; CTAM, Clinical Trial Assessment Measure.

▲ Hedges’ g; ▲ SMD, standard mean difference.
TAU with similar ESs for negative symptom improvement. There were insufficient studies to conduct a subanalysis of anaerobic exercise. A more recent medium-quality meta-analysis replicated a beneficial effect of yoga for negative symptoms in patients suffering from a schizophrenia spectrum disorder [160]. However, the heterogeneity between the included studies was substantial.

13.2 Cognitive Behavioural Therapy (CBT)
Negative symptoms are associated with low self-esteem and negative beliefs such as low expectations regarding pleasure and success [161]. There is no convincing evidence of the efficacy of CBT for primary negative symptoms in schizophrenia spectrum disorders (see Table 13). Research on CBT for primary negative symptoms is confounded by the failure to control for sources of secondary negative symptoms. While early research data concerning efficacy of CBT for negative symptoms were inconsistent [162–166], a recent high-quality meta-analysis of 30 RCTs (N=2,312) failed to affirm a positive effect [167]. Subanalyses revealed no significant effect of CBT interventions in 28 RCTs with negative symptoms as a secondary outcome (Hedges’ g=0.093, p=0.130), nor in two RCTs with negative symptoms as the primary outcome in patients with prominent negative symptoms. However, this subanalysis of primary negative symptoms was not controlled for positive, depressive and extrapyramidal symptoms. In a more recent, but smaller and medium-quality meta-analysis a small beneficial effect of CBT on negative symptoms (without primary/secondary distinction) was found, but there was substantial heterogeneity between the included studies [160].

13.3 Cognitive remediation (CR)
CR may affect negative symptoms by influencing working memory, reward sensitivity and executive functions [11]. Moreover, CR may result in a self-esteem boost by challenging defeatist beliefs, avoidance behaviour and poor motivation. A high-quality meta-analysis on the efficacy of CR in patients with schizophrenia spectrum disorders revealed a small reduction of negative symptoms (without primary/secondary distinction) at post-therapy compared to TAU and active treatment [168] (see Table 14). This beneficial effect of CR was maintained at follow-up. Moreover, studies with more robust methodology (Clinical Trial Assessment Measure [CTAM]≥65) showed a larger negative symptom reduction com-
Table 15. Meta-analyses of double-blind, randomised trials evaluating the efficacy of other psychological and psychosocial interventions for negative symptom improvement.

<table>
<thead>
<tr>
<th>Study</th>
<th>Inclusion criteria</th>
<th>Specification study population, NSS and analysis</th>
<th>Treatment</th>
<th>Comparison</th>
<th>k</th>
<th>ESₙₐ Hedges' g</th>
<th>P</th>
<th>Heterogeneity</th>
<th>SA</th>
<th>Funding</th>
<th>AMSTAR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fusar-Poli et al. 2015 [4]</td>
<td>schizophrenia spectrum disorder</td>
<td>aged ≥18 heterogeneous I/II NSS post-treatment</td>
<td>psychological interventions&lt;sup&gt;+&lt;/sup&gt;</td>
<td>TAU</td>
<td>27</td>
<td>0.40</td>
<td>&lt;0.001</td>
<td>I&lt;sup&gt;2&lt;/sup&gt; =58%</td>
<td>+</td>
<td>independent</td>
<td>10</td>
</tr>
<tr>
<td>Roder et al. 2011 [169]</td>
<td>schizophrenia</td>
<td>heterogeneous I/II NSS post-treatment</td>
<td>integrated psychological therapy</td>
<td>TAU placebo-attention</td>
<td>11</td>
<td>0.42&lt;sup&gt;△&lt;/sup&gt;</td>
<td>&lt;0.01</td>
<td>Q=12</td>
<td>+</td>
<td>none</td>
<td>4</td>
</tr>
<tr>
<td>Khoury et al. 2013 [170]</td>
<td>schizophrenia spectrum disorder</td>
<td>heterogeneous I/II NSS post-treatment</td>
<td>mindfulness</td>
<td>TAU active treatment wait list</td>
<td>7</td>
<td>0.41&lt;sup&gt;▲&lt;/sup&gt;</td>
<td>&lt;0.0001</td>
<td>moderate to high</td>
<td>+</td>
<td>none</td>
<td>4</td>
</tr>
<tr>
<td>Khoury et al. 2013 [170]</td>
<td>schizophrenia spectrum disorder</td>
<td>heterogeneous I/II NSS follow-up</td>
<td>mindfulness</td>
<td>TAU active treatment wait list</td>
<td>3</td>
<td>0.55&lt;sup&gt;▲&lt;/sup&gt;</td>
<td>0.08</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lugtens et al. 2017 [160]</td>
<td>schizophrenia spectrum disorder</td>
<td>heterogeneous I/II NSS post-treatment</td>
<td>skills-based training occupational therapy</td>
<td>TAU active treatment</td>
<td>13</td>
<td>0.44&lt;sup&gt;▲&lt;/sup&gt;</td>
<td>*</td>
<td>I&lt;sup&gt;2&lt;/sup&gt; =66%</td>
<td>+</td>
<td>none</td>
<td>7</td>
</tr>
<tr>
<td>Lugtens et al. 2017 [170]</td>
<td>schizophrenia spectrum disorder</td>
<td>heterogeneous I/II NSS post-treatment</td>
<td>family-based intervention</td>
<td>TAU active treatment</td>
<td>3</td>
<td>0.19&lt;sup&gt;▲&lt;/sup&gt;</td>
<td>NS</td>
<td>I&lt;sup&gt;2&lt;/sup&gt; =65%</td>
<td>+</td>
<td>none</td>
<td>7</td>
</tr>
</tbody>
</table>

NSS, negative symptoms of schizophrenia; SA, sensitivity analysis; I/II, no primary/secondary distinction; TAU, treatment as usual; CTAM, Clinical Trial Assessment Measure; NS, non-significant.
<sup>+</sup>cognitive behavioural therapy, cognitive rehabilitation and music therapy.
<sup>△ESₙₐ</sup>, weighted effect size; <sup>▲Hedges'g</sup>; <sup>▲SMD</sup>, standard mean difference.
* significant effect, but not stated specifically.
Table 16. Cochrane review and meta-analyses of double-blind, randomized trials evaluating the efficacy of art and music therapy for negative symptom improvement.

| Study | Inclusion criteria | Treatment | Specification | Finding | Funding | AMSTAR
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mössler et al. 2011 [171]</td>
<td>Schizophrenia spectrum disorder</td>
<td>TAU</td>
<td>Low-dose music therapy</td>
<td>2</td>
<td>0.69</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Schizophrenia spectrum disorder</td>
<td>TAU</td>
<td>High-dose music therapy</td>
<td>2</td>
<td>0.97</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>Schizophrenia spectrum disorder</td>
<td>TAU</td>
<td>No treatment</td>
<td>2</td>
<td>0.96</td>
<td>+</td>
</tr>
<tr>
<td>Tseng et al. 2016 [172]</td>
<td>Schizophrenia spectrum disorder</td>
<td>TAU</td>
<td>No treatment</td>
<td>4</td>
<td>0.74</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>Schizophrenia spectrum disorder</td>
<td>TAU</td>
<td>Music therapy</td>
<td>4</td>
<td>0.40</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Schizophrenia spectrum disorder</td>
<td>TAU</td>
<td>Music therapy</td>
<td>4</td>
<td>0.74</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>Schizophrenia spectrum disorder</td>
<td>TAU</td>
<td>Music therapy</td>
<td>4</td>
<td>0.40</td>
<td>+</td>
</tr>
<tr>
<td>Lutgens et al. 2017 [160]</td>
<td>Schizophrenia spectrum disorder</td>
<td>TAU</td>
<td>Music therapy</td>
<td>4</td>
<td>0.74</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>Schizophrenia spectrum disorder</td>
<td>TAU</td>
<td>Music therapy</td>
<td>4</td>
<td>0.40</td>
<td>+</td>
</tr>
</tbody>
</table>

NSS: Negative symptoms of schizophrenia; SA: Sensitivity analysis; I/II: No primary/secondary distinction; TAU: Treatment as usual.
pared to studies with a CTAM score below 65. A medium-quality meta-analysis also found a small beneficial effect of neurocognitive therapy compared to standard care and active treatment in patients with a schizophrenia spectrum disorder, but primary negative symptoms were not distinguished from secondary negative symptoms and a substantial level of heterogeneity across studies was an important limitation [160]. In conclusion, more research is needed to affirm a beneficial effect of CR in patients suffering from prominent negative symptoms.

13.4 Other forms of psychotherapy

For psychological interventions other than cognitive remediation the mode of action is unclear and the evidence is based on single meta-analyses with high levels of heterogeneity across the primary studies, which failed to discriminate between primary and secondary negative symptoms and included patients with a schizophrenia spectrum disorder (see Table 15). Based on a high-quality meta-analysis [4], compared with standard care psychological treatments (cognitive behavioural therapy, cognitive rehabilitation and music therapy) provide significant benefit for negative symptoms in adults suffering from a schizophrenia spectrum disorder.

Integrated psychological therapy (IPT) combines neurocognitive and social cognitive interventions with social skills and problem-solving approaches, which was found to be superior over placebo-attention conditions and standard care in a medium-quality meta-analysis [169].

For post-treatment mindfulness interventions a moderately beneficial effect for negative symptoms was found compared to standard care, active treatment or no treatment in a medium-quality meta-analysis with moderate to high heterogeneity between primary studies [170]. At follow-up the effect was no longer superior.

A medium-quality meta-analysis comparing skills-based training, occupational therapy and cognitive adaptation therapy with standard care and active treatment revealed a beneficial effect for negative symptoms of these interventions, which was largely driven by studies using TAU as a control [160].

A medium-quality meta-analysis of three studies with a marginally significant heterogeneity did not favour family-based interventions over standard care or active treatment [160].

Based on the findings of a Cochrane review and two medium and high-quality meta-
Table 17. Meta-analyses of double-blind, randomised trials evaluating the efficacy of repetitive transcranial magnetic stimulation compared to sham stimulation for negative symptom improvement.

<table>
<thead>
<tr>
<th>Study</th>
<th>Specification of schizophrenia/neurodevelopmental disorder</th>
<th>Treatment</th>
<th>n</th>
<th>SMD</th>
<th>Heterogeneity</th>
<th>p</th>
<th>k</th>
<th>Population and NSS</th>
<th>Inclusion criteria</th>
<th>Treatment and NSS</th>
<th>Population and NSS</th>
<th>Inclusion criteria</th>
<th>Treatment and NSS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Freitas et al. 2009</td>
<td>schizophrenia/Neurodevelopmental disorder</td>
<td>rTMS</td>
<td>7</td>
<td>0.41</td>
<td>ns</td>
<td>7</td>
<td>0.41</td>
<td>NSS</td>
<td>7</td>
<td>He et al. 2017 (178)</td>
<td>rTMS</td>
<td>schizophrenia</td>
<td></td>
</tr>
<tr>
<td>Dlabac-de Lange et al. 2010</td>
<td>schizophrenia/Neurodevelopmental disorder</td>
<td>rTMS</td>
<td>10</td>
<td>0.23</td>
<td>0.413</td>
<td>9</td>
<td>0.23</td>
<td>NSS</td>
<td>9</td>
<td>Shi et al. 2010 (177)</td>
<td>NSS</td>
<td>schizophrenia</td>
<td></td>
</tr>
<tr>
<td>Shi et al. 2013 (179)</td>
<td>schizophrenia/Neurodevelopmental disorder</td>
<td>rTMS</td>
<td>5</td>
<td>0.03</td>
<td>0.005</td>
<td>13</td>
<td>0.03</td>
<td>NSS</td>
<td>13</td>
<td>Fusar-Poli et al. 2015 (4)</td>
<td>NSS</td>
<td>schizophrenia</td>
<td></td>
</tr>
<tr>
<td>Fusar-Poli et al. 2015 (4)</td>
<td>schizophrenia/Neurodevelopmental disorder</td>
<td>rTMS</td>
<td>5</td>
<td>0.43</td>
<td>0.03</td>
<td>9</td>
<td>0.43</td>
<td>NSS</td>
<td>9</td>
<td>He et al. 2017 (178)</td>
<td>rTMS</td>
<td>schizophrenia</td>
<td></td>
</tr>
</tbody>
</table>

NSS, negative symptoms of schizophrenia; SA, sensitivity analysis; I/II, no primary/secondary distinction; I, primary; rTMS, repetitive transcranial magnetic stimulation; NS, non-significant.
analyses of studies in patients with a schizophrenia spectrum disorder, with substantial heterogeneity between the included studies which did not discriminate between primary and secondary negative symptoms, music therapy is a promising intervention (see Table 16). Compared with standard care, placebo treatment or no treatment, music therapy proved to be efficacious for negative symptoms in a Cochrane review of four RCTs which also showed a larger reduction in high-dose music therapy compared to low-dose music therapy [171]. A high-quality meta-analysis (nine RCTs and one non-RCT) on adjunct music therapy to standard treatment revealed a significant and large beneficial effect for negative symptom improvement compared with standard treatment alone [172]. In another smaller, medium-quality meta-analysis music therapy compared to standard care was found to moderately improve negative symptoms [160].

14. Repetitive transcranial magnetic stimulation (rTMS)

High-frequency bilateral rTMS uses strong magnetic pulses and increases brain activity in the dorsolateral prefrontal cortex and the medial frontal gyrus [173]. For rTMS evidence for negative symptom improvement is not convincing, based on the inconsistent findings of five meta-analyses comparing rTMS with sham stimulation (see Table 17). In an early, low-quality meta-analysis rTMS did not differ from sham stimulation as regards negative symptom improvement in chronic and stable schizophrenia patients [174]. A more recent, medium-quality meta-analysis found low-to-moderate efficacy of rTMS for negative symptoms in a heterogeneous study population of patients with a schizophrenia spectrum disorder [175], which prompted additional research [176]. While these two meta-analyses failed to distinguish primary from secondary negative symptoms [174,175], confounding factors were not assessed in another medium-quality, larger meta-analysis with moderate heterogeneity across studies [177]. A moderate improvement of primary negative symptoms was found in schizophrenia patients with prominent negative symptoms [177]. The optimal treatment duration was at least 3 consecutive weeks. However, two more recent high and medium-quality meta-analyses with significant heterogeneity between primary studies and no discrimination between primary and secondary negative symptoms did not affirm the potential of rTMS for the treatment of negative symptoms [4,178].
15. Discussion

At present there is little robust and consistent evidence to support interventions for primary negative symptoms [179–185]. Moreover, no known treatment ameliorates both primary and secondary negative symptoms to such an extent that clinically significant improvement is achieved as measured on the Clinical Global Impression Scale (CGI-S) [4]. However, the evidence for several interventions found in studies that did not focus on primary negative symptoms is more favourable.

We recommend that for patients with moderate to severe primary negative symptoms psychiatrists should consider several possible interventions that are supported by some evidence from meta-analytic research and are relevant to clinical practice. These potential treatment options are discussed below. However, these recommendations should be treated with caution and both the possible benefits and the risks – such as potential adverse effects – should be discussed in a shared decision-making process with the patient.

The first step towards preventing or improving primary negative symptoms is early identification and treatment of psychosis [47–51]. Since antipsychotic medication in higher doses may contribute to secondary negative symptoms, an important recommendation is to use the lowest possible dosage of antipsychotic medication in order to improve subjective experiences and prevent neuroleptic-induced parkinsonism [28–33]. Furthermore, for every patient without sufficient physical activity, frequent exercise with aerobic training or yoga is recommended [27].

Secondary negative symptoms should be prevented or treated adequately. Interventions aimed at diminishing the causal conditions of secondary negative symptoms are thought to be helpful and clinically relevant in reducing these symptoms. If positive symptoms persist in the absence of dose-related side effects, the dosage of antipsychotic should be increased or a switch to a different antipsychotic is recommended. If depression is present, psychotherapy and/or antidepressants are appropriate interventions [17]. If chronic abuse of illicit drugs and alcohol is problematic, we suggest motivational interviewing to reduce substance abuse, although at present evidence of the efficacy of this intervention is scarce [19–24]. Family interventions can be especially helpful if desolation and demoralisation are prominent [26]. Rehabilitation reduces fear and anxiety, and improves self-image, self-esteem, the ability to cope with stress and willingness to establish interpersonal relationships [25]. Since prominent negative symptoms have a large impact on the social and professional life of schizophrenia patients, causing social withdrawal, loss
of autonomy, less employment or even long-term hospitalisation, psychiatric rehabilitation and a hope-oriented approach may contribute in an important way to the treatment of secondary negative symptoms.

Although SGAs were considered to have more potential for treating both primary and secondary negative symptoms, evidence of clear benefits of SGAs versus FGAs remains inconclusive for unspecified negative symptoms [3,4,58]. There is also no solid evidence for the superiority of any single antipsychotic for negative symptom improvement (without primary/secondary distinction) [59,60,56]. No consistently greater benefits of clozapine versus other antipsychotic treatment for unspecified negative symptoms have been demonstrated either [64–68,60].

Aripiprazole augmentation seems beneficial for negative symptoms (without discrimination between primary and secondary negative symptoms) and is well tolerated in both patients receiving non-clozapine antipsychotics and in clozapine-treated patients [73,76]. Most studies on antidepressants failed to specify primary negative symptoms by eliminating confounding factors. Although some SSRIs have been found to have beneficial effects, in particular fluvoxamine and citalopram [84,85,91] and the alpha-2 receptor antagonists mirtazapine and mianserin [89–91], recent clinical guidelines for persistent negative symptoms state that SSRI or serotonin-norepinephrine reuptake inhibitor (SNRI) add-on treatment should be used with caution, since the reporting of adverse events is poor and inconsistent across all studies on the efficacy of add-on treatment with these antidepressants for persistent negative symptoms. There was no difference between the groups as regards neurological adverse events, and psychotic symptoms neither worsened nor improved with SSRI therapy. Obvious adverse reactions such as serotonergic and sexual side effects and suicidal ideation were mentioned a few of the studies, but poorly reported. No mention was made in any of the studies of suicide or suicide attempts [186]. Despite this, we believe that adjunctive antidepressants may be considered, since no study has found positive symptoms worsening as a result of antidepressants. Among glutamate antagonists as add-on therapy to both non-clozapine and clozapine antipsychotics, the most robust evidence favours topiramate for negative symptom improvement (without primary/secondary distinction), good tolerability and the additional advantage of weight maintenance [100–102].

Music therapy seems a viable treatment option for unspecified negative symptoms, without any adverse effects. It requires further research in patients with prominent primary negative symptoms.

Our review of evidence gathered over the past decade reveals that most RCTs were
short-term, conducted in chronically ill patients or patients in both the acute phase and residual phase of their illness. Unfortunately, the data required to distinguish primary from secondary negative symptoms, such as information demonstrating a stable disease course prior to the start of the study and levels of positive, depressive and extrapyramidal symptoms at baseline and endpoint, are not available. Fewer studies included patients at the time of an acute psychotic episode and therefore more focused on secondary negative symptoms. Persistent primary negative symptoms were often not the primary outcome variable. Recent meta-analyses and subanalyses of RCTs in patients with predominant persistent negative symptoms did not adequately control for confounding factors and have only been conducted for amisulpride [69], methylphenidate [83], antidepressants [85], extract of ginkgo biloba [141], oxytocin [155], CGT [167] and rTMS [177]. The finding of a slight advantage of amisulpride over other SGAs or placebo should therefore be interpreted with caution [69]. Single studies on the efficacy of memantine [108], NAC [127], raloxifene [145], DHEA [149] and testosterone [150] in chronic schizophrenia patients with residual negative symptoms controlled for confounding factors and with the exception of DHEA found a beneficial effect on primary negative symptoms.

The methodological quality of the included reviews and meta-analyses is generally medium to high. There were independent sources of funding or no financial support in the included meta-analyses and reviews, except for one low-quality review on psychostimulants [83]. Comparative research of different pharmacological treatments is hampered by small sample sizes, non-equivalent dosages, a range of concomitant antipsychotic regimens, variable duration of treatment, the nature of the inclusion criteria and outcome measures used, and funding bias. There are few comparisons of augmentation strategies for clozapine and non-clozapine antipsychotics.

In future research the scientific quality of primary studies included in reviews and meta-analyses should be documented more extensively and considerations of scientific quality should be made explicit when formulating conclusions. Future reviews and meta-analyses should also improve as regards reporting excluded studies and conflict of interest for each of the included studies. A study duration of at least six months is recommended to reasonably anticipate a clinically relevant improvement in primary negative symptoms [183]. Long-term studies that focus on prominent primary negative symptoms in the non-acute phase and control for confounders such as positive symptoms, depressive symptoms, and extrapyramidal side effects should be conducted in order to increase evidence for interventions for primary negative symptoms of schizophrenia. The inclusion
of patients suffering from schizophrenia and especially TRS in treatment studies may be hampered not only by negative symptoms, but also by paranoia, cognitive impairments, impaired decisional capacity [187], compromised ability to appreciate risk information [188] and fear of experiencing adverse events [189]. In the future, long-term, prospective naturalistic studies on the efficacy of interventions in everyday practice may have more success in including such patients and may therefore yield clinically relevant evidence that is more generalisable than that gained in double-blind RCTs [190]. Moreover, a better understanding of the pathogenesis of negative symptoms may help to identify potential new treatment targets in order to develop more specific interventions for primary negative symptoms. Perhaps in the future we will be able to determine the specific pathogenesis of primary negative symptoms in individual patients using hormone levels, neuroimaging of dopamine synthesis, frontal glutamate levels and activation of microglial cells [191,192]. Personalised medicine may be possible in which treatment targets the specific underlying mechanism in individual patients. Moreover, it is conceivable that certain interventions are efficacious in a particular phase of schizophrenia. Currently we lack evidence for such individualised and phase-specific interventions.

16. Conclusion

Negative symptoms remain an important treatment challenge. It is clear that we currently lack convincing evidence that patients with primary negative symptoms can be effectively treated with targeted interventions. There is also no consistent evidence indicating a preferential intervention to treat primary negative symptoms. However, there is evidence for modest short-term efficacy of several interventions in patients with unspecified negative symptoms. Potential side effects should be considered carefully in the shared decision-making process regarding medication. We urgently need more robust evidence. This review highlights the need for large, well-designed long-term studies.
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The glutamate hypothesis: a pathogenic pathway from which pharmacological interventions have emerged

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How does the glutamate hypothesis identify potential new treatment targets for schizophrenia?

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Abstract

We discuss the relevance of the glutamate hypothesis in explaining cognitive disturbances and negative symptoms in schizophrenia. 4 lines of evidence support the hypothesis that glutamate deregulation, mainly through dysfunction of the N-methyl-D-aspartate (NMDA) receptor is an important underlying mechanism of schizophrenia. Glutamate pathways are promising sites for intervention. Glutamate agonists combined with non-clozapine antipsychotics and glutamate antagonists augmented to clozapine show interesting clinical benefits in refractory schizophrenia. We illustrate how unique properties of the NMDA receptor antagonist memantine in addition to clozapine, may cause improvement of positive, negative and cognitive symptoms of schizophrenia.
Introduction

The natural course of schizophrenia is heterogeneous. Negative symptoms contribute to functional impairment, reduced quality of life and predict a worse social outcome [1]. Social and cognitive impairments usually develop many years before the onset of positive symptoms [2]. Various pathophysiological hypotheses have been put forward to explain the development of schizophrenia. Altered receptor functions of several neurotransmitters could be either the primary deficit or secondary to other neuropathology. Dysregulation of the glutamatergic system through hypofunction of the N-methyl-D-aspartate (NMDA) receptor is thought to contribute to the development of schizophrenia [3,4]. The glutamate hypothesis describes the pathophysiology of cognitive disturbances in schizophrenia and elaborates the dopamine hypothesis, which attributes dysregulation of dopaminergic function as a possible cause of negative and positive symptoms in schizophrenia. We explain how both hypotheses are closely entwined through reciprocal relationships between glutamatergic pathways and mesolimbic dopaminergic projections.

If we could fully comprehend the underlying mechanism of schizophrenia, one day we might be able to intervene in the critical or possibly in the prodromal period with neuroprotective medication in order to limit cognitive decline or even prevent the development of negative and positive symptoms. In case of therapy-resistant schizophrenia, when usual dopamine blockers do not alleviate schizophrenic symptoms sufficiently, clozapine is indicated. Unfortunately 40–70% of patients fail to benefit from clozapine monotherapy or only partially respond [5]. Even after controlling for clozapine plasma level 30% of patients still do not respond [6]. For this subgroup of clozapine-refractory patients the development of innovative pharmacological strategies is imperative. Novel avenues of research are needed for improved drug treatment of schizophrenia. Following functional psychopharmacological principles, abovementioned knowledge concerning glutamatergic neurotransmission in schizophrenia results in a novel strategy: modulation of the glutamatergic system [7]. Glutamatergic medication may prevent neurodegenerative changes in early psychosis and subsequently disease progression. Moreover, it is conceivable that glutamatergic modulators are useful in treatment of patients with refractory schizophrenia, especially with prevailing negative symptoms and invalidating severe cognitive symptoms.

To understand the link between the occupancy of various receptors by glutamate agonists or antagonists and the resulting therapeutic effect of these glutamate modulators, we first describe the glutamate system and relationships between different glutamatergic
receptors. We summarize evidence for altered glutamatergic neurotransmission in schizophrenia. We clarify the mechanism and function of different glutamate modulators. We review recent clinical findings concerning glutamatergic agents in combination with antipsychotic medication, limited to randomized double-blind placebo-controlled trials. We explain the potency of clozapine, which has glutamate agonistic activity in several ways. We illustrate how the combination of clozapine and memantine, a voltage dependent glutamate antagonist restores imbalanced glutamatergic homeostasis in schizophrenia. Finally we describe a protocol of a proof-of-concept study on memantine augmentation to clozapine in refractory schizophrenia.

Methods

We review the literature on the pathophysiological hypothesis of deregulation of the glutamatergic system in schizophrenia to give a possible explanation of the beneficial effect of glutamatergic modulators. We review randomized double-blind studies on glutamatergic augmentation strategies in patients with clozapine-resistant schizophrenia, which were found after a search of the electronic databases PsycINFO, EMBASE, EBM reviews-Cochrane Database of Systematic Reviews, EBM reviews-Cochrane Central Register of Controlled Trials with the keywords “schizophrenia”, “clozapine”, “augmentation/combination”, “treatment resistant/refractory”, “randomized”, and “glutamate”. Titles, abstracts and related articles were examined. There were no language or year of publication restrictions.

Results

Glutamate System

Glutamate is the primary excitatory neurotransmitter in the brain [8]. Glutamatergic receptors play a pivotal role in regulating neuronal migration, neural growth, synaptogenesis and the pruning of neurons by apoptosis. 2 different types of glutamate receptors can be distinguished. (i) Ionotropic receptors mediate fast excitatory postsynaptic potentials throughout the brain. The kainate receptor and the amino-3-hydroxy-5-methyl-isoxazoloe-4-propionic acid (AMPA) receptor play an important role in excitatory neurotransmission by mediating fast postsynaptic potentials. The N-methyl-D-aspartate (NMDA) receptor is a third ionotropic
receptor playing a primary role in long-term potentiation (LTP), which is a major form of use-dependent synaptic plasticity. Each time the NMDA receptor is activated, stimulation of pyramidal neurons becomes easier. Therefore, the synaptic efficacy increases persistently, resulting in LTP. In LTP preferential routes for impulses develop in the brain. This is the physiological foundation of conditioned reaction and thus learning. In the hippocampus these neuroplastic effects play a key role in formation of long-term memory. The NMDA receptor is activated by 2 distinct mechanisms. For the first mechanism several steps are necessary to ensure activation of the NMDA receptor (Fig. 1). At resting membrane potential Mg\(^{2+}\) blocks the NMDA channel, prohibiting Ca\(^{2+}\) influx. This second messenger causes an increased sensitivity of the synapse, resulting in LTP. A brief period of high-intensity excitatory synaptic activity results in a fall of membrane potential, which removes the Mg\(^{2+}\) block of the NDMA receptor. A second mechanism of activation of the NMDA receptor is the occupation of glutamate at 2 binding sites, which occurs in case of excessive glutamate release [9,10]. After sufficient depolarization of the postsynaptic membrane Mg\(^{2+}\) no longer blocks the channel, causing Ca\(^{2+}\) influx. However, excessive glutamate in the synapse leads to overstimulation of NMDA receptors. Ca\(^{2+}\)-influx increases, resulting in elevation of intracellular Ca\(^{2+}\) levels. Thus toxic metabolic processes are triggered that may lead to

![Activation of NMDA receptors](image)

**Fig. 1** Activation of NMDA receptors. Inotropic receptors gate cation channels, which are permeable to Na\(^{+}\), K\(^{+}\) and Ca\(^{2+}\). Glutamate in the synaptic cleft activates the kainate and/or AMPA receptor on the postsynaptic membrane, resulting in substantial neuronal depolarization. After partial depolarization of the postsynaptic membrane both glutamate and glycine or D-serine, which are endogenous co-agonists need to bind simultaneously to the tetrameric structure of the NMDA receptor. The voltage-dependent Mg\(^{2+}\) block releases, the NDMA channel opens and Ca\(^{2+}\) flows into the postsynaptic neuronal cell.
neuronal cell death.

Glutamate metabotropic receptors (mGluRs) affect intracellular metabolic processes [11]. mGluRs are G-protein-coupled receptors with a relatively slow modulating mechanism. After activation of mGluRs, the G-protein is activated, followed by the conversion of ATP to cyclic AMP by adenylyl cyclase. MGlur5 receptors modulate the NMDA receptor, potentiating NMDA receptor function in forebrain regions [12]. MGlur2 and MGlur3 receptors belong to the subgroup II family of mGluRs. High levels of MGlur2 receptors are found in almost all regions of the limbic system, including the prefrontal cortex, thalamus and amygdala [13]. Expression of MGlur3 receptors is also seen in other limbic regions, including the hippocampus. Distribution of MGlur3 receptors is more diffuse than distribution of MGlur2 receptors. Activation of MGlur2 and MGlur3 receptors on presynaptic nerve terminals inhibits presynaptic glutamate release, modulating synaptic plasticity and LTP.

Altered Glutamate System in Schizophrenia

The hypothesis that an imbalanced dopaminergic neurotransmission is a fundamental underlying mechanism of schizophrenia is supported by the fact that people at high risk of psychosis who subsequently develop psychosis show elevated dopaminergic function in the brainstem region using positron emission tomography [14]. Furthermore, dopamine agonists such as levodopa induce psychosis and all known antipsychotic drugs block dopamine receptors. However, the dopamine hypothesis merely explains the development of positive symptoms and negative symptoms. The glutamate hypothesis elaborates the dopamine hypothesis, describing synaptic relationships between glutamatergic systems and dopaminergic projections. More importantly, the glutamate hypothesis offers an explanation for the pathophysiology of cognitive disturbances in schizophrenia.

There are several lines of evidence which link glutamate and specifically NMDA receptor hypofunction to the pathogenesis of schizophrenia. Firstly, hypofunctional NMDA receptors caused by chronic administration of phenylcyclohexylpiperidine (PCP) and to a lesser extent ketamine lead to transient schizophrenia-like psychosis (including positive, negative and cognitive symptoms) in healthy subjects [3,15,16]. Contrary to dopamine agonists like amphetamines and levodopa, PCP and ketamine do not only cause positive symptoms, but also induce prominent emotional blunting, anhedonia and social withdrawal, similar to negative symptoms of schizophrenia. Cognitive disruptions associated with
the prefrontal cortex, such as working memory impairments, which are characteristic for schizophrenia, are caused as well by these non-competitive NMDA receptor antagonists. The primary site of action of PCP and ketamine is proposed to be the NMDA receptors on GABA-ergic (gamma-aminobutyric acid) interneurons in the thalamus, the basal forebrain and the hippocampus. Because these drugs block the NMDA channel completely and induce symptoms such as psychosis, social withdrawal and executive function deficits, it is proposed that NMDA receptor agonists could reduce the symptoms of schizophrenia.

The second line of evidence is provided by genetic linkage studies and confirmed by animal models and studies of post-mortem brain tissue of patients with schizophrenia [3]. Highly replicated findings concern several different genes, associated with schizophrenia and linked to hypoactivity of glutamatergic and particularly NMDA-receptor-mediated activity. Examples of candidate genes are G72 and deaminooxidase (DAAO), associated with metabolism of D-serine, which is an agonist of the NMDA receptor. Other candidate genes are involved in glutamate cysteine ligase, which is involved in glutamate metabolism and the synthesis of glutathione, an important anti-oxidant. Recently Japanese researchers examined genetic data from several Asian populations and identified a rare variant in GRIN3A, associated with schizophrenia. GRIN3A is a gene that codes for the GluN3A subunit of NMDA receptor [17].

The third line of evidence that NMDA hypofunction is implicated in the pathogenesis of schizophrenia is the clinical presentation of anti-NMDA-receptor encephalitis, first described in 2007 in women with ovarian teratoma and autoantibodies targeting specifically the NMDA receptor [18]. Generally the clinical course of this severe neuropsychiatric syndrome is a non-specific flu-like prodrome, followed by a psychotic stage with bizarre behaviour, disorientation, confusion, paranoid thought, visual or auditory hallucinations and memory deficits. The following phase is characterized by decreased consciousness, hypoventilation, lethargy, seizures, autonomous instability and the development of dyskinesia. A recent discovery is the presence of increased antibodies against the NMDA receptor in approximately 10% of patients with schizophrenia, which sustains the hypothesis that schizophrenia may be associated with dysfunctional reaction to infectious illness with a genetic predisposition in which the NMDA receptor is a target for the immune system [19].

The fourth line of evidence has been presented recently in a meta-analysis of 24 ¹H-MRS (magnetic resonance spectroscopy) studies demonstrating altered frontal glutamate concentration in schizophrenia patients [20]. Glutamatergic metabolites seem to increase between the age of 20 and 30 years in healthy control subjects, followed by a
gradual decline. These findings suggest age-related alterations in neuronal and particularly in glutamatergic metabolism in the normal human brain. In early schizophrenia and even in people with prodromal symptoms of psychosis glutamatergic metabolites in frontal brain areas show a higher peak increase than in healthy controls, suggesting an excitotoxic process and neuronal cell death. As described above excessive glutamate levels in the synaptic cleft lead to overstimulation of the NMDA receptors, followed by increased Ca\(^{2+}\) influx and subsequently neuronal cell death. This could explain why negative symptoms and cognitive deficits are the early signs of schizophrenia and cognitive decline is most prominent in this critical period. Thus glutamatergic metabolites seem to peak during the early course of schizophrenia, but glutamate and glutamine levels decrease more progressively thereafter than in healthy control subjects. This decrease of frontal region glutamate may be a reflection of progressive loss of synaptic activity and brain volume reductions or the effect of antipsychotic medication. Besides changes in membrane metabolism, altered expression of intracellular and extracellular glutamate transporters as well as dysfunction of glutamate transport in the synaptic cleft or inside presynaptic neurons can all be involved in altered glutamatergic levels.

Diminished activation of the NMDA receptor is believed to be an important underlying mechanism of schizophrenia. While the basal activity of pyramidal neurons is not directly regulated by NMDA receptors, the activity of cortical GABA-ergic interneurons is highly sensitive to tonic regulation by NMDA receptors [21] (Fig. 2). Reciprocal synaptic relationships between glutamatergic systems and mesolimbic dopaminergic projections explain how NMDA hypofunction results in dopaminergic hyperfunction in the amygdala, causing positive symptoms [22] (Fig. 2). Studies in rodents have shown that NMDA hypofunction has a disinhibitory effect on glutamatergic transmission in the prefrontal cortex [23]. A sustained firing rate potentiation of prefrontal cortex (PFC) neurons is proposed to lead to an increased number of irregularly discharged single spikes. This increase in spike activity results in cortical noise and transmission of disinformation. Increase in disorganized spike activity also causes significant reduction in organized bursting activity, which reduces transmission efficacy of cortical neurons. Abnormal cortical signal-to-noise patterns cause negative symptoms and impairment of frontal-lobe related cognitive functions. In the beginning NMDA hypofunction results in too little excitation [9,15]. However, the glutamate concentration in the synaptic cleft increases, resulting in overstimulation of the NDMA receptor. Neurotoxic Ca\(^{2+}\) influx leads to neuronal cell death and subsequently neurodegenerative changes as seen in schizophrenia.
Fig. 2  Reciprocal relationship between glutamatergic synapses and dopaminergic axons. Normally activation of NMDA receptors by glutamate stimulates cortical inhibitory interneurons, which release GABA to excitatory pyramidal neurons, resulting in inhibition of release of dopamine from the mesolimbic dopamine pathway. In schizophrenia NMDA hypofunction results in decreased activity of cortical GABA-ergic interneurons. Reduced release of GABA leads to diminished inhibitory control of pyramidal neurons, resulting in increased dopamine release. Due to NMDA hypofunction glutamate accumulates in the synaptic space. Ca²⁺ influx increases, triggering an excitotoxic cascade.
Glutamate modulators

NMDA-receptor based treatment approaches include agonists at the NMDA receptor glycine site (clozapine, glycine, D-serine, D-cycloserine and N-acetylcysteine), a glycine transport inhibitor (sarcosine) and non-competitive open-channel blockers of the NMDA receptor (amantadine and memantine) [24]. Potential sites for intervention also include glutathione synthesis (N-acetylcysteine). An allosteric modulator of the AMPA receptor enhances glutamate-mediated synaptic transmission (ampakine CX516) [25]. Other pathways regulating glutamate involve agents resulting in inhibition of presynaptic glutamate release, which include mixed mGluR2/3 agonists, mGluR2 positive allosteric modulators (PAMs), an antagonist for postsynaptic kainate receptors and AMPA receptors (topiramate) and an antagonist for postsynaptic voltage sensitive sodium channels (lamotrigine) [16,26].

Metabotropic glutamate agonists

Recent animal studies provide strong evidence that specific metabotropic glutamate agonists are effective in the treatment of different symptom domains of schizophrenia [26]. Animal studies of mGluR5 agonists show improvement of positive, negative and cognitive symptoms. MGlu5 receptor activation even reverses cognitive dysfunction in preclinical studies. However, this selective agonist has not yet been tested in a clinical trial. Group II mGluR agonists demonstrated efficacy in multiple animal models for schizophrenia and were considered a promising novel approach in the treatment of schizophrenia, selectively targeting downstream glutamate increase due to NMDA receptor hypofunction [13,27].

Pomaglumetad methionil (LY2140023 monohydrate) is a methionine amide prodrug of the active compound LY404039, acting as a selective and potent orthosteric agonist at both mGluR2 and mGluR3 [11,26]. Initially this mixed mGluR2/3 agonist showed promise as potential monotherapy for schizophrenia in the proof-of-concept study, consistent with the predictions from pre-clinical animal studies [28]. In this randomized, 3-armed, double-blind, placebo-controlled trial an intention to treat (ITT) analysis was performed on 97 patients with schizophrenia receiving 40 mg pomaglumetad methionil twice daily, 34 patients receiving 15 mg olanzapine once daily and 62 patients receiving placebo for 4 weeks. Although less efficacious than olanzapine, pomaglumetad methionil showed significant improvement in positive, negative and overall symptoms of schizophrenia.
without side effects such as weight gain, extrapyramidal symptoms and elevated prolactin. However, antipsychotic properties of pomaglumetad methionil were not confirmed in the second phase 2 clinical trial, which was a 4-week double-blind placebo-controlled dose-ranging study [29]. While in the olanzapine (15 mg/day) treatment group (N=62) positive symptoms significantly improved, none of the pomaglumetad methionil dosages, varying from twice daily either 5 mg (N=121), 10 mg (N=122), 40 mg (N=120) to 80 mg (N=122) were found to be superior to placebo (N=122) for positive, negative and overall symptoms of schizophrenia. The second disappointing result was shown in another dose-ranging study [30]. After 6 weeks of treatment no significant differences in overall symptoms of schizophrenia were found between pomaglumetad methionil and placebo in patients with an acute exacerbation. Risperidone 2 mg twice daily significantly improved Positive and Negative Syndrome Scale (PANSS) total score, while efficacy of pomaglumetad methionil in different dosages (twice daily 40 mg and 80 mg) was similar to placebo. The first long-term open-label phase 2 study without a placebo treatment arm was designed to study safety and tolerability rather than efficacy [31]. Patients with prominent negative symptoms were randomized to either pomaglumetad methionil 40 mg twice daily or a second generation antipsychotic (olanzapine, risperidone or aripiprazole). After 24 weeks of treatment a second generation antipsychotic was significantly superior to pomaglumetad methionil for total symptom severity and both treatment groups showed comparable improvement in negative symptoms. In a 24-week double-blind randomized phase 3 study pomaglumetad methionil was found to be significantly inferior to aripiprazole (dosage varying from 10 to 30 mg/day) for total symptom severity [32]. This study was discontinued prematurely, because of disappointing results in the above mentioned phase 2 trials and the early cessation of a double-blind placebo-controlled dose-ranging phase 3 trial in acutely ill patients due to lack of efficacy.

Selective mGlur2 PAMs may be a more preferred approach than a mixed mGlur2/3 agonist like pomaglumetad methionil [13]. PAMs are small molecules, which bind at an alternative site to orthosteric agonists and enhance the agonistic activity in the presence of the endogenous ligand glutamate. MGlur2 PAMs modulate excessive synaptic glutamate release in almost all regions of the limbic system. Contrary to orthosteric agonism, positive allosteric modulation does not induce overactivation or desensitization via downregulation of mGlur2 receptors [33]. Therefore, normal or basal glutamate release remains stable.

2 PAMs advanced from animal models of psychosis to clinical trials. The MGlur2 PAM AZD8529 was tested in a phase 1 clinical trial in 2008 [33]. However, AZD8529 did
not meet the high expectations of an alternative treatment for schizophrenia. The phase 2 clinical trial in patients with schizophrenia, which started in 2009, discontinued in 2011 without further details. Another mGluR2 PAM, called addex (ADX71149) was studied in a phase 2 clinical trial in 2011 [33,34]. 15 patients with subacute psychosis, who were not treated with antipsychotic medication, received ADX71149 as monotherapy during 12 weeks with a dose range from 50 mg twice daily titrated up to 150 mg twice daily. Safety and tolerability were confirmed. However, results of this open-label trial, investigating efficacy of addex as monotherapy in patients with (sub)acute positive symptoms, have not yet been reported.

I onotrophic glutamate agonists

Most glutamate agonists are NMDA receptor modulators at the glycine site [10]. The following compounds have glutamate agonistic properties. Glycine and D-serine are full agonists at the glycine site of the postsynaptic NMDA receptor [35]. D-cycloserine is a partial agonist at the NMDA receptor glycine site with approximately 60% activity [36]. In the presence of low concentrations of glycine, D-cycloserine acts as an agonist, while in the presence of high concentrations of glycine, D-cycloserine acts as an antagonist. N-Acetyl-cysteine provides cysteine for glutathione synthesis and is a NMDA receptor modulator. Sarcosine acts as an inhibitor of type 1 glycine transporter [37]. Ampakine CX516 is a positive modulator of the postsynaptic AMPA receptor [25].

There seems to be a differential effect of NMDA-receptor based interventions depending on the type of antipsychotic (clozapine or non-clozapine). This may be the result of effects of clozapine on glutamatergic homeostasis and antagonism at GABA receptors.

C lozapine

Clozapine is a highly efficacious second-generation agent, which has preferential antagonist activity at 5-HT2 receptors, followed by activity at adrenergic, cholinergic, histamine and muscarine receptors with high affinity to dopamine 4 receptors in specifically the frontal cortex and amygdala and only modest activity at dopamine 1, dopamine 2 and dopamine 5
receptors [38–40]. The exact mode of action by which clozapine exerts its superior efficacy for both positive and negative symptoms is unknown, but clozapine is hypothesized to interact with GABA as antagonist and to improve the glutamatergic homeostasis in different ways. Clozapine blocks dopamine 4 receptors resulting in upregulation (increase in number) of AMPA receptors [41]. This way clozapine enhances depolarization of the postsynaptic membrane and facilitates NMDA receptor activation. Clozapine activates astrocyte glial cells, star-shaped support cells which are not able to fire like neurons but release, absorb or transport neurotransmitters [42]. Activated astrocyte glial cells release D-serine, followed by NMDA receptor activation. Clozapine first induces release of D-serine, shortly followed by release of glutamate by astrocytes. Glutamate activates metabotropic glutamate receptors, resulting in increased expression of NMDA receptors by increasing brain-derived neurotrophic factor (BDNF).

Glutamate agonists in combination with antipsychotic medication

Therapeutic effects of modulation of the glutamate system depend on the type of co-medication. There is limited evidence to support augmentation of non-clozapine antipsychotics with glutamate agonists.

A disappointing result on a mixed mGlur2/3 agonist was shown in a 16-week double-blind placebo-controlled randomized trial in patients with prevailing negative symptoms of schizophrenia [30]. Pomaglumetad methionil as add-on therapy to a second generation antipsychotic did not show significant change in negative symptoms compared to placebo. The mGlur2 PAM addex seems a more promising augmentation strategy in patients with residual negative symptoms. In a double-blind phase 2 clinical trial 92 patients received either addex 50 mg twice daily, addex 150 mg twice daily or placebo in addition to antipsychotic medication for 4 weeks. Efficacy was only demonstrated in patients with residual negative symptoms (N=47) and not in patients with residual positive symptoms (N=25) or in patients with insufficient response to clozapine (N=20) [33,34].

A meta-analysis by Singh and Singh (2011) of 1253 cases from 29 placebo-controlled, double-blind randomized clinical trials on the efficacy of adjunctive NMDA receptor modulators to antipsychotic treatment confirmed additional therapeutic benefits of glutamate agonists in combination with non-clozapine antipsychotics [24]. Negative symptoms (Positive and Negative Syndrome Scale negative subscale or Scale for
the Assessment of Negative Symptoms) improved with D-serine (standardized mean difference=–0.54), N-acetyl-cysteine (SMD=–0.45) and sarcosine (SMD=–0.39) as adjuncts to non-clozapine antipsychotics. Overall symptoms of schizophrenia (total PANSS score or total Brief Psychiatric Symptom Scale score) improved in combination with D-serine (SMD=–0.45), N-acetyl-cysteine (SMD=–0.64), sarcosine (SMD=–0.53). Combination therapy of non-clozapine antipsychotics and glycine or NMDA receptor modulators as a group improved positive symptoms (PANSS positive subscale or BPRS positive subscale SMD=–0.54 and –0.14, respectively) and overall symptoms of schizophrenia (total PANSS score or total BPRS score SMD=–1.12 and –0.38, respectively).

As adjuvant to clozapine, these glutamate agonists had no favourable effect on all symptoms of schizophrenia, while glycine even worsened positive symptoms (PANSS SMD=0.56) [24]. 6 placebo-controlled, double-blind randomized clinical trials on NMDA receptor agonists showed no beneficial effects in addition to clozapine [43]. A possible explanation for the absence of favourable effects of NMDA receptor agonists in addition to clozapine is downregulation of NMDA receptors [44]. The glutamate agonist ampakine CX516 with its allosteric agonistic action at the AMPA receptor may be an exception, because ampakine CX516 combined with clozapine does not induce downregulation of NMDA receptors [25]. This hypothesis is supported by a single study on ampakine CX516 combined with clozapine, which showed favourable effects on negative, overall clinical symptoms and cognitive functioning [25].

Glutamate antagonists

Lamotrigine is an anticonvulsant drug that acts through voltage sensitive sodium channels antagonism and reduces presynaptic glutamate release. Lamotrigine is assumed to augment the antipsychotic efficacy of clozapine by antagonizing overactive kainate receptors [35,45]. Topiramate has a mixed profile with both GABA-ergic and glutamatergic actions. This anticonvulsant potentiates GABA-ergic neurotransmission and acts as an antagonist for postsynaptic kainate receptors and AMPA receptors, decreasing the presynaptic release of glutamate [46,47]. Amantadine is an indirect dopaminergic agonist and a non-competitive open-channel blocker of the NMDA receptor with weak antagonist action [48]. Amantadine presumably exerts its neuroprotective effect through reducing the release of pro-inflammatory factors from activated microglia cells (the main cells for immune defence
in the brain) and increasing the expression of neurotrophic factors from astrocyte glial cells [49]. Memantine acts as a low to moderate-affinity type of uncompetitive, nonselective NMDA receptor antagonist [50]. Whereas PCP and ketamine are non-competitive NMDA receptor antagonists and induce schizophrenia-like symptoms, memantine is a voltage dependent antagonist. Memantine exerts its subtle effect on the NMDA receptor by binding at or near the Mg²⁺ site within the ion channel. Memantine binds somewhat stronger than Mg²⁺, decreasing Ca²⁺ influx. Through reduction of overstimulation of NMDA receptors in the presence of excessive glutamate in the synaptic cleft, a homeostatic state is restored.

**Glutamate antagonists in combination with antipsychotic medication**

At the present moment the effect of glutamate antagonists as augmentation of antipsychotics is not clear because of contradictory results. There is one study with lamotrigine augmentation to conventional and atypical antipsychotics (4 patients with clozapine) in patients with treatment-resistant schizophrenia [51]. The last observation carried forward (LOCF) analysis did not show significant differences in all domains at the end of 10 weeks of treatment between 25 inpatients receiving lamotrigine (titrated up to 400 mg/day) and 13 inpatients receiving placebo. Because of the number of early drop-outs in both groups an analysis was performed in which only patients who completed the trial were included. The completer analysis showed significant improvement of positive symptoms (ES=–0.82), general psychopathology symptoms (ES=–0.85) and total PANSS score (ES=–0.92), but no significant improvement of negative symptoms (Scale for the Assessment of Negative Symptoms ES=–0.55), overall clinical symptoms (Brief Psychiatric Rating Scale ES=–0.52) or affective symptoms (21-item Hamilton Rating Scale for Depression ES=–0.05). Because these positive findings were not demonstrated in the LOCF analysis, this study had insufficient power to investigate the efficacy of lamotrigine as adjunctive agent to non-clozapine antipsychotics.

A meta-analysis by Kishi and Iwata (2013) on amantadine and memantine included 8 double-blind placebo-controlled trials across 406 patients (347 patients with schizophrenia related disorder and 59 patients with bipolar disorder) [52]. Amantadine (5 trials with 220 patients) and memantine (3 trials with 186 patients) as adjunctive therapy were not superior to placebo in positive symptoms, negative symptoms, overall symptoms of schizophrenia and symptom severity (Clinical Global Impression Severity scale) (effect sizes were not
stated). In 3 cross-over studies on 74 patients with schizophrenia [53–55] amantadine addition to antipsychotics in patients with schizophrenia did not show a significant beneficial effect on overall symptoms of schizophrenia after a very short treatment duration, varying from 2 weeks to 7 weeks. Angus et al. (1997) included 16 patients with schizophrenia, receiving first generation antipsychotics (6 patients fluphenazine decanoate, 3 patients flupentixole decanoate, 3 patients trifluoperazine, 2 patients chlorpromazine, one patient thioridazine and one patient haloperidol) [53]. Amantadine or placebo were administered during 7 weeks (amantadine 100 mg/day in the first week, 200 mg/day in the second week, 300 mg/day during the third, fourth and fifth week, 200 mg/day in the sixth week and 100 mg/day in the seventh week) followed by a washout period of one week before cross-over to placebo or amantadine. This study did not show significant beneficial effect of amantadine on overall symptoms of schizophrenia (Psychiatric Assessment Scale for Rating Chronic Psychiatric Patients).

Silver et al. (2005) included 36 patients (32 patients with schizophrenia and 4 patients with schizoaffective disorder) in a 6-week cross-over trial [54]. A completer analysis was performed on 3 patients receiving clozapine, 9 patients receiving other second-generation antipsychotics and 17 patients receiving first-generation antipsychotics. After 3 weeks amantadine add-on therapy (200 mg/day) showed no significant effect on positive symptoms (Scale for the Assessment of Positive Symptoms), negative symptoms (SANS) and overall symptoms of schizophrenia (PANSS) or cognitive functions (including attention, memory, emotion perception and executive functions). Cognitive assessments were performed with an elaborate test battery. These disappointing results are not surprising because of the short treatment duration of merely 3 weeks. However, in this study, amantadine improved visuomotor coordination and symptom severity (CGI) compared to placebo [52]. Pappa et al. (2010) included 22 patients with schizophrenia, receiving olanzapine with random assignment to either amantadine 100 mg/day or placebo during 2 weeks, followed by a washout period of 4 days before cross-over to placebo or amantadine [55]. Whereas overall symptoms (BPRS) and cognition (Mini-Mental State Examination) did not change compared to placebo, symptom severity (CGI) did improve significantly after merely 2 weeks [40].

Only one of 3 trials on memantine in combination with non-clozapine antipsychotics showed clinical benefits. In a small 8-week placebo-controlled trial 20 patients were randomly assigned to risperidone 6 mg/day combined with memantine (titrated up to 20 mg in 1 week) and 20 patients received risperidone plus placebo [56]. In this trial meman-
tine proved to be an efficacious adjunct for the treatment of primary negative symptoms (ES=−1.5), total symptoms (ES=−1.6) and general psychopathological symptoms of schizophrenia (ES=−1.0) (PANSS), but not for positive symptoms (ES=−0.1) or affective symptoms (ES=0.0) (Hamilton Depression Rating Scale). Lieberman et al. (2009) compared 70 patients with persistent residual positive symptoms of schizophrenia receiving memantine (titrated up to 20 mg in 3 weeks) and 68 patients receiving placebo as an adjunct to other atypical antipsychotics than clozapine (olanzapine, risperidone, quetiapine, aripiprazole and ziprasidone) during 8 weeks [57]. Memantine showed no therapeutic benefits on total symptoms, positive symptoms, negative symptoms (PANSS), affective symptoms (Calgary Depression Scale for Schizophrenia) or cognitive functioning (Brief Assessment of Cognition in Schizophrenia). Auditory hallucinations occurred only in the memantine group (5.8%), suggesting an increase of psychotic symptoms due to memantine. These contradictory results may be explained by the shorter treatment duration with the maximum memantine dosage in the negative study in comparison to the positive study (5 weeks instead of 7 weeks). However, Lee et al. (2012) did not find beneficial effects of adjunctive memantine therapy to non-clozapine antipsychotics either [58]. 15 patients received memantine and 11 patients received placebo during 12 weeks as adjunct to conventional antipsychotic treatment. Results showed no significant differences regarding cognitive functioning (Korean MMSE, Hopkins Verbal Learning Test, Rey Complex Figure Test, Digit Span Forward and Backward Test, Digit Symbol Substitution Test, Stroop test, Trail Making Test (Part A), the Verbal Fluency Test and the Boston Naming Test), general psychopathology, positive, negative symptoms (PANSS) and affective symptoms (Hamilton Rating Scale for Depression). Treatment duration with memantine 20 mg/day was 9 weeks. Accordingly, there is still controversy regarding the role of memantine as adjunctive agent to non-clozapine antipsychotics. When the study by Rezaei et al. (2013) is considered an outlier and is removed from the analysis [56], the trend toward superior effect of memantine over placebo in addition to non-clozapine antipsychotics disappears.

Contrary to non-clozapine antipsychotics, clozapine augmentation with glutamate antagonists has therapeutic potential. Combined results of 6 lamotrigine trials show a trend towards reducing positive and negative symptoms [43]. Topiramate add-on treatment to clozapine demonstrated therapeutic benefits in 2 out of 4 small studies, but a meta-analysis showed no significant efficacy nor trend in reducing symptoms [43]. Research on amantadine add-on therapy to clozapine is limited to 3 patients in the study by Silver et al. (2005), in which it is not stated whether these 3 patients were suffering from schizo-
phrenia or schizoaffective disorder [54]. No subanalysis was performed on the efficacy of amantadine addition to clozapine compared with non-clozapine antipsychotics. Memantine addition to clozapine showed substantial beneficial effects on negative symptoms, positive symptoms, overall clinical symptoms (BPRS) and cognitive functioning (MMSE) in clozapine-resistant patients with schizophrenia in a small Brazilian study [50].

Taken together, we conclude that glutamate antagonists may have beneficial effects in combination with clozapine in refractory schizophrenia, but not in combination with non-clozapine antipsychotics.

Discussion

Here, we reviewed the relevance of the glutamate hypothesis in explaining cognitive disturbances and negative symptoms in schizophrenia and the evidence in support of this hypothesis. Based on this information glutamate pathways are promising sites for intervention. mGlu5 receptors are a potential target for novel drug development, because activation of mGlu5 receptors enhances NMDA receptor function [26]. Highly selective mGluR2 PAMs, inhibiting presynaptic glutamate release, are more promising than mixed mGluR2/3 agonists in clinical studies [30–34]. However, no conclusion regarding efficacy of highly selective mGluR2 PAMs are currently justified based on the preliminary conclusions of one phase 2 clinical trial [33,34]. Medication trials suggest that ionotrophic glutamate agonists combined with non-clozapine antipsychotics and ionotropic glutamate antagonists augmented to clozapine show interesting clinical benefits in refractory schizophrenia.

Memantine is especially promising as augmentation strategy to clozapine and perhaps even to non-clozapine antipsychotics, but evidence is limited to 4 trials (one study by Lucena et al., 2009 on augmentation to clozapine shows significant beneficial effect [50] and one out of 3 studies on augmentation to non-clozapine antipsychotics by Rezaei et al., 2013 shows significant beneficial effect [56]).

Preliminary conclusions regarding favourable effects of clozapine augmentation with glutamate antagonists (lamotrigine, topiramate and memantine) are based on a small number of trials with a small sample size (5 out of 12 studies show a significant beneficial effect) [43]. Notably the memantine trial shows impressive effect sizes. Therefore additional studies in larger samples are needed.

The glutamate hypothesis concerning NMDA hypofunction may explain why
memantine as an adjunct to clozapine is a logical treatment approach in refractory schizophrenia. Memantine in combination with clozapine contributes to upregulation of NMDA receptors [44]. Moreover, memantine prevents an excitotoxic cascade in the presence of glutamate spill over in the synaptic cleft, which is a result of NMDA hypofunction in schizophrenia. Because memantine is a voltage dependent NMDA receptor antagonist, the NMDA receptor is only activated by a strong stimulus. Thus memantine reduces abnormal cortical signal-to-noise patterns and prefrontal noise in schizophrenia. Glutamatergic transmission efficacy and dopaminergic neurotransmission in the frontal cerebral cortex improves. These 2 mechanisms of action explain why memantine has potential as add-on treatment for primary negative symptoms in combination with non-clozapine antipsychotics as well.

To test this hypothesis we recently started a 26-week randomized, placebo-controlled cross-over study to evaluate the clinical efficacy, tolerability and safety of memantine versus placebo in combination with ongoing clozapine treatment in 52 outpatients with refractory schizophrenia. Participants fail to achieve the remission criteria, defined as simultaneous ratings of mild or less (≤3 points) on 8 of the PANSS items evaluating the core symptoms of schizophrenia [59] after adequate treatment with clozapine for at least 6 months. Participants are recruited from Flexible Assertive Community Treatment (FACT) teams, specialized in the treatment of patients with severe mental illness. Memantine starts with 10 mg during the first week, builds up in the second week to the maximum dosage of 20 mg daily as add-on therapy. 20 mg is the ordinary study dosage in trials into Alzheimer’s disease and other indications of memantine. To enhance compliance, the dosing regimen in this study is simplified and accelerated. No substantial withdrawal due to adverse events is anticipated, since memantine tends to cause a similar range of side effects as placebo [60,61]. Expectations are that poor-outcome patients, who suffer from debilitating cognitive disturbances and negative symptoms as well as persistent positive symptoms will have clinical relevant advantages with memantine as add-on therapy to clozapine, reflected in improvement of daily functioning and quality of life. To improve the original proof-of-concept study by Lucena et al. (2009) cognitive functioning will be assessed by the Cambridge Neuropsychological Test Automated Battery (CANTAB), a sensitive and extensively validated cognitive testing battery [62]. Severity of psychopathology (measured with Clinical Global Impression Severity Scale [63] and PANSS [64]) is an additional primary parameter. Secondary study parameters are social cognition (Emotion Recognition Task of the CANTAB and Reading the Mind in the Eyes test [65]), psychosocial functioning (Health of the National Outcome Scales [66]) and quality of life (Manchester Short Assessment
of Quality of Life [67]). Depressive and obsessive-compulsive symptoms are frequent in schizophrenia. Since memantine may have a beneficial effect on these symptoms [68–70] they are measured as additional secondary parameters (Calgary Depression Scale for Schizophrenia [71] and Yale-Brown Obsessive-Compulsive Scale [72]). Safety measures are laboratory tests (fasting plasma glucose, triglycerides, LDL, HDL and total cholesterol, liver enzymes, renal function, white blood cell count and differentiation, plasma clozapine level), blood pressure and waist circumference. Neuroleptic induced side-effects are assessed by the Liverpool University Neuroleptic Side-Effect Rating Scale [73]. All parameters are assessed at baseline, week 12, week 14 (after a wash-out period of 2 weeks) and week 26 (or drop-out). If this second proof-of-concept study replicates and extends findings concerning relevant clinical improvement in patients with clozapine-resistant schizophrenia, clinical investigation should be the follow-up for this augmentation strategy.

Conclusion

Glutamate agonists are hypothesized to restore glutamatergic neurotransmission in schizophrenia, which is imbalanced due to NMDA receptor hypofunction. Focusing on the early diagnosis and treatment of psychosis, glutamatergic modulators may prevent neurodegenerative changes in the early stage of schizophrenia and subsequent cognitive decline. Inhibition of mesolimbic dopaminergic neurotransmission by activation of GABA interneurons may ameliorate positive symptoms. Negative symptoms improve by reduction of cortical noise and enhancement of efficacy of neuronal transmission in the prefrontal cortex. Cognitive deficits ameliorate due to this decrease of prefrontal noise and improvement of organized bursting activity, as well as reduction of neuronal excitotoxicity.

Drug development based on positive allosteric modulation of mGlu5 receptors, potentiating NMDA receptor function, has not yet advanced from animal studies to clinical studies. Evidence regarding efficacy and tolerability of glutamate modulators in addition to antipsychotics is limited. A novel mGluR2 PAM (addex) shows potential as the first non-dopaminergic drug that may address negative symptoms of schizophrenia, based on a single double-blind placebo-controlled augmentation study. Ionotropic glutamate agonists in addition to non-clozapine antipsychotics appear to render clinically meaningful benefits for patients with residual positive symptoms and debilitating negative symptoms. The addition of a NMDA receptor agonist to clozapine does not improve therapeutic response, possibly
due to downregulation of NMDA receptors. Ampakine CX516 does not induce downregulation of NMDA receptors and seems to be the only glutamate agonist to have favourable effects combined with clozapine. Glutamate antagonists seem to have therapeutic potential as add-on treatment for patients, who respond unsatisfactorily to clozapine. Perhaps lamotrigine and memantine have clinical value in addition to non-clozapine antipsychotics as well, but evidence regarding efficacy is limited and not convincing at the present moment.

The superior efficacy of clozapine is probably due to its glutamate agonistic actions. Clozapine restores glutamate dysfunction in schizophrenia, especially when combined with the voltage dependent NMDA receptor antagonist memantine through upregulation of NMDA receptors. Memantine blocks the effects of excessive glutamate in schizophrenia, while preserving physiological activation of NMDA receptors required for long term potentiation. The combination of clozapine and memantine is believed to create a specific glutamatergic environment, in which neurotransmission is improved and clinical improvement is sustained. Based on the glutamate hypothesis, functional psychopharmacological characteristics of memantine and evidence from one proof-of-concept study, memantine as an add-on treatment to clozapine is expected to have favourable effects on cognitive functioning, negative and positive symptoms, reflected in improvement of daily functioning and quality of life in patients with refractory schizophrenia. If a second proof-of-concept study replicates and extends the findings of the earlier study memantine is a promising novel augmentation strategy in patients with clozapine-refractory schizophrenia.

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Non-glutamatergic clozapine augmentation strategies: a review and meta-analysis

Selene R.T. Veerman, Peter F.J. Schulte, Marieke J.H. Begemann, Lieuwe de Haan

Is the addition of non-glutamatergic agents to clozapine efficacious for negative, positive, and overall symptoms of schizophrenia and affective symptoms?

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Abstract

Persistent negative symptoms and cognitive impairment are major clinical problems in the treatment of schizophrenia. There is no convincing evidence regarding the efficacy of augmentation of clozapine with a second antipsychotic, ethyl eicosapentaenoic acid (E-EPA), an antidepressant, a mood stabilizer or extract of Ginkgo biloba in clozapine-resistant schizophrenia. We present an overview of studies in which the potential clinical utility of the addition of non-glutamatergic agents to clozapine is assessed. We performed a meta-analysis on the efficacy of both risperidone and aripiprazole compared to placebo. We compared the effects of the addition of a second antipsychotic or an antidepressant to clozapine on positive, negative, overall and affective symptoms of schizophrenia in double-blind placebo-controlled trials.
Introduction

Treatment resistance in schizophrenia is a major health problem [1]. In patients with an incomplete response to treatment with typical and atypical antipsychotics, clozapine has proven superior efficacy in several domains of dysfunction above all other antipsychotics. However, in up to 70% of patients treated with clozapine, refractory symptoms continue, ranging from severe positive symptoms or debilitating negative symptoms to cognitive dysfunction or affective symptoms [2]. Only a few clinical trials have tested the efficacy of augmentation strategies in this patient population with an incomplete response to clozapine [3]. 5 meta-analyses of randomized, double-blind, placebo-controlled clozapine augmentation trials found no solid evidence for any effective clozapine augmentation therapy [4–8]. The aim of the present review is to summarize the results of studies of the efficacy of non-glutamatergic medication as an adjunctive agent in clozapine-resistant schizophrenia. Non-glutamatergic agents which have been studied in combination with clozapine and compared to placebo are antipsychotics, antidepressants, ethyl eicosapentaenoic acid (E-EPA), lithium and extract of Ginkgo biloba.

Methods

A search was carried out in the electronic databases PsycINFO, EMBASE, EBM reviews-Cochrane Database of Systematic Reviews, EBM reviews-Cochrane Central Register of Controlled Trials. Keywords were “schizophrenia,” “clozapine,” “augmentation or combination,” “treatment resistant or refractory,” “randomized”. Titles, abstracts and related articles were examined, and randomized double-blind studies in patients with clozapine-resistant schizophrenia and clozapine augmentation with non-glutamatergic medication selected. There were no language or year of publication restrictions. Open-label studies were not included in the current review. We excluded studies in which non-glutamatergic agents were added to antipsychotics other than clozapine as well as studies where data on augmentation in a subset of patients using clozapine could not be retrieved.

If possible, efficacy was determined regarding 3 symptom domains of schizophrenia, i.e., positive symptoms, negative symptoms and affective symptoms. Hedges’s $g$ was used to quantify effect sizes (ES) for the mean difference between change scores (end of treatment minus baseline) of the augmentation group versus control [9]. Change
scores were preferred over pre- and post-treatment scores to avoid overestimation of the true effect size because of the pre-treatment-post-treatment correlation. If change scores were not reported, pre- and post-treatment means, or exact F, t or p values for main effect of treatment group (augmentation or placebo) were used. When possible, meta-analyses were conducted on the total Positive and Negative Syndrome Scale (PANSS) score as well as on the positive and negative subscores of the PANSS. If PANSS scores were not available, Brief Psychiatric Rating Scale (BPRS) scores were used instead. To assess negative symptoms we preferred Scale for the Assessment of Negative Symptoms (SANS) above PANSS negative subscale or BPRS-negative subscale. For the study by Freundenreich et al. (2007) we used the PANSS negative subscale instead of the SANS, because no data on change score of the SANS were reported [10].

When more than 2 studies were available studies were combined in meta-analysis. A random effects model was deemed most appropriate for this research area given the heterogeneity of the methods applied and the limited number of studies of some augmentation strategies. Although a random effects model is typically more conservative than a fixed effects model, it allows for greater generalization [11,12]. A homogeneity statistic $I^2$ was calculated to test whether the studies could be taken together to share a common population effect size [13]. High heterogeneity (i.e., $I^2 \geq 50\%$; 30–50% was considered moderate) indicates heterogeneity of the individual study effect sizes, which poses a limitation to reliable interpretation of the results. Although the random effects model allows variance between studies, it is important to investigate potential outlier studies, which were excluded from the meta-analysis one by one (standardized residuals $>2.5$). Potential moderator variables were also examined. When meta-analytic outcomes are interpreted, the possibility of an upward bias of the calculated effect sizes due to the omission of unpublished, non-significant studies must be taken into account. The fact that studies with null effects are often not published generates a publication bias, also known as the “file drawer problem” [14]. Potential publication bias was investigated by means of a visual inspection of the funnel plot, and the fail-safe number of studies ($N_{fr}$) was calculated, providing an estimate of the number of non-significant or missing studies that would be needed to reduce an observed overall significant result to non-significance. All calculations were executed using Comprehensive Meta-Analysis Version 2.0 by Biostat [15].

In the study by Gunduz-Bruce et al. (2013) we found a discrepancy in significance of SANS score, because the authors used a mixed effects model comparing the pimozide and placebo group at different time points [16]. For the study by Fleischhacker et al. (2010)
and Peet and Horrobin (2002) we calculated the ES using the P-value and treatment difference based on analysis of covariance [17,18], although neither of these papers reports whether initial baseline differences were significant. In the study by Freundreich et al. (2007) [10] we found a discrepancy in significance of the effect of risperidone on the total PANSS score, because rather than using a linear regression analysis we calculated the ES and P-value on the basis of the mean change scores. In the study by Weiner et al. (2010) we found a discrepancy in significance of the effect of risperidone on SANS score [19], because we did not adjust for baseline scores using mixed model analysis of covariance (ANCOVA), as no baseline differences were reported. We received original data from 4 authors, mentioned in the acknowledgements.

Results

We present an overview of 22 randomized double-blind studies of augmentation with non-glutamatergic agents in patients with schizophrenia with a partial or non-response to clozapine.

Antipsychotic medication

We found 13 double-blind, placebo-controlled randomized trials of antipsychotics as adjunctive treatment to clozapine in clozapine-resistant schizophrenia (Table 1A–D). There are 2 small trials of clozapine augmentation with typical antipsychotics with a treatment duration varying from 10 to 12 weeks (Table 1A) [16,20]. A single trial of haloperidol 4 mg/day did not show any significant change in overall symptoms of schizophrenia compared to placebo [20]. A single trial of pimozide 4 mg/day showed a significant improvement in negative symptoms in the placebo group compared to the pimozide group [16].

11 placebo-controlled randomized trials of clozapine add-on therapy with atypical antipsychotics were included with a treatment duration varying from 6 to 24 weeks. A significant benefit of adjunctive treatment with sulpiride 600 mg/day for positive, negative and overall clinical symptoms was found in a double-blind study [21] (Table 1B). Amisulpride was found to have a significant beneficial effect in a dosage of 600 mg/day (but not in a
### Table 1A  Double-blind, placebo-controlled randomized trials of clozapine augmentation with typical antipsychotics in refractory schizophrenia.

<table>
<thead>
<tr>
<th>Study</th>
<th>N</th>
<th>Trial duration (weeks)</th>
<th>Outcome of measure</th>
<th>ES</th>
<th>P-Value</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mossaheb et al., 2006</strong></td>
<td>2</td>
<td>10</td>
<td>Positive symptoms (PANSS-P)</td>
<td>0.257</td>
<td>0.712</td>
<td>=</td>
</tr>
<tr>
<td>Completer analysis</td>
<td>4</td>
<td></td>
<td>Negative symptoms (PANSS-N)</td>
<td>-0.308</td>
<td>0.659</td>
<td>=</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Overall clinical symptoms (Total PANSS)</td>
<td>-0.635</td>
<td>0.376</td>
<td>=</td>
</tr>
<tr>
<td><strong>Gunduz-Bruce et al., 2013</strong></td>
<td>14</td>
<td>12</td>
<td>Positive symptoms (BPRS-P)</td>
<td>-0.680</td>
<td>0.072</td>
<td>=</td>
</tr>
<tr>
<td>Completer analysis</td>
<td>14</td>
<td></td>
<td>Negative symptoms (SANS)</td>
<td>-1.119</td>
<td>0.005</td>
<td>=</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Overall symptoms (BPRS)</td>
<td>-0.693</td>
<td>0.067</td>
<td>=</td>
</tr>
</tbody>
</table>

**PANSS, Positive and Negative Syndrome Scale; SANS, Scale for the Assessment of Negative Symptoms; BPRS, Brief Psychiatric Rating Scale**

+ Significant positive effect
- Significant negative effect
= No significant effect

### Table 1B  Double-blind, placebo-controlled randomized trials of clozapine augmentation with sulpiride and amisulpride in refractory schizophrenia.

<table>
<thead>
<tr>
<th>Study</th>
<th>N</th>
<th>Trial duration (weeks)</th>
<th>Outcome of measure</th>
<th>ES</th>
<th>P-Value</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Shiloh et al., 1997</strong></td>
<td>16</td>
<td>10</td>
<td>Positive symptoms (SAPS)</td>
<td>0.770</td>
<td>0.045</td>
<td>+</td>
</tr>
<tr>
<td>Completer analysis*</td>
<td>12</td>
<td></td>
<td>Negative symptoms (SAPS)</td>
<td>0.760</td>
<td>0.048</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Overall clinical symptoms (BPRS)</td>
<td>0.830</td>
<td>0.032</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Affective symptoms (HAM-D)</td>
<td>0.401</td>
<td>0.285</td>
<td>=</td>
</tr>
<tr>
<td><strong>Assion et al., 2008</strong></td>
<td>6</td>
<td>6</td>
<td>Overall clinical symptoms (BPRS)</td>
<td>x</td>
<td>x</td>
<td>=</td>
</tr>
<tr>
<td>LOCF analysis</td>
<td>7</td>
<td></td>
<td>Affective symptoms (MADRS)</td>
<td>x</td>
<td>x</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Nielsen et al., 2012</strong></td>
<td>25</td>
<td>12</td>
<td>Positive symptoms (PANSS-P)</td>
<td>0.028</td>
<td>0.921</td>
<td>=</td>
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<tr>
<td>LOCF analysis</td>
<td>25</td>
<td></td>
<td>Negative symptoms (PANSS-N)</td>
<td>-0.014</td>
<td>0.961</td>
<td>=</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Overall symptoms of schizophrenia (Total PANSS)</td>
<td>0.003</td>
<td>0.992</td>
<td>=</td>
</tr>
</tbody>
</table>

**SAPS, Scale for Assessment of Positive Symptoms; HAM-D, Hamilton Rating Scale for Depression; MADRS, Montgomery Asberg Depression Rating Scale**

* ES and P-value could not be calculated with reported data
* * Not explicitly stated by the authors
* + * Significant beneficial effect in amisulpride group with maximum dosage 600 mg, not in amisulpride group with maximum dosage 400 mg

### Table 1C  Double-blind, placebo-controlled randomized trials of clozapine augmentation with aripiprazole in refractory schizophrenia.

<table>
<thead>
<tr>
<th>Study</th>
<th>N</th>
<th>Trial duration (weeks)</th>
<th>Outcome of measure</th>
<th>ES</th>
<th>P-Value</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Chang et al., 2008</strong></td>
<td>29</td>
<td>8</td>
<td>Positive symptoms (BPRS-P)</td>
<td>-0.254</td>
<td>0.318</td>
<td>=</td>
</tr>
<tr>
<td>LOCF analysis</td>
<td>32</td>
<td></td>
<td>Negative symptoms (SAPS)</td>
<td>0.690</td>
<td>0.008</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Overall clinical symptoms (BPRS)</td>
<td>0.107</td>
<td>0.674</td>
<td>=</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Affective symptoms (MADRS)</td>
<td>0.297</td>
<td>0.244</td>
<td>=</td>
</tr>
<tr>
<td><strong>Fleischhacker et al., 2010</strong></td>
<td>108</td>
<td>16</td>
<td>Positive symptoms (PANSS-P)</td>
<td>0.125</td>
<td>0.369</td>
<td>=</td>
</tr>
<tr>
<td>LOCF analysis</td>
<td>99</td>
<td></td>
<td>Negative symptoms (PANSS-N)</td>
<td>0.126</td>
<td>0.364</td>
<td>=</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Overall symptoms of schizophrenia (Total PANSS)</td>
<td>0.121</td>
<td>0.386</td>
<td>=</td>
</tr>
<tr>
<td><strong>Muscatello et al., 2011</strong></td>
<td>14</td>
<td>24</td>
<td>Positive symptoms (SAPS)</td>
<td>1.177</td>
<td>0.002</td>
<td>+</td>
</tr>
<tr>
<td>Completer analysis</td>
<td>17</td>
<td></td>
<td>Negative symptoms (SAPS)</td>
<td>0.358</td>
<td>0.312</td>
<td>=</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Overall clinical symptoms (BPRS)</td>
<td>0.694</td>
<td>0.056</td>
<td>=</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Affective symptoms (CDSS)</td>
<td>0.371</td>
<td>0.295</td>
<td>=</td>
</tr>
</tbody>
</table>

**BPRS-P, BPRS- positive symptom scale; PANSS-P, PANSS-positive symptom scale; PANSS-N, PANSS-negative symptom scale**

For more abbreviations see Table 1A

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lower dose of 400 mg/day) on affective symptoms [22]. Another trial of adjunctive sertindole 16 mg/day did not demonstrate clinical benefits for symptoms of schizophrenia [23].

3 studies on aripiprazole addition to clozapine were found to have discrepant results, which could be explained by limited trial duration in one study and small sample size in the other two studies [17,24,25] (Table 1C). In one trial aripiprazole 30 mg/day compared to placebo significantly improved negative symptoms after 8 weeks [24]. No significant changes in negative symptoms were found in 2 other trials [17,25]. While a large-scale 16-week trial of aripiprazole 15 mg/day showed no significant changes compared to placebo, aripiprazole 15 mg/day was significantly more efficacious than placebo in reducing positive symptoms after a treatment duration of 24 weeks [17,25]. The combined results of these 3 studies (N=297) showed that adjunctive aripiprazole has similar effects to placebo for positive symptoms (ES=0.311, p=0.322; 95% CI=–0.304 to 0.926). However, the studies were highly heterogeneous (I²=79.466%). The ES for negative symptoms showed a trend in favour of aripiprazole, with an ES of 0.340 (p=0.069; 95% CI=–0.026 to 0.705; I²=46.175%) (Fig. 1). Aripiprazole was not found to be superior to placebo treatment for total symptom severity (ES=0.190, p=0.144; 95% CI=–0.065 to 0.445; I²=11.011%).

3 out of 5 risperidone trials found that this drug as add-on therapy had significant clinical benefits [10,19,26] (Table 1D). Risperidone 6 mg/day resulted in significant improvement on positive, negative and overall symptoms of schizophrenia after a
treatment duration of 12 weeks [26]. A smaller study of the same dosage of risperidone found significantly greater improvement in positive symptoms in the placebo group after 6 weeks of treatment [27]. However, another 6-week study found that risperidone 4 mg/day resulted in substantial improvement in overall symptoms [10]. In a 16-week trial of risperidone 4 mg/day the intention-to-treat analysis showed no significant improvement in the risperidone group [19]. In the completer analysis significant improvement was found in positive symptoms (ES=0.27, \( p=0.02 \)) and overall clinical symptoms (ES=0.27, \( p=0.03 \)). A daily dosage of 3 mg of risperidone did not significantly change clinical symptoms compared to placebo after a short treatment duration of 8 weeks [28]. We were able to combine the results of 4 intention-to-treat studies with last observation carried forward (LOCF), including a total of 188 patients [10,19,27,28] in a meta-analysis. Risperidone did not significantly change positive symptoms compared to placebo (ES=0.000, \( p=1.000; \) 95% CI=–0.472 to 0.472). However, heterogeneity was high (\( I^2=59.685\% \)). After excluding the outlier study by Anil Yagcioglu et al. (2005), the ES increased to 0.209 but did not reach significance (\( p=0.187; \) 95% CI=–0.102 to 0.520; \( I^2=0\% \)). Risperidone did not differ from placebo with regard to negative symptoms (ES=0.193, \( p=0.356; \) 95% CI=–0.217 to 0.604). Heterogeneity was moderate (\( I^2=47.247\% \)). For total symptom severity risperidone showed no superior efficacy over placebo treatment (ES=0.305, \( p=0.406; \) 95% CI=–0.414 to 1.023). Although heterogeneity was high (\( I^2=81.699\% \)), no outlier study could be identified.

**Meta-analysis of antipsychotic medication**

We analyzed the efficacy of adjunctive antipsychotic medication for positive, negative and overall symptoms of schizophrenia in 11 studies, including a total of 599 patients [10,16,17,
Antipsychotic medication did not significantly improve positive symptoms compared to placebo (ES=0.112, \( p=0.438 \); 95% CI=–0.171 to 0.395). However, heterogeneity was high (\( I^2=60.668\% \)). The funnel plot did not indicate publication bias. After excluding the outlier study by Muscatello et al. (2011), the ES was reduced to 0.030 and was not found to be significant (\( p=0.818 \); 95% CI=–0.224 to 0.284; \( I^2=48.567\% \)). Antipsychotics did not differ from placebo with regard to reducing negative symptoms (ES=0.162, \( p=0.248 \); 95% CI=–0.113 to 0.436). However, heterogeneity was high (\( I^2=58.134\% \)). After excluding the outlier study by Gunduz-Bruce et al. (2013), the ES increased to 0.249, thereby showing a significant superior effect over placebo (\( p=0.023 \); 95% CI=0.035 to 0.463) (Fig. 2a). Heterogeneity was reduced to moderate (\( I^2=30.156\% \)), with no indications of publication bias and 10 studies needed to render this positive result non-significant. For total symptom severity clozapine add-on treatment with antipsychotics compared to placebo showed no superior efficacy (ES=0.174, \( p=0.257 \); 95% CI=–0.127 to 0.475). However, heterogeneity was high (\( I^2=64.818\% \)) although no publication bias was indicated. Again, the study by Freudenreich et al. (2007) was identified as an outlier study. After its exclusion, the ES was reduced to –0.082 (\( p=0.523 \); 95% CI=–0.170 to 0.335; \( I^2=48.820\% \)).

As treatment duration differed greatly between studies (\( M=11.64 \text{ weeks} \)), we compared the 6 studies with a treatment duration of 11 weeks or shorter [10,20,21,24,27,28] to five studies with a treatment duration longer than 11 weeks [16,17,19,23,25]. We found no significant differences between shorter or longer trials as regards effect on positive, negative or total symptom severity.
We analyzed 4 studies of antipsychotic medication in relation to affective symptoms, including a total of 150 patients [21,25,27]. Antipsychotics did not differ from placebo, but a trend towards reducing affective symptoms was found (ES=0.296, \( p=0.068 \); 95% CI=–0.022 to 0.613) (Fig. 2b). The studies were homogeneous (\( I^2=0.000\% \)).

**Antidepressant medication**

Evidence concerning antidepressants as add-on therapy to clozapine is limited to 5 trials (Table 2A, B) [29–33]. Fluoxetine 80 mg/day did not result in any significant change in positive, negative or affective symptoms compared to placebo [29]. A first study of 8 weeks of mirtazapine 30 mg/day showed significant improvement in overall clinical symptoms and negative symptoms (avolition/apathy and anhedonia/asociality) [30]. After a treatment period of just 6 weeks in a second study of the same dosage of mirtazapine, no significant change was found in any symptoms of schizophrenia or affective symptoms in the few patients using clozapine [31]. A potential role in the treatment of negative and overall symptoms of schizophrenia was found for citalopram 20 mg/day and duloxetine 60 mg/day based on single studies [32,33]. Duloxetine compared to placebo also improved affective symptoms significantly.

**Meta-analysis of antidepressant medication**

For positive and negative symptom severity, we performed a meta-analysis of 4 studies of antidepressant medication (\( N=111 \)) [29,30,31,33]. Antidepressants did not significantly
improve positive symptoms compared to placebo (ES=0.102, p=0.607; 95% CI=−0.288 to 0.493). Heterogeneity was low ($I^2=8.393\%$). Antidepressant medication showed a trend towards improving negative symptoms when compared to placebo (ES=0.868, p=0.062; 95% CI=−0.042 to 1.779) (Fig. 3). High heterogeneity was found ($I^2=79.649\%$), although no outlier study could be identified. For total symptom severity, we analyzed 3 studies of antidepressant medication, including a total of 78 patients [30,31,33]. For total symptom severity, add-on treatment with antidepressants compared to placebo showed no superior efficacy (ES=2.016, p=0.119; 95% CI=−0.521 to 4.552). However, heterogeneity was very high ($I^2=93.870\%$), probably due to the very large ES found by Zoccali et al. (2004). Again, the study by Freudenreich et al. (2007) was identified as an outlier study. After its exclusion, ES was reduced to −0.082 (p=0.523; 95% CI=−0.170 to 0.335; $I^2=48.820\%$). We analyzed

### Table 2A

<table>
<thead>
<tr>
<th>Study</th>
<th>Adjunctive agent (maximum dose)</th>
<th>N</th>
<th>Trial duration (weeks)</th>
<th>Outcome of measure</th>
<th>ES</th>
<th>P Value</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buchanan et al., 1996</td>
<td>Fluoxetine (80 mg) Placebo</td>
<td>15</td>
<td>8</td>
<td>Positive symptoms (BPRS-P)</td>
<td>0.120</td>
<td>0.726</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Negative symptoms (SANS)</td>
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<td>0.587</td>
<td>=</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Affective symptoms (HAM-D)</td>
<td>−0.115</td>
<td>0.737</td>
<td>=</td>
</tr>
<tr>
<td>Zoccali et al., 2004</td>
<td>Mirtazapine (30 mg) Placebo</td>
<td>12</td>
<td>8</td>
<td>Positive symptoms (SAPS)</td>
<td>0.252</td>
<td>0.557</td>
<td>=</td>
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<td></td>
<td></td>
<td></td>
<td>Negative symptoms (SANS)</td>
<td>1.959</td>
<td>&lt;0.001</td>
<td>+</td>
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<tr>
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<td></td>
<td>Overall clinical symptoms (BPRS)</td>
<td>5.836</td>
<td>&lt;0.001</td>
<td>+</td>
</tr>
<tr>
<td>Berk et al., 2009</td>
<td>Mirtazapine (30 mg) Placebo</td>
<td>7</td>
<td>6</td>
<td>Positive symptoms (PANSS-P)</td>
<td>−0.736</td>
<td>0.156</td>
<td>=</td>
</tr>
<tr>
<td></td>
<td></td>
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<td></td>
<td>Negative symptoms (PANSS-N)</td>
<td>−0.085</td>
<td>0.866</td>
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<td>Overall symptoms of schizophrenia (Total PANSS)</td>
<td>−0.561</td>
<td>0.273</td>
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<tr>
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<td>Affective symptoms (CDSS)</td>
<td>−0.584</td>
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### Table 2B

<table>
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<tr>
<th>Study</th>
<th>Adjunctive agent (maximum dose)</th>
<th>N</th>
<th>Trial duration (weeks)</th>
<th>Outcome of measure</th>
<th>ES</th>
<th>P-Value</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
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<td>Lan et al, 2006</td>
<td>Citalopram (20 mg) Placebo</td>
<td>20</td>
<td>12</td>
<td>Positive symptoms (PANSS-P)</td>
<td>x</td>
<td>x</td>
<td>=</td>
</tr>
<tr>
<td>Not known</td>
<td></td>
<td></td>
<td></td>
<td>Negative symptoms (PANSS-N)</td>
<td>x</td>
<td>x</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Overall symptoms of schizophrenia (Total PANSS)</td>
<td>x</td>
<td>x</td>
<td>+</td>
</tr>
<tr>
<td>Mico et al., 2010</td>
<td>Duloxetine (60 mg) Placebo</td>
<td>20</td>
<td>16</td>
<td>Positive symptoms (PANSS-P)</td>
<td>0.333</td>
<td>0.286</td>
<td>=</td>
</tr>
<tr>
<td>LOCF analysis</td>
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<td></td>
<td></td>
<td>Negative symptoms (PANSS-N)</td>
<td>1.466</td>
<td>&lt;0.001</td>
<td>+</td>
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<tr>
<td></td>
<td></td>
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<td></td>
<td>Overall symptoms of schizophrenia (Total PANSS)</td>
<td>1.319</td>
<td>&lt;0.001</td>
<td>+</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Affective symptoms (CDSS)</td>
<td>1.339</td>
<td>&lt;0.001</td>
<td>+</td>
</tr>
</tbody>
</table>

\(\cap\) Not stated in the English abstract how many of 61 patients were randomized to each group

For abbreviations see Table 1A–D

129
3 studies of antidepressant medication in relation to affective symptoms, including a total of 87 patients [29,30,33]. Co-medication with antidepressants did not differ from placebo in reducing affective symptoms (ES=0.249, \( p=0.671 \); 95% CI=–0.897 to 1.395). Again, heterogeneity was high (\( I^2=85.065\% \)).

Other non-glutamatergic medication

The efficacy of E-EPA as an adjunctive to clozapine in refractory schizophrenia was evaluated in only 2 double-blind placebo-controlled randomized trials (Table 3) [18,34]. In a 12-week trial Emsley et al. (2002) found no significant changes in positive, negative or overall symptoms of schizophrenia in patients using clozapine after treatment with E-EPA 3g/day (original data were retrieved from Emsley) [34]. The potential efficacy of E-EPA in combination with antipsychotics and especially as a clozapine adjunct is supported in only one randomized clinical trial [18]. Peet and Horrobin (2002) conducted a 12-week placebo-controlled dose-ranging exploratory study (1 g/day, 2 g/day and 4 g/day) of 115 patients with persistent symptoms of schizophrenia, receiving either clozapine (31 patients), atypical antipsychotics (48 patients) or first-generation antipsychotics (36 patients). In the clozapine group only, a post-hoc analysis showed a clinically important and significant effect on all PANSS subscales and MADRS, which was most profound at an E-EPA dosage of 2 g/day (ES and P-value in Table 3 are for 2 g/dag).

Lithium was added to clozapine in the triple crossover study by Small et al. (2003) of hospitalized patients with schizoaffective disorder (mean plasma lithium levels 0.61
The results of the study by Small suggest that lithium addition to clozapine has a positive effect in patients with a schizoaffective disorder, but a deleterious effect on negative symptoms and cognitive functioning (auditory verbal learning subtests, visual recognition, letter-number score) in patients with schizophrenia (Table 4). Extracts of Ginkgo biloba 120 mg/day added to clozapine showed a substantial and significant improvement in negative symptoms compared to placebo in one study [36] (Table 4).
Discussion

Comparing the efficacy of aripiprazole and risperidone, we found that neither antipsychotic differed significantly from placebo as regards reducing positive, negative or overall symptoms of schizophrenia. However, reduction of negative symptoms was more pronounced in those treated with aripiprazole than risperidone as adjunctive agents in clozapine-resistant schizophrenia. The meta-analysis of antipsychotic medication in general demonstrated no significant changes in positive, negative or overall symptoms of schizophrenia, regardless of treatment duration. However, after exclusion of one outlier study a second antipsychotic as an adjunct to clozapine showed a small but significant beneficial effect on negative symptoms.

Antidepressants significantly improved negative and overall symptoms of schizophrenia in three out of five trials of clozapine add-on therapy. However, in the meta-analysis of antidepressant medication no significant beneficial effect on positive, negative, affective and overall symptoms of schizophrenia was found compared to placebo.

Significant clinical benefits were observed in one out of two studies of E-EPA as augmentation of clozapine [18], but the evidence is unsatisfactory due to contradictory results. No conclusions can be based on single trials of lithium and extract of ginkgo biloba with small sample sizes [35,36].

Conclusions as to the efficacy of the addition of a second antipsychotic to clozapine are hampered by the limited trial duration in most studies (10 weeks or less in 7 studies, 12 weeks in 3 studies, 16 weeks in 2 studies and 24 weeks in one study) and by the absence of clozapine level monitoring. The small sample size in most studies is also an obvious restriction. On the basis of single studies of haloperidol, pimozide, sulpiride, amisulpride and sertindole, no conclusions about the efficacy of these drugs are justified [16,20–23]. Double-blind, placebo-controlled trials of aripiprazole in addition to clozapine are limited to three studies [17,24,25]. Most research on clozapine augmentation involves risperidone. Unfortunately, a meta-analysis could only be performed of four risperidone studies [10,19,27,28].

Comparing the efficacy of clozapine augmentation strategies is complicated, because statistical analyses vary among studies. In 2 aripiprazole studies and four risperidone studies an intention-to-treat analysis was used [10,17,19,24,27,28] which included data on subjects with noncompliance, protocol deviations and withdrawal. This approach avoids bias associated with non-random loss of participants. For missing data the last observation
was carried forward (LOCF analysis). In one aripiprazole study and one risperidone study only patients who completed the protocol were evaluated [25,26]. A completer analysis may bias the trial results. Weiner *et al.* (2010) conducted both analyses and found a significant change in the completer analysis only [19]. The latter showed a significant improvement in positive symptoms and overall clinical symptoms in the risperidone group, whereas the intention-to-treat analysis did not demonstrate a significant difference between groups.

It is difficult to make evidence-based recommendations regarding the combination of clozapine with non-glutamatergic agents in clozapine-refractory schizophrenia. For residual positive symptoms, meta-analyses of studies of both antipsychotic medication and antidepressant medication in general show no significant changes compared to placebo. The evidence is somewhat more positive for adjunctive E-EPA: one trial significantly improved positive symptoms with a dosage of 2 g/day, but the other trial was negative. Therefore a definite conclusion cannot be drawn.

A meta-analysis showed that in patients with prevailing negative symptoms after treatment with clozapine the addition of antipsychotics results in a small but significant effect on negative symptoms. Aripiprazole may be the best choice, because it is well tolerated and may also have the additional advantage of improvement in physical health through reduction of the metabolic risk factors (body weight and metabolic variables) associated with clozapine treatment [17,37–40]. *Gingko biloba* and E-EPA show positive effects in single trials, but are unreplicated (*Gingko biloba*) or in contradiction with a second, negative trial.

For affective symptoms, the small amount of research that exists on clozapine augmentation shows limited efficacy. Although no significant differences compared to placebo were found, antipsychotics seem to reduce affective symptoms more than antidepressants. In one out of 2 trials of E-EPA affective symptoms were assessed and were found to improve significantly in that trial [18].

Further study is necessary of pharmacological augmentation strategies to boost clozapine treatment in all domains of dysfunctions, including cognitive dysfunction, which strongly influences quality of life and social functioning.
Conclusion

Treatment of patients with clozapine-resistant schizophrenia is challenging since there is no established next step. Meta-analyses of both aripiprazole and risperidone studies failed to demonstrate any significant differences between placebo and aripiprazole or risperidone as regards changes in positive, negative or overall symptoms of schizophrenia. Although effect sizes were not significant, aripiprazole seems to be more efficacious in reducing negative symptoms than risperidone. When double-blind, placebo-controlled studies of antipsychotics and antidepressants were compared, these clozapine augmentation strategies did not differ from placebo as regards effect on positive and overall symptoms of schizophrenia. Treatment of negative symptoms in clozapine-resistant schizophrenia favours antipsychotics above antidepressants, since antipsychotics were found to have a significant beneficial effect, whereas only a positive trend was found for antidepressants. Curiously, for antipsychotic medication we found a positive trend in reducing affective symptoms, whereas antidepressant medication did not significantly influence affective symptoms compared to placebo. There is no evidence of any positive effect of lithium as an adjunct to clozapine therapy; in a single trial, a deleterious effect was found in refractory schizophrenia. E-EPA addition to clozapine was favourable on all outcome measures in one trial, but this effect was not replicated in a second trial. Extract of *Ginkgo biloba* appears to afford potential benefit, but evidence is limited to a single trial. Clearly, these 2 substances are good candidates for further research into treatment strategies of clozapine-resistant schizophrenia.

Acknowledgements

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Chapter 5

Clozapine augmented with glutamate modulators in refractory schizophrenia: a review and meta-analysis

Selene R.T. Veerman, Peter F.J. Schulte, Marieke J.H. Begemann, Fabiana Engelsbel, Lieuwe de Haan

Is glutamatergic medication in addition to clozapine efficacious for negative, positive, and overall symptoms of schizophrenia?

Pharmacopsychiatry 2014;47(6):185-194
Abstract

Clozapine is an efficacious antipsychotic drug for patients with treatment-resistant schizophrenia, but does not sufficiently improve these symptoms in a substantial proportion of this population. There is no convincing evidence for the efficacy of any clozapine augmentation strategy. New evidence suggests that glutamate receptors are a candidate target for therapeutic effects in schizophrenia. We present an overview of studies assessing the potential clinical utility of adding glutamatergic agents to clozapine. We conducted 3 metaanalyses of data on positive, negative and overall symptoms of schizophrenia, analysing results from 3 studies on clozapine augmentation with glycine, 6 studies on lamotrigine add-on therapy to clozapine and 4 studies on topiramate addition to clozapine.
Introduction

Clozapine has proven efficacy and is the only antipsychotic drug licensed for treatment-resistant schizophrenia [1]. Patients with an incomplete response to treatment are referred to as ‘treatment-resistant’ or ‘treatment-refractory’. Findings of several cost-effectiveness studies consistently favour clozapine over first-generation antipsychotics on measures of clinical efficacy, cost and cost-effectiveness, reflected in gains in life expectancy and quality-adjusted life expectancy [2]. In spite of its superior efficacy, 4% of all patients with schizophrenia do not respond to clozapine at all and approximately one third to one half of patients treated with clozapine still present with significant residual psychotic symptoms and negative symptoms [2–4]. The number of poor-outcome patients who respond partially or not at all to clozapine is substantial and the cost of their medical care is high.

There is no convincing evidence regarding the efficacy of clozapine add-on therapy with non-glutamatergic medication, such as a second antipsychotic, ethyl eicosapentaenoic acid (E-EPA), an antidepressant or a mood stabilizer [5,6]. However, the glutamate hypothesis of schizophrenia suggests that drugs modulating disrupted glutamate pathways may reduce the symptoms of schizophrenia [7]. Functional psychopharmacotherapy offers a solution for developing new medications for schizophrenia. Glutamatergic modulators specifically target psychopathological dysfunctions in schizophrenia by reversing the NMDA receptor deficiency or reducing excessive presynaptic glutamate release [8]. Although the exact mechanism of clozapine is still unknown, clozapine is hypothesized to influence glutamatergic neurotransmission in different ways [9,10]. To determine the efficacy of several clozapine augmentation strategies with glutamatergic modulators in refractory schizophrenia, first we reviewed glutamate agonists as adjunctive therapy to clozapine. The results of 3 trials on glycine are combined to assess efficacy for positive, negative and overall symptom severity. We then reviewed glutamate antagonists as add-on therapy to clozapine and conducted metaanalyses of the effects of lamotrigine and topiramate on positive, negative and overall symptoms of schizophrenia.

Methods

A search was carried out in the electronic databases PsycINFO, EMBASE, EBM reviews-Cochrane Database of Systematic Reviews, and EBM reviews-Cochrane Central Register
of Controlled Trials. Keywords were “schizophrenia”, “clozapine”, “augmentation or combination”, “treatment resistant or refractory”, “randomized”. Titles, abstracts and related articles were examined and randomized double-blind studies of patients with clozapine-resistant schizophrenia and clozapine augmentation with glutamatergic medication were selected. There were no language or year of publication restrictions. Open-label studies were not included in the current review. In some studies on glutamate modulators patients received other second-generation antipsychotics than clozapine and first-generation antipsychotics. Those studies were excluded from this review if we were unable to retrieve data on the efficacy of clozapine augmentation. We will discuss the efficacy of glutamate modulators in combination with antipsychotics in general in a review on the glutamate hypothesis [11] and present the results of post-hoc analyses on patients receiving clozapine in 3 lamotrigine trials by Kremer et al., 2004, studies 464 and 926 by Goff et al., 2007 [12,13] and one topiramate trial by Tiihonen et al., 2005 [14].

2 independent reviewers extracted data from the articles and assessed efficacy. Disagreements were resolved by consensus. Calculations were executed using Comprehensive Meta-Analysis Version 2.0 by Biostat [15]. Effect sizes (ES) and P-values were calculated for the mean difference between change scores (endpoint minus baseline scores) of the augmentation group versus control using Hedges’s g [16]. In order to avoid overestimation of the true effect size, change scores were preferred over pre- and post-treatment scores. Pre- and post-treatment means or exact F, t or p values for main effect of treatment group (augmentation or placebo) were used when change scores were not reported. For both lamotrigine and topiramate, calculations of effect sizes and P-values were based on the data used by Sommer et al., (2011) from studies by Tiihonen et al. (2003), Kremer et al. (2004), Zoccali et al. (2007), study 464 by Goff et al., (2007) and Tiihonen et al. (2005) [17,12,18,14]. A post-hoc analysis was performed with the original data on patients receiving clozapine in study 926 by Goff et al. (2007) [13].

We conducted 3 metaanalyses on the total Positive and Negative Syndrome Scale (PANSS) score, the positive and negative subscores of the PANSS. We used Brief Psychiatric Rating Scale (BPRS) scores if PANSS scores were not available. We preferred Scale for the Assessment of Negative Symptoms (SANS) above PANSS negative subscale or BPRS-negative subscale to assess negative symptoms. In all 3 analyses Hedges’s g was used as a formulation for the standard mean difference (SMD). Because of the heterogeneity in the methods applied and the limited number of studies, a random effects model was chosen, allowing for greater generalization [19,20]. We calculated homogeneity
statistic $I^2$ to determine whether studies could be taken together to share a common population effect size [21]. High heterogeneity (i.e., $I^2 \geq 50\%$; 30–50% was considered moderate) indicates heterogeneity of the individual study effect sizes, posing a limitation to a reliable interpretation of the results. Potential outlier studies were excluded from the metaanalysis. The first metaanalysis was applied in order to pool the findings of 3 glycine studies. The other 2 metaanalyses were performed to assess the effect of lamotrigine and topiramate as add-on therapy to clozapine on overall, positive and negative symptoms of schizophrenia.

Results

Glutamate agonists

Glutamate agonists in combination with clozapine have no effect on symptoms of schizophrenia, cognitive functioning or affective symptoms (Table 1A, B) [22–28], with the apparent exception of ampakine CX516, a positive modulator of the AMPA receptor: with this agent, negative, overall clinical symptoms and cognitive functioning all improved significantly after 4 weeks [27]. However, these findings must be regarded as preliminary because of the small sample size and the fact that the ampakine CX516 groups were substantially and significantly more impaired on cognitive function than the placebo group. Ampakine CX516 was well tolerated. The short trial duration in 6 of 7 randomized placebo-controlled trials of clozapine augmentation with glutamate agonists is a limitation in this analysis.

Table 1A Double-blind, placebo-controlled randomized trials of clozapine augmentation with glutamate agonists in refractory schizophrenia.

<table>
<thead>
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<th>Study</th>
<th>Statistical analysis</th>
<th>Adjunctive agent (maximum dose)</th>
<th>N (phase 1/2)</th>
<th>Trial duration (weeks)</th>
<th>Outcome of measure</th>
<th>ES</th>
<th>P-Value</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potkin et al., 1999</td>
<td>Completer analysis</td>
<td>Glycine (30 g)</td>
<td>9</td>
<td>12</td>
<td>Positive symptoms (BPRS-P)</td>
<td>-1.052</td>
<td>0.025</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Placebo</td>
<td>10</td>
<td></td>
<td>Negative symptoms (SANS)</td>
<td>-0.228</td>
<td>0.605</td>
<td>=</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Overall clinical symptoms (BPRS)</td>
<td>-0.437</td>
<td>0.326</td>
<td>=</td>
</tr>
<tr>
<td>Evins et al., 2000</td>
<td>Completer analysis</td>
<td>Glycine (60 g)</td>
<td>14</td>
<td>8</td>
<td>Positive symptoms (PANSS-P)</td>
<td>-0.545</td>
<td>0.152</td>
<td>=</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Placebo</td>
<td>13</td>
<td></td>
<td>Negative symptoms (SANS)</td>
<td>0.186</td>
<td>0.619</td>
<td>=</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Overall symptoms of schizophrenia (Total PANSS)</td>
<td>-0.075</td>
<td>0.840</td>
<td>=</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Cognitive functioning (*)</td>
<td>x</td>
<td>x</td>
<td>=</td>
</tr>
<tr>
<td>Diaz et al., 2005</td>
<td>Completer analysis</td>
<td>Glycine (60 g)</td>
<td>5/6</td>
<td>14 + 14 (cross-over)</td>
<td>Positive symptoms (PANSS-P)</td>
<td>-0.444</td>
<td>0.286</td>
<td>=</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Placebo</td>
<td>6/5</td>
<td></td>
<td>Negative symptoms (PANSS-N)</td>
<td>-0.237</td>
<td>0.564</td>
<td>=</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Overall symptoms of schizophrenia (Total PANSS)</td>
<td>-0.022</td>
<td>0.956</td>
<td>=</td>
</tr>
</tbody>
</table>

BPRS, Brief Psychiatric Rating Scale; BPRS-P, BPRS-positive symptom scale; PANSS, Positive and Negative Syndrome Scale; PANSS-P, PANSS-positive symptom scale; PANSS-N, PANSS-negative symptom scale; SANS, Scale for the Assessment of Negative Symptoms

(*) Stroop, WAIS vocabulary, information, digit span, and block design subtests, California Verbal Learning Test, finger tapping and judgment of line orientation

Not explicitly stated whether treatment or placebo group showed greater improvement

x ES and P-Value could not be calculated with reported data

* Significant positive effect

- Significant negative effect

= No significant effect
Metaanalysis of glycine

We analyzed the efficacy of glycine for positive, negative and overall symptoms of schizophrenia in all 3 studies, including a total of 57 patients [22–24]. Glycine treatment was associated with a significant worsening of positive symptoms compared to placebo (ES=–0.644, p=0.008; 95% CI=–1.117 to –0.171) (Fig. 1a). Studies were homogeneous ($I^2=0.000\%$). For negative symptoms glycine did not differ from placebo treatment (ES=–0.069, p=0.770; 95% CI=–0.528 to 0.391; $I^2=0.000\%$) (Fig. 1b). For total symptom severity no significant differences were found between glycine and placebo (ES=–0.159, p=0.499; 95% CI=–0.619 to 0.301; $I^2=0.000\%$) (Fig. 1c).

**Figure 1a.** Meta-analysis of glycine augmentation of clozapine for positive symptom score (PANSS-P / BPRS-P)

**Figure 1b.** Meta-analysis of glycine augmentation of clozapine for negative symptom score (PANSS-N / SANS)
Glutamate antagonists

We found twelve randomized controlled trials of glutamate antagonists in combination with clozapine in refractory schizophrenia (Table 2–4).

Lamotrigine

Lamotrigine is an antagonist of postsynaptic voltage-sensitive sodium channels, decreasing presynaptic release of glutamate [17]. Of 6 trials on lamotrigine addition to clozapine (Table 2A, B) [17,12,18,13,29], 2 studies show promising clinical improvement in outpatients [18,13]. In all 6 studies lamotrigine was well tolerated and no significant differences in adverse events were observed in the lamotrigine group versus placebo group.

In a 14-week crossover trial by Tiihonen et al. (2003) in hospitalized patients with clozapine-resistant schizophrenia, lamotrigine with a maximum dosage of 200 mg/day did not significantly improve positive or negative symptoms [17]. 21% of patients in the lamotrigine group showed a reduction of at least 3 points in the PANSS positive symptoms scale vs. 3% in the placebo group. Subanalyses suggest that those patients with a shorter duration of illness and those patients who are most resistant to clozapine treatment (having high PANSS positive symptom scores) benefit most from this augmentation strategy [30].

In a randomized controlled trial by Kremer et al. (2004) 25 hospitalized patients with treatment-resistant schizophrenia were allocated to lamotrigine in addition to their
<table>
<thead>
<tr>
<th>Study</th>
<th>Adjunctive agent</th>
<th>ES</th>
<th>P-Value</th>
<th>Outcome of measure</th>
<th>Total duration (weeks)</th>
<th>N (phase 1/2)</th>
<th>Adjunctive agent (maximum dose)</th>
<th>P-Value</th>
<th>ES</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.996</td>
<td>0.022</td>
<td>Overall symptoms (PANSS)</td>
<td>0.277</td>
<td>4/6/8</td>
<td>Glycine (60 g)</td>
<td>0.605</td>
<td>0.84</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.954</td>
<td>0.044</td>
<td>Positive symptoms (PANSS-P)</td>
<td>0.196</td>
<td>4/6/8</td>
<td>Placebo</td>
<td>0.326</td>
<td>0.44</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.075</td>
<td>x</td>
<td>Cognitive functioning (PANSS)</td>
<td>0.152</td>
<td>4/6</td>
<td>Placebo</td>
<td>0.025</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.196</td>
<td>0.052</td>
<td>Negative symptoms (PANSS-N)</td>
<td>0.228</td>
<td>4/6</td>
<td>Glycine (60 g)</td>
<td>0.025</td>
<td>0.19</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.128</td>
<td>0.052</td>
<td>Positive symptoms (BPRS-P)</td>
<td>0.228</td>
<td>4/6</td>
<td>Placebo</td>
<td>0.025</td>
<td>0.19</td>
</tr>
</tbody>
</table>

**Table 1A**

Double-blind, placebo-controlled, randomized trials of clozapine augmentation with glutamate agonists in refractory schizophrenia.
Table 1B  Double-blind, placebo-controlled randomized trials of clozapine augmentation with glutamate agonists in refractory schizophrenia.

<table>
<thead>
<tr>
<th>Study</th>
<th>Adjunctive agent (maximum dose)</th>
<th>N (phase 1/2)</th>
<th>Trial duration (weeks)</th>
<th>Outcome of measure</th>
<th>ES</th>
<th>P Value</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tsai et al., 1999</td>
<td>D-serine (30 mg/kg) Placebo</td>
<td>10 10</td>
<td>6</td>
<td>Positive symptoms (PANSS-P)</td>
<td>0.395</td>
<td>0.361</td>
<td>=</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Negative symptoms (SANS)</td>
<td>0.326</td>
<td>0.450</td>
<td>=</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Overall symptoms of schizophrenia (Total PANSS)</td>
<td>x</td>
<td>x</td>
<td>=</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Affective symptoms (HDRS)</td>
<td>x</td>
<td>x</td>
<td>=</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Cognitive functioning (Wisconsin Card Sorting Test)</td>
<td>1.059</td>
<td>0.021</td>
<td>=</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(Percentage of perseverative errors)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Goff et al., 1999</td>
<td>D-cycloserine (50 mg) Placebo</td>
<td>6/5 11/6</td>
<td>6 + 1 + 6 (cross-over)</td>
<td>Positive symptoms (PANSS-P)</td>
<td>x</td>
<td>x</td>
<td>=</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Negative symptoms (SANS)</td>
<td>-0.393</td>
<td>0.168</td>
<td>=</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Overall symptoms of schizophrenia (Total PANSS)</td>
<td>x</td>
<td>x</td>
<td>=</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Affective symptoms (HAM-D)</td>
<td>x</td>
<td>x</td>
<td>=</td>
</tr>
<tr>
<td>Goff et al., 2001</td>
<td>Ampakine CX516 (3600 mg) Placebo</td>
<td>12 6</td>
<td>4</td>
<td>Positive symptoms (PANSS-P)</td>
<td>0.200</td>
<td>0.675</td>
<td>=</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Negative symptoms (PANSS-N)</td>
<td>1.754</td>
<td>0.002</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Overall symptoms of schizophrenia (Total PANSS)</td>
<td>1.352</td>
<td>0.010</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Affective symptoms (HAM-D)</td>
<td>0.043</td>
<td>0.927</td>
<td>=</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Cognitive functioning (Wisconsin Card Sorting Test, no. correct)</td>
<td>-0.169</td>
<td>0.723</td>
<td>=</td>
</tr>
<tr>
<td>Lane et al., 2006</td>
<td>Sarcosine (2 g) Placebo</td>
<td>10 10</td>
<td>6</td>
<td>Positive symptoms (PANSS-P)</td>
<td>0.075</td>
<td>0.862</td>
<td>=</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Negative symptoms (PANSS-N)</td>
<td>-0.068</td>
<td>0.873</td>
<td>=</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Overall symptoms of schizophrenia (Total PANSS)</td>
<td>-0.214</td>
<td>0.619</td>
<td>=</td>
</tr>
</tbody>
</table>

HDRS, Hamilton Depression Rating Scale
^ ES was based on the standardized mean difference, calculated by Tihonen et al. (2003)
For more abbreviations see Table 1A
Double-blind, placebo-controlled randomized trials of clozapine augmentation with lamotrigine in refractory schizophrenia.

### Table 2A

#### Significance

<table>
<thead>
<tr>
<th>Study</th>
<th>Statistical analysis</th>
<th>Adjunctive agent (maximum dose)</th>
<th>N</th>
<th>Treatment duration (weeks)</th>
<th>Outcome of measure</th>
<th>P-Value</th>
<th>ES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tiihonen et al., 2003</td>
<td>LOCF analysis</td>
<td>Lamotrigine (400 mg)</td>
<td>12</td>
<td>14</td>
<td>Positive symptoms (PANSS-P)</td>
<td>0.049</td>
<td>0.49</td>
</tr>
<tr>
<td>Kremer et al., 2004*</td>
<td>LOCF analysis</td>
<td>Lamotrigine (400 mg)</td>
<td>22</td>
<td>24</td>
<td>Negative symptoms (PANSS-N)</td>
<td>0.182</td>
<td>0.013</td>
</tr>
<tr>
<td>Zoccali et al., 2007</td>
<td>Completer analysis</td>
<td>Lamotrigine (400 mg)</td>
<td>26</td>
<td>26</td>
<td>Overall symptoms of schizophrenia (Total PANSS)</td>
<td>0.454</td>
<td>0.300</td>
</tr>
<tr>
<td>Goff et al., 2007 (Study 464)</td>
<td>LOCF analysis</td>
<td>Lamotrigine (400 mg)</td>
<td>9</td>
<td>0.079</td>
<td>Positive symptoms (PANSS-P)</td>
<td>0.929</td>
<td>0.481</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Negative symptoms (PANSS-N)</td>
<td>0.726</td>
<td></td>
</tr>
</tbody>
</table>

**For more abbreviations see Table 1**

SAPS: Scale for the Assessment of Positive Symptoms; SANS: Scale for the Assessment of Negative Symptoms; CDSS: Calgary Depression Scale for Schizophrenia; GOAT: Global Assessment of Treatment; PANSS: Positive and Negative Syndrome Scale; BPRS: Brief Psychiatric Rating Scale.
Table 2B  Double-blind, placebo-controlled randomized trials of clozapine augmentation lamotrigine in refractory schizophrenia.

<table>
<thead>
<tr>
<th>Study</th>
<th>Adjunctive agent (maximum dose)</th>
<th>N</th>
<th>Trial duration (weeks)</th>
<th>Outcome of measure</th>
<th>ES</th>
<th>P-Value</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Goff et al., 2007</td>
<td>Lamotrigine (400 mg)</td>
<td>21</td>
<td>12</td>
<td>Positive symptoms (PANSS-P)</td>
<td>0.101</td>
<td>0.738</td>
<td>=</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>21</td>
<td></td>
<td>Negative symptoms (SANS)</td>
<td>0.860</td>
<td>0.034</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Overall symptoms of schizophrenia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(Total PANSS)</td>
<td>0.465</td>
<td>0.130</td>
<td>=</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Affective symptoms (CDSS)</td>
<td>−0.111</td>
<td>0.713</td>
<td>=</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Cognitive functioning (BACS)</td>
<td>−0.565</td>
<td>0.068</td>
<td>=</td>
</tr>
<tr>
<td>LOCF analysis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vayisoglu et al., 2013</td>
<td>Lamotrigine (200 mg)</td>
<td>16</td>
<td>12</td>
<td>Positive symptoms (PANSS-P)</td>
<td>0.034</td>
<td>0.921</td>
<td>=</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>17</td>
<td></td>
<td>Negative symptoms (PANSS-N)</td>
<td>−0.459</td>
<td>0.193</td>
<td>=</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Overall symptoms of schizophrenia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(Total PANSS)</td>
<td>−0.717</td>
<td>0.041</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Affective symptoms (CDSS)</td>
<td>−0.250</td>
<td>0.464</td>
<td>=</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Cognitive functioning (Wisconsin Card Sorting Test, perseverative error)</td>
<td>0.533</td>
<td>0.124</td>
<td>=</td>
</tr>
</tbody>
</table>

∞ From this trial of lamotrigine addition to atypical antipsychotic medication, we extracted data from 42 patients receiving clozapine
CGI, CGI-S, Clinical Global Impression Severity of Illness scale; BACS, Brief Assessment of Cognition in Schizophrenia
For more abbreviations see Table 1
Table 3

Double-blind, placebo-controlled randomized trials of clozapine augmentation with topiramate in refractory schizophrenia.

<table>
<thead>
<tr>
<th>Study</th>
<th>Adjunctive agent (maximum dose)</th>
<th>Outcome of measure (weeks)</th>
<th>N (Trial duration)</th>
<th>Adjunctive agent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tiihonen et al., 2005</td>
<td>Topiramate (300 mg)</td>
<td>Positive symptoms (PANSS-P)</td>
<td>35</td>
<td>placebo</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Negative symptoms (PANSS-N)</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Total symptoms (PANSS)</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>Afshar et al., 2008</td>
<td>No drop outs</td>
<td>Positive symptoms (PANSS-P)</td>
<td>16</td>
<td>Topiramate (300 mg)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Negative symptoms (PANSS-N)</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>Muscatello et al., 2010</td>
<td>Topiramate (200 mg-300 mg)</td>
<td>Positive symptoms (PANSS-P)</td>
<td>24</td>
<td>Topiramate (300 mg)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Negative symptoms (PANSS-N)</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Total symptoms (PANSS)</td>
<td>24</td>
<td></td>
</tr>
</tbody>
</table>

**Outcome of measure**

- Positive symptoms (PANSS-P)
- Negative symptoms (PANSS-N)
- Total symptoms (PANSS)

**Significance**

- T-test

**ES (Effect Size)**

- 0.82

**P-Value**

- <0.001

**Note**

For more abbreviations see Table 1.
Table 4 Double-blind, placebo-controlled randomized trials of clozapine augmentation with memantine in refractory schizophrenia.

<table>
<thead>
<tr>
<th>Study</th>
<th>Adjunctive agent (maximum dose)</th>
<th>N</th>
<th>Trial duration (weeks)</th>
<th>Outcome of measure</th>
<th>ES</th>
<th>P-Value</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lucena et al., 2009</td>
<td>Memantine (20 mg)</td>
<td>10</td>
<td>12</td>
<td>Positive symptoms (BPRS-P)</td>
<td>1.325</td>
<td>0.002</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>11</td>
<td></td>
<td>Negative symptoms (BPRS-N)</td>
<td>3.197</td>
<td>&lt;0.001</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>No drop outs</td>
<td></td>
<td></td>
<td>Overall clinical symptoms (BPRS)</td>
<td>2.640</td>
<td>&lt;0.001</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Cognitive functioning (MMSE)</td>
<td>1.267</td>
<td>0.003</td>
<td>+</td>
</tr>
</tbody>
</table>

For more abbreviations see Table 1
ongoing treatment with antipsychotics, while 13 patients received placebo [12]. Tiihonen et al. (2009) extracted the data of 4 patients who were on clozapine (1 of these 4 patients completed the trial) [31]. Lamotrigine was gradually titrated up to 400 mg/day during the last two weeks of the 10-week trial. No significant benefits could be determined in these few patients.

In a study by Zoccali et al. (2007), placebo or lamotrigine with a maximum dosage of 200 mg/day was gradually added to ongoing clozapine treatment during 8 weeks in outpatients with clozapine-resistant schizophrenia [18]. Lamotrigine proved to have beneficial effects on positive, negative and overall symptoms of schizophrenia. Cognitive functions did not significantly improve in the lamotrigine group compared with the placebo group, except for semantic fluency at week 24 (ES=1.01).

Goff et al. (2007) conducted 2 trials (study 464 and study 926) in predominantly outpatients with residual psychotic symptoms [13]. Lamotrigine was gradually titrated up to 200 mg/day in the first 6 weeks, and in some cases increased up to a maximum of 400 mg/day (mean lamotrigine doses achieved 205 mg/day in study 464 and 241 mg/day in study 926). Trial duration was 12 weeks. Unlike study 464, which did not find significant differences in positive, negative and overall symptoms of schizophrenia compared with the placebo group, study 926 found a significant positive effect on negative symptoms.

Vayısoğlu et al. (2013) found no significant effect of lamotrigine as an add-on to clozapine compared with placebo on clinical symptoms of schizophrenia, affective symptoms or cognitive functioning [29].

Perhaps a lamotrigine dose of 200 mg/day is insufficient: in 2 out of 3 studies this dosage was not efficacious [17,29]. A post-hoc comparison of the 200 mg group with the 400 mg group of the Tiihonen et al. (2003) and Kremer et al. (2004) studies found a superior effect for the higher dose [32], but a problem with this analysis is the inclusion of 19 patients on non-clozapine antipsychotics in the Kremer et al. (2004) study. In studies on lamotrigine as an adjunct to clozapine, lamotrigine dosages of both 200 mg/day and 400 mg/day showed beneficial effects in two separate studies. Therefore, no conclusions regarding optimal dosage can be given. Different titration schemes in the above-mentioned studies limit the effective treatment duration with the maximum dose to 2 weeks [12], 4 weeks [17], 6 weeks [13], 7 weeks [29] to 8 weeks [18]. Two 12-week trials showed no beneficial effect of lamotrigine [13,29]. However, after the same treatment duration of 12 weeks in the slightly larger study 926 by Goff et al. (2007) the lamotrigine group showed moderate significant improvement in overall symptoms of schizophrenia [13]. A closer look reveals that two trials
with longer treatment duration (study 926 by Goff et al. and Zoccali et al., 2007) show more positive effects than the trials with shorter treatment duration [13,18].

Metaanalysis of lamotrigine

Results on positive, negative and overall symptom severity were combined in a metaanalysis of all 6 studies, including a total of 185 patients [17,12,18,13,29]. Lamotrigine in addition to clozapine did not significantly change positive symptoms compared to placebo, but a trend towards reducing positive symptoms was found (ES=0.314, \(p=0.065\); 95% CI=--0.020 to 0.648) (Fig. 2a). Studies were homogeneous (\(I^2=26.260\%\)). Lamotrigine did not differ from placebo with regard to reducing negative symptoms (ES=0.367, \(p=0.163\); 95% CI=--0.148 to 0.883) (Fig. 2b). Heterogeneity was high (\(I^2=67.572\%\)). After excluding 2 outlier studies by Zoccali et al. (2007) and Vayısoğlu et al. (2013), the ES bordered on significant 0.352 (\(p=0.065\); 95% CI=--0.021 to 0.725; \(I^2=0.000\%\)) [18,29]. Lamotrigine did not significantly improve total symptom severity compared to placebo treatment (ES=0.315, \(p=0.297\); 95% CI=--0.277 to 0.906) (Fig. 2c). However, heterogeneity was high (\(I^2=75.141\%\)). Again, the studies by Zoccali et al. (2007) and Vayısoğlu et al. (2013) were identified as outlier studies; after their exclusion ES was reduced to 0.308 (\(p=0.104\); 95% CI=--0.063 to 0.679; \(I^2=0.000\%\)). For affective symptoms we analyzed 3 studies (N=126) [18,13,29]. Clozapine add-on treatment with lamotrigine showed no superior efficacy compared to placebo (ES=0.070, \(p=0.765\); 95% CI=--0.389 to 0.529). Heterogeneity was moderate (\(I^2=42.723\%\)).

Topiramate

Topiramate has a mixed profile of both gamma-aminobutyric acid (GABA)-ergic and antiglutamatergic actions [14,33]. Presynaptic glutamate release is reduced because topiramate is an antagonist for postsynaptic kainate receptors and amino-3-hydroxy-5-methyl-isoxazoloe-4-propionic acid (AMPA) receptors. 2 out of 4 trials with topiramate added to clozapine show beneficial effects in refractory schizophrenia (Table 3) [34,35]. In 3 trials topiramate augmentation was found to be well tolerated and adverse events did not differ significantly between the topiramate group and the placebo group [14,34,35], but in another study 30% of topiramate subjects failed to complete the trial due to side effects.
such as sleepiness, ataxia, psychomotor slowness and exacerbation of positive symptoms [33]. This difference may be due to the short titration scheme in this trial, with a 50 mg/day increment every 2 days (in 6 days increase of topiramate to 200 mg or in ten days increase of topiramate to 300 mg).

One out of 3 studies found clinical improvement in hospitalized patients with treatment-resistant schizophrenia after topiramate augmentation [14,34,33]. In a study by Afshar et al. (2008) topiramate proved to be efficacious, with a significant decrease in negative and positive symptoms and an impressive clinical response (defined as >20% reduction in PANSS) in 50% of topiramate-treated subjects versus 12.5% in controls [34]. In Tiihonen et al. (2005) 22 patients received topiramate in addition to their ongoing atypical antipsychotic medication and 23 patients received placebo over two 12-week crossover treatment periods [14]. A subanalysis of 14 patients receiving clozapine did not show significant improvement in positive, negative and overall symptoms of schizophrenia compared to placebo. The study by Behdani et al. (2011) even found more improvement in positive and negative symptoms of schizophrenia in the placebo group than in the topiramate group; however, this difference was not significant [33].

The efficacy of topiramate augmentation of clozapine was confirmed in a single study of outpatients by Muscatello et al. (2010) [35]. Positive and negative symptoms improved significantly after a relatively long treatment duration of 24 weeks with a topiramate dosage of only 200 g/day compared to placebo.

**Metaanalysis of topiramate**

The combined results of all 4 studies (N=152) showed topiramate to have similar effects to placebo for positive symptoms (ES=0.412, p=0.153; 95% CI=–0.153 to 0.977) (Fig. 3a) [14,33–35]. Studies showed large heterogeneity ($I^2=64.549\%$). Topiramate was not superior to placebo treatment for negative symptoms either (ES=0.400, p=0.321; 95% CI=–0.390 to 1.189) (Fig. 3b). Studies were highly heterogeneous ($I^2=81.395\%$). For overall symptom severity we analyzed 3 studies (N=9) [14,34,35]. Topiramate studies yielded no significant difference between topiramate and placebo for total symptom score (ES=0.754, p=0.068; 95% CI=–0.055 to 1.564) (Fig. 3c). Studies showed high heterogeneity ($I^2=69.439\%$).
**Memantine**

Memantine is a voltage-dependent antagonist of the NMDA receptor, which binds more strongly than Mg\(^{2+}\) [36]. A small proof-of-concept study by Lucena et al. (2009) is the only randomized controlled trial on clozapine augmentation with memantine (Table 4). Interestingly, negative symptoms in particular were reduced, with a very high effect size (ES=−3.33) in this study population of outpatients with refractory schizophrenia and prevailing negative symptoms. The effect size of therapeutic effects on positive symptoms, overall clinical symptoms, global clinical impression and cognitive functions was also impressive after 3 weeks of titration and 9 weeks of treatment with the maximum dosage of 20 mg of memantine (range ES=−2.75 to ES=1.32). A remarkable improvement of cognitive function was found, using the Mini-Mental State Examination (MMSE). In future research a sensitive and extensively validated cognitive testing battery should be used for cognitive assessment. Due to the small sample size there are insufficient data to justify a conclusion about the safety and tolerability of memantine in combination with clozapine in patients with schizophrenia, although Lucena et al. (2009) found no adverse events, no significant changes in weight and no extrapyramidal symptoms.

**Discussion**

With NMDA receptor hypofunction as underlying pathological mechanism of schizophrenia in mind, addition of a glutamate agonist seems a reasonable treatment approach. Addition of NMDA receptor modulators to antipsychotics has additional therapeutic benefits due to agonistic activity at the NMDA receptor [37]. Clozapine is itself a glutamate agonist, which is probably why it has superior antipsychotic efficacy to dopamine antagonists in treatment-refractory schizophrenia. Clozapine improves glutamatergic neurotransmission in several different ways. Clozapine has preferential antagonist activity at dopamine 4 receptors, resulting in upregulation of AMPA receptors and consequently improvement of glutamatergic neurotransmission [9]. Clozapine enhances activation of the NMDA receptor through induction of release of D-serine by glial cells. Clozapine also stimulates glial cells to release glutamate, resulting in activation of postsynaptic metabotropic glutamate (mGlu) receptors and upregulation of NMDA receptors [10]. This leads to improvement in glutamatergic tonus in brain areas such as the prefrontal, thalamic and cerebro-cerebellar regions,
which may explain the unique therapeutic effect of clozapine.

However, when clozapine is combined with a NMDA receptor agonist, selective and concurrent activation of mGlu receptors and NMDA receptors leads to downregulation of NMDA receptors [38]. This explains we found no favourable effects of NMDA receptor agonists in combination with clozapine in 6 double-blind placebo-controlled randomized trials. The combined results of 3 glycine studies even show significant worsening of positive symptoms compared to placebo. We found one single positive study with a glutamate agonist – ampakine CX516 [27]. This can be explained by the differential effect of ampakine CX516: unlike NMDA receptor agonists, ampakine CX516 is a positive modulator of the AMPA receptor and does not activate the NMDA receptor directly. This is why the combination of clozapine and ampakine CX516 does not lead to downregulation of NMDA receptors.

We found a significant therapeutic effect of lamotrigine augmentation of clozapine in 2 out of 6 trials. Perhaps a lamotrigine dose of 200 mg/day is adequate, since this dosage was efficacious in reducing positive, negative and overall symptoms of schizophrenia after an effective treatment duration of eight weeks [18]. However, no conclusions are justified on the basis of this small study. 2 shorter trials with a treatment duration of 4 and 7 weeks respectively, with the same maximum lamotrigine dose of 200mg/day, failed to replicate these positive results [17,29]. The combined results of 6 studies showed a trend towards reduction in positive symptoms and negative symptoms. There is scarce and conflicting evidence of the efficacy of topiramate addition in clozapine-resistant patients. A minimum topiramate dosage of 200 mg/day seems necessary. In 1 out of 2 trials showing beneficial effect the topiramate dosage was 200 mg/day [35]. A metaanalysis of topiramate studies showed no significant change or trend compared to placebo. Both lamotrigine and topiramate antagonize excitotoxic actions of glutamate by reduction of presynaptic glutamate release [17,14,33].

Memantine emerges as a superior augmentation strategy, with very large effect sizes on positive symptoms, negative symptoms, overall clinical symptoms of schizophrenia and cognitive functioning [36]. However, one small trial does not allow us to draw a definitive conclusion. This possible unique therapeutic effect of memantine addition to clozapine may be due to the fact that as an antagonist for NMDA receptors memantine prevents simultaneous activation of mGlu receptors and NMDA receptors and blocks induction of downregulation of NMDA receptors [38]. Therefore, the combination of clozapine and memantine results in upregulation of NMDA receptors. Clozapine combined with memantine
may ameliorate positive symptoms through enhancement of the projection of glutamate from the orbitofrontal and prefrontal cortex on the amygdala [39]. In schizophrenia NMDA hypofunction has a disinhibitory effect, causing abnormal cortical signal-to-noise patterns with prefrontal noise and reduction of transmission efficacy of cortical neurons, resulting in negative symptoms and cognitive deficits, associated with frontal lobe dysfunction [40]. Memantine is hypothesized to induce a decrease of prefrontal noise. Only a strong stimulus results in activation of the NMDA receptor, resulting in improved glutamatergic tonus and dopaminergic neurotransmission in the frontal cerebral cortex, explaining decrease of negative symptoms and improvement of prefrontal cortex-dependent cognitive functions [41]. Excessive glutamate spillover due to dysfunction of the NMDA system leads to reduced synaptic connections and neuronal excitotoxicity in the PFC, also causing cognitive impairment. In the presence of glutamate spillover in the synaptic cleft, memantine is considered to be a neuroprotective drug, decreasing neuronal cell death [40]. By preventing the neurotoxic Ca\(^{2+}\) influx, memantine might even be able to attenuate progressive cognitive impairment [6].

**Conclusion**

A growing body of evidence indicates that the glutamatergic system is implicated in the pathophysiology of schizophrenia and may represent a target for intervention. Clozapine is a glutamate agonist, affecting the glycine site of the NMDA receptor by inducing the release of D-serine by glial cells and activating mGlu receptors through subsequent release of glutamate by glial cells. Hence clozapine results in upregulation of NMDA receptors. Through antagonist activity at dopamine 4 receptors clozapine induces upregulation of AMPA receptors. Treatment-resistant patients do not benefit from the combination of clozapine and a NMDA receptor-related agonist, as shown by 6 negative trials. Ongoing synaptic activity results in persistent downregulation of NMDA receptors, which are hypothesized to be hypofunctional in schizophrenia. Ampakine CX516, in one small trial, seems to be the only glutamate agonist to have significant benefits, especially on negative symptoms, as an adjunct to clozapine. Presumably this AMPA receptor agonist improves glutamatergic neurotransmission and does not activate the NMDA receptor directly, which is why ampakine CX516 does not lead to downregulation of NMDA receptors like other NMDA receptor agonists. However, this preliminary positive result awaits replication.

Glutamate antagonists are promising as adjunctive therapy to clozapine, because this particular combination modulates glutamatergic neurotransmission at multiple levels,
acting at the AMPA (clozapine), NMDA (clozapine, memantine and amantadine) and mGlu receptors (clozapine) or affecting presynaptic glutamate release (lamotrigine and topiramate). Lamotrigine and topiramate reduce presynaptic glutamate release and antagonize excitotoxic actions of glutamate. To create an improved glutamatergic balance in the brain, study results indicate that sufficient duration of combination therapy of a glutamate antagonist and clozapine is necessary to achieve efficacious response. Limited evidence suggests that a minimum of 200 mg/day lamotrigine and a minimum of 200 mg/day topiramate may be necessary to achieve response. A metaanalysis of lamotrigine studies showed only a trend towards reduction of residual positive symptoms and negative symptoms, whereas a metaanalysis on topiramate showed no significant difference between topiramate and placebo. Memantine is a voltage dependent low-to-moderate-affinity channel-blocking NMDA receptor antagonist. Due to these neuroprotective properties memantine is hypothesized to improve cortical signal-to-noise patterns and transmission efficacy of cortical neurons in schizophrenia. In the presence of glutamate spillover in the synaptic cleft due to NMDA receptor hypofunction, memantine reduces neurotoxic Ca\(^{2+}\) influx. In combination with clozapine memantine blocks concurrent activation of mGlu receptors and NMDA receptors and thus causes upregulation of NMDA receptors. Early intervention with clozapine combined with memantine might interfere with the excitotoxic process, limiting cognitive impairment in schizophrenia. In one small study memantine was found to have an impressive effect size after a treatment duration of only nine weeks with a maximum dosage of 20 mg/day. A subgroup of schizophrenia patients with prevailing negative symptoms might benefit specifically from the combination of clozapine and NMDA receptor antagonists.

At present there is no solid evidence indicating how patients with schizophrenia suffering from clozapine-resistant symptoms can be helped [5,6]. Glutamate antagonists are a promising augmentation strategy. Memantine seems the most promising candidate, because it improves the expression and regulation of NMDA receptors. Significant efficacy (with large effect sizes) for all symptoms of schizophrenia, including cognitive functioning, was demonstrated in one small study. Large-scale, placebo-controlled trials are required to determine the efficacy, safety and sufficient duration of adjunctive therapy with glutamate antagonists in clozapine-resistant patients.
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Chapter 6

Memantine augmentation in clozapine-refractory schizophrenia: a randomized, double-blind, placebo-controlled crossover study

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What is the short-term efficacy and tolerability of memantine in clozapine-refractory schizophrenia?

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Abstract

Background.
Dysfunction of neuroplasticity due to N-methyl-D-aspartate (NMDA) receptor hypofunction may be a causal factor for memory and executive dysfunctioning in schizophrenia. Deregulation of NMDA transmission in the prefrontal cortex may also explain negative and positive symptoms. Clozapine augmentation with memantine targets altered NMDA receptor-mediated neurotransmission in schizophrenia and showed substantial beneficial effects on several symptom domains in a small proof-of-concept study. We evaluate effects of memantine add-on treatment to clozapine for memory and executive function, negative and positive symptoms in schizophrenia.

Method.
Clozapine-treated patients with refractory schizophrenia were randomly assigned to 12 weeks of double-blind adjunctive treatment with memantine (n=26) or placebo (n=26). Crossover occurred after a 2-week placebo wash-out period. Primary endpoints were change from baseline to 12 weeks treatment and 14 weeks to 26 weeks treatment on memory and executive function using the Cambridge Neuropsychological Test Automated Battery (CANTAB), Positive and Negative Syndrome Scale (PANSS), and Clinical Global Impression Severity Scale (CGI-S). Side effects were assessed using the Liverpool University Neuroleptic Side-Effect Rating Scale (LUNSERS).

Results.
When compared with placebo, memantine improved a composite memory score comprising verbal recognition memory and paired associates learning task scores on the CANTAB (effect size=0.30) and PANSS negative subscale score (effect size=0.29). Side effects were mild and transient.

Conclusions.
In patients with clozapine-treated refractory schizophrenia, memantine addition significantly improved verbal and visual memory and negative symptoms without serious adverse effects. These results justify further investigations on long-term memantine augmentation to clozapine in treatment-resistant schizophrenia.
Introduction

Although clozapine is efficacious for treatment-resistant schizophrenia patients, as many as 70% of patients show only a partial response (Hasan et al. 2012). Polypharmacy is frequently used, however evidence concerning additional pharmacological treatment of refractory schizophrenia is limited (Muscatello et al. 2014; Veerman et al. 2014a, b). Novel avenues of research are needed to bring about improved drug treatment of schizophrenia.

On the basis of the glutamate hypothesis, with hypofunction of the glutamate N-methyl-D-aspartate (NMDA) receptor as an underlying mechanism for schizophrenia (Stone et al. 2007; Kantrowitz & Javitt, 2010), glutamate modulators can be seen as promising antipsychotic agents (Veerman et al. 2014c). The glutamate hypothesis of schizophrenia stipulates that hypofunction of the NMDA receptor is responsible for excitotoxic neurodegeneration, dysfunction of neuroplasticity and dysregulation of downstream neurons in response to glutamate release, resulting in cognitive impairment and negative symptoms (Javitt & Zukin, 1991; Bressan & Pilowsky, 2000; Howes & Kapur, 2009, Orellana & Slachevsky, 2013). Positive symptoms may develop through disinhibition of prefrontal cortical γ-aminobutyric acid (GABA) interneurons, which are responsible for recurrent inhibition of pyramidal neurons (Homayoun & Moghaddam, 2007).

Memantine acts as a low-affinity type, uncompetitive, nonselective and voltage dependent NMDA receptor antagonist (Parsons et al. 2007). Memantine is licensed for treatment of moderate-to-severe Alzheimer’s disease (AD) (Areosa et al. 2005). Efficacy in patients with moderate-to-severe AD was demonstrated in a meta-analysis of six randomized placebo-controlled trials showing modest beneficial effects on global status and cognition after treatment with memantine (Winblad et al. 2007). Memantine has a favorable safety and tolerability profile (Farlow et al. 2008).

Favorable effects of memantine addition to non-clozapine antipsychotics described in case reports and open studies were replicated in only one of three placebo-controlled trials of memantine in combination with non-clozapine antipsychotics (Lieberman et al. 2009; Lee et al. 2012; Rezaei et al. 2013). However, memantine is thought to be more promising as an adjunctive therapy to clozapine than to non-clozapine antipsychotics. One small 12-week randomized, placebo-controlled trial (n=21) demonstrated efficacy of memantine augmentation in patients with partial remission of negative symptoms of schizophrenia on clozapine treatment with large effect sizes (ESs) for overall symptoms (ES=2.75), positive symptoms (ES=1.38), negative symptoms (ES=3.33) and global cognitive functioning
The favorable effects of memantine augmentation to clozapine may be related to their conjunct action on NMDA receptors. This particular combination modulates glutamatergic neurotransmission at multiple levels (Veerman et al. 2014c): clozapine induces both upregulation of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors and NMDA receptors (Yeun et al. 2010; Tanahashi et al. 2012), and memantine may enhance further upregulation of NMDA receptors causing activation in the presence of a strong stimulus (Joshi et al. 2007). NMDA receptors are highly expressed in the hippocampus (Bliss & Collingridge, 1993). Improvement of hippocampal dysfunction and functional connectivity between brain circuits, involving the prefrontal cortex (PFC) through NMDA-receptor mediated neuroplasticity explains why combination therapy of clozapine and memantine possibly targets two specific cognitive domains: impaired memory and executive function.

Inspired by the unique functional psychopharmacological characteristics of the memantine–clozapine combination and the substantial positive findings of the first proof-of-concept study, we conducted a second, larger and more elaborate trial studying effects of adjunctive memantine therapy on memory, executive function and symptom severity in clozapine-treated patients suffering from refractory schizophrenia.

Method

Study design

The study was approved by the Central Committee on Research Involving Human Subjects and the Medical Research Ethics Committee (MREC) of Alkmaar Medical Center and was conducted in accordance with the Declaration of Helsinki (World Medical Association, 2013). The study was a 26-week single-center, double-blind trial that was randomized and placebo controlled. The trial consisted of two crossover, 12-week treatment phases and a placebo wash-out period of 2 weeks in the 13th and 14th week to avoid carryover effects (Fig. 1). Clozapine dosage and use of concomitant medications were at the discretion of the treating psychiatrist and remained as much unaltered as possible throughout the study. Subjects were randomly assigned to receive an identical number of either memantine or placebo tablets. During the memantine phase a dosage of 10 mg taken once daily was built up after 1 week to 20 mg taken once daily during 11 weeks as add-on therapy to ongoing
Fig. 1. Patient Disposition in a double-blind, randomized, placebo-controlled trial of memantine as adjunctive treatment to clozapine in refractory schizophrenia. SAE, Serious adverse event; AE, adverse event.
clozapine treatment. The dose of 20 mg/day was similar to the dosage used in all four randomized placebo-controlled trials in patients with schizophrenia.

Randomisation to starting with either memantine or placebo was designated on a 1:1 basis in blocks of four. The allocation sequence was produced independently by the pharmacist of the VU Medical Center in Amsterdam. The code was concealed for patients, care providers, raters, and investigators until all subjects completed the trial and data were entered into a computer data file.

Study population, inclusion and exclusion criteria

The study was performed from August 2013 until August 2014, at 12 Flexible Assertive Community Treatment (FACT) facilities of the Mental Health Service Organization North Holland North (Netherlands) (van Veldhuizen, 2007). The original eligibility criterium of ‘out-patients’ was broadened to patients living either independently or in a sheltered home and patients admitted to open long-stay wards, receiving care from an out-patient facility. This was reported to the MREC of Alkmaar Medical Center. Eligible subjects were between ages 18 and 60 years, met Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-IV) criteria for schizophrenia on the Mini International Neuropsychiatric Interview Plus (MINI-Plus) (Overbeek et al. 1999), and failed to achieve remission criteria proposed by Andreasen et al. (2005), defined as simultaneous ratings of mild or less (≤3 points) on eight of the following Positive and Negative Syndrome Scale (PANSS) items: P1 delusions, G9 unusual thought content, P3 hallucinatory behaviour, P2 conceptual disorganization, G5 mannerisms and posturing, N1 blunted affect, N4 passive or apathetic social withdrawal, N6 lack of spontaneity and flow of conversation. At inclusion, duration of clozapine therapy was at least 6 months with a minimum of 12 weeks with a clozapine plasmalevel above 350 ng/ml or intolerability to achieve this threshold (Schulte, 2003). Patients with a recent deterioration needing treatment in an acute treatment ward were not included. Other exclusion criteria included pregnancy, lactating women, and female subjects without adequate contraception, known hypersensitivity to memantine, comedication with glutamate modulators, lactose intolerance, uncontrolled epilepsy, myocardial infarction, uncontrolled hypertension, renal insufficiency, liver failure, or AD (Wesemann et al. 1983). The sample size was calculated at 52, based on an ES of 0.55 (α=0.05, power=0.80) and accounting for an estimated discontinuation rate of 20%. After complete description of the
study to the subjects, written informed consent was obtained by the principal investigator. Care providers distributed study medication and monitored compliance on a daily basis in patients in sheltered homes and open long-stay wards and on a weekly basis in outpatients.

Clinical assessments

We used the Cambridge Neuropsychological Test Automated Battery (CANTAB), a computerized, nonlinguistic cognitive testing battery (Levaux et al. 2007). Test selection was based on 6 cognitive domains, recommended by the Measurement and Treatment Research to Improve Cognition in Schizophrenia (MATRICS): reaction time and psychomotor speed, sustained visual attention, verbal memory, visuo-spatial memory, learning and association ability, working visuo-spatial memory and strategy use, spatial planning and motor control and emotion recognition (Nuechterlein et al. 2008, Barnett et al. 2010). One cognitive domain of the MATRICS Consensus Cognitive Battery (MCCB) was not assessed, because Intra/Extra-Dimensional Set-Shifting (IED) for reasoning and problem solving was too difficult for our patient population with severe cognitive disturbances.

Two cognitive domains were selected as primary outcomes: memory and executive function (see Table 2†). Memory was assessed by computing a composite score of the sum of the CANTAB scores of four tasks: verbal recognition memory (VRM) free recall and VRM recognition, and paired associates learning (PAL) total errors and PAL first trial memory score. To reduce practice effects a parallel form of the VRM task, equivalent in difficulty was used for the second and fourth measurement. Executive function was assessed by computing a composite of three CANTAB task scores: One Touch Stockings of Cambridge (OTS) problems solved on first choice, and spatial working memory (SWM) strategy and SWM between errors.

The PANSS was used to assess severity of positive, negative, and total symptoms of schizophrenia (Kay et al. 1987). We assessed the effect of memantine on two subdomains of negative symptoms: (1) expressive deficits [flat affect (N1), poor rapport (N3), lack of spontaneity and flow of conversation (N6), mannerisms and posturing (G5), motor retardation (G7) and avolition (G13)]; and (2) social amotivation [emotional withdrawal (N2), passive/apathetic social withdrawal (N4) and active social avoidance (G16)] (Liemburg et al. 2013; Millan et al. 2014). Global severity of psychopathology was determined by using

1 † The notes appear after the main text.
the Clinical Global Impression Severity Scale (CGI-S) (Guy, 1976).

Careful clinical procedures were performed to assess safety and tolerability of memantine add-on therapy to clozapine. Physical examination included measurements of waist circumference and blood pressure. Regular controls of white blood cell count and differentiation were combined with measurements of liver and renal function, blood glucose, lipids, and plasma clozapine level (12 ± 0.5 h after ingestion). The occurrence and intensity of side effects were assessed by self-rating on the Liverpool University Neuroleptic Side-Effect Rating Scale (LUNERS) (Day et al. 1995) augmented with rating of Likert scales for possible side effects of memantine (thrombosis, dyspnoea, and mycosis).

All outcomes were rated before treatment initiation, after 12 weeks, after 14 weeks, and after 26 weeks. Two raters were trained in diagnostic interviewing and all clinical assessments. Interrater exact and adjacent agreement (within one scale point) was 96% for the PANSS, based on seven assessments (two live patient interviews and five videotaped patient interviews).

Adverse events (AEs) were defined as any undesirable experience occurring to a subject during the study, whether or not they were considered to be related to memantine ingestion. All AEs, reported by either the subject or treatment staff, were recorded. Admission to a psychiatric hospital was no reason to break the code or for withdrawal from the study. A medical emergency was the only reason to break the code and withdraw the subject from the study.

Statistical analysis

To determine the effects of memantine on the hypothesized cognitive functions, schizophrenia symptoms, safety measures, and side effects, the two phases (memantine or placebo) of the crossover trial were compared using a linear mixed-effects model conducted in SPSS Statistics version 22.0.0 (SPSS Inc., 2014). This analytic approach can estimate random and fixed effects simultaneously (Putt & Chinchilli, 1994). A natural log transformation was applied to cognitive function scores assessed via the CANTAB, which are non-normally distributed, so that estimates from the linear mixed-effects models would be trustworthy.

We conducted the analyses using an intention-to-treat (ITT) analytic approach and a per-protocol analytic approach, which included only protocol completers. Protocol completion was defined as having completed both treatment phases without a serious
protocol violation in the memantine phase. There was no significant difference in study completion rate by random group assignment (group 1=23/26, group 2=21/26; $\chi^2_1=1.209$, $p=0.47$). We also tested a number of covariates in the model, which are potentially related to the dependent variables. Covariates included patient age and gender, years of education, age of onset, duration of psychosis, and duration of untreated psychosis. We followed the backwards trimming method described by Singer and Willet to construct our models (Singer & Willett, 2003): As covariates were entered into the model, one at a time, those that were significantly related to one of the model’s parameters were retained. Only the patient’s years of education variable was related to the slope parameter; thus, this variable was retained in the final model and the others were discarded.

We first tested a model with random intercepts. However, the models failed to converge or produced errors in the Hessian matrix, so the intercept parameter was fixed to ensure trustworthy parameter estimates. The slope parameter was treated as a fixed effect after it was found that models with random slopes resulted in worse model fit. This indicates that models with fixed intercepts and slopes (i.e. constraining these parameters to be equal across participants) do not significantly differ from models in which these parameters are individually estimated. All tests of significance were two tailed, and $\alpha$ was set to 0.05. Standardized ESs (Cohen’s $d$) were calculated (Cohen, 1988). We performed a post-hoc analysis to assess whether memantine had a more pronounced effect on expressive deficits or social amotivation. To evaluate possible differences between inpatients admitted to long-stay wards and other included patients we performed a post-hoc analysis of demographic variables and baseline characteristics and also conducted a post-hoc analysis of outpatients, excluding patients admitted to long-stay wards.

Reliability of analyses

To include the full, randomized sample in the analyses, restricted maximum likelihood estimation was used, which has been shown to provide unbiased estimates when data are missing at random or missing completely at random (MCAR) (Little & Rubin, 2002). There was some degree of missing data in our sample (see Tables 2 and 3 and online Supplementary Tables S1 and S2 for valid n’s), but the data were determined to be MCAR (39) ($\chi^2_{737}=144.40$, $p=1.00$), so the missing data did not introduce bias into the analyses.

Two steps were taken in the analytic strategy to address unique sources of potential
bias in the crossover design: (1) a period effect parameter and a period x treatment interaction were tested to determine whether the order in which memantine was received was related to the outcome and whether there was a larger effect in one of the phases of the trial; and (2) carryover effects were controlled for in all analyses.

Other possible confounders were analyzed, such as change in clozapine dosage, concomittant medication, substance use, and use of psychotherapy during the study. The element of expectation of the placebo response was tested by examining whether pretrial expectations of positive benefits of memantine moderated the effect on the outcomes of active drug and placebo. Further, we analyzed the degree of successful blinding by examining the degree of accurate appraisal of receipt of memantine from the perspective of the patient and the rater.

Results

Baseline characteristics

Fig. 1 presents participant screening and enrollment flow data. A total of 134 patients were screened, of whom 116 patients met inclusion criteria; 64 eligible patients refused participation. The remaining 52 patients gave informed consent and were enrolled. Table 1 presents demographic and clinical characteristics of the study population. Omnibus tests revealed that the two groups did not differ on any parameter except for significantly higher expectations for improvement in daily activities and living conditions in the group first assigned to placebo, compared with the group first assigned to memantine. A post-hoc analysis showed no significant differences between the six in-patients and the 46 out-patients on any of the demographic variables or baseline characteristics.

Clinical efficacy results

Analysis of effects using an ITT approach, presented in Table 2, indicated that all effects were in the direction of desired effect. The following primary outcome variables significantly improved during the memantine phase in comparison with the placebo phase: memory composite ($F_{4,655} = 0.30$, $p=0.32$); PANSS negative symptoms ($F_{1,84} = 4.170$, ES=0.29, $p=0.043$). Other primary outcomes did not significantly improve after addition of meman-
### Table 1. Patient demographics and baseline characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>n (%)</th>
<th>Mean (s.d.)</th>
<th>$\chi^2$ / $F$</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>13 (25)</td>
<td></td>
<td>$\chi^2 = 0.923$</td>
<td>0.52</td>
</tr>
<tr>
<td>Male</td>
<td>39 (75)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, years (s.d.)</td>
<td>42.35 (9.55)</td>
<td>$F_{1,51} = 3.165$</td>
<td>0.08</td>
<td></td>
</tr>
<tr>
<td>Years of education (s.d.)</td>
<td>12.23 (1.79)</td>
<td>$F_{1,51} = 0.596$</td>
<td>0.44</td>
<td></td>
</tr>
<tr>
<td>Education level</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>28 (53.8)</td>
<td></td>
<td>$\chi^2 = 0.202$</td>
<td>0.90</td>
</tr>
<tr>
<td>Middle</td>
<td>17 (32.7)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>28 (13.5)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Living conditions</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Independently</td>
<td>30 (57.7)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sheltered home</td>
<td>16 (30.8)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Long-stay department</td>
<td>6 (11.5)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age of onset, years</td>
<td>19.46 (4.68)</td>
<td>$F_{1,51} = 2.848$</td>
<td>0.10</td>
<td></td>
</tr>
<tr>
<td>Duration untreated psychosis, months</td>
<td>35.04 (39.80)</td>
<td>$F_{1,51} = 0.818$</td>
<td>0.37</td>
<td></td>
</tr>
<tr>
<td>Duration of illness, years</td>
<td>22.88 (7.99)</td>
<td>$F_{1,51} = 1.24$</td>
<td>0.27</td>
<td></td>
</tr>
<tr>
<td>Alcohol use</td>
<td>18 (34.6)</td>
<td></td>
<td>$\chi^2 = 0.000$</td>
<td>1.00</td>
</tr>
<tr>
<td>Cannabis use</td>
<td>9 (17.3)</td>
<td></td>
<td>$\chi^2 = 1.209$</td>
<td>0.47</td>
</tr>
<tr>
<td>Cocaine use</td>
<td>1 (1.9)</td>
<td></td>
<td>$\chi^2 = 1.020$</td>
<td>1.00</td>
</tr>
<tr>
<td>Amphetamine use</td>
<td>1 (1.9)</td>
<td></td>
<td>$\chi^2 = 1.020$</td>
<td>1.00</td>
</tr>
<tr>
<td>Clozapine daily dose, mg</td>
<td>350.00 (182.84)</td>
<td>$F_{1,51} = 0.171$</td>
<td>0.68</td>
<td></td>
</tr>
<tr>
<td>Clozapine monotherapy (single antipsychotic)</td>
<td>36 (69.2)</td>
<td></td>
<td>$\chi^2 = 2.342$</td>
<td>0.22</td>
</tr>
<tr>
<td>Combination clozapine + second antipsychotic $^a$</td>
<td>16 (30.8)</td>
<td></td>
<td>$\chi^2 = 3.250$</td>
<td>0.13</td>
</tr>
<tr>
<td>Combination clozapine + antidepressant</td>
<td>30 (57.7)</td>
<td></td>
<td>$\chi^2 = 0.315$</td>
<td>0.78</td>
</tr>
<tr>
<td>Combination clozapine + mood stabilizer $^b$</td>
<td>6 (11.5)</td>
<td></td>
<td>$\chi^2 = 3.014$</td>
<td>0.19</td>
</tr>
<tr>
<td>Combination clozapine + benzodiazepine</td>
<td>18 (34.6)</td>
<td></td>
<td>$\chi^2 = 0.000$</td>
<td>1.00</td>
</tr>
<tr>
<td>Psychotherapy during past 6 months</td>
<td>4 (7.7)</td>
<td></td>
<td>$\chi^2 = 0.000$</td>
<td>1.00</td>
</tr>
<tr>
<td>Family members or partners involved in the study</td>
<td>26 (50.0)</td>
<td></td>
<td>$\chi^2 = 4.600$</td>
<td>0.47</td>
</tr>
<tr>
<td>Expected improvement due to memantine</td>
<td>3.50 (0.92)</td>
<td>$F_{1,51} = 0.818$</td>
<td>0.37</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>2 (3.8)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Very little</td>
<td>3 (5.8)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Little</td>
<td>20 (38.5)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Much</td>
<td>21 (40.4)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Very much</td>
<td>6 (11.5)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Expected improvement in personal hygiene</td>
<td>2.19 (1.37)</td>
<td>$\chi^2 = 4.671$</td>
<td>0.46</td>
<td></td>
</tr>
<tr>
<td>Expected improvement in personal relationships</td>
<td>2.75 (1.17)</td>
<td>$\chi^2 = 3.444$</td>
<td>0.49</td>
<td></td>
</tr>
<tr>
<td>Expected improvement in daily activities</td>
<td>3.00 (1.21)</td>
<td>$\chi^2 = 12.600$</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>Expected improvement in living conditions</td>
<td>2.35 (1.27)</td>
<td>$\chi^2 = 11.200$</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>Expected improvement in finances</td>
<td>1.83 (1.18)</td>
<td>$\chi^2 = 3.586$</td>
<td>0.17</td>
<td></td>
</tr>
</tbody>
</table>

s.d., Standard deviation.

$^a$ Eleven patients received aripiprazole, two patients received quetiapine, one patient received olanzapine and one patient received zuclopenthixol acetate.

$^b$ Four patients received valproate and two patients received lithium.
tine compared to placebo: executive function composite ($F_{1,84}=4.665$, ES=0.12, $p=0.395$); PANSS positive symptoms ($F_{1,84}=1.008$, ES=0.15, $p=0.299$); PANSS total symptoms ($F_{1,84}=1.869$, ES=0.19, $p=0.174$); CGI ($F_{1,84}=0.591$, ES=0.11, $p=0.443$). A post-hoc analysis on PANSS negative symptoms showed that memantine had a more pronounced effect on the expressive deficits subscale score (ES=0.17) compared to the social amotivation subscale score (ES=0.01). Table 2 contains the results of analyses on the individual CANTAB tasks comprising the two composite scores for reference only. No specific hypotheses were tested concerning the individual CANTAB tasks.

This pattern of significant results in the ITT approach was consistent with the per protocol analyses: memory composite ($F_{4,150}=4.665$, ES=0.12, $p=0.043$); and PANSS negative symptoms ($F_{1,72}=3.514$, ES=0.21, $p=0.043$). Tables 2 and 3 and online Supplementary Tables S1 and S2 summarize results and statistics of primary outcomes, side effects, and safety measures.

A post-hoc analysis showed no evidence to suggest that the six patients admitted to long-stay wards responded differently to memantine than the 46 out-patients. In comparison to the full sample of 52 patients, the ESs were nearly identical in magnitude (data available on request).

### Table 2. Intention-to-treat analysis of primary treatment effects

<table>
<thead>
<tr>
<th>Variable</th>
<th>CO</th>
<th>$n$</th>
<th>$F$ test</th>
<th>ES</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cognitive domains and CANTAB tasks</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Verbal memory</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VRM free recall - total correct (phase 1)</td>
<td>0.00</td>
<td>40</td>
<td>11.818</td>
<td>0.48*</td>
<td>0.001</td>
</tr>
<tr>
<td>VRM recognition - total correct</td>
<td>0.09</td>
<td>40</td>
<td>1.421</td>
<td>0.17</td>
<td>0.235</td>
</tr>
<tr>
<td>Associative learning and short term visuo-spatial memory</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PAL total errors (adjusted)</td>
<td>0.04</td>
<td>37</td>
<td>1.219</td>
<td>–0.15</td>
<td>0.271</td>
</tr>
<tr>
<td>PAL first trial memory score</td>
<td>0.09</td>
<td>37</td>
<td>5.778</td>
<td>0.34*</td>
<td>0.017</td>
</tr>
<tr>
<td>Memory Composite</td>
<td>0.00</td>
<td>37</td>
<td>4.655</td>
<td>0.30*</td>
<td>0.032</td>
</tr>
<tr>
<td>Working visuo-spatial memory and strategy use</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SWM strategy</td>
<td>0.32</td>
<td>37</td>
<td>0.159</td>
<td>–0.06</td>
<td>0.690</td>
</tr>
<tr>
<td>SWM between errors</td>
<td>0.47</td>
<td>37</td>
<td>1.522</td>
<td>–0.17</td>
<td>0.219</td>
</tr>
<tr>
<td>Visual planning, reasoning and impulsivity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OTS problems solved on first choice</td>
<td>0.02</td>
<td>39</td>
<td>0.048</td>
<td>0.03</td>
<td>0.827</td>
</tr>
<tr>
<td>Executive Function Composite</td>
<td>0.47</td>
<td>37</td>
<td>0.726</td>
<td>0.12</td>
<td>0.395</td>
</tr>
<tr>
<td>CGI-S</td>
<td>0.32</td>
<td>47</td>
<td>0.591</td>
<td>0.11</td>
<td>0.443</td>
</tr>
<tr>
<td>PANSS-P</td>
<td>0.02</td>
<td>44</td>
<td>1.088</td>
<td>0.15</td>
<td>0.299</td>
</tr>
<tr>
<td>PANSS-N</td>
<td>0.01</td>
<td>44</td>
<td>4.170</td>
<td>0.29*</td>
<td>0.043</td>
</tr>
<tr>
<td>PANSS total</td>
<td>0.00</td>
<td>44</td>
<td>1.869</td>
<td>0.19</td>
<td>0.174</td>
</tr>
</tbody>
</table>

CO, Carryover effect ($p$ value of paired $t$ test); ES, effect size (Cohen’s $d$); CANTAB, Cambridge Neuropsychological Test Automated Battery; VRM, verbal recognition memory-immediate (free recall) and verbal recognition memory-delayed (recognition); PAL, paired associates learning; OTS, One Touch Stockings of Cambridge; SWM, spatial working memory; CGI-S, Clinical Global Impression Severity Scale; PANSS, Positive and Negative Syndrome Scale; PANSS-P, PANSS positive subscale; PANSS-N, PANSS negative subscale; PANSS total, PANSS total symptom score.

* All effects were in the direction of desired effect.

* Significant beneficial effect.
### Table 3. Side effects and safety measures

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline</th>
<th>Memantine 1</th>
<th>Placebo 1</th>
<th>After wash-out</th>
<th>Memantine 2</th>
<th>Placebo 2</th>
<th>$\chi^2$</th>
<th>$F$</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Memantine-related AE mycosis, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4.563</td>
<td></td>
<td>0.03</td>
</tr>
<tr>
<td>None</td>
<td>46 (67.3)</td>
<td>23 (92)</td>
<td>20 (83.3)</td>
<td>35 (81.4)</td>
<td>12 (57.1)</td>
<td>22 (88.5)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Very little</td>
<td>2 (3.8)</td>
<td>0 (0.0)</td>
<td>3 (12.5)</td>
<td>3 (7.0)</td>
<td>6 (26.6)</td>
<td>1 (4.2)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Little</td>
<td>4 (7.7)</td>
<td>2 (7.7)</td>
<td>1 (4.2)</td>
<td>4 (9.3)</td>
<td>2 (9.5)</td>
<td>1 (4.2)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Much</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>1 (2.3)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Very much</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>1 (4.8)</td>
<td>0 (0.0)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean LUNSERS scores (S.D.)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Extrapyramidal symptoms</td>
<td>13.37 (4.06)</td>
<td>13.08 (4.18)</td>
<td>13.33 (5.31)</td>
<td>12.34 (3.94)</td>
<td>13.00 (4.30)</td>
<td>12.4 (3.12)</td>
<td>1.598</td>
<td>0.21</td>
<td></td>
</tr>
<tr>
<td>Anticholinergic side effects</td>
<td>9.87 (3.46)</td>
<td>9.13 (3.66)</td>
<td>9.92 (3.67)</td>
<td>9.26 (3.04)</td>
<td>9.80 (3.86)</td>
<td>8.33 (2.79)</td>
<td>1.323</td>
<td>0.25</td>
<td></td>
</tr>
<tr>
<td>Other autonomic side effects</td>
<td>9.27 (3.16)</td>
<td>8.42 (3.28)</td>
<td>9.33 (3.69)</td>
<td>8.95 (2.85)</td>
<td>9.11 (3.04)</td>
<td>8.57 (2.59)</td>
<td>0.066</td>
<td>0.80</td>
<td></td>
</tr>
<tr>
<td>Allergic reactions</td>
<td>5.57 (1.66)</td>
<td>5.17 (1.83)</td>
<td>6.26 (2.49)</td>
<td>6.58 (2.29)</td>
<td>6.70 (3.01)</td>
<td>5.91 (2.52)</td>
<td>4.391</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>Psychic side effects</td>
<td>23.63 (6.34)</td>
<td>22.36 (7.40)</td>
<td>24.58 (6.90)</td>
<td>22.44 (7.42)</td>
<td>22.75 (7.00)</td>
<td>22.08 (7.11)</td>
<td>1.295</td>
<td>0.26</td>
<td></td>
</tr>
<tr>
<td>Hormonal side effects</td>
<td>8.20 (3.03)</td>
<td>7.96 (2.22)</td>
<td>8.33 (2.22)</td>
<td>8.54 (2.68)</td>
<td>8.72 (3.12)</td>
<td>7.38 (2.27)</td>
<td>0.066</td>
<td>0.80</td>
<td></td>
</tr>
<tr>
<td>Miscellaneous side effects</td>
<td>6.43 (1.95)</td>
<td>6.54 (2.19)</td>
<td>6.96 (1.76)</td>
<td>6.86 (2.04)</td>
<td>7.37 (1.80)</td>
<td>6.83 (2.10)</td>
<td>1.694</td>
<td>0.20</td>
<td></td>
</tr>
<tr>
<td>Red herring</td>
<td>14.52 (4.72)</td>
<td>14.83 (4.35)</td>
<td>14.92 (4.49)</td>
<td>15.14 (4.94)</td>
<td>15.65 (5.21)</td>
<td>14.78 (4.73)</td>
<td>0.801</td>
<td>0.37</td>
<td></td>
</tr>
<tr>
<td>Red herring ≥ 20</td>
<td>0.13 (0.35)</td>
<td>0.17 (0.38)</td>
<td>0.21 (0.95)</td>
<td>0.21 (0.41)</td>
<td>0.20 (0.41)</td>
<td>0.17 (0.39)</td>
<td>0.446</td>
<td>0.51</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>92.80 (21.06)</td>
<td>90.90 (23.95)</td>
<td>95.74 (0.12)</td>
<td>93.63 (23.27)</td>
<td>96.00 (27.27)</td>
<td>87.50 (21.94)</td>
<td>0.041</td>
<td>0.84</td>
<td></td>
</tr>
<tr>
<td>Total without red herring items</td>
<td>78.41 (17.12)</td>
<td>75.95 (20.16)</td>
<td>80.70 (20.47)</td>
<td>78.69 (19.37)</td>
<td>80.43 (21.96)</td>
<td>72.85 (17.66)</td>
<td>0.477</td>
<td>0.73</td>
<td></td>
</tr>
<tr>
<td>Laboratory measurement</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean clozapine plasma level, ng/ml (S.D.)</td>
<td>421.27 (224.61)</td>
<td>515.16 (240.43)</td>
<td>376.48 (185.11)</td>
<td>461.71 (219.61)</td>
<td>403.06 (179.90)</td>
<td>436.1 (157.07)</td>
<td>1.387</td>
<td>0.24</td>
<td></td>
</tr>
<tr>
<td>Physical examination</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>110.04 (13.82)</td>
<td>108.32 (14.71)</td>
<td>104.04 (11.72)</td>
<td>105.50 (13.89)</td>
<td>104.41 (12.16)</td>
<td>107.88 (14.86)</td>
<td>1.205</td>
<td>0.27</td>
<td></td>
</tr>
<tr>
<td>Systolic blood pressure, mmHg (S.D.)</td>
<td>134.57 (18.77)</td>
<td>169.00 (170.28)</td>
<td>130.26 (12.59)</td>
<td>130.48 (15.94)</td>
<td>125.68 (12.82)</td>
<td>130.75 (11.84)</td>
<td>1.954</td>
<td>0.16</td>
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</tr>
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<td>Diastolic blood pressure, mmHg (S.D.)</td>
<td>88.00 (9.77)</td>
<td>86.20 (10.57)</td>
<td>87.04 (8.10)</td>
<td>85.95 (9.41)</td>
<td>86.14 (9.70)</td>
<td>83.88 (7.60)</td>
<td>1.084</td>
<td>0.30</td>
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</table>

AE, Adverse event; LUNSERS, Liverpool University Neuroleptic Side-Effect Rating Scale; S.D., standard deviation.

*Extrapyramidal symptoms: item 19, 29, 34, 37, 40, 43, 48; anticholinergic side effects = item 6, 10, 32, 38, 51; other autonomic side effects = item 15, 16, 20, 27, 36; allergic reactions = item 1, 3, 47, 49; psychic side effects = 2, 4, 9, 14, 18, 21, 23, 26, 31, 41; hormonal side effects = item 7, 13, 17, 24, 46, 50; miscellaneous side effects = 5, 12, 39, 44; red herring = 3, 8, 11, 12, 25, 28, 30, 33, 42, 45.*
Possible confounders

Tests for period effects and period × treatment interaction were non-significant across all outcomes. When matched-pair $t$ tests were used to compare scores from the first baseline to the second baseline of the trial, carryover effects were significant for the memory composite from the CANTAB, PANSS positive, negative, and total scores. $t$ Tests and a repeated-measures analysis of variance indicated no significant differences in clozapine levels across the four assessment times in the trial, suggesting that memantine has no effect on clozapine levels. Clozapine dosage (mean 350 mg, range = 75 mg–1000 mg) remained unaltered in all subjects except in one subject in the placebo phase whose clozapine dosage was increased with 175 mg because of agitation and verbal aggression. Alterations in concomitant medications throughout the study were limited to changes in benzodiazepines in one subject in the placebo phase. Use of substances or psychotherapy did not significantly change during the trial (see online Supplementary Table S3). Tests of moderation based on low and high levels of expectation for improvement prior to beginning the trial revealed that the element of expectation did not contribute to the placebo effect. Blinding was successful in that 45.7% of patients guessed correctly which group they had been randomized to. The raters were correct in 21.7% of cases.

Safety and tolerability

No significant changes in liver and renal function were observed. Compared with placebo, memantine did not significantly affect metabolic parameters, such as waist circumference, blood pressure, blood glucose, or lipids (see Table 3). The only significant increase in reported side effects while taking memantine was found on the Allergic Reactions subscale of the LUNSERS (rash, sensitivity to sun, unusual skin marks, and itchy skin).

Of all AEs (see Table 4), one report of dizziness, a common side effect of memantine in elderly patients with AD, was probably related to memantine. Complaints of dizziness were alleviated within 5 days of discontinuation of study medication. There were two reports of temporary increase in constipation, which were rated as possibly related to memantine. Both participants had been already treated with laxatives because of clozapine-induced constipation. We observed one serious AE (a suicide attempt during the placebo phase).
Inspired by the unique psychopharmacological characteristics of the memantine–clozapine combination and the substantial positive findings of the first proof-of-concept study we conducted a second proof-of-concept study with a larger sample size and a computerized cognitive test battery to ensure accurate and objective study data with minimized interrater variability to evaluate the efficacy of memantine as an adjunct to clozapine in refractory schizophrenia.

Memantine treatment added to clozapine was associated with significant improvement in memory (ES=0.30). Memory enhancing effects of the combination therapy clozapine and memantine may be a result of upregulation of synaptic NMDA receptor currents in the hippocampus, facilitating induction of long term potentiation and therefore learning and memory (Kornmeier & Sosic-Vasic, 2012).

Memantine did not significantly improve executive function (ES=0.12). Executive function is a central cognitive process, involving the PFC, corticocortical and corticosubcortical networks (Evans et al. 1997; Lesh et al. 2011). Enhancement of executive function would require effects on more elaborate networks and several cognitive domains including planning, working memory, strategy use, cognitive flexibility and ability to suppress...
impulsivity. Apparently, memantine addition does not improve the functioning in these networks, or alternatively longer treatment duration is needed.

Negative symptoms significantly improved with a small ES (ES=0.29). Memantine affected diminished expression to a larger extent than social amotivation. Improvement of expressive deficits may be a result of increased signal transmission with an enhanced signal-to-noise ratio in the PFC (Geerts & Grossberg, 2006; Hasan, 2013) due to the particular combination of clozapine and memantine.

Memantine and placebo did not differ significantly with respect to adverse effects, except for mild and transient allergic symptoms.

Improvement of cognitive disturbances and negative symptoms is an important goal for treatment-resistant schizophrenia. Together these symptoms have a more pronounced impact on psychosocial functioning and quality of life than positive symptoms (Ventura et al. 2015). Clinical impairment of memory is one of the major disabilities in schizophrenia. Specifically, verbal memory is a strong predictor of functional outcome (Green, 1996). Favorable effects of memantine in combination with clozapine may be based on the neuroprotective properties and pharmacodynamic activities of this combination. In a recent proton magnetic resonance spectroscopy study anterior cingulate cortex glutamate levels were elevated in patients with treatment-resistant schizophrenia compared to patients with treatment-responsive schizophrenia, endorsing our hypothesis that memantine is specifically efficacious in refractory schizophrenia (Mouchlianitis et al. 2015).

The effect of memantine augmentation we found is in line with the improvement of negative symptoms that has been found in five trials of addition of a glutamate antagonist to clozapine in partially responding schizophrenia patients (Muscatello et al. 2010; Afshar et al. 2008; Zoccali et al. 2007; Goff et al. 2007 (study 926); de Lucena et al. 2009). Topiramate and lamotrigine both showed favorable effects on negative symptoms in each of two trials, with ESs varying from 0.76 to 1.37 and 0.66 to 1.21, respectively. Cognitive functioning had been assessed with different cognitive test batteries in five double-blind, placebo-controlled randomized trials of clozapine augmentation with glutamate antagonists. In one trial of topiramate (Muscatello et al. 2010) and two trials of lamotrigine add-on therapy to clozapine (Goff et al. 2007 (study 926); Vayısoğlu et al. 2013), cognitive functions did not significantly change compared with placebo. However, two trials showed favorable results on cognition (Zoccali et al. 2007; de Lucena et al. 2009). In the study by Zoccali et al. (2007), the only cognitive function that significantly improved was semantic fluency after 24 weeks addition of lamotrigine 200 mg daily.
The differences and similarities between our results and that of the 12-week memantine add-on study by de Lucena et al. (2009) is striking. De Lucena et al. found exceptionally large ESs on all treatment outcome parameters. Most striking was the large ES of 3.33 concerning negative symptoms (de Lucena et al. 2009). But also the effect on global cognitive functioning (ES=-1.32), as measured by the Mini-Mental State Examination (MMSE) (Folstein et al. 1975), was substantial. Caution is necessary, for efficacy of memantine was perhaps overestimated partly by chance in this relatively small sample study (21 patients) (Sinclair & Adams, 2014; Tajika et al. 2015). The crossover design of our larger double-blind randomized clinical trial eliminated the influence of between-subject variability on effect. The MMSE, used by de Lucena et al. (2009), does not measure executive functioning and detects cognitive deficits only at an advanced stage, because tasks for language and memory functions are extremely simple (Feher et al. 1992). We used a more sensitive and comprehensive cognitive test battery developed for assessment of cognitive impairments in schizophrenia intervention studies (Levaux et al. 2007). The Brief Psychiatric Rating Scale (BPRS), used by de Lucena et al. (2009), merely covers three negative symptom items (blunted affect, emotional withdrawal and psychomotor retardation). We used the more sensitive PANSS scale with seven items on the negative symptom subscale (Eckert et al. 1996) and four additional items on the general symptom subscale in the post-hoc analysis (Liemburg et al. 2013). Our patient population differed compared with that of the first memantine augmentation to clozapine study in mean age (42.35 v. 34.67 years) and mean duration of illness (22.88 years v. 17.84 years), CGI-S scores (6.15 v. 5.34) and mean total PANSS and total BPRS scores (81.21 and 14.38, respectively) suggesting that our patient population was more severely ill than were patients in the study by de Lucena et al. (2009). Although in our patients the severity of residual negative symptoms was comparable to the severity of persistent positive symptoms (mean PANSS negative subscale=22.12, S.D.=5.86; mean PANSS positive subscale=21.02, S.D.=6.34), negative symptoms prevailed in the study by de Lucena et al. (2009). While mean total BPRS score (14.38) corresponds with ‘markedly ill’ according to CGI-S (score 5) in the de Lucena study, our patient population was rated as severely ill (mean CGI-S score 6.15) due to prominent cognitive impairment. These differences between studies may partly explain the more moderate beneficial effects of memantine addition in our study. However, the difference in ES for all symptom domains between the de Lucena study and ours is very large. It has been demonstrated that among randomized trials, initially stronger effects are not unusual (Ioannidis, 2005; Tajika et al. 2015).
Memantine was generally well tolerated in both studies. Although there were no drop-outs in the first study, one participant discontinued in our trial because of dizziness in the memantine phase.

The results of our study are limited by the short memantine treatment duration of 12 weeks. A longer treatment duration may result in more pronounced treatment effects associated with an improved glutamatergic balance, as was found in patients with AD. In a meta-analysis of six randomized, placebo-controlled trials of memantine treatment in 2311 patients with AD, symptoms of delusion were more improved after 24 to 28 weeks compared with 12 weeks (Puangthong & Hsiung, 2009). Furthermore, the crossover design resulted in carryover effects on several measures, including verbal memory and all PANSS subscales, despite a 2 week placebo wash-out period. Although the model controls for carryover effects, these cannot be discarded completely. Practice effects were minimized due to the fact that the number of subjects randomized to the placebo and memantine group before crossover was the same and a parallel form was used for verbal memory. Tests on executive function, depending on strategy show strong practice effects and low test-retest reliability (Lowe & Rabbitt, 1998). However, there is no research on practice effects using the CANTAB in patients with severe cognitive disturbances suffering from refractory schizophrenia. Although our study included more patients than the first investigation by de Lucena et al. (2009), our sample size is still relatively small (Sinclair & Adams, 2014). The results of our study need to be validated in a randomized multicenter long-term treatment study with a large sample size and enough power to clearly show a reduction of at least 25% of the baseline score in order to help further evaluate pro-cognitive properties of memantine in combination with clozapine in refractory patients and its potential to reduce negative symptoms associated with schizophrenia (Leucht et al. 2009).

In conclusion, we found evidence that addition of memantine may be a well-tolerated treatment option for cognitive impairments and negative symptoms in patients with clozapine refractory schizophrenia, deserving further study.

Acknowledgments

We thank the participants of this study and their care providers for their assistance, enthusiasm and support. We also thank M. Monden and Dr J.B. Deijen for their contribution to
the training of the raters. This research received no specific grant from any funding agency, commercial or not-for-profit sectors. This work was supported by the Community Mental Health Division of Mental Health Service Organization North Holland North. The study was partially funded by the 2012 Care Innovation Prize of Mental Health Service Organization North Holland North. Both verum and placebo tablets were provided by H. Lundbeck A/S at no cost. H. Lundbeck A/S did not play any role in the design, conduct, analysis or interpretation of the trial.

Notes

1 The full results of the study are available by request from the first author (S.R.T.V.).
2 A period effect was included as a control only as the study does not have sufficient power to test for such an effect directly. Further, a period effect was not hypothesized.

Supplementary Table S1. Per protocol analysis of primary treatment effects

<table>
<thead>
<tr>
<th>Variable</th>
<th>Per protocol (n=44)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
</tr>
<tr>
<td><strong>Cognitive domains and CANTAB tasks</strong></td>
<td></td>
</tr>
<tr>
<td>Verbal memory</td>
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</tr>
<tr>
<td>VRM free recall - total correct (phase 1)</td>
<td>38</td>
</tr>
<tr>
<td>VRM recognition - total correct</td>
<td>38</td>
</tr>
<tr>
<td><strong>Associative learning and short term visuo-spatial memory</strong></td>
<td></td>
</tr>
<tr>
<td>PAL total errors (adjusted)</td>
<td>35</td>
</tr>
<tr>
<td>PAL first trial memory score</td>
<td>35</td>
</tr>
<tr>
<td>Memory Composite</td>
<td>35</td>
</tr>
<tr>
<td><strong>Working visuo-spatial memory and strategy use</strong></td>
<td></td>
</tr>
<tr>
<td>SWM strategy</td>
<td>35</td>
</tr>
<tr>
<td>SWM between errors</td>
<td>35</td>
</tr>
<tr>
<td><strong>Visual planning, reasoning and impulsivity</strong></td>
<td></td>
</tr>
<tr>
<td>OTS problems solved on first choice</td>
<td>37</td>
</tr>
<tr>
<td>Executive function Composite</td>
<td>35</td>
</tr>
<tr>
<td>CGI-S</td>
<td>42</td>
</tr>
<tr>
<td>PANSS-P</td>
<td>41</td>
</tr>
<tr>
<td>PANSS-N</td>
<td>41</td>
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<tr>
<td>PANSS total</td>
<td>41</td>
</tr>
</tbody>
</table>

* ES = effect size (Cohen’s d); p, p-value; CANTAB, Cambridge Neuropsychological Test Automated Battery; VRM, verbal recognition memory-immediate (free recall) and verbal recognition memory-delayed (recognition); PAL, paired associates learning; OTS, One Touch Stockings of Cambridge; SWM, spatial working memory; CGI-S, Clinical Global Impression Severity Scale; PANSS = Positive and Negative Syndrome Scale; PANSS-P = PANSS positive subscale; PANSS-N, PANSS negative subscale; PANSS total, PANSS total symptom score.

* Significant beneficial effect.
### Supplementary Table S2: Results of primary treatment effects

<table>
<thead>
<tr>
<th>Variable</th>
<th>Memantine 0</th>
<th>Placebo 0</th>
<th>Memantine 1</th>
<th>Placebo 1</th>
<th>After wash-out Memantine 2</th>
<th>Placebo 2</th>
<th>Memantine 2</th>
<th>Placebo 2</th>
<th>Memantine 1</th>
<th>Placebo 1</th>
<th>After wash-out Memantine 2</th>
<th>Placebo 2</th>
<th>Memantine 2</th>
<th>Placebo 2</th>
<th>Memantine 2</th>
<th>Placebo 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cognitive Functioning (CANTAB)</td>
<td></td>
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<tr>
<td>Verbal memory (VRM) total correct</td>
<td>3.39 (1.67)</td>
<td>3.81 (1.39)</td>
<td>3.74 (1.88)</td>
<td>4.32 (1.94)</td>
<td>4.73 (1.82)</td>
<td>4.76 (2.00)</td>
<td>4.43 (1.89)</td>
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<tr>
<td>VRM recognition – total correct</td>
<td>19.78 (2.45)</td>
<td>19.58 (4.00)</td>
<td>21.05 (2.46)</td>
<td>20.09 (3.70)</td>
<td>21.05 (2.41)</td>
<td>20.43 (5.05)</td>
<td>20.62 (1.63)</td>
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<tr>
<td>Associative learning and short term visuospatial memory</td>
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<tr>
<td>PAL total errors (adjusted)</td>
<td>74.14 (45.96)</td>
<td>64.92 (45.70)</td>
<td>73.83 (51.40)</td>
<td>56.00 (45.61)</td>
<td>56.63 (41.05)</td>
<td>60.95 (44.33)</td>
<td>73.57 (45.64)</td>
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<tr>
<td>PAL first trial memory score</td>
<td>9.68 (6.77)</td>
<td>10.62 (6.42)</td>
<td>9.33 (7.00)</td>
<td>10.05 (4.98)</td>
<td>11.74 (6.15)</td>
<td>10.55 (5.82)</td>
<td>9.24 (6.39)</td>
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<tr>
<td>Memory Composite</td>
<td>–38.48 (53.12)</td>
<td>–30.92 (53.44)</td>
<td>–36.32 (59.83)</td>
<td>–19.45 (50.66)</td>
<td>–15.83 (47.90)</td>
<td>–22.81 (49.92)</td>
<td>–39.29 (52.60)</td>
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<td>Working visuo-spatial memory and strategy use</td>
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<tr>
<td>SWM strategy</td>
<td>25.55 (11.69)</td>
<td>22.00 (9.68)</td>
<td>22.00 (12.91)</td>
<td>18.00 (9.68)</td>
<td>18.00 (9.30)</td>
<td>17.20 (4.20)</td>
<td>17.63 (4.09)</td>
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<tr>
<td>SWM between errors</td>
<td>19.35 (11.57)</td>
<td>19.35 (11.57)</td>
<td>19.05 (12.80)</td>
<td>19.10 (12.31)</td>
<td>19.45 (9.85)</td>
<td>19.45 (9.85)</td>
<td>22.74 (11.49)</td>
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<td>Visual planning, reasoning and impulsivity</td>
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<tr>
<td>OTS problems solved on first choice</td>
<td>7.55 (3.70)</td>
<td>7.12 (4.47)</td>
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<td>7.05 (3.80)</td>
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<tr>
<td>CGI-S</td>
<td>6.15 (0.68)</td>
<td>6.15 (0.78)</td>
<td>6.20 (0.71)</td>
<td>6.20 (0.71)</td>
<td>6.20 (0.71)</td>
<td>6.15 (0.78)</td>
<td>6.12 (0.68)</td>
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<tr>
<td>PANSS-P</td>
<td>20.23 (7.20)</td>
<td>21.81 (5.37)</td>
<td>18.48 (6.21)</td>
<td>18.71 (5.47)</td>
<td>19.52 (5.82)</td>
<td>19.05 (6.07)</td>
<td>17.92 (5.85)</td>
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<tr>
<td>PANSS total</td>
<td>80.04 (18.02)</td>
<td>82.38 (15.59)</td>
<td>74.36 (17.47)</td>
<td>74.38 (18.07)</td>
<td>77.11 (17.28)</td>
<td>74.82 (17.74)</td>
<td>70.96 (15.64)</td>
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</table>

S.D., standard deviation; CANTAB, Cambridge Neuropsychological Test Automated Battery; VRM, verbal recognition memory-immediate (free recall) and verbal recognition memory-delayed (recognition); PAL, paired associates learning; OTS, One Touch Stockings of Cambridge; SWM, spatial working memory; CGI-S, Clinical Global Impression Severity Scale; PANSS, Positive and Negative Syndrome Scale; PANSS-P, PANSS positive subscale; PANSS-N, PANSS negative subscale; PANSS total, PANSS total symptom score.
Supplementary Table S3. Treatment expectations, experienced improvement, substance use, and psychotherapy

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline</th>
<th>Evaluation</th>
<th>$\chi^2$ / $F$</th>
<th>$\beta$</th>
<th>$P$</th>
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</thead>
<tbody>
<tr>
<td>Mean expected improvement in personal hygiene (s.d.)</td>
<td>2.19 (1.37)</td>
<td></td>
<td>$\chi^2_4 = 4.671$</td>
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<td>Mean expected improvement in personal relationships (s.d.)</td>
<td>2.75 (1.17)</td>
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<td>$\chi^2_4 = 3.444$</td>
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<tr>
<td>Mean expected improvement in daily activities (s.d.)</td>
<td>3.00 (1.21)</td>
<td></td>
<td>$\chi^2_4 = 12.600$</td>
<td>0.01</td>
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<td>Mean expected improvement in living conditions (s.d.)</td>
<td>2.35 (1.33)</td>
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<td>$\chi^2_4 = 11.200$</td>
<td>0.04</td>
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<td>Mean expected improvement in finances (s.d.)</td>
<td>1.83 (1.18)</td>
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<td>$\chi^2_4 = 3.586$</td>
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<td>Mean experienced improvement in personal hygiene (s.d.)</td>
<td>1.65 (1.08)</td>
<td>B = 0.57 (SE = 0.32)</td>
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<td>0.08</td>
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<tr>
<td>Mean experienced improvement in personal relationships (s.d.)</td>
<td>1.78 (1.19)</td>
<td>B = 0.66 (SE = 0.33)</td>
<td>0.28</td>
<td>0.05</td>
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<tr>
<td>Mean experienced improvement in daily activities (s.d.)</td>
<td>1.87 (1.05)</td>
<td>B = 0.45 (SE = 0.32)</td>
<td>0.22</td>
<td>0.17</td>
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<tr>
<td>Mean experienced improvement in living conditions (s.d.)</td>
<td>1.46 (1.01)</td>
<td>B = 0.26 (SE = 0.32)</td>
<td>0.13</td>
<td>0.42</td>
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<tr>
<td>Mean experienced improvement in finances (s.d.)</td>
<td>1.35 (0.71)</td>
<td>B = 0.42 (SE = 0.21)</td>
<td>0.30</td>
<td>0.05</td>
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<tr>
<td>Alcohol use, n (%)</td>
<td>18 (34.6)</td>
<td>18 (39.1)</td>
<td>$F_{1,45} = 0.328$</td>
<td>0.57</td>
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<td>Cannabis use, n (%)</td>
<td>9 (17.3)</td>
<td>7 (13.5)</td>
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<tr>
<td>Cocaine use, n (%)</td>
<td>1 (1.9)</td>
<td>1 (1.9)</td>
<td>a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amphetamine use, n (%)</td>
<td>1 (1.9)</td>
<td>1 (1.9)</td>
<td>a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Psychotherapy during trial, n (%)</td>
<td>4 (7.7)</td>
<td>6 (13.0)</td>
<td>$F_{1,45} = 2.731$</td>
<td>0.11</td>
<td></td>
</tr>
</tbody>
</table>

a Cannot be computed as there was no change in substance use status from baseline to evaluation for available data. Omnibus tests for expected improvements compared differences by random assignment to the treatment group. Omnibus tests for experienced improvements compared differences by random assignment to the treatment group, controlling for baseline expectation of improvement in the same domain. Omnibus tests of substance use and psychotherapy compared baseline to evaluation measures.
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Adjunctive memantine in clozapine-treated refractory schizophrenia: an open-label 1-year extension Study

Selene R.T. Veerman, Peter F.J. Schulte, Jan Berend Deijen, Lieuwe de Haan

What is the long-term efficacy and tolerability of memantine in clozapine-refractory schizophrenia?

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Abstract

Background.
In a recent placebo-controlled, double-blind crossover trial (n=52), significant beneficial effects on memory (d=0.30) and negative symptoms (d=0.29) were found after 12 weeks of memantine augmentation in patients with clozapine-refractory schizophrenia. In this open-label 1-year extension study we report the long-term effects and tolerability of memantine add-on therapy to clozapine.

Method.
Completers of the first trial who experienced beneficial effects during 12 weeks of memantine treatment received memantine for one year. Primary endpoints were memory and executive function using the Cambridge Neuropsychological Test Automated Battery (CANTAB), the Positive and Negative Syndrome Scale (PANSS), and the Clinical Global Impression Severity Scale (CGI-S).

Results.
Of 31 RCT completers who experienced beneficial effects from memantine, 24 received memantine for one year. The small improvement in memory found in the memantine condition in the placebo-controlled trial remained stable in the extension study. Executive function did not improve. After 26 weeks of memantine add-on therapy to clozapine, PANSS negative symptoms (r=0.53), PANSS positive symptoms (r=0.50), and PANSS total symptoms (r=0.54) significantly improved. Even further significant improvement in all these measures was observed between 26 weeks and 52 weeks memantine, with effect sizes varying from 0.39 to 0.51. CGI-S showed a non-significant moderate improvement at 26 weeks (r=0.36) and 52 weeks (r=0.34). Memantine was well tolerated without serious adverse effects.

Conclusions.
In the one-year extension phase the favourable effect of adjunctive memantine on memory was sustained and we observed further improvement of negative, positive, and overall symptoms in patients with clozapine-treated refractory schizophrenia.
Introduction

Treatment-resistant schizophrenia (TRS) is a major clinical problem, since in approximately a third of schizophrenia patients psychotic symptoms persist despite appropriate antipsychotic treatment (Elkis, 2007). In TRS negative symptoms are prominent (de Bartolomeis et al. 2013) and associated with impaired working memory (Zierhut et al. 2013), impaired verbal memory (Anderson et al. 2015; de Bartolomeis et al. 2013) and deficits in cognitive flexibility, processing speed, executive functions, and verbal fluency (Frydecka et al. 2016).

Patients with TRS may be suffering from a normodopaminergic subtype of schizophrenia (Howes & Kapur, 2014; Kahn & Sommer, 2015; Lawrie et al. 2016) since in patients with TRS the striatal dopamine synthesis capacity is not increased (Demjaha et al. 2012). Moreover, anterior cingulate cortex glutamate levels are elevated in TRS compared to patients with treatment-responsive schizophrenia (Demjaha et al. 2014; Mouchlianitis et al. 2015). Therefore glutamatergic alterations may be a plausible explanation for the lack of response to dopamine-blocking antipsychotic medication in TRS patients. Glutamate receptors (N-methyl-D-aspartate; NMDA) are therefore a candidate target for intervention in TRS (Veerman et al. 2014b). Clozapine acts as a glutamate agonist and has proven superior efficacy for positive symptoms relative to other antipsychotic medication (Leucht et al. 2013). However, up to 70% of patients treated with clozapine show insufficient response (Buckley et al. 2001; Agid et al. 2010) and clozapine is not consistently associated with improvement of cognitive impairments (Bourque et al. 2013).

Memantine targets the NMDA receptor and has been shown to improve cognition in patients with Alzheimer’s disease (McShane et al. 2006). Findings of two proton magnetic resonance spectroscopy (¹H-MRS) studies showed elevated γ-amino butyric acid (GABA) and glutamate levels in the medial prefrontal cortex (Kegeles et al. 2012; de la Fuente-Sandoval et al. 2015) and in the dorsal caudate (de la Fuente-Sandoval et al. 2015) in antipsychotic-naive patients with schizophrenia and subjects at ultra-high risk. In a ¹H-MRS study memantine decreased hippocampal glutamate levels, which supports the hypothesis that memantine protects against excitotoxicity (Glodzik et al. 2008). In a functional magnetic resonance imaging (fMRI) study memantine augmentation of atypical antipsychotics normalized brain activity in the inferior frontal gyrus (Cerullo et al. 2007), which is altered in patients with schizophrenia (Fletcher et al. 1998; Thermenos et al. 2005) and is involved in working memory (Lawrence et al. 2003; Tomasi et al. 2006).

de Lucena et al. (2009) found substantial effects of adjunctive memantine to cloza-
pine in TRS on global cognitive functioning, negative symptoms, positive symptoms and overall symptoms with exceptionally large effect sizes (ESs) varying from 1.32 to 3.33. In a larger and more elaborate crossover study (n=52) we found more modest favourable effects of memantine augmentation in TRS on memory (ES=0.30) and negative symptoms (ES=0.29). Executive function (ES=0.12), positive symptoms (ES=0.15), overall symptoms (ES=0.19), and global severity of disease (ES=0.11) did not significantly improve (Veerman et al. 2016).

Given the paucity of effective interventions to ameliorate cognitive dysfunction, negative and positive symptoms in TRS these results are clinically relevant. There are several indications that longer treatment than the mean trial duration of 8–12 weeks may be needed for an optimal treatment effect (Keefe et al. 2013). In order to show an enduring effect on cognition the Measurement and Treatment Research to Improve Cognition in Schizophrenia (MATRICS) guidelines recommend a trial duration of at least 26 weeks (Buchanan et al. 2005). And, for instance, Zoccali et al. (2007) showed a robust improvement of negative and positive symptoms (ESs 1.21 and 0.97, respectively) after 24 weeks lamotrigine treatment, while in 4 trials with a treatment duration varying from 12 to 14 weeks those symptoms did not significantly improve (Veerman et al. 2014a).

We therefore performed a 1-year extension study of our former 26-week randomized, double-blind placebo-controlled crossover study evaluating memantine augmentation in clozapine-refractory schizophrenia. Since there is some preliminary evidence that memantine has beneficial effects on depressive and obsessive-compulsive symptoms (Zdanys & Tampi, 2008; Sani et al. 2012; Gameiro & Esteves, 2015), we explored the effects of memantine on these comorbid symptoms as well. We also evaluated the long-term effects on severity of impairments of psychosocial functioning, quality of life and tolerability of memantine add-on therapy to clozapine.

**Method**

**Study design**

A 1-year open follow-up study in which all patients who had completed a previous double-blind, placebo-controlled randomised trial (RCT) were asked to participate (see Fig. 1). Participants who experienced beneficial effects during 12 weeks of memantine in the trial
were allowed to be treated with memantine for 1 year. Those without beneficial effect on 12 weeks memantine could participate without memantine augmentation.

**Study population, inclusion and exclusion criteria**

The study was conducted from August 2014 to September 2015 at 11 Flexible Assertive Community Treatment (FACT) facilities of the Mental Health Service Organisation Noord-Holland Noord (the Netherlands) (van Veldhuizen, 2007).

In the original single-centre, double-blind, placebo-controlled crossover study 52 severely ill patients were included [mean Clinical Global Impression Severity Scale (CGI-S) score 6.15, mean age 42.35 and mean duration of illness 22.88 years], both male ($n=39$) and female ($n=13$), who met Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV) criteria for schizophrenia on the Mini International Neuropsychiatric Interview Plus (MINI-Plus) (Overbeek et al. 1999). At inclusion participants suffered from persistent residual psychopathology (Andreasen et al. 2005) after treatment with clozapine for at least 6 months (mean clozapine plasma level 421 ng/ml, range 50–878 ng/ml) (Veerman et al. 2016). All participants received care from an out-patient facility, while living independently ($n=30$), in a sheltered home ($n=16$) or in an open long-stay ward ($n=6$).

Adjunctive memantine treatment was allowed provided that more beneficial effects were established in the memantine phase compared to the placebo phase on primary outcomes after 12 weeks memantine add-on therapy in the placebo-controlled trial. Exclusion criteria included admission to an acute treatment ward, pregnancy, lactating women and female subjects without adequate contraception, known hypersensitivity to memantine, comedication with glutamate modulators, lactose intolerance, uncontrolled epilepsy, myocardial infarction, uncontrolled hypertension, renal insufficiency, liver failure, or Alzheimer’s disease (Wesemann et al. 1983). Substance use was not an exclusion criterion, but needed to remain as stable as possible, while changes were monitored throughout the study as a possible confounder.

All subjects gave informed consent after receiving a complete description of the extension study from the principal investigator. Compliance was monitored on a daily basis in patients in sheltered homes and open long-stay wards and was at the discretion of the care providers in patients who lived independently.
Clinical assessments

Outcome measures and assessments were identical to those in the double-blind trial. Key primary outcomes were memory and executive function. We used the MATRICS Consensus Cognitive Battery (MCCB) to assess six cognitive domains using the Cambridge Neuropsychological Test Automated Battery (CANTAB) (Levaux et al. 2007): reaction time and psychomotor speed, sustained visual attention, verbal memory, visuospatial memory, learning and association ability, working visuospatial memory and strategy use, spatial planning and motor control and emotion recognition (Nuechterlein et al. 2008; Barnett et al. 2010). A composite memory score was assessed by computing the sum of the CANTAB scores of four tasks: verbal recognition memory (VRM) free recall and VRM recognition, and paired associates learning (PAL) total errors and PAL first trial memory score. A parallel form of the VRM task, equivalent in difficulty was used for the second measurement in order to reduce practice effects. Executive function was assessed by computing a composite score of the sum of three CANTAB task scores: spatial working memory (SWM) strategy, SWM between errors and One Touch Stockings of Cambridge (OTS) problems solved on first choice.

Other primary outcomes included effects on the severity of negative, positive and total symptoms of schizophrenia, using the Positive and Negative Syndrome Scale (PANSS) (Kay et al. 1987), and on global severity of psychopathology, using the Clinical Global Impression Severity Scale (CGI-S) (Guy, 1976).

Secondary outcome measures related to social cognition (facial emotion recognition and theory of mind, as assessed with the Emotion Recognition Task of the CANTAB and the Reading the Mind in the Eyes test; Baron-Cohen et al. 1997), severity of depressive symptoms (Calgary Depression Scale for Schizophrenia; Addington et al. 1990), severity of obsessive-compulsive symptoms (Yale-Brown Obsessive-Compulsive Scale; Goodman et al. 1989), severity of impairments of psychosocial functioning [Health of the Nation Outcome Scales (HoNOS); Wing et al. 1999] and quality of life (Manchester Short Assessment of Quality of Life; Priebe et al. 1999).

Analyses of the safety and tolerability of memantine augmentation to clozapine included measurements of blood pressure and waist circumference, laboratory tests of white blood cell count and differentiation, liver and renal function, blood glucose, lipids, and clozapine plasma level (12 $\pm$ 0.5 h after ingestion). Subjects used the self-rating Liverpool University Neuroleptic Side-Effect Rating Scale (LUNSERS) to determine the occurrence
and severity of side effects (Day et al. 1995). Three possible side effects of memantine (thrombosis, dyspnoea, and mycosis) were assessed with separate Likert scales.

All primary and secondary outcomes were rated at baseline (t0), after 26 weeks (t26), and after 52 weeks (t52). In one patient the baseline measurement was conducted directly after the 12-week memantine treatment phase of the double-blind study and the second and third assessments were conducted after a treatment period of 38 weeks and 64 weeks continuous treatment with memantine. The trainers in the instruments to assess severity of psychopathology in the first study also trained the three raters of the extension study. Interrater agreement between the first two raters was moderate, as indicated by the Spearman correlation coefficient (ρ=0.42, p=0.001) based on four live patient interviews under supervision (Mukaka, 2012). Interrater agreement between the second and third rater was high, (ρ=0.81, p<0.0005), based on seven assessments of joint interviews with subjects. Interrater agreement between the first and third rater was high (ρ=0.78, p<0.0005) based on three interviews under supervision.

Any undesirable experience occurring to a subject during the study, whether or not considered to be related to memantine, was defined as adverse event (AE). AEs were reported by either the subject or his or her case manager, community mental health nurse or psychiatrist. Alternative causes of AEs were explored through physical examination by a general practitioner and additional laboratory measurements. Criteria for premature termination of the study were withdrawal of consent, lack of motivation of the participant to complete the study, or a medical emergency.

Statistical analysis

We conducted the analyses of the effects of memantine using a per-protocol analytic approach including protocol completers. To analyse the three repeated measurements we used the Skillings-Mack test (Chatfield & Mander, 2009). We chose this within-subject nonparametric statistical test because it can be used to analyse longitudinal data when some data are missing completely at random.

Post hoc analyses for t26 vs. t0 and t52 vs. t26 were conducted by means of Wilcoxon signed-rank tests. The ES r’s of significant differences were calculated as Z/sqrt N (Tomczak & Tomczak, 2014).

We applied the highly conservative Holm-Bonferroni method (Holm, 1979) to control
for the family-wise error rate inherent in multiple comparisons with the following criteria:

Primary cognitive parameters (CANTAB memory composite score, CANTAB executive function composite score): Skillings-Mack test [Holm-Bonferroni correction for two parameters ($\alpha=0.05/2=0.025$)] and Wilcoxon [Holm-Bonferroni correction for two parameters and two post hoc tests ($\alpha=0.05/4=0.0125$)].

Primary rating scale parameters (PANSS negative symptoms, PANSS positive symptoms, PANSS total symptoms, and CGI-S): Skillings-Mack test [Holm-Bonferroni correction for four primary parameters ($\alpha=0.05/4=0.0125$)] and Wilcoxon [Holm-Bonferroni correction for four parameters and two post hoc tests ($\alpha=0.05/8=0.0062$)].

Secondary parameter (HoNOS total): Skillings-Mack test [no Holm-Bonferroni correction ($\alpha=0.05$)] and Wilcoxon [Holm-Bonferroni correction for two post hoc tests ($\alpha=0.05/2=0.025$)].

Memantine-related side effects and all subscales of the LUNSERS of the entire group were analyzed. Changes in laboratory measurements, waist circumference, and blood pressure were analyzed in individual subjects.

In accordance with the first study we conducted a post hoc analysis on the effect of memantine on two subdomains of primary negative symptoms. We used a two-factor model of the PANSS constructed by Liemburg et al. (2013): (1) expressive deficits [flat affect (N1), poor rapport (N3), lack of spontaneity and flow of conversation (N6), mannerisms and posturing (G5), motor retardation (G7) and avolition (G13)] and (2) social amotivation [emotional withdrawal (N2), passive/apathetic social withdrawal (N4) and active social avoidance (G16)].

We conducted a second post hoc analysis using the five-factor model of the PANSS constructed by van der Gaag et al. (2006) to explore effects of memantine on specific PANSS subscales.

Patients who did not receive memantine add-on therapy to clozapine ($n=3$) were not analyzed due to the small sample size of this control group.

Reliability of analyses

Change in clozapine dosage, concomitant medication, substance use, and psychotherapy during the study were analyzed in individual subjects as possible confounders.

The element of expectation was analyzed by examining a correlation between
pre-trial expectations of positive effects of memantine and difference scores (t26–t0 and t52–t26) of the PANSS and the HoNOS.

The original RCT was approved by the Central Committee on Research Involving Human Subjects and the Medical Research Ethics Committee (MREC) of Alkmaar Medical Centre. The MREC was informed about the 1-year open-label extension study, which did not require official approval, because the study was conducted in accordance with the post-trial provisions of the Declaration of Helsinki (World Medical Association, 2013), enabling all participants in an RCT to be treated with memantine augmentation (see Fig. 1).

Results

Baseline characteristics

Fig. 1 presents participant screening and enrolment flow data. Of 31 patients who had experienced beneficial effects of memantine, three patients did not participate in the extension study for various reasons and two patients chose to participate without adjunctive memantine, one patient withdrew consent, leaving 25 patients to be included in the extension study. Table 1 presents demographic and clinical characteristics of the study population of these 25 patients (mean age 42 years and mean duration of illness 19.6 years). Online Supplementary Table S1 shows demographic and clinical characteristics of the three patients who participated without adjunctive memantine, but were not included in the analysis. Subjects received care from an out-patient facility while living independently (n=14) or in a sheltered home (n=9), or had been admitted to an open long-stay ward (n=2).

After the crossover RCT, the interval without memantine treatment varied (range = 4–35 weeks). Memantine augmentation was restarted with a dose of 10 mg memantine taken orally once daily and titrated to the full dose of 20 mg after one week. In one patient memantine treatment was not interrupted between the RCT and the current extension study: the memantine phase of the crossover study was followed directly by the extension study. As much as possible, all medication remained unaltered throughout the study. Treating psychiatrists notified the principal investigator of any changes in medication.
Fig. 1. Patient disposition in an open-label extension study of adjunctive memantine for clozapine-resistant schizophrenia. MAOTC, Memantine add-on therapy to clozapine.
Clinical efficacy results

Table 2 summarizes results and statistics on all primary and secondary outcomes. Analysis of effects using the Skillings-Mack test indicated no significant differences between the 3 repeated measurements for the CANTAB composite scores for executive function and memory. Significant differences were found between the three repeated measurements for the remaining primary outcome variables: PANSS negative symptoms (Q=32.23, p<0.0001), PANSS positive symptoms (Q=20.89, p<0.0001), PANSS total symptoms (Q=31.33, p<0.0001), and CGI-S (Q=9.16, p=0.01). Post hoc Wilcoxon signed-rank tests revealed a significant improvement between t0 and t26 in PANSS negative symptoms (r=0.53), PANSS positive symptoms (r=0.50), and PANSS total symptoms (r=0.54). Improvement of CGI-S did not survive the Holm-Bonferroni correction and showed a positive trend with a moderate ES (r=0.36). After Wilcoxon signed-rank post hoc tests
Table 2. Results and analysis of primary and secondary treatment effects of memantine addition to clozapine

<table>
<thead>
<tr>
<th>Variable</th>
<th>Time=0 - 26 - 52 weeks (n=24)</th>
<th>Time=0 weeks (n=24)</th>
<th>Time=26 weeks (n=24)</th>
<th>Time=52 weeks (n=23)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (S.D.)</td>
<td>ES</td>
<td>Mean (S.D.)</td>
<td>ES</td>
</tr>
<tr>
<td>Qp</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Verbal memory</td>
<td>VRM free recall - total correct (phase 1)</td>
<td>3.39   (0.18)</td>
<td>4.70 (1.87)</td>
<td>N.A.   N.A.</td>
</tr>
<tr>
<td></td>
<td>VRM recognition - total correct</td>
<td>1.22   (0.54)</td>
<td>21.30 (1.92)</td>
<td>N.A.   N.A.</td>
</tr>
<tr>
<td>Associative learning and short term visuo-spatial memory</td>
<td>PAL total errors (adjusted)</td>
<td>0.31   (0.86)</td>
<td>60.41 (40.92)</td>
<td>N.A.   N.A.</td>
</tr>
<tr>
<td></td>
<td>PAL first trial memory score</td>
<td>0.16   (0.92)</td>
<td>10.64 (6.64)</td>
<td>N.A.   N.A.</td>
</tr>
<tr>
<td>Memory Composite</td>
<td>2.47 (0.29)</td>
<td>–0.39 (2.32)</td>
<td>–0.29 (2.52)</td>
<td>N.A.   N.A.</td>
</tr>
<tr>
<td>Working visuospatial memory / strategy use</td>
<td>SWM strategy</td>
<td>0.07   (0.97)</td>
<td>17.64 (4.19)</td>
<td>N.A.   N.A.</td>
</tr>
<tr>
<td></td>
<td>SWM between errors</td>
<td>0.74   (0.69)</td>
<td>21.36 (10.51)</td>
<td>N.A.   N.A.</td>
</tr>
<tr>
<td>Visual planning, reasoning and impulsivity</td>
<td>OTS problems solved on first choice</td>
<td>0.33   (0.85)</td>
<td>6.70 (3.57)</td>
<td>N.A.   N.A.</td>
</tr>
<tr>
<td>Executive function Composite</td>
<td>–0.21 (2.48)</td>
<td>–0.27 (2.52)</td>
<td>–0.24 (3.17)</td>
<td>N.A.   N.A.</td>
</tr>
<tr>
<td>CGI-S</td>
<td>9.16   (0.01)</td>
<td>5.54 (0.78)</td>
<td>5.21 (0.78)</td>
<td>0.36   0.011</td>
</tr>
<tr>
<td>PANSS-N</td>
<td>32.23 (0.0001)</td>
<td>17.33 (6.23)</td>
<td>15.17 (6.12)</td>
<td>0.53 &lt;0.001*</td>
</tr>
<tr>
<td>PANSS-P</td>
<td>20.89 (0.0001)</td>
<td>15.00 (3.65)</td>
<td>12.96 (2.93)</td>
<td>0.50 &lt;0.001*</td>
</tr>
<tr>
<td>PANSS total</td>
<td>31.33 (0.0001)</td>
<td>60.25 (14.16)</td>
<td>53.42 (14.07)</td>
<td>0.54 &lt;0.001*</td>
</tr>
<tr>
<td>Secondary outcome parameters</td>
<td>Reading the Mind in the Eyes test</td>
<td>1.17   (0.56)</td>
<td>18.55 (3.43)</td>
<td>N.A.   N.A.</td>
</tr>
<tr>
<td></td>
<td>CANTAB ERT % correct</td>
<td>0.30   (0.86)</td>
<td>48.54 (11.14)</td>
<td>N.A.   N.A.</td>
</tr>
<tr>
<td></td>
<td>CDSS</td>
<td>4.55   (0.10)</td>
<td>2.88 (3.55)</td>
<td>N.A.   N.A.</td>
</tr>
<tr>
<td></td>
<td>Y-BOCS total</td>
<td>1.41   (0.49)</td>
<td>3.46 (6.44)</td>
<td>N.A.   N.A.</td>
</tr>
<tr>
<td></td>
<td>HoNOS total</td>
<td>18.99 (0.0001)</td>
<td>13.58 (8.04)</td>
<td>0.33  0.018†</td>
</tr>
<tr>
<td>MANSA</td>
<td>1.25   (0.53)</td>
<td>63.79 (10.9)</td>
<td>64.75 (8.68)</td>
<td>N.A.   N.A.</td>
</tr>
</tbody>
</table>

Q, test statistic Skillings-Mack; S.D., standard deviation; ES, effect size (r) = Z/sqrt(N); CANTAB, Cambridge Neuropsychological Test Automated Battery; VRM, verbal recognition memory-immediate (free recall) and verbal recognition memory-delayed (recognition); PAL, paired associates learning; SWM, spatial working memory; OTS, One Touch Stockings of Cambridge; N.A., not applicable, since the Skillings-Mack test indicated no significant differences between the three repeated measurements. CGI-S = Clinical Global Impression Severity Scale; PANSS, Positive and Negative Syndrome Scale; PANSS-N, PANSS negative subscale; PANSS+P, PANSS positive subscale; PANSS total, PANSS total score; CANTAB, Cambridge Neuropsychological Test Automated Battery; VRM, verbal recognition memory; Skillings-Mack test indicated significant differences between the three repeated measurements at baseline, t=26 weeks and t=52 weeks. * Significant beneficial effect after Holm-Bonferroni correction for two post hoc tests and 4 primary observation scales (α=0.0062). † Significant beneficial effect after Holm-Bonferroni correction for two post hoc tests (α=0.025).
the same primary outcome variables showed significant improvement between t26 and t52: negative symptoms ($r=0.51$) PANSS positive symptoms ($r=0.39$), and PANSS total symptoms ($r=0.51$). Once again improvement of CGI-S did not survive the Holm-Bonferroni correction ($r=0.34$). However, between t0 and t52 a post hoc Wilcoxon signed-rank test revealed a large significant improvement of CGI-S ($r=0.67$, $p=0.001$).

A post hoc analysis of the two dimensions of negative symptoms (Liemburg et al. 2013) showed a substantial significant effect of memantine add-on therapy on expressive deficits between t0 and t26 ($r=0.40$, $p=0.006$) and between t26 and t52 ($r=0.34$, $p=0.022$). Similar results were found for social amotivation at t26 ($r=0.35$, $p=0.016$) and t52 ($r=0.48$, $p=0.001$).

A post hoc analysis of PANSS subscales (van der Gaag et al. 2006) showed moderate significant improvement of excitement ($r=0.37$, $p=0.009$) and emotional distress ($r=0.47$, $p=0.001$) after post hoc Wilcoxon signed-rank tests between t0 and t26. Post hoc Wilcoxon signed-rank tests revealed substantial improvement of disorganization between t0 and t26 ($r=0.54$, $p<0.001$) and between t26 and t52 ($r=0.38$, $p=0.011$).

The Skillings-Mack test indicated significant differences between the three repeated measurements for a single secondary outcome variable: the HoNOS. After Wilcoxon signed-rank post hoc tests the HoNOS showed significant improvement between t0 and t26 ($r=0.33$) and again between t26 and t52 ($r=0.49$). Social cognition, depressive symptoms, obsessive-compulsive symptoms and subjective quality of life did not significantly change during the study.

One patient discontinued memantine at t26 after a period of noncompliance. Discontinuation was not related to memantine treatment.

Possible confounders

Clozapine dosage (mean 333 mg, range = 75 mg–700 mg) remained unaltered in all but 4 subjects and alterations in concomitant medications were limited to 7 subjects (see online Supplementary Table S2). During the extension study psychotherapy and use of substances did not change significantly. One patient started using ecstasy on a weekly basis after 26 weeks, whereas he had not used this psychoactive substance during the first 6 months of the extension study. External factors which were deemed to be not related to memantine treatment triggered ecstasy abuse.
Safety and tolerability

Memantine did not affect liver and renal function nor metabolic parameters, such as waist circumference, blood pressure, blood glucose, or lipids. There was no significant increase in reported side effects assessed by LUNSERS (see Table 3).

Table 4 represents AEs. Only two AEs were possibly related to memantine: both regurgitation and mycosis are rare side effects of memantine (≥1/1000 and ≤1/100). An alternative cause for regurgitation was a high plasma clozapine level in this patient. A hazard for the development of transient mycosis in the other patient was labour in an animal shelter. No serious AEs were observed.

Discussion and conclusions

Table 3. Side effects and safety measures

<table>
<thead>
<tr>
<th>Variable</th>
<th>Time=0 weeks (n=24)</th>
<th>Time=26 weeks (n=24)</th>
<th>Time=52 weeks (n=23)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Memantine-related AE mycosis, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>18 (75)</td>
<td>19 (79.2)</td>
<td>15 (65.2)</td>
</tr>
<tr>
<td>Very little</td>
<td>2 (8.3)</td>
<td>5 (20.8)</td>
<td>4 (17.4)</td>
</tr>
<tr>
<td>Little</td>
<td>3 (12.5)</td>
<td>0</td>
<td>2 (8.7)</td>
</tr>
<tr>
<td>Much</td>
<td>0</td>
<td>0</td>
<td>2 (8.7)</td>
</tr>
<tr>
<td>Very much</td>
<td>1 (4.2)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Memantine-related AE dyspnea, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>10 (41.7)</td>
<td>11 (45.8)</td>
<td>14 (60.9)</td>
</tr>
<tr>
<td>Very little</td>
<td>2 (8.3)</td>
<td>5 (20.8)</td>
<td>3 (13.0)</td>
</tr>
<tr>
<td>Little</td>
<td>8 (33.3)</td>
<td>4 (16.7)</td>
<td>4 (17.4)</td>
</tr>
<tr>
<td>Much</td>
<td>1 (4.2)</td>
<td>3 (12.5)</td>
<td>2 (8.7)</td>
</tr>
<tr>
<td>Very much</td>
<td>3 (12.5)</td>
<td>1 (4.2)</td>
<td>0</td>
</tr>
<tr>
<td>LUNSERS: mean (s.d.)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Extrapyramidal symptoms</td>
<td>13.5 (5.1)</td>
<td>12.6 (4.1)</td>
<td>12.5 (4.8)</td>
</tr>
<tr>
<td>Anticholinergic side effects</td>
<td>8.6 (4.0)</td>
<td>8.7 (3.6)</td>
<td>9.0 (4.0)</td>
</tr>
<tr>
<td>Other autonomic side effects</td>
<td>9.3 (3.6)</td>
<td>9.1 (3.7)</td>
<td>9.4 (4.0)</td>
</tr>
<tr>
<td>Allergic reactions</td>
<td>5.1 (2.0)</td>
<td>5.3 (1.9)</td>
<td>5.5 (2.2)</td>
</tr>
<tr>
<td>Psychological side effects</td>
<td>24.5 (8.7)</td>
<td>22.8 (7.5)</td>
<td>21.4 (6.7)</td>
</tr>
<tr>
<td>Hormonal side effects</td>
<td>6.5 (2.4)</td>
<td>6.9 (2.9)</td>
<td>6.6 (2.7)</td>
</tr>
<tr>
<td>Miscellaneous side effects</td>
<td>6.8 (1.9)</td>
<td>6.7 (1.8)</td>
<td>6.8 (1.9)</td>
</tr>
<tr>
<td>Red herrings</td>
<td>14.9 (4.5)</td>
<td>15.2 (4.4)</td>
<td>15.0 (5.2)</td>
</tr>
<tr>
<td>Red herrings ≥ 20</td>
<td>0.8 (0.28)</td>
<td>0.17 (0.38)</td>
<td>0.22 (0.4)</td>
</tr>
<tr>
<td>Total</td>
<td>94.0 (24.7)</td>
<td>91.6 (23.9)</td>
<td>90.6 (26.2)</td>
</tr>
<tr>
<td>Total without red herring items</td>
<td>79.1 (20.9)</td>
<td>76.4 (20.1)</td>
<td>75.6 (21.4)</td>
</tr>
<tr>
<td>Laboratory measurement (s.d.)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clozapine daily dose, mg</td>
<td>338.5 (160.7)</td>
<td>332.3 (166.4)</td>
<td>322.8 (163.4)</td>
</tr>
<tr>
<td>Clozapine plasma level, ng/ml</td>
<td>512.6 (247.8)</td>
<td>453.3 (196.3)</td>
<td>433.6 (216.0)</td>
</tr>
<tr>
<td>Range, ng/ml</td>
<td>90 – 1050</td>
<td>80 – 794</td>
<td>53 – 857</td>
</tr>
<tr>
<td>Physical examination (s.d.)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>106.2 (16.4)</td>
<td>104.9 (15.3)</td>
<td>105.1 (15.0)</td>
</tr>
<tr>
<td>Systolic blood pressure, mmHg</td>
<td>127.3 (17.8)</td>
<td>126.5 (13.5)</td>
<td>120.5 (11.1)</td>
</tr>
<tr>
<td>Diastolic blood pressure, mmHg</td>
<td>81.4 (9.4)</td>
<td>82.9 (9.2)</td>
<td>80.9 (8.7)</td>
</tr>
</tbody>
</table>

AE, Adverse event; LUNSERS, Liverpool University Neuroleptic Side-Effect Rating Scale; s.d., standard deviation.
In our former placebo-controlled crossover study (n=52) we found a small improvement of memory and negative symptoms after 12 weeks of memantine treatment (Veerman et al. 2016). The current 1-year extension study provided an opportunity to find an indication whether further improvement can be expected from long-term memantine treatment of clozapine-refractory schizophrenia.

In the former placebo-controlled trial memantine showed a small significant improvement of memory after 12 weeks of treatment. This effect was sustained in the extension study without further improvement. Both the placebo-controlled trial and the extension study failed to show any effect of memantine on executive function.

While in the placebo-controlled trial negative symptoms significantly improved with a small ES, in the extension study negative symptoms ameliorated further, with a large ES between t0 and t26 and between t26 and t52. In the placebo-controlled trial a non-significant small effect was found for one subdomain: diminished expression (d=0.17). An equal and moderate effect of memantine on expressive deficits and social amotivation was found at t26 and t52.

Although positive and overall symptoms of schizophrenia did not significantly improve in the placebo-controlled trial, these symptoms showed substantial significant improvement after 26 weeks of memantine adjunctive treatment, followed by even further improvement at t52. The ESs ranged from moderate to large.

Whereas in the primary placebo-controlled study only memory and negative symptoms significantly improved, in the 1-year extension study most outcomes (negative

<table>
<thead>
<tr>
<th>Adverse events</th>
<th>Memantine add-on therapy</th>
<th>No add-on therapy</th>
<th>Time, weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Have adverse event</td>
<td>15</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Increase in menopausal hot flashes</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mycosis (vagina/ feet/hands)</td>
<td>1</td>
<td></td>
<td>4 / 24 / 34</td>
</tr>
<tr>
<td>Increase in paranoia</td>
<td>2</td>
<td></td>
<td>16 / 20</td>
</tr>
<tr>
<td>Nausea and vomiting</td>
<td>1</td>
<td></td>
<td>17</td>
</tr>
<tr>
<td>Reflux and dysphagia/urinary tract infection</td>
<td>1</td>
<td></td>
<td>25 / 36</td>
</tr>
<tr>
<td>Flu-like symptoms / urinary tract infection</td>
<td>1</td>
<td></td>
<td>26 / 47</td>
</tr>
<tr>
<td>Collapse/hemifacial spasm/vitamin D deficiency</td>
<td>1</td>
<td></td>
<td>27 / 39 / 39</td>
</tr>
<tr>
<td>Dizziness</td>
<td>1</td>
<td></td>
<td>28</td>
</tr>
<tr>
<td>Disorientation and incoherent thought</td>
<td>1</td>
<td></td>
<td>30</td>
</tr>
<tr>
<td>Elevated triglycerides and cholesterol</td>
<td>1</td>
<td></td>
<td>37</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>1</td>
<td></td>
<td>42</td>
</tr>
</tbody>
</table>

Table 4. Adverse events
symptoms, positive symptoms, overall symptoms, and impairments in psychosocial functioning) showed improvement, with moderate to large ESs. Long-term beneficial effects of memantine were also reflected in a moderate positive trend of the CGI-S at t26 and t52.

Memantine was well-tolerated without significant change in side effects or metabolic parameters. No serious AEs were reported.

In summary, positive long-term effects of memantine add-on therapy to clozapine were found on nearly all aspects of interest, except executive function, social cognition, depressive symptoms, obsessive-compulsive symptoms and subjective quality of life.

A possible explanation for the more substantial beneficial effects in the extension study compared to the placebo-controlled study is the longer treatment duration. It is conceivable that the 12-week treatment period with memantine in the placebo-controlled trial was too short and that clinical improvement would have occurred in more subjects in the placebo-controlled trial if memantine add-on therapy had been extended for a longer period. After 1 year of memantine addition to clozapine we found moderate to large beneficial effects for most outcomes. These effects were more substantial than in the double-blind trial, but still not as large as the very large ESs of the short-term RCT by de Lucena et al. (2009). This discrepancy may be explained by the differences in patient population. Our participants were older (mean 7.7 years older), more severely ill (CGI-S scores 6.15 vs. 5.34) due to severe cognitive impairments, and had been suffering from schizophrenia for a longer period of time (mean 5.04 years longer). Therefore, substantial improvement of symptoms may have been more difficult to reach in our patient population.

Our results must be interpreted within their limitations. Because only three patients chose to participate without adjunctive memantine treatment, we were unable to compare the intervention group with a control group. Moreover, the open-label design is susceptible to bias such as observer and participant expectancy effects, which may have resulted in overestimation of the beneficial effects of memantine (Sackett, 1979; Crow et al. 1999; Fisher & Greenberg, 1993). However, these biases are thought to have a more modest influence on CANTAB results (Barnett et al. 2010). Furthermore, subjective quality of life was not rated as significantly improved, at least showing that expectancy was not pervasive to all outcome domains. This is supported by measurements of expectations in participants before the extension phase. In our patient population the measured expectations of efficacy of memantine were very small to small in approximately 50%. Furthermore, no association was found between expectation scores and experienced effect of memantine (data not shown). Another potential bias is participation in a medication trial, which might possibly
lead to better care and adherence which in turn might mediate better outcomes (Kinon et al. 2011). However, participating patients did not receive any extra attention or treatment apart from being given memantine and participating in three assessment sessions – at baseline, after six months and after one year. Substantial improvement due only to these three sessions seems unlikely. Practice effects for the CANTAB due to serial cognitive assessments are another possible limitation. In healthy subjects some CANTAB tests show low test-retest reliability, in particular tests on executive function (Lowe & Rabbit, 1998). Test-retest stability is lower in patients with schizophrenia (Beglinger et al. 2003). Practice effects in patients with severe cognitive impairments suffering from refractory schizophrenia are even more improbable. In our study comparisons of verbal memory were even less compromised than other cognitive domains, because the second VRM task differed from the first and third VRM task. Because the interval was 26 weeks between three cognitive assessments, significant practice effects in this patient population were highly unlikely.

Considering that robust evidence is limited for treatment options for residual symptoms in clozapine refractory schizophrenia, future studies are imperative to find effective approaches. The presently available results on memantine in combination with clozapine in refractory schizophrenia should encourage a large, double-blind, placebo-controlled trial with a treatment duration of at least 26 weeks in order to ascertain any beneficial effects of memantine. In patients with less severe residual symptoms memantine may have even stronger beneficial effects after a shorter treatment period, given the large ESs de Lucena et al. (2009) found in this group. Because renal clearance of memantine is superior in adult patients compared to elderly, a higher dosage of 30–40 mg is thought to be safe (Schwenkreis et al. 2005; Areosa & Sherriff, 2003; Beister et al. 2004; Schifitto et al. 2007; Ferguson & Shingleton, 2007; Wiech et al. 2001; Wiech et al. 2004) and may prove to be more efficacious. It is also conceivable that memantine has more efficacy in patients in the early stages of schizophrenia when neurodegenerative changes are more limited. At present no dose-ranging study has been conducted in schizophrenia patients. It would be interesting to investigate dose-dependent effects of memantine, because a higher dosage than 20 mg may yield more improvement. Future study could also include younger clozapine-treated patients with TRS. In our study as well as previous clinical trials in patients with TRS the effect of memantine on parameters of central glutamatergic transmission and brain activity was not assessed. In future studies it would be interesting to use 1H-MRS and fMRI to investigate whether memantine normalizes glutamate levels and brain activity in TRS and whether this is related to treatment effects.
Given the paucity of efficacious pharmacological options for clozapine-refractory schizophrenia patients, adjunctive memantine is an interesting treatment option based on the glutamate hypothesis, two positive double-blind, placebo-controlled RCTs, and the current open-label extension study.

Acknowledgments
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**Supplementary Table S1. Demographics and baseline characteristics of the group without add-on therapy**

<table>
<thead>
<tr>
<th>Variable</th>
<th>No add-on therapy (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, n (%)</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>1 (33.3)</td>
</tr>
<tr>
<td>Male</td>
<td>2 (66.7)</td>
</tr>
<tr>
<td>Education level, n (%)</td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>1 (33.3)</td>
</tr>
<tr>
<td>Middle</td>
<td>0</td>
</tr>
<tr>
<td>High</td>
<td>2 (66.7)</td>
</tr>
<tr>
<td>Living conditions, n (%)</td>
<td></td>
</tr>
<tr>
<td>Independently</td>
<td>3 (100.0)</td>
</tr>
<tr>
<td>Sheltered home</td>
<td>0</td>
</tr>
<tr>
<td>Long-stay ward</td>
<td>0</td>
</tr>
<tr>
<td>Alcohol use, n (%)</td>
<td>2 (66.7)</td>
</tr>
<tr>
<td>Cannabis use, n (%)</td>
<td>0</td>
</tr>
<tr>
<td>Cocaine use, n (%)</td>
<td>1 (33.3)</td>
</tr>
<tr>
<td>Clozapine use, n (%)</td>
<td></td>
</tr>
<tr>
<td>Clozapine (single antipsychotic)</td>
<td>3 (100.0)</td>
</tr>
<tr>
<td>Combination clozapine + second / third antipsychotic</td>
<td>0</td>
</tr>
<tr>
<td>Combination clozapine + antidepressant</td>
<td>0</td>
</tr>
<tr>
<td>Combination clozapine + mood stabiliser</td>
<td>0</td>
</tr>
<tr>
<td>Combination clozapine + benzodiazepine</td>
<td>0</td>
</tr>
<tr>
<td>Combination clozapine + E-EPA</td>
<td>0</td>
</tr>
<tr>
<td>Combination clozapine + fish oil</td>
<td>0</td>
</tr>
<tr>
<td>Psychotherapy during past 6 months, n (%)</td>
<td>1 (33.3)</td>
</tr>
<tr>
<td>Mean age, years (S.D.)</td>
<td>39.3 (8.3)</td>
</tr>
<tr>
<td>Mean years of education (S.D.)</td>
<td>15 (2.0)</td>
</tr>
<tr>
<td>Mean age of onset, years (S.D.)</td>
<td>15 (1.0)</td>
</tr>
<tr>
<td>Mean duration untreated psychosis, months (S.D.)</td>
<td>88 (121.4)</td>
</tr>
<tr>
<td>Mean duration of illness, years (S.D.)</td>
<td>15.0 (1.0)</td>
</tr>
<tr>
<td>Mean clozapine daily dose, mg (S.D.)</td>
<td>350 (100)</td>
</tr>
</tbody>
</table>

E-EPA, Ethyl eicosapentaenoic acid; S.D., standard deviation.
## Supplementary Table S2. Medication changes

<table>
<thead>
<tr>
<th>Time, weeks</th>
<th>Medication change</th>
<th>Immediate cause</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Clozapine dosage</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>200 mg decrease</td>
<td>cessation of smoking</td>
</tr>
<tr>
<td>16</td>
<td>50 mg increase</td>
<td>increasing paranoia due to alcohol abuse</td>
</tr>
<tr>
<td>20</td>
<td>50 mg increase</td>
<td>increasing paranoia due to stress</td>
</tr>
<tr>
<td>25</td>
<td>50 mg decrease</td>
<td>dysphagia and regurgitation</td>
</tr>
<tr>
<td></td>
<td>Concomitant medication</td>
<td></td>
</tr>
<tr>
<td>4*</td>
<td>antifungal cream</td>
<td>vaginal mycosis</td>
</tr>
<tr>
<td>21</td>
<td>opioid</td>
<td>cervical hernia</td>
</tr>
<tr>
<td>24*</td>
<td>antifungal cream</td>
<td>mycosis of the feet</td>
</tr>
<tr>
<td>25</td>
<td>pantoprazole</td>
<td>dysphagia and regurgitation</td>
</tr>
<tr>
<td>28</td>
<td>cessation of beta blocker</td>
<td>dizziness and hypotension</td>
</tr>
<tr>
<td>34*</td>
<td>steroidal anti-inflammatory cream</td>
<td>mycosis of the hands</td>
</tr>
<tr>
<td>35</td>
<td>reduction of benzodiazepines</td>
<td>dependence of benzodiazepines</td>
</tr>
<tr>
<td>36</td>
<td>statin</td>
<td>elevation of cholesterol and triglycerides</td>
</tr>
<tr>
<td>42</td>
<td>ranitidine hydrochloride</td>
<td>abdominal pain</td>
</tr>
</tbody>
</table>

All medication changes are for different subjects, except for events marked by * which belong to one single subject.
References


Buckley P, Miller A, Olsen J, Garver D, Miller DD, Csernansky J (2001). When symp-


tyric acid and glutamate-glutamine levels in schizophrenia measured in vivo with proton magnetic resonance spectroscopy. Archives of General Psychiatry 69, 449-459.


Chapter 8

Summary and general discussion
Pharmacological Interventions in Clozapine-Refractory Schizophrenia

Summary and general discussion

The main focus of this thesis is on treatment of clozapine-resistant schizophrenia. Robust evidence is limited as regards efficacious treatment for residual symptoms in clozapine-resistant schizophrenia. According to the glutamate hypothesis (Kantrowitz & Javitt, 2010), the disconnection hypothesis (Stephan et al. 2009) and the immune hypothesis (Muller & Schwarz, 2006) the NMDA receptor is affected in schizophrenia. In TRS glutamatergic alterations may be responsible for a lack of response to dopamine-blocking antipsychotic medication (Demjaha et al. 2014; Mouchlianitis et al. 2015). We therefore investigated whether an agent which specifically targets the NMDA receptor might have clinical benefits in combination with clozapine. In a 26-week single-centre double-blind crossover trial 52 clozapine-treated patients with refractory schizophrenia were randomly assigned to twelve weeks of adjunctive treatment with memantine or placebo. Memory and executive function, negative, positive, and overall symptoms and global severity of psychopathology were primary endpoints. Secondary endpoints were social cognition, depressive and obsessive-compulsive symptoms, psychosocial functioning, subjective quality of life and adverse events. Twenty-four completers of the primary placebo-controlled study who experienced beneficial effects from memantine received memantine during one year in an open-label extension study. Long-term effects with the same endpoints and also tolerability were assessed to establish whether this clozapine augmentation strategy has potential and deserves further study.

Main findings

To begin with, I will summarize the main findings of Chapters Two to Seven. I will then present an overview of efficacious and safe interventions in treatment-resistant schizophrenia (TRS) for the three symptom domains of schizophrenia: cognitive dysfunction, negative symptoms, and positive symptoms.
1. Treatment for negative symptoms in schizophrenia: a comprehensive review

In the second chapter we investigated whether any convincing evidence currently exists to support interventions for unspecified negative symptoms in schizophrenia (Veerman et al. 2017b). We explored whether efficacious pharmacological treatment options for negative symptoms (without distinguishing between primary and secondary negative symptoms) are different in schizophrenia patients receiving non-clozapine antipsychotics compared to clozapine-treated patients with TRS. There is no convincing evidence regarding efficacy for treatment of primary negative symptoms, since little research has been conducted in patients suffering predominantly from persistent negative symptoms in the non-acute phase which also controls for confounders such as positive symptoms, depressive symptoms, and extrapyramidal side effects. Based on reviews and meta-analyses of randomized, double-blind, controlled trials (RCTs) that did not focus on primary negative symptoms we found evidence for modest short-term efficacy of several interventions in patients with unspecified negative symptoms. Below I present an overview of the main findings and my recommendations for clinical care.

First of all, we emphasized the importance of early detection and treatment of psychosis to prevent or reduce primary negative symptoms (Perkins et al. 2005; Boonstra et al. 2012; Murru & Carpiniello, 2016) and to improve functional outcome (Perkins et al. 2005; Penttilä et al. 2014; Souaiby et al. 2016). Secondly, we recommended using the lowest possible dosage of antipsychotic medication in order to prevent iatrogenic negative symptoms and improve subjective experiences (de Haan et al. 2000, 2003, 2004; Mizrahi et al. 2007). Furthermore, we encouraged psychiatrists to motivate their patients to exercise frequently and vigorously (Firth et al. 2015) with aerobic training or yoga (Dauwan et al. 2016). We also suggested music therapy to treat unspecified negative symptoms (Mössler et al. 2011; Tseng et al. 2016, Lutgens et al. 2017).

We also found that meta-analytic comparisons of antipsychotic medication did not reveal any clear benefits of SGAs versus FGAs (Leucht et al. 2009b; Fusar-Poli et al. 2015; Zhang et al. 2013) or consistent superior efficacy of any single antipsychotic for improvement in negative symptoms (without discrimination between primary and secondary negative symptoms) (Leucht et al. 2009c; Samara et al. 2016; Harvey et al. 2016). Even for clozapine no consistent evidence of greater benefits for negative symptoms (without primary/secondary distinction) was found (Essali et al. 2009; Asenjo et al. 2010; Souza et
Aripiprazole (Zheng et al. 2016a; Galling et al. 2017), antidepressants (Singh et al. 2010; Hecht & Landy, 2012; Kishi & Iwata, 2014, Terevnikov et al. 2015; Helfer et al. 2016) and topiramate (Correll et al. 2016; Okuyama et al. 2016; Zheng et al. 2016b) are adjunctive agents which showed convincing modest efficacy for the treatment of unspecified negative symptoms. Although in general it is better to avoid treatment with two antipsychotic drugs, aripiprazole combined with another antipsychotic seems to be an exception (Galling et al. 2017).

A final important recommendation is that potential side effects should be considered carefully in the shared decision-making process regarding medication.

2. The glutamate hypothesis: a pathogenic pathway from which pharmacological interventions have emerged

In Chapter Three we explained how the NMDA receptor plays a key role in learning and memory, facilitated by a cellular mechanism called long-term potentiation (LTP) which causes long-lasting enhancement of signal transmission between two neurons (Kornmeier & Sosic-Vasic, 2012; Veerman et al. 2014a).

We presented four lines of evidence supporting the hypothesis that dysfunction of the NMDA receptor is an important underlying mechanism of schizophrenia (Tiihonen et al. 2006; Javitt et al. 2006; Takata et al. 2013; Wandinger et al. 2001; Marsman et al. 2011).

We discussed how compromised glutamate uptake in NMDA receptor hypofunction causes glutamate spill-over in the synaptic cleft, which triggers cell death (Goff & Coyle, 2001; Papanastasiou et al. 2013). We explained how increased apoptotic activity in the early stages of schizophrenia may account for neurodevelopmental abnormalities, which contribute to cognitive disturbances and negative symptoms (Marsman et al. 2011). We illustrated a second mechanism for impaired frontal lobe-related cognitive function and negative symptoms due to abnormal cortical signal-to-noise patterns, which are caused by deregulated glutamatergic transmission in the prefrontal cortex (PFC) (Homayoun et al. 2007; Jackson et al. 2004). Furthermore, we clarified how positive symptoms are caused by NMDA receptor hypofunction, resulting in dopaminergic hyperfunction in the amygdala through reciprocal synaptic relationships between glutamatergic systems and mesolimbic dopaminergic projections (Gordon, 2010).

We reviewed how glutamate agonists combined with non-clozapine antipsychotics
show interesting clinical benefits in refractory schizophrenia, while glutamate antagonists have favourable effects when added to clozapine.

We illustrated how the combination of clozapine and the voltage-dependent NMDA receptor antagonist memantine may indirectly modulate LTP through upregulation of NMDA receptors (Joshi et al. 2007). Clozapine may also potentiate neuroprotective effects of memantine for NMDA-induced excitotoxicity. Hence, this synergistic effect of clozapine combined with memantine may improve cognitive functioning in TRS. Together these ligands may cause an improved signal-to-noise ratio, which is associated with improved regulation of NMDA transmission in the PFC and improvement of negative symptoms (Goff & Coyle, 2001; Javitt, 2006; Stone et al. 2007). A long-term beneficial effect on positive symptoms may be caused by improved inhibition of prefrontal cortical GABA interneurons, which are responsible for recurrent inhibition of pyramidal neurons (Homayoun & Moghaddam, 2007).

Based on my review of the literature I proposed a study design for a proof-of-concept study on memantine augmentation to clozapine in TRS.

### 3. Non-glutamatergic clozapine augmentation strategies: a review and meta-analysis

In Chapter Four we presented an overview of 22 randomized, double-blind, placebo-controlled trials assessing the potential clinical utility of the addition of non-glutamatergic agents to clozapine: a second antipsychotic, an antidepressant, ethyl eicosapentaenoic acid (E-EPA), lithium and extract of ginkgo biloba (Veerman et al. 2014b).

We concluded that there is little robust evidence for the efficacy of any of these clozapine augmentation strategies in treatment-resistant schizophrenia. Clozapine augmentation with a second antipsychotic showed a small but significant beneficial effect (ES=0.25, p=0.023) on negative symptoms after exclusion of the outlier study by Gunduz-Bruce et al. (2013). Although comparisons with placebo were not significant, aripiprazole (ES=0.34, p=0.069) resulted in a more pronounced reduction of negative symptoms than risperidone (ES=0.19, p=0.356). Other arguments in favour of aripiprazole are tolerability and potential reduction of the metabolic risk factors associated with clozapine after comedication with aripiprazole (Newcomer, 2005; Brixner et al. 2006; Englisch & Zink, 2008; Fleischhacker et al. 2010; Fan et al. 2013). For residual positive symptoms, antipsychotic medication or antidepressant medication as adjunctive treatment to clozapine did not
show significant changes compared to placebo. Antidepressants showed no significant clinical beneficial effect compared to placebo, not even for affective symptoms (ES=0.25, \( p=0.671 \)). Surprisingly, antipsychotics showed a trend towards improving affective symptoms (ES=0.30, \( p=0.068 \)), suggesting a more beneficial effect on affective symptoms when compared to antidepressants.

In conclusion, augmentation with a second antipsychotic may slightly improve negative symptoms, but we found no robust evidence regarding the efficacy of augmentation of clozapine with an antidepressant, E-EPA, lithium or extract of ginkgo biloba in clozapine-resistant schizophrenia.

4. Clozapine augmented with glutamate modulators in refractory schizophrenia: a review and meta-analysis

In Chapter Five we sought evidence for the clinical utility of augmentation of glutamate agonists and antagonists in clozapine-resistant schizophrenia (Veerman et al. 2014c). We presented an overview of 18 double-blind, placebo-controlled RCTs on adjunctive glutamatergic agents to clozapine with data on negative, positive, and overall symptoms of schizophrenia.

In six studies on NMDA receptor agonists combined with clozapine we found no favourable effects. In a meta-analysis of three studies on clozapine combined with glycine, we found significant worsening of positive symptoms compared to placebo. Ongoing synaptic activity caused by the combination of two glutamate agonists possibly results in persistent downregulation of NMDA receptors, which explains the absence of efficacy and even worsening of symptoms due to this combination therapy. In one single positive study ampakine CX516 improved negative, overall clinical symptoms and cognitive functioning compared to placebo after four weeks (Goff et al. 2001). The combination of clozapine and this positive allosteric modulator of the \( \alpha \)-amino-3-hydroxy-5-methyl-4-isoxazole proprionic acid (AMPA) receptor may have favourable effects, because this combination does not lead to downregulation of NMDA receptors.

A meta-analysis of four studies on topiramate add-on therapy to clozapine did not show significant difference between topiramate and placebo as regards negative symptom improvement. In a meta-analysis of six studies on lamotrigine addition to clozapine we found a trend towards improving negative symptoms (ES=0.35, \( p=0.065 \)) after exclusion
of two outlier studies by Zoccali et al. (2007) and Vayısoğlu et al. (2013). Lamotrigine also showed a positive trend compared to placebo (ES=0.31, p=0.065) towards reducing positive symptoms.

We emphasized the promising beneficial effects of memantine with exceptionally large effect sizes in all symptom domains in the first proof-of-concept study by de Lucena et al. (2009) in clozapine-resistant patients with prevailing negative symptoms.

5. Memantine augmentation in clozapine-refractory schizophrenia: a randomized, double-blind, placebo-controlled crossover study

In Chapter Six we investigated the efficacy and tolerability of memantine addition in clozapine-treated refractory schizophrenia (Veerman et al. 2016). Memantine, which is licensed for the treatment of moderate-to-severe Alzheimer’s disease (Areosa et al. 2005), is a pro-cognitive drug which targets the NMDA receptor. The NMDA receptor is highly expressed in the hippocampus (Bliss & Collingridge, 1993) and is essential in functional connectivity between brain circuits, involving the PFC through neuroplasticity. Combination therapy of clozapine and memantine targets altered NMDA receptor-mediated neurotransmission in schizophrenia and may therefore have beneficial effects on all symptom domains, including memory and executive function (Javitt & Zukin, 1991; Bressan & Pilowsky, 2000; Homayoun & Moghaddam, 2007; Howes & Kapur, 2009; Orellana & Slachevsky, 2013).

We randomly assigned 52 clozapine-treated patients with refractory schizophrenia to twelve weeks of double-blind adjunctive treatment with memantine or placebo. After a 2-week placebo wash-out period crossover occurred. A composite memory score consisting of the Cambridge Neuropsychological Test Automated Battery (CANTAB) verbal recognition memory and paired associated learning task scores (ES=0.30, p=0.032) and the Positive and Negative Syndrome Scale (PANSS) negative subscale score (ES=0.29, p=0.043) were the only primary endpoints which significantly improved compared to placebo. This study yielded no significant difference between memantine and placebo for executive function, positive symptoms, overall symptoms of schizophrenia and global clinical impression after a treatment period of twelve weeks. Memantine was generally well tolerated with mild and transient allergic side effects. In one participant dizziness in the memantine phase was the reason for discontinuation.

In our placebo-controlled study we found more moderate beneficial effects of me-
mantine add-on therapy than de Lucena et al. (2009). However, the results of this first proof-of-concept study should be interpreted with caution, since initially stronger effects are not unusual among RCTs (Ioannidis, 2005; Tajika et al. 2015). Furthermore, the efficacy of memantine may have been overestimated by chance due to the relatively small sample of 21 patients (Sinclair & Adams, 2014; Tajika et al. 2015). Moreover, our patient population was older, more severely ill due to severe cognitive impairments and had been suffering from schizophrenia for a longer period of time compared with the patients included in the first proof-of-concept study.

Our study provided further evidence supporting memantine as an adjunct to clozapine, since we found beneficial effects on memory and negative symptoms in patients with TRS and severe cognitive impairments after a memantine treatment duration of twelve weeks. An extension study on the long-term effects of this novel approach to treating clozapine-resistant schizophrenia was therefore justified.

6. Adjunctive memantine in clozapine-treated refractory schizophrenia: an open-label 1-year extension study

In Chapter Seven we investigated the long-term effects and tolerability of memantine augmentation in patients with clozapine-resistant schizophrenia in completers of the placebo-controlled trial who experienced beneficial effects during twelve weeks of memantine treatment (Veerman et al. 2017a). Of 25 patients who were included in the open-label, one-year extension study, one patient discontinued memantine at 26 weeks after a period of noncompliance, deemed unrelated to memantine treatment. While executive function still did not improve, the small significant improvement of memory after twelve weeks of memantine treatment in the former study (Veerman et al. 2016) was sustained in the extension study without further improvement. We observed further improvement of negative symptoms with large effect sizes between baseline and 26 weeks ($r=.53$, $p<0.001$) and between 26 weeks and 52 weeks ($r=0.51$, $p<0.001$). Although positive and overall symptoms of schizophrenia did not significantly improve in the twelve weeks treatment phase during the RCT, long-term beneficial effects of memantine were reflected in substantial improvement of positive symptoms ($r=0.50$, $p=0.001$) and overall symptoms ($r=0.54$, $p<0.001$) at 26 weeks. We observed even further improvement of positive symptoms ($r=0.39$, $p=0.006$) and overall symptoms ($r=0.51$, $p<0.001$) at 52 weeks.
Global clinical impression showed a moderate positive trend after 26 weeks and 52 weeks. Impairments of psychosocial functioning also showed significant improvement between baseline and week 26 week ($r=0.33$, $p=0.018$) and again between week 26 and week 52 ($r=0.49$, $p<0.001$). We found no significant positive long-term effects of adjunctive memantine to clozapine on social cognition, depressive symptoms, obsessive-compulsive symptoms or subjective quality of life. Memantine was well tolerated without serious adverse effects.

In conclusion, memantine seems a promising treatment option for clozapine-resistant schizophrenia patients, based on the glutamate hypothesis, two positive short-term double-blind, placebo-controlled RCTs and our open-label, one-year extension study.

**Main findings concerning cognitive, negative and positive symptom domains in TRS**

**Cognitive dysfunction**

At present there are no known satisfactory pharmacological treatment options for cognitive impairments in patients suffering from schizophrenia (Javitt et al. 2015), let alone TRS. Clozapine may have beneficial effects on several cognitive domains (Meltzer & McGurk, 1999; Molina et al. 2014; Spagna et al. 2015). However, there is no robust evidence supporting the superiority of clozapine for cognitive symptoms. Memantine addition to clozapine may benefit memory in TRS patients, based on the favourable results of two proof-of-concept studies (de Lucena et al. 2009; Veerman et al. 2016). The small short-term effect of adjunctive memantine in clozapine-treated patients suffering from severe cognitive impairments was sustained in the open-label extension study without further improvement or decline of memory (Veerman et al. 2017a). However, the evidence is too limited to permit firm conclusions regarding the benefits of memantine for cognitive functioning in clozapine-resistant schizophrenia.

Non-pharmacological interventions such as neurocognitive and social cognitive interventions seem to have small to moderate favourable effects on neurocognition and social cognition (Roder et al. 2011; Kurtz & Richardson, 2012; Lindenmayer et al. 2013; Pinkham & Harvey, 2013; Mueller et al. 2015). The same applies to a high level of weekly exercise (Firth et al. 2016). In addition, yoga may specifically improve long-term memory (Dauwan et al. 2016).
Negative symptoms

For persistent negative symptoms in TRS patients adjunctive aripiprazole may have a small beneficial effect (Veerman et al. 2014b; Zheng et al. 2016a). Antidepressants as an adjunct to clozapine are a second option to consider (Veerman et al. 2014b, 2017b). A third treatment option is the addition of glutamate antagonists to clozapine (Veerman et al. 2014c). The evidence supporting adjunctive topiramate in clozapine-resistant schizophrenia is the most convincing, with a moderate effect size in a recent meta-analysis (Zheng et al. 2016b). The most impressive efficacy for primary negative symptoms was found in a small 12-week RCT on memantine addition to clozapine (de Lucena et al. 2009), but this finding was confounded by a large improvement of positive symptoms, while depressive symptoms were not assessed. We did not replicate this huge improvement of negative symptoms in our second proof-of-concept study. We found only a small significant effect on negative symptoms compared to placebo after twelve weeks of memantine treatment in clozapine-refractory patients (Veerman et al. 2016). The substantial improvement of negative symptoms after 26 weeks and between 26 weeks and 52 weeks of memantine treatment was demonstrated in an open-label trial (Veerman et al. 2017a) and therefore requires further exploration in an RCT with a duration of six months to one year.

Two non-pharmacological treatment options for which meta-analytic comparisons have demonstrated some evidence of improvement of negative symptoms (without primary/secondary distinction) are music therapy (Mössler et al. 2011; Tseng et al. 2016, Lutgens et al. 2017) and physical exercise (Firth et al. 2015; Dauwan et al. 2016), with similar efficacy for aerobic exercise and yoga (Dauwan et al. 2016).

Positive symptoms

At present the only evidence-based pharmacological treatment for clozapine-resistant positive symptoms is topiramate, with a moderate significant effect (Zheng et al. 2016b). The evidence supporting memantine in a double-blind RCT is limited to the first, small proof-of-concept study (de Lucena et al. 2009) in which a substantial improvement in positive symptoms was found compared to placebo. Evidence of the substantial improvement in positive symptoms after 26 weeks and between 26 weeks and 52 weeks memantine treatment was demonstrated in an open-label trial (Veerman et al. 2017a) and therefore requires further
exploration in an RCT with a duration of six months to one year.

Two non-pharmacological interventions with small to moderate beneficial effects compared to treatment as usual are cognitive behavioural therapy, with a particularly favourable effect on hallucinations (Jauhar et al. 2014), and physical exercise (Firth et al. 2015; Dauwan et al. 2016). Aerobic exercise and yoga seem to be equally efficacious for positive symptoms (Dauwan et al. 2016).

Electroconvulsive therapy was tested in one single-blind RCT ($n=39$) and found to result in at least a 40% reduction of positive symptoms in approximately 50% of patients (Petrides et al. 2015). It should therefore also be considered in TRS patients suffering from persistent positive symptoms.

**Methodological considerations**

Firstly, I will discuss the limitations of the reviews and meta-analyses I presented in Chapters Two to Five. Secondly, I will examine the strengths and limitations of the proof-of-concept study and open-label study on adjunctive memantine to clozapine as described in Chapters Six and Seven.

1. **Reviews and meta-analyses**

Most evidence regarding interventions for negative symptoms, discussed in Chapter Two, is based on short-term RCTs with negative symptoms as a primary or secondary outcome, conducted in chronically ill patients stable on medication and in heterogeneous study groups of patients in the acute and chronic phase of their illness with a schizophrenia spectrum disorder. A distinction between primary and secondary negative symptoms is not possible, since adequate data regarding a stable disease course prior to the start of the study and confounding factors (positive, depressive and extrapyramidal symptoms) are not available. In most meta-analyses and reviews on the efficacy of interventions for negative symptoms there is a substantial degree of heterogeneity between primary studies. Future research should therefore focus on long-term efficacy and be conducted in patients suffering predominantly from persistent negative symptoms. The scope of methods should perhaps be broadened to include both double-blind RCTs and long-term prospective naturalistic
studies (Leichsenring, 2004). In addition, evidence regarding the efficacy of clozapine is hampered by the small number of double-blind RCTs, the short treatment duration, rapid clozapine titration, and non-equivalent dosing in comparisons of clozapine versus FGAs or other SGAs.

Comparative research on different non-glutamatergic clozapine augmentation strategies, a topic addressed in Chapter Four, is hampered by the limited number of RCTs on the efficacy of specific pharmacological agents in clozapine-resistant schizophrenia, small sample sizes, limited duration of treatment and different statistical analyses. In Chapter Five we found that for glutamate antagonists as well, large-scale, long-term, placebo-controlled trials are required to determine their efficacy and tolerability in clozapine-resistant patients.

2. Memantine studies

One strength of our memantine studies is that we improved and built on the methods used in the study by de Lucena et al. (2009). We used the MATRICS Consensus Cognitive Battery (MCCB) (Nuechterlein et al. 2008) instead of the Mini-Mental State Examination (MMSE) (Folstein et al. 1975) and the PANSS negative subscale with seven items instead of the Brief Psychiatric Rating Scale (BPRS) which covers only three negative symptom items (blunted affect, emotional withdrawal and psychomotor retardation) (Eckert et al. 1996). We also used additional measures in order to assess social cognition, depressive symptoms, obsessive compulsive symptoms, psychosocial functioning, and subjective quality of life. While de Lucena et al. (2009) used the Simpson-Angus Scale (SAS) to assess extrapyramidal symptoms (Simpson & Angus, 1970), we used more elaborate self-ratings of the Liverpool University Neuroleptic Side-Effect Rating Scale (LUNSERS) (Day et al. 1995) with additional rating of Likert scales for possible side effects of memantine (thrombosis, dyspnoea, and mycosis).

However, there are several important limitations to our placebo-controlled study. The main limitation is the treatment duration, which may have been too short to achieve the optimal treatment effect. Studies in Alzheimer’s disease show a greater effect after 24 to 28 weeks of treatment in comparison to twelve weeks of treatment (Puangthong & Hsiung, 2009). Secondly, the wash-out period of two weeks also proved to be too short, since carry-over effects were significant for the CANTAB composite memory score and the positive, negative and total scores of the PANSS.
Another important issue to address is the relatively small sample size (Sinclair & Adams, 2014), which does not generate enough power to show response to memantine, defined as a reduction of at least 25% of the baseline score (Leucht et al. 2009c). In our open-label extension study the sample size was even smaller than in our primary study. We were unable to compare the intervention group with the control group without adjunctive memantine treatment, since this group consisted of only three patients.

A potential bias is selection, which may have influenced our results. It is possible that among the 64 patients suffering from TRS at the FACT facilities of the Mental Health Service Noord-Holland Noord who declined to participate there was a high level of paranoia. During the screening, education sessions about interventional human subject research or just the term ‘information for experimental subjects’ triggered some patients to abruptly cease the conversation and decline participation in the study. Perhaps our study population was not representative of patients with TRS in general and only patients with a relatively low level of paranoia were included in the study.

It is important to note that the beneficial effects of memantine in our extension study may be overestimated due to the open-label design (Sackett, 1979; Fisher & Greenberg, 1993; Crow et al. 1999). Although observer bias is a relevant shortcoming in our secondary study for all measurements except the CANTAB results (Barnett et al. 2010), participant expectancy effects seem likely to be limited, firstly because before the extension phase expectations of efficacy of memantine were low to very low in approximately 50% of our study population and secondly, because we found no association between measured expectation scores and the experienced effect of memantine. Thirdly, expectancy effects were not consistent, because not all outcomes showed significant improvement.

Furthermore, participation in our trials may have led to better care and improved adherence, with a favourable effect on study outcomes (Kinon et al. 2011). This potential bias is limited to the extension phase and seems likely to be minimal, since no extra attention or treatment was given to participating patients apart from three assessment sessions over a period of one year.

We chose the CANTAB to assess cognitive domains relevant to schizophrenia, because this is a comprehensive, validated cognitive battery which is sensitive and easy to administer (Levaux et al. 2007). However, although test-retest stability is lower in patients with schizophrenia compared to healthy subjects (Beglinger et al. 2003), serial cognitive assessments can result in practice effects (Lowe & Rabbitt, 1998). Considering the short interval between cognitive assessments varying from two to twelve weeks, practice effects
seem a more likely limitation in the placebo-controlled study than in the extension study, with an interval of 26 weeks between measurements. Regrettfully, we only used a parallel version of the test for verbal memory. However, practice effects seem a less relevant limitation. The placebo-controlled design makes it possible to control for practice effects. Moreover, since our study population suffered from severe cognitive disturbances it seems less probable that the patients could have improved their performance to such a degree that substantial practice effects resulted.

A shortcoming of our studies is the fact that we did not differentiate primary from secondary negative symptoms, using the Clinical Assessment Interview for Negative Symptoms (CAINS) or the Brief Negative Symptom Scale (BNSS) (Carpenter et al. 2016). Anhedonia and attention deficits are important primary negative symptoms which are not included in the PANSS. We therefore performed a post-hoc exploratory factor analysis of the PANSS, which yielded a two-factor structure of negative symptoms comparable to the two subdomains of the negative syndrome – expressive deficits and social amotivation (Liemburg et al. 2013; Millan et al. 2014).

**Clinical implications**

Since cognitive and negative symptoms mainly account for functional and social outcome (Bowie & Harvey, 2006; Lepage et al. 2014; Ventura et al. 2015), these specific symptoms warrant more attention in daily clinical practice and future research. Antipsychotics have proven efficacy for positive symptoms and to a lesser extent for unspecified negative symptoms, but are without clinically relevant efficacy for cognitive deficits (Javitt, 2015; Nielsen et al. 2015; Takeuchi et al. 2017).

The available evidence indicates that a combination of neurocognitive and social cognitive interventions and a high level of weekly physical exercise may be more efficacious in improving cognitive functioning in schizophrenia than any pharmacological treatment. Comparisons of the efficacy of antipsychotic medication for cognitive functioning are limited (Nielsen et al. 2015; Takeuchi et al. 2017). We therefore cannot identify any single antipsychotic with superior efficacy for cognitive impairments associated with schizophrenia. However, when cognitive deficits are prominent, clozapine is a viable treatment option, since favourable effects on verbal fluency, executive function (Meltzer & McGurk, 1999), verbal and working memory (Molina et al. 2014) and the orienting function of attention (Spagna et
al. 2015) have been demonstrated in single studies. To further enhance memory, I would recommend memantine addition to clozapine, since verbal and visual memory improved in the placebo-controlled study after twelve weeks of memantine add-on therapy to clozapine in patients who suffered from severe cognitive deficits (Veerman et al. 2016) and this improvement was sustained in the 52-week open-label extension study (Veerman et al. 2017a). Moreover, memantine is well tolerated in combination with clozapine (Veerman et al. 2016; Veerman et al. 2017a).

In order to treat negative symptoms adequately, primary negative symptoms should be distinguished from secondary negative symptoms (Murphy et al. 2006). Based on the favourable results of two double-blind placebo-controlled RCTs (de Lucena et al. 2009; Veerman et al. 2016) and an open-label extension study (Veerman et al. 2017a), memantine may have a beneficial effect in clozapine-treated patients with prominent persistent negative symptoms. Moreover, memantine has a better safety and tolerability profile than other clozapine augmentation strategies with demonstrated efficacy such as aripiprazole (Zheng et al. 2016a; Galling et al. 2017), antidepressants (Singh et al. 2010; Hecht & Landy, 2012; Kishi & Iwata, 2014, Terevnikov et al. 2015; Helfer et al. 2016) and topiramate (Correll et al. 2016; Okuyama et al. 2016; Zheng et al. 2016b).

If possible, the efficacy of medication for negative symptoms should be evaluated after a treatment period of at least 26 weeks. Based on the small improvement of negative symptoms after twelve weeks of memantine treatment in the placebo-controlled study and the large effect size between baseline and 26 weeks in the open-label extension study, a treatment period exceeding twelve weeks is probably necessary for optimal treatment efficacy.

When positive symptoms persist in clozapine-treated patients with TRS, memantine add-on therapy may have a beneficial effect. However, given the absence of a significant beneficial effect compared to placebo in the crossover study, a memantine treatment duration of twelve weeks seems likely to be too short for positive symptoms to improve. Since we found a large effect size for positive symptoms between baseline and 26 weeks in the extension study, any evaluation of the efficacy of memantine for positive symptoms should take place after approximately 26 weeks.

For clinical global functioning to improve, an even longer treatment duration may be necessary. Whereas the Clinical Global Impression-Severity scale (CGI-S) did not significantly change compared to placebo in the RCT and the CGI-S showed only a trend towards improvement between baseline and 26 weeks and between 26 weeks and 52
weeks in the extension phase, we found a large significant improvement of CGI-S between baseline and week 52.

In patients who show intolerance to other medication or who are susceptible to complaints of dizziness I recommend a 3-week memantine titration scheme, starting with a dose of 5 mg memantine once daily, followed by a weekly increase with an increment of 5 mg to a dose of 20 mg memantine. However, in most patients an accelerated 1-week memantine titration scheme is well tolerated starting with 10 mg memantine once daily.

Finally, it should be emphasized that at present the evidence for adjunctive memantine to clozapine in TRS is sparse and further research is needed. This should be made clear in the shared decision-making process in daily clinical practice.

Future directions

In order to replicate this limited evidence of beneficial effects of memantine on severe residual symptoms in clozapine-resistant schizophrenia, a large-scale long-term double-blind placebo-controlled trial with a treatment duration of at least 26 weeks should be conducted.

Although a crossover design eliminates the influence of between-subject variability on effect and requires a smaller sample size since each patient serves as his or her own control, this is not an option in a long-term medication trial due to biases caused by carry-over effects. However, in my experience patients are more inclined to participate in a medical trial with a crossover design in which both verum and placebo are prescribed. It is often more difficult to motivate patients to participate in an RCT in which there is a 50% chance of receiving the experimental intervention. An alternative option for replication of this limited evidence for the efficacy of adjunctive memantine to clozapine is a 52-week RCT in which the first group is randomized to receive memantine treatment and the second group is randomized to receive placebo during the first 26 weeks and memantine treatment during the second 26 weeks. Another option is a long-term, prospective naturalistic study (Leichsenring, 2004).

Therefore, in order to find sufficient motivated participants for a large, long-term RCT, extensive informed consent procedures are recommended. Although informed consent obtained at baseline is also valid in this particular patient population, in a long-term trial with a duration exceeding 26 weeks, it would be wise to assess sustained informed consent throughout the course of a trial of this kind. After all, schizophrenia patients with
severe cognitive impairments who are partially responsive to clozapine are bound to have more difficulty retaining study information for the duration of their involvement in the research (Prentice et al. 2007). This way the fact that participation is voluntary is stressed, sustained informed consent is guaranteed and drop-out from the study is limited.

In my opinion, in our studies on the efficacy and tolerability of memantine, inclusion was successful and patients were genuinely motivated due to extensive screening and the fact that they were repeatedly given information on the study design, the rationale, and the potential risks and benefits in the presence of care providers of the FACT team preceding informed consent. Although expectations of the efficacy of memantine were low, discontinuation of study medication due to lack of motivation was limited to only one participant. Before the initiation of the placebo-controlled study the mean expectations in approximately 50% of participants varied from little to no improvement resulting from memantine treatment. In approximately 50% of completers of the first trial in whom a beneficial effect of memantine had already been established, expectations of the efficacy of memantine were still low to very low. While the patients consistently stated that the slight chance of benefiting from this novel combination treatment was a motivation to participate in the two trials, another important reason for participation was the desire to contribute to scientific research for schizophrenia patients.

In future research cognition and primary negative symptoms are essential primary endpoints. The MATRICS Consensus Cognitive Battery (MCCB) is recommended by the National Institute of Mental Health (NIMH) for assessing seven relevant cognitive domains in clinical trials of cognition-enhancing drugs for schizophrenia (Nuechterlein et al. 2008). For the assessment of primary negative symptoms the NIMH recommends the Clinical Assessment Interview for Negative Symptoms (CAINS) or the Brief Negative Symptom Scale (BNSS) (Carpenter et al. 2016). I would also recommend the WHO Disability Assessment Schedule II (WHODAS II) as a primary endpoint (Chopra et al. 2008), a self-report tool which has replaced Axis V in the DSM 5. In terms of recovery social disability is more important than symptomatic remission.

Several other aspects are of particular interest in future research on adjunctive memantine in clozapine-resistant schizophrenia. Firstly, inclusion of younger clozapine-treated patients with TRS could show whether memantine has a more favourable effect in the early stages of schizophrenia with less longstanding dysfunction.

Secondly, dose-dependent effects of memantine could be assessed in a dose-ranging study to investigate whether a dosage of 30 to 40 mg memantine (Wiech et al.
2001, 2004; Areosa & Sherriff, 2003; Beister et al. 2004; Schwenkreis et al. 2005; Ferguson & Shingleton, 2007; Schifitto et al. 2007) is more efficacious than the regular dosage of 20 mg memantine.

Thirdly, since memantine may decrease hippocampal glutamate levels (Glodzik et al. 2008) and adjunctive memantine to atypical antipsychotics may normalize brain activity in the inferior frontal gyrus (Cerullo et al. 2007), it would be of great interest to include parameters of central glutamatergic transmission and brain activity using proton magnetic resonance spectroscopy (1H-MRS) and functional MRI (fMRI) in a future clinical study of the effect of memantine in TRS.

The participation of TRS patients in pharmacological trials remains essential. However, it is important to emphasise that in this patient population paranoia complicates inclusion in an RCT. Moreover, there are challenges in the assessment of decisional capacity. Patients suffering from schizophrenia and especially TRS are at risk of losing their personal autonomy on the basis of their diagnosis, because of biases regarding their compromised ability to appreciate risk information and their reduced decision-making capacity (Prentice et al. 2007). Cognitive functioning appears to be more relevant than psychotic symptoms in predicting decisional capacity in schizophrenia patients (Carpenter et al. 2000). Since cognitive impairments do not always cause an enduring inability to understand information relevant to a study, repeated additional education about research procedures improves patients’ ability to provide informed consent. Based on a comparative study in 30 patients with TRS and 24 healthy adults, patients suffering from TRS and severe cognitive impairments are competent to give informed consent provided they are offered additional opportunities to learn the information about the study that is required to do so (Carpenter et al. 2000).

In addition, schizophrenia patients seem to show less optimism about experiencing adverse events compared to healthy adults, which may be an obstacle to inclusion into a treatment study (Prentice et al. 2005). To conclude, in this vulnerable patient population in which severe cognitive impairments and residual negative symptoms with feelings of hopelessness are common a more extensive informed consent procedure might compensate for a tendency towards negative beliefs and reduced capacity for informed consent.

More research is imperative if we are to take significant steps forward in schizophrenia treatment. In this thesis, I have explained the importance of exploring innovative interventions for patients with schizophrenia, notably patients with TRS. Additionally, I have provided evidence that even the most severely ill patients, with cognitive impairments such as disorganized thinking and poor memory, and residual negative and positive symptoms,
benefit from participation in a clinical trial. After all, in both the placebo and the memantine phase, symptoms of schizophrenia improved in all participants of our study population. Awakening hope of recovery and a better future is therefore a potent intervention. Although research in patients with TRS is more complicated than in patients with a less severe mental illness, we should not be discouraged and should continue our search for effective interventions. Finally, we should promote hope in the pursuit of optimal recovery from schizophrenia.
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Chapter 9

Dutch summary / Nederlandse samenvatting
Farmacologische interventies bij clozapineresistente schizofrenie

Dutch summary / Nederlandse samenvatting en algemene discussie

Dit proefschrift concentreert zich op clozapineresistente schizofrenie. Er is weinig sterk bewijs voor effectieve behandeling van restsymptomen bij clozapineresistente schizofrenie. Volgens de glutamaathypothese (Kantrowitz & Javitt, 2010), de disconnectivtheotypose (Stephan e.a. 2009) en de ontstekingshypothese (Muller & Schwarz, 2006) is de NMDA receptor disfunctioneel bij schizofrenie. Bij therapieresistente schizofrenie zijn glutamaterge veranderingen mogelijk verantwoordelijk voor het ontbreken van behandelrespons op dopamineblokkerende antipsychotica (Demjaha e.a. 2014; Mouchlianitis e.a. 2015). Daarom hebben we onderzocht of een middel, dat specifiek aangrijpt op de NMDA receptor, klinische voordelen heeft in combinatie met clozapine. In een monocenter, dubbelblind crossover onderzoek van 26 weken werden 52 patiënten met clozapineresistente schizofrenie gerandomiseerd behandeld met twaalf weken memantine of placebo. Geheugen, executief functioneren, negatieve, positieve en totale symptomen van schizofrenie en de globale ernst van de aandoening waren primaire uitkomsten. Secundaire uitkomsten waren sociale cognitie, depressieve en obsessief-compulsieve symptomen, psychosociaal functioneren, subjectieve kwaliteit van leven en ongewenste voorvallen en bijwerkingen. In een open-label extensieonderzoek werden vierentwintig patiënten, die het eerste placebogecontroleerde onderzoek hadden afgerond en een gunstig effect van memantine hadden ondervonden, gedurende een jaar met memantine behandeld. Langetermijneffecten met dezelfde uitkomsten en verdraagbaarheid werden geobserveerd om vast te stellen of deze clozapine augmentatiestrategie veelbelovend is en nader onderzoek verdient.

Belangrijkste bevindingen

Allereerst zal ik de belangrijkste bevindingen van voorgaande hoofdstukken samenvatten. Ik zal vervolgens een overzicht presenteren van werkzame en veilige interventies bij therapieresistente schizofrenie voor alle drie de symptoomdomeinen van schizofrenie: cognitief disfunctioneren, negatieve symptomen en positieve symptomen.
1. Aanbevelingen en rationale voor behandeling van negatieve symptomen bij schizofrenie: een uitgebreide review

In het tweede hoofdstuk onderzochten we of er momenteel overtuigend bewijs bestaat ter ondersteuning van interventies voor ongespecificeerde negatieve symptomen bij schizofrenie (Veerman e.a. 2017b). We gingen na of werkzame farmacologische behandelopties voor negatieve symptomen (zonder onderscheid tussen primaire en negatieve symptomen) verschillend zijn bij schizofreniepatiënten, die met andere antipsychotica dan clozapine worden behandeld vergeleken met clozapinegebruikende patiënten met therapieresistente schizofrenie. Er is geen overtuigend bewijs met betrekking tot de werkzaamheid van behandeling voor primaire negatieve symptomen, omdat weinig onderzoek is verricht bij patiënten met overwegend persistenderende negatieve symptomen in de niet-acute fase, waarbij is gecorrigeerd voor confounders zoals positieve symptomen, depressieve symptomen en extrapiramidale bijwerkingen. Gebaseerd op reviews en meta-analyses van gerandomiseerde, dubbelblinde, gecontroleerde onderzoeken (RCT) die niet gericht waren op primaire negatieve symptomen, hebben we bewijs gevonden voor een bescheiden kortetermijneffect van diverse interventies bij patiënten met ongespecificeerde negatieve symptomen. Hier geef ik een overzicht van de belangrijkste bevindingen en mijn aanbevelingen voor de klinische zorg.


Verder bespraken we dat meta-analytische vergelijkingen van antipsychotica geen duidelijke voordelen lieten zien van atypische antipsychotica versus klassieke antipsychotica (Leucht e.a. 2009b; Fusar-Poli e.a. 2015; Zhang e.a. 2013) noch consistente superieure

Een laatste belangrijke aanbeveling is om potentiële bijwerkingen zorgvuldig in overweging te nemen in gedeelde besluitvorming met betrekking tot medicatie.

2. De glutamaathypothese: een pathogeen mechanisme waaruit farmacologische interventies zijn voortgekomen

In hoofdstuk drie legden we uit op welke wijze de NMDA receptor een belangrijke rol speelt bij het leren en het geheugen, gefaciliteerd door een cellulair mechanisme waarbij langdurige bevordering van de signaaloverdracht tussen twee neuronen wordt veroorzaakt, genaamd langetermijnpotentiëring (LTP) (Kornmeier & Sosic-Vasic, 2012; Veerman e.a. 2014a).

We presenteerden vier bewijzen ter ondersteuning van de hypothese dat disfunctie van de NMDA receptor een belangrijk onderliggend mechanisme is van schizofrenie (Tiihonen e.a. 2006; Javitt e.a. 2006; Takata e.a. 2013; Wandinger e.a. 2001; Marsman e.a. 2011).

We bespraken hoe gecompromitteerde glutamatopname door hypofunctie van de NMDA receptor glutamaat spill-over in de synaptische spleet veroorzaakt, waardoor celleldood wordt geïnduceerd (Goff & Coyle, 2001; Papanastasiou e.a. 2013). We legden uit hoe verhoogde apoptotische activiteit in de vroege stadia van schizofrenie misschien neuroontwikkelingsstoornissen verklaart, die bijdragen aan cognitieve stoornissen en negatieve symptomen (Marsman e.a. 2011 bijdragen). We verduidelijkten een tweede mechanisme
voor verminderde cognitieve functies, gerelateerd aan de frontale kwab, waarbij ook negatieve symptomen een gevolg zijn van abnormale corticale signaal-ruisverhoudingen, veroorzaakt door de gedisreguleerde glutamaterge transmissie in de prefrontale cortex (PFC) (Homayoun e.a. 2007; Jackson e.a. 2004). Verder verhelderden we hoe positieve symptomen worden veroorzaakt door verminderde functie van de NMDA receptor, resulterend in dopaminerge hyperfunctie in de amygdala via reciproke synaptische relaties tussen glutamaterge systemen en mesolimbische, dopaminerge projecties (Gordon, 2010).

We behandelde hoe glutamaatagonisten, gecombineerd met andere antipsychotica dan clozapine, interessante klinische voordelen bij therapiresistente schizofrenie laten zien, terwijl glutamaatantagonisten gunstige effecten hebben als toevoeging aan clozapine.

We verduidelijkten hoe de combinatie van clozapine en de voltage-afhankelijke NMDA receptorantagonist memantine LTP mogelijk indirect kan moduleren via upregulatie van NMDA receptoren (Joshi e.a. 2007). Clozapine kan ook neuroprotectieve effecten van memantine voor NMDA-geïnduceerde excitotoxiciteit versterken. Om die reden kan dit synergetische effect van clozapine gecombineerd met memantine het cognitief functioneren verbeteren bij therapiresistente schizofrenie. Samen kunnen deze middelen leiden tot een verbeterde signaal-ruisverhouding, hetgeen geassocieerd wordt met verbeterde regulatie van NMDA transmissie in de PFC en verbetering van negatieve symptomen (Goff & Coyle, 2001; Javitt, 2006; Stone e.a. 2007). Een langdurig gunstig effect op positieve symptomen kan worden veroorzaakt door verbeterde remming van de prefrontale corticale GABA interneuronen, die verantwoordelijk zijn voor negatieve feedback van de piramidale neuronen (Homayoun & Moghaddam, 2007).

Gebaseerd op mijn review van de literatuur schreef ik een onderzoeksopzet voor een proof-of-concept onderzoek naar memantinetoevoeging aan clozapine bij therapiresistente schizofrenie.

3. Niet-glutamaterge clozapine augmentatiestrategieën: een review en meta-analyse

In hoofdstuk vier presenteerden we een overzicht van 22 gerandomiseerde, dubbelblinde, placebogecontroleerde onderzoeken, waarbij het potentiële klinische nut wordt beoordeeld van de toevoeging van niet-glutamaterge middelen aan clozapine: een tweede antipsychoticum, een antidepressivum, ethyl-eicosapentaeenzuur (E-EPA), lithium en een extract
van ginkgo biloba (Veerman e.a. 2014b).

We voerden drie meta-analyses uit waarbij de effecten op negatieve symptomen, positieve symptomen en totale symptomen van schizofrenie en affectieve symptomen werden vergeleken van de volgende adjuvante middelen bij clozapineresistente schizofrenie: 1. antipsychotica, 2. aripiprazol versus risperidon, en 3. antidepressiva. We waren niet in staat om E-EPA, lithium en extract van ginkgo biloba adequaat te vergelijken, vanwege het geringe aantal dubbelblinde, placebogecontroleerde RCTs naar de werkzaamheid van deze middelen.

We concludeerden dat er weinig robuust bewijs is voor de werkzaamheid van elk van deze clozapine augmentatiestrategieën bij therapieresistente schizofrenie. Augmentatie van clozapine met een tweede antipsychoticum liet een klein, maar significant gunstig effect zien (ES=0.25, p=0.023) voor negatieve symptomen na uitsluiting van het onderzoek door Gunduz-Bruce e.a. (2013), dat een uitschieter was. Hoewel vergelijkingen met placebo niet significant waren, resulteerde aripiprazol (ES=0.34, p=0.069) in een uitgesprokenere vermindering van negatieve symptomen dan risperidon (ES=0.19, p=0.356). Andere argumenten voor de voorkeur voor het gebruik van aripiprazol zijn de verdraagbaarheid en de potentiële vermindering van met clozapine geassocieerde metabole risicofactoren na gecombineerd gebruik met aripiprazol (Newcomer, 2005; Brixner e.a. 2006; Englisch & Zink, 2008; Fleischhacker e.a. 2010; Fan e.a. 2013). Voor resterende positieve symptomen lieten antipsychotica of antidepressiva als adjuvante behandeling aan clozapine geen significante veranderingen zien ten opzichte van placebo. Antidepressiva toonden geen significant klinisch gunstig effect in vergelijking met placebo, zelfs niet voor affectieve symptomen (ES=0.25, p=0.671). Verrassend was dat antipsychotica wel een trend tot verbetering van affectieve symptomen toonden (ES=0.30, p=0.068), waarbij een gunstiger effect op affectieve symptomen wordt gesuggereerd in vergelijking met antidepressiva.

Kortom, augmentatie met een tweede antipsychoticum kan negatieve symptomen enigszins verbeteren, maar we vonden geen robuust bewijs met betrekking tot de werkzaamheid van augmentatie van clozapine met een antidepressivum, E-EPA, lithium of extract van ginkgo biloba bij clozapineresistente schizofrenie.
4. Clozapine augmentatie met glutamaatmodulatoren bij therapieresistente schizofrenie: een review en meta-analyse

In hoofdstuk vijf zochten we bewijs voor klinisch nut van augmentatie van glutamaatagonisten en -antagonisten bij clozapineresistente schizofrenie (Veerman e.a. 2014c). We presenteerden een overzicht van 18 dubbelblinde, placebogecontroleerde RCTs naar adjuvante glutamaterge middelen aan clozapine met gegevens over negatieve, positieve en de totale symptomen van schizofrenie. Bij zes onderzoeken naar NMDA receptor agonisten gecombineerd met clozapine vonden we geen gunstige effecten. In een meta-analyse van drie onderzoeken naar clozapine gecombineerd met glycine vonden we zelfs een aanzienlijke verslechtering van positieve symptomen in vergelijking met placebo. Voortdurende synaptische activiteit, veroorzaakt door de combinatie van twee glutamaatagonisten, resulteert mogelijk in persistender downregulatie van NMDA receptoren, hetgeen het ontbreken van de werkzaamheid en zelfs verslechtering van symptomen verklaart als gevolg van deze combinatiebehandeling. In een enkel onderzoek verbeterde ampakine CX516 negatieve, totale klinische symptomen en cognitief functioneren ten opzichte van placebo na vier weken (Goff e.a. 2001). De combinatie van clozapine en deze positieve allostere modulator van de α-amino-3-hydroxy-5-methyl-4-isoxazolpropionzuur (AMPA) receptor heeft mogelijk gunstige effecten, omdat deze combinatie niet tot downregulatie van NMDA-receptoren leidt.

Een meta-analyse van vier onderzoeken naar topiramaatadditietherapie bij clozapine toonde geen significant verschil tussen topiramaat en placebo met betrekking tot verbetering van negatieve symptomen. In een meta-analyse van zes onderzoeken naar lamotriginetoevoeging aan clozapine vonden we een trend tot verbetering van de negatieve symptomen (ES=0.35, p=0.065) na uitsluiting van onderzoeken door Zoccali e.a. (2007) en Vayısoğlu e.a. (2013), die uitschieters waren. Lamotrigine liet ook een positieve trend zien in vergelijking met placebo (ES=0.31, p=0.065) wat betreft vermindering van positieve symptomen.

We benadrukten de veelbelovende positieve effecten van memantine met een uitzonderlijk grote effectgrootte voor alle symptoomdomeinen in het eerste proof-of-concept onderzoek door de Lucena e.a. (2009) bij clozapineresistente patiënten met overheersende negatieve symptomen.
5. Memantineaugmentatie bij clozapineresistente schizofrenie: een gerandomiseerde, dubbelblinde, placebogecontroleerde crossover studie

In hoofdstuk zes onderzochten we de werkzaamheid en verdraagbaarheid van memantine-toevoeging aan clozapinebehandeling bij therapieresistente schizofrenie (Veerman e.a. 2016). Memantine, dat geregistreerd is voor de behandeling van matige tot ernstige ziekte van Alzheimer (Areosa e.a. 2005), is een procognitief medicijn dat gericht is op de NMDA receptor. De NMDA receptor komt sterk tot expressie in de hippocampus (Bliss & Collingridge, 1993) en is essentieel bij functionele verbindingen tussen hersenencircuits, waarbij de PFC is betrokken via neuroplasticiteit. Combinatiebehandeling van clozapine en memantine is gericht op veranderde NMDA receptor-gemederde transmissie bij schizofrenie en heeft wellicht daarom gunstige effecten op alle symptoomdomeinen, met inbegrip van geheugen en executief functioneren (Javitt & Zukin, 1991; Bressan & Pilowsky, 2000; Homayoun & Moghaddam, 2007; Howes & Kapur, 2009; Orellana & Slachevsky, 2013).

We willekeurig bij 52 patiënten met clozapinebehandelde, therapieresistente schizofrenie twaalf weken dubbelblinde adjuvante behandeling met memantine of placebo toe. Na een tweeweekse placebo washout periode vond crossover plaats. Een samengestelde geheugenscore, bestaande uit Cambridge Neuropsychological Test Automated Battery (CANTAB) scores voor verbaal en visueel geheugen (ES=0.30, p=0.032), en de score van negatieve subschaal van de Positive and Negative Syndrome Scale (PANSS) (ES=0.29, p=0.043) waren de enige primaire uitkomsten, die significant verbeterd waren ten opzichte van placebo. Dit onderzoek leverde geen significant verschil op tussen memantine en placebo voor executief functioneren, positieve symptomen, totale symptomen van schizofrenie en de globale ernst van de aandoening na een behandelperiode van twaalf weken. Memantine werd in het algemeen goed verdragen met milde en voorbijgaande allergische bijwerkingen. Bij één deelnemer was duizeligheid in de memantinefase reden om te stoppen met het onderzoek.

In ons placebogecontroleerde onderzoek vonden we bescheidenere gunstige effecten van memantineadditietherapie dan de Lucena e.a. (2009). De resultaten van dit eerste proof-of-concept onderzoek dienen echter met de nodige voorzichtigheid te worden geïnterpreteerd, aangezien initiële sterkere effecten niet ongewoon zijn bij RCTs (Ioannidis, 2005, Tajika e.a. 2015). Verder is de werkzaamheid van memantine mogelijk overschat door toeval als gevolg van de relatief kleine steekproef van 21 patiënten (Sinclair & Adams,
2014; Tajika e.a. 2015). Bovendien was onze populatie ouder, ernstiger ziek als gevolg van ernstige cognitieve beperkingen en leed gedurende een langere periode aan schizofrenie in vergelijking met de patiënten die waren geïncludeerd in het eerste proof-of-concept onderzoek.

Ons onderzoek leverde aanvullend bewijs voor memantine als toevoeging aan clozapine, omdat we gunstige effecten vonden op geheugen en negatieve symptomen bij patiënten met therapiereisente schizofrenie en ernstige cognitieve beperkingen na een duur van twaalf weken memantinebehandeling. Een extensieonderzoek naar de langetermijneffecten van deze nieuwe aanpak voor de behandeling van clozapineresistente schizofrenie was derhalve gerechtvaardigd.

6. Toegevoegde memantine bij clozapinebehandelde therapiereisente schizofrenie: een open-label, 1-jarig extensieonderzoek

In hoofdstuk zeven onderzochten we de langetermijneffecten en verdraagbaarheid van memantineaugmentatie bij patiënten met clozapineresistente schizofrenie bij de proefpersonen, die het placebogecontroleerde onderzoek hadden afgerond en gunstige effecten hadden ondervonden gedurende twaalf weken van memantinebehandeling (Veerman e.a. 2017a). Van de 25 patiënten, die waren geïncludeerd in het open-label, eenjarige extensieonderzoek, staakte één patiënt memantine bij 26 weken na een periode van therapietrouw, hetgeen niet gerelateerd werd geacht aan de memantinebehandeling. Terwijl het executief functioneren nog steeds niet verbeterde, bleef de kleine significante verbetering van het geheugen na twaalf weken memantinebehandeling van het vorig onderzoek (Veerman e.a. 2016) gehandhaafd in het extensieonderzoek zonder verdere verbetering. We observeerden verdere verbetering van negatieve symptomen met een forse effectgrootte tussen de aanvang van het onderzoek en 26 weken ($r=0.53$, $p<0.001$) en tussen 26 weken en 52 weken ($r=0.51$, $p<0.001$). Hoewel positieve en totale symptomen van schizofrenie geen aanzienlijke verbetering lieten zien in de behandelfase van twaalf weken tijdens de RCT, kwamen gunstige langetermijneffecten van memantine tot uiting in een aanzienlijke verbetering van positieve symptomen ($r=0.50$, $p=0.001$) en totale symptomen ($r=0.54$, $p<0.001$) bij 26 weken. We observeerden nog verdere verbetering van positieve symptomen ($r=0.39$, $p=0.006$) en totale symptomen ($r=0.51$, $p<0.001$) bij 52 weken. De globale ernst van de aandoening toonde een matige, positieve trend na 26 weken en 52 weken.
Beperkingen in psychosociaal functioneren lieten ook een aanzienlijke verbetering zien tussen de aanvang van het onderzoek en week 26 ($r=0.33$, $p=0.018$) en opnieuw tussen week 26 en week 52 ($r=0.49$, $p<0.001$). We vonden geen significante, positieve lange-termijn effecten van memantine toegevoegd aan clozapine op sociale cognitie, depressieve symptomen, obsessief-compulsieve symptomen of subjectieve kwaliteit van leven. Memantine werd goed verdragen zonder ernstige ongewenste voorvallen of bijwerkingen.

Kortom, memantine lijkt een veelbelovende behandeloptie voor patiënten met clozapineresistente schizofrenie, gebaseerd op de glutamaathypothese, twee positieve korte termijn dubbelblinde, placebogecontroleerde RCTs en ons open-label, eenjarig extensieonderzoek.

Belangrijkste bevindingen met betrekking tot cognitieve, negatieve en positieve symptoomdomeinen bij therapieresistente schizofrenie

**Cognitief disfunctioneren**


Niet-farmacologische interventies zoals neurocognitieve en sociaal cognitieve interventies lijken kleine tot matige gunstige effecten te hebben op neurocognitie en sociale cognitie (Roder *et al.* 2011; Kurtz & Richardson, 2012; Lindenmayer *et al.* 2013; Pinkham & Harvey, 2013; Mueller *et al.* 2015). Hetzelfde geldt voor een hoog niveau van weke-
lijkse lichaamsbeweging (Firth e.a. 2016). Bovendien verbetert yoga mogelijk het lange-
termijngeheugen (Dauwan e.a. 2016).

**Negatieve symptomen**

Voor aanhoudende negatieve symptomen bij patiënten met therapieresistente schizo-
frenie heeft wellicht adjuvante aripiprazol een klein positief effect (Veerman e.a. 2014b; Zheng e.a. 2016a). Adjuvante antidepressiva aan clozapine zijn een tweede overwe-
ging (Veerman e.a. 2014b, 2017b). Een derde behandelloptie is de augmentatie van glu-
tamaantagonisten aan clozapine (Veerman e.a. 2014c). Het bewijs voor adjuvante
topiramaat bij clozapineresistente schizofrenie is het meest overtuigend met een matige
effect size in een recente meta-analyse (Zheng e.a. 2016b). De meest indrukwekkende
werkzaamheid voor primaire negatieve symptomen werd gevonden in een kleine RCT
met een duur van twaalf weken naar memantine toevoeging aan clozapine (de Lucena
e.a. 2009), maar deze bevinding was niet betrouwbaar door een grote verbetering van
positieve symptomen, terwijl depressieve symptomen niet waren onderzocht. We hebben
deze enorme verbetering van negatieve symptomen niet gerepliceerd in ons tweede
proof-of-concept onderzoek. We vonden alleen een klein significant effect voor negatieve
symptomen in vergelijking met placebo na twaalf weken behandeling met memantine
bij clozapineresistente patiënten (Veerman e.a. 2016). De aanzienlijke verbetering van
negatieve symptomen na 26 weken en tussen 26 weken en 52 weken memantinebehan-
deling werd aangetoond in een open-label onderzoek (Veerman e.a. 2017a) en behoeft
dus verdere exploratie in een RCT met een duur van zes maanden tot één jaar.

Twee niet-farmacologische behandelopties, waarvoor meta-analytische vergelij-
kingen enig bewijs hebben aangetoond betreffende verbetering van negatieve symptomen
(zonder onderscheid primair/secundair) zijn muziektherapie (Mössler e.a. 2011; Tseng e.a.
2016, Lutgens e.a. 2017) en lichaamsbeweging (Firth e.a. 2015; Dauwan e.a. 2016) met
gelijke werkzaamheid voor aerobe oefening en yoga (Dauwan e.a. 2016).
**Positieve symptomen**

Op dit moment is de enige op bewijs gebaseerde farmacologische behandeling voor clozapineresistente positieve symptomen topiramaat met een matig significant effect (Zheng e.a. 2016b). Het bewijs voor memantine in een dubbelblinde RCT is beperkt tot het eerste, kleine proof-of-concept onderzoek (de Lucena e.a. 2009), waarbij een grote verbetering werd gevonden van positieve symptomen in vergelijking met placebo. Er zijn aanwijzingen gevonden voor een grote verbetering van positieve symptomen na 26 weken en tussen 26 weken en 52 weken memantinebehandeling in een open-label onderzoek (Veerman e.a. 2017a) en dus is verdere exploratie in een RCT met een duur van zes maanden tot één jaar nodig.

Twee niet-farmacologische interventies met kleine tot matige gunstige effecten in vergelijking met reguliere behandeling zijn cognitieve gedragstherapie met vooral een gunstig effect op hallucinaties (Jauhar e.a. 2014) en lichaamsbeweging (Firth e.a. 2015; Dauwan e.a. 2016). Aerobe oefening en yoga lijken even werkzaam voor positieve symptomen (Dauwan e.a. 2016).

Elektroconvulsieve therapie (ECT) werd getest in een enkelvoudig blinde RCT (n=39) (Petrides e.a. 2015). Ongeveer 50% van de patiënten toonde minimaal 40% vermindering van positieve symptomen. Daarom dient ECT ook te worden overwogen bij patiënten met therapieresistente schizofrenie en aanhoudende positieve symptomen.

**Methodologische overwegingen**

Ten eerste zal ik ingaan op de beperkingen van de reviews en meta-analyses, die ik heb beschreven in hoofdstuk twee tot vijf. Ten tweede zal ik de sterke punten en beperkingen van het proof-of-concept onderzoek en het open-label onderzoek naar adjuvante memantine aan clozapine bespreken, beschreven in hoofdstuk zes en zeven.

**1. Reviews en meta-analyses**

Het meeste bewijs voor interventies voor negatieve symptomen, beschreven in hoofdstuk twee, is gebaseerd op korte termijn RCTs met negatieve symptomen als primaire of
secundaire uitkomst, uitgevoerd bij chronisch zieke, met medicatie gestabiliseerde patiënten en bij heterogene studiegroepen van patiënten in de acute en chronische fase van hun ziekte met een schizofrenie spectrum stoornis. Het onderscheid tussen primaire en secundaire symptomen is niet mogelijk, omdat de passende gegevens betreffende een stabiel ziektebeloop voorafgaande aan de start van de studie en confounders (positieve, depressieve en extrapiramidale symptomen) niet schikbaar zijn. Bij de meeste meta-analyses en reviews, waarbij de effectiviteit van interventies voor negatieve symptomen wordt onderzocht, bestaat een aanzienlijke mate van heterogeniteit tussen de primaire studies. Derhalve dient toekomstig onderzoek te worden gericht op langetermijnwerkzaamheid en te worden uitgevoerd bij patiënten met overwegend persistierende negatieve symptomen, waarbij eventueel onderzoeksmethoden worden uitgebreid van dubbelblinde RCTs naar lange termijn, prospectieve, naturalistische onderzoeken (Leichsenring, 2004). Bewijs voor de werkzaamheid van clozapine wordt bovendien belemmerd door het kleine aantal dubbelblinde RCTs, de korte behandelduur, snelle titratie van clozapine en ongelijke dosering bij vergelijkingen van clozapine versus klassieke antipsychotica of andere atypische antipsychotica.

Vergelijkend onderzoek naar verschillende niet-glutamaterge clozapine augmentatiestrategieën, die we in hoofdstuk vier bespraken, wordt belemmerd door het beperkte aantal RCTs naar de werkzaamheid van bepaalde farmacologische middelen bij clozapineresistente schizofrenie, kleine steekproeven, beperkte duur van de behandeling en verschillende statistische analyses. In hoofdstuk vijf constateerden we dat ook voor glutamaantagonisten grootschalige, lange termijn, placebogecontroleerde onderzoeken nodig zijn om de werkzaamheid en verdraagbaarheid bij clozapineresistente patiënten vast te stellen.

2. Memantineonderzoeken

Een sterk punt van onze memantineonderzoeken is dat we de methoden die in het onderzoek van de Lucena e.a. (2009) waren gebruikt, hebben verbeterd en uitgebreid. We gebruikten de MATRICS Consensus Cognitive Battery (MCCB) (Nuechterlein e.a. 2008) in plaats van de Mini-Mental State Examination (MMSE) (Folstein e.a. 1975). We gebruikten de PANSS negatieve subschaal in plaats van de Brief Psychiatric Rating schaal (BPRS), die betrekking heeft op slechts drie negatieve symptoom items (afgestompt affect, emo-

Er zijn echter enkele belangrijke beperkingen aan ons placebogecontroleerde onderzoek. De belangrijkste beperking is de duur van de behandeling, die vermoedelijk te kort was om het optimale behandeleffect te bereiken. Onderzoeken naar de ziekte van Alzheimer tonen een groter effect na 24 tot 28 weken behandeling in vergelijking met twaalf weken behandeling (Puangthong & Hsiung, 2009). Ten tweede bleek de washout periode van twee weken te kort, aangezien carry-over effecten van de samengestelde CANTAB geheugen score en de positieve, negatieve en totale scores van de PANSS significant waren.

Een andere belangrijke kwestie is de relatif kleine steekproef (Sinclair & Adams, 2014), die onvoldoende kracht genereert om respons op memantine te tonen, gedefinieerd als een vermindering van ten minste 25% van de uitgangswaarde (Leucht e.a. 2009c). In ons open-label extensieonderzoek was de grootte van de steekproef zelfs kleiner dan in ons primaire onderzoek. We waren niet in staat om de interventiegroep te vergelijken met de controlegroep zonder adjuvante memantinebehandeling, omdat deze groep uit slechts drie patiënten bestond.

Een mogelijke bias is selectie, hetgeen onze resultaten kan hebben beïnvloed. Het is denkbaar dat er bij de 64 patiënten met therapieresistente schizofrenie in zorg bij de FACT teams van GGZ Noord-Holland-Noord, die weigerden om deel te nemen, een hoog niveau van achterdocht was. Tijdens de screening werden sommige patiënten door voorlichting over experimenteel onderzoek bij mensen of alleen al de term ‘proefpersoneninformatie’ getriggerd om abrupt het gesprek te beëindigen en deelname aan het onderzoek te weigeren. Misschien is onze studiepopulatie niet representatief voor patiënten met therapieresistente schizofrenie in het algemeen en zijn alleen patiënten met een relatief laag niveau van achterdocht geïncludeerd in het onderzoek.

Het is belangrijk om te vermelden dat de gunstige effecten van memantinie in ons extensieonderzoek mogelijk zijn overschat door het open-label karakter van dit onderzoek (Sackett, 1979; Fisher & Greenberg, 1993; Crow e.a. 1999). Hoewel observer bias een
relevante tekortkoming is in ons tweede onderzoek bij alle metingen, met uitzondering van de resultaten van de CANTAB (Barnett e.a. 2010), lijkt het effect van de verwachtingen van de deelnemers beperkt. Ten eerste, omdat vóór het extensieonderzoek de verwachtingen van werkzaamheid van memantine zeer klein tot klein waren bij ongeveer 50% van onze studiepopulatie. Ten tweede, omdat we geen associatie hebben gevonden tussen gemeten verwachtingsscores en ervaren effect van memantine. Ten derde waren verwachtings-effecten niet consistent, omdat niet alle uitkomsten significant verbeterden.

Deelname aan onze onderzoeken veroorzaakte bovendien eventueel betere zorg en therapietrouw met een gunstig effect op de onderzoeksresultaten (Kinon e.a. 2011). Deze potentiële bias is beperkt tot het extensieonderzoek en lijkt minimaal, aangezien er geen extra aandacht of behandeling is gegeven aan deelnemende patiënten afgezien van drie meetsessies in een periode van één jaar.

We kozen de CANTAB om de bij schizofrenie relevante cognitieve domeinen te beoordelen omdat zij een uitgebreide, gevalidateerde cognitieve batterij is, die gevoelig is en eenvoudig om af te nemen (Levaux e.a. 2007). Echter, hoewel test-hertest stabilité lager is bij patiënten met schizofrenie ten opzichte van gezonde proefpersonen (Beglinger e.a. 2003), kunnen serieuze cognitieve metingen resulteren in oefeneffecten (Lowe & Rab-bitt, 1998). Gezien het korte interval tussen de cognitieve metingen, variërend van twee tot twaalf weken, lijken oefeneffecten eerder in het placebogecontroleerde onderzoek een beperking dan in het extensieonderzoek met een interval van 26 weken tussen de me-tingen. Helaas hebben we alleen een parallelle versie van de test voor verbale geheugen gebruikt. Oefeneffecten lijken echter een minder relevante beperking. Het placebogecon-rollederde studieontwerp maakt het mogelijk om te controleren voor oefeneffecten. Boven-dien, omdat onze studiepopulatie aan ernstige cognitieve stoornissen leed, lijkt het minder waarschijnlijk dat de patiënten hun prestaties in een aanzienlijke mate alleen door oefen-effecten kunnen leren verbeteren.

Een tekortkoming van onze onderzoeken is het feit dat we primaire negatieve symptomen niet hebben onderscheiden van secundaire negatieve symptomen met behulp van de Clinical Assessment Interview for Negative Symptoms (CAINS) of de Brief Negative Symptom Scale (BNSS) (Carpenter e.a. 2016). Anhedonie en aandacht zijn belangrijke primaire negatieve symptomen, die ontbreken in de PANSS. We hebben om die reden een post-hoc verkennende factoranalyse van de PANSS uitgevoerd, die een twee-factor structuur van negatieve symptomen opleverde, vergelijkbaar met de twee subdomeinen van de negatieve syndroom, nl. expressieve tekorten en sociale amotivatie (Liemburg e.a. 2013; Millan e.a. 2014).
Klinische implicaties

Omdat voornamelijk de cognitieve en negatieve symptomen de functionele en sociale uitkomsten bepalen (Bowie & Harvey, 2006; Lepage e.a. 2014; Ventura e.a. 2015), verdienen deze specifieke symptomen meer aandacht in de dagelijkse klinische praktijk en toekomstig onderzoek. Antipsychotica hebben bewezen werkzaamheid voor positieve symptomen en in mindere mate voor ongespecificeerde negatieve symptomen, maar geen klinische relevante werkzaamheid voor cognitieve beperkingen (Javitt, 2015; Nielsen e.a. 2015; Takeuchi e.a. 2017).

Uit het beschikbare bewijs blijkt dat een combinatie van neurocognitieve en sociaal cognitieve interventies of een hoog niveau van wekelijkse lichaamsbeweging mogelijk werkzamer is voor het cognitief functioneren bij schizofrenie dan een farmacologische behandeling. Vergelijkingen van de werkzaamheid van antipsychotica voor het cognitief functioneren zijn beperkt (Nielsen e.a. 2015; Takeuchi e.a. 2017). Om die reden kunnen we geen antipsychoticum met superieure werkzaamheid voor cognitieve beperkingen in het kader van schizofrenie identificeren. Echter, wanneer cognitieve stoornissen prominent zijn, is clozapine een geschikte behandeloptie, aangezien gunstige effecten op verbale vloeiendheid, executief functioneren (Meltzer & McGurk, 1999), verbaal geheugen en werkgeheugen (Molina e.a. 2014), en het richten van de aandacht (Spagna e.a. 2015) zijn aangetoond. Om het geheugen verder te verbeteren, zou ik memantinetoevoeging aan clozapine aadviseer, omdat het verbale en visuele geheugen in het placebogecontroleerde onderzoek verbeterde na twaalf weken memantineadditietherapie bij clozapine bij patiënten, die ernstige cognitieve stoornissen hadden (Veerman e.a. 2016). Deze verbetering bleef stabiel in het open-label extensieonderzoek met een duur van 52 weken (Veerman e.a. 2017a). Bovendien is memantine goed verdraagbaar in combinatie met clozapine (Veerman e.a. 2016; Veerman e.a. 2017a).

Om negatieve symptomen adequaat te behandelen, dienen primaire negatieve symptomen te worden onderscheiden van secundaire negatieve symptomen (Murphy e.a. 2006). Op basis van de gunstige resultaten van twee dubbelblinde, placebogecontroleerde RCTs (de Lucena e.a. 2009; Veerman e.a. 2016) en een open-label extensieonderzoek (Veerman e.a. 2017a) heeft memantine wellicht een gunstig effect bij clozapinebehandelde patiënten met prominente, persisterende negatieve symptomen. Memantine heeft bovendien een gunstiger bijwerkingenprofiel dan andere clozapine augmentatiestrategieën met bewezen werkzaamheid, zoals aripiprazol (Zheng e.a. 2016a; Galling e.a. 2017), antide-

Bij voorkeur wordt de werkzaamheid van medicatie voor negatieve symptomen na een behandelperiode van ten minste 26 weken geëvalueerd. Op basis van de kleine verbetering van negatieve symptomen na twaalf weken behandeling met memantine in het placebogecontroleerde onderzoek en de grote effect size tussen de uitgangswaarde en 26 weken in het open-label extensieonderzoek, is een behandelperiode van meer dan twaalf weken waarschijnlijk noodzakelijk voor optimale werkzaamheid van de behandeling.

Wanneer positieve symptomen aanhouden bij clozapinebehandelde patiënten met therapieresistente schizofrenie, kan memantine additietherapie een gunstig effect hebben. Echter, gebaseerd op het ontbreken van een significant gunstig effect in vergelijking met placebo in het crossover onderzoek, lijkt een memantine behandelduur van twaalf weken te kort om positieve symptomen te verbeteren. Omdat we een grote effect size voor positieve symptomen tussen de aanvang van het onderzoek en 26 weken in het extensieonderzoek hebben gevonden, dient evaluatie van de werkzaamheid van memantine voor positieve symptomen na ongeveer 26 weken plaats te vinden.

Om de globale ernst van de aandoening te verbeteren, kan een nog langere behandelduur nodig zijn. Terwijl de Clinical Global Impression-Severity scale (CGI-S) niet significant veranderde ten opzichte van placebo in de RCT en de CGI-S een trend tot verbetering toonde tussen de uitgangswaarde en 26 weken en tussen 26 weken en 52 weken in het extensieonderzoek, vonden we een grote significante verbetering van de CGI-S tussen de aanvang van het extensieonderzoek en week 52.

Bij patiënten, die andere medicatie niet goed kunnen verdragen of gevoelig zijn voor duizeligheidsklachten, raad ik een titratieschema van drie weken aan, te beginnen met een dosis van 5 mg memantine eenmaal daags, gevolgd door een wekelijkse verhoging met een toename van 5 mg naar een dosis van 20 mg memantine. Bij de meeste patiënten wordt echter een versneld memantine titratieschema van een week goed verdragen, beginnend met memantine 10 mg eenmaal daags.

Tot slot dient benadrukt te worden dat op dit moment het bewijs schaars is voor adjuvante memantine aan clozapine bij therapieresistente schizofrenie en verder onderzoek nodig is, hetgeen vermeld moet worden bij gedeelde besluitvorming in de dagelijkse klinische praktijk.
Toekomstig onderzoek

Om dit beperkte bewijs van gunstige effecten van memantine voor ernstige restsymptomen bij clozapineresistente schizofrenie te repliceren en uit te breiden, dient een groot, lange termijn, dubbelblind, placebogecontroleerd onderzoek met een behandelduur van tenminste 26 weken plaats te vinden. Hoewel een crossover ontwerp de invloed van variabiliteit tussen deelnemers op het effect elimineert en een kleinere steekproefgrootte vereist is met elke patiënt dienends als zijn eigen controle, is dit niet een optie bij langetermijngeneesmiddelenonderzoek, vanwege vertekeningen veroorzaakt door carry-over effecten. Mijn ervaring is echter dat patiënten meer geneigd zijn om deel te nemen aan een medisch onderzoek met een crossover ontwerp, waarbij zowel verum als placebo worden voorgeschreven. Het lijkt moeilijker om patiënten te motiveren om deel te nemen aan een RCT met 50% kans op het krijgen van de experimentele interventie. Een alternatief voor replicatie van dit beperkte bewijs voor de werkzaamheid van adjuvante memantine aan clozapine is een RCT van 52 weken, waarbij de eerste groep willekeurig memantinebehandeling toegewezen krijgt en de tweede groep met placebo wordt behandeld tijdens de eerste 26 weken en met memantine tijdens de tweede 26 weken. Een andere optie is lange termijn, prospectief naturalistisch onderzoek (Leichsenring, 2004).

Om voldoende gemotiveerde deelnemers te verkrijgen voor een grote en langdurige RCT, zijn uitgebreide procedures rondom informed consent aan te bevelen. Hoewel informed consent bij de aanvang van een onderzoek ook geldig is bij deze specifieke patiëntenpopulatie, zou het verstandig zijn om, bij een langetermijnonderzoek met een duur van meer dan 26 weken, opnieuw informed consent te beoordelen in de loop van dit onderzoek. Schizofreniepatiënten met ernstige cognitieve beperkingen, die een partiële respons hebben op clozapine, zullen beslist meer moeilijkheden ondervinden bij het onthouden van studie-informatie gedurende de duur van hun betrokkenheid bij het onderzoek (Prentice e.a. 2007). Op deze manier wordt vrijwillige deelneming benadrukt, is aanhoudend informed consent gegarandeerd en wordt drop-out uit het onderzoek beperkt.

Ik ben ervan overtuigd dat in onze onderzoeken naar de werkzaamheid en verdraagbaarheid van memantine, inclusie succesvol was en patiënten oprecht gemotiveerd waren als gevolg van uitgebreide screening met herhaalde informatie over het ontwerp van het onderzoek, de rationale, en de potentiële risico’s en voordelen in het bijzijn van de behandelaars van het FACT team voorafgaande aan informed consent. Hoewel verwachtingen omtrent de werkzaamheid van memantine laag waren, was het staken
van de studiemedicatie wegens gebrek aan motivatie beperkt tot slechts één deelnemer. Vóór de aanvang van het placebogecontroleerde onderzoek varieerden de gemiddelde verwachtingen bij ongeveer 50% van de deelnemers van weinig tot geen verbetering als gevolg van memantine. Bij ongeveer 50% van de deelnemers, die het eerste onderzoek hadden afgerond en bij wie al een gunstig effect van memantine was aangetoond, waren verwachtingen van de werkzaamheid van memantine nog steeds zeer klein tot klein. Terwijl de patiënten consequent een kleine mogelijkheid om voordeel te ondervinden van deze nieuw geteste combinatiebehandeling als een motivatie vermeldden om deel te nemen aan deze twee onderzoeken, was een belangrijke reden voor deelname om bij te dragen aan wetenschappelijk onderzoek voor schizofreniepatiënten.

Bij toekomstig onderzoek zijn cognitie en primaire negatieve symptomen essentiële primaire uitkomsten. De MATRICS Consensus Cognitive Battery (MCCB) wordt aanbevolen door National Institute of Mental Health (NIMH) om de zeven relevante cognitieve domeinen te beoordelen bij klinische onderzoeken naar medicijnen, die de cognitie bij schizofrenie verbeteren (Nuechterlein e.a. 2008). Om primaire negatieve symptomen te beoordelen, raadt de NIMH de Clinical Assessment Interview for Negative Symptoms (CAINS) of de Brief Negative Symptom Scale (BNSS) aan (Carpenter e.a. 2016). Ik zou ook als primaire uitkomst de WHO Disability Assessment Schedule II (WHODAS II) aanbevelen (Chopra e.a. 2008), een instrument dat as V in de DSM-5 heeft vervangen. In termen van herstel zijn sociale beperkingen belangrijker dan symptomatische remissie.

Verschillende andere aspecten zijn van bijzonder belang bij toekomstig onderzoek naar adjuvante memantine bij clozapineresistente schizofrenie. Ten eerste zou bij inclusie van jongere clozapinebehandelde patiënten met therapieresistente schizofrenie kunnen worden onderzocht of memantine een gunstiger effect heeft in vroege stadia van schizofrenie met minder langdurig disfunctioneren.

Ten tweede zouden dosisafhankelijke effecten van memantine kunnen worden bepaald in een dosisvergelijkend onderzoek om te onderzoeken of een dosering van 30 tot 40 mg memantine (Wiech e.a. 2001, 2004; Areosa & Sherriff, 2003; Beister e.a. 2004; Schwenkreis e.a. 2005; Ferguson & Shingleton, 2007; Schifitto e.a. 2007) werkzamer is dan de reguliere dosering van 20 mg memantine.

Ten derde zou het van groot belang zijn, aangezien memantine mogelijk glutamaatniveaus in de hippocampus verlaagt (Glodzik e.a. 2008) en adjuvante memantine aan aty-
pische antipsychotica mogelijk in de inferieure frontale gyrus de hersenactiviteit normaliseert (Cerullo e.a. 2007), om parameters als centrale glutamaterge transmissie en hersenenactiviteit met behulp van Proton Magnetische Resonantie Spectroscoopie (1H-MRS) en functionele Magnetische Resonantie Imaging (fMRI) op te nemen in toekomstig klinisch onderzoek naar het effect van memantine bij therapieresistente schizofrenie.

Het blijft van essentieel belang dat patiënten met therapieresistente schizofrenie deelnemen aan geneesmiddelenonderzoek. Het is echter belangrijk om te benadrukken dat in deze patiëntenpopulatie achterdocht de inclusie in een RCT bemoeilijkt. Bovendien zijn er uitdagingen bij de beoordeling van wilsbekwaamheid. Bij patiënten die lijden aan schizofrenie en vooral therapieresistente schizofrenie wordt vaak gedacht dat zij minder goed in staat zouden zijn om informatie omtrent risico’s te kunnen waarderen en daarom beperkt wilsbekwaam zouden zijn (Prentice e.a. 2007). Niet zozeer psychotische symptomen, maar cognitieve functioneren lijkt relevant bij het voorspellen van wilsbekwaamheid bij schizofreniepatiënten (Carpenter e.a. 2000). Aangezien cognitieve beperkingen niet altijd leiden tot een blijvend onvermogen om voor een onderzoek relevante informatie te begrijpen, verbetert herhaaldelijke, extra voorlichting over de procedures van het onderzoek de competentie om informed consent te geven. Gebaseerd op een vergelijkend onderzoek bij 30 patiënten met therapieresistente schizofrenie en 24 gezonde volwassenen, zijn patiënten die lijden aan therapieresistente schizofrenie en ernstige cognitieve beperkingen bekwaam om informed consent te geven, mits extra mogelijkheden worden aangeboden om de nodige studie-informatie te begrijpen (Carpenter e.a. 2000).

Bovendien lijken schizofreniepatiënten minder optimistisch over het ervaren van ongewenste voorvallen of bijwerkingen in vergelijking met gezonde volwassenen, hetgeen inclusie in interventieonderzoek kan belemmeren (Prentice e.a. 2005). Kortom, in deze kwetsbare patiëntenpopulatie bij wie ernstige cognitieve beperkingen en resterende negatieve symptomen met gevoelens van hopeloosheid vaak voorkomen, is een uitgebreidere informed consent procedure nodig, zodat patiënten het deelnemen aan onderzoek zorgvuldig en realistisch kunnen afwegen.

Meer onderzoek is absoluut noodzakelijk om in de behandeling van schizofrenie een sprong voorwaarts te maken. In dit proefschrift heb ik het belang uitgelegd van het onderzoeken van innovatieve interventies voor patiënten met schizofrenie en met name patiënten met therapieresistente schizofrenie. Daarnaast heb ik aangetoond dat zelfs de meest ernstig zieke patiënten met cognitieve beperkingen, zoals gedesorganiseerd denken en een slecht geheugen, en resterende negatieve en positieve symptomen baat heb-
Symptomen van schizofrenie verbeterden immers zowel in de placebofase als in de memantinefase bij alle deelnemers van onze onderzoeks-populatie. Aanwakkeren van hoop op herstel en een betere toekomst blijkt daarom een krachtige interventie. Hoewel onderzoek bij patiënten met therapiereistente schizofrenie ingewikkelder is dan bij patiënten met een minder ernstige psychiatrische aandoening, moeten we ons niet laten ontmoedigen en onze zoektocht naar werkzame interventies voortzetten. Het is haalbaar en noodzakelijk om te blijven streven naar een optimaal herstel van patiënten met therapiereistente schizofrenie.
Dankwoord
Dankwoord

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Mijn intervisiegroep heeft de afgelopen jaren met mij meegeleefd.

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Selene Roxane Tamasine Veerman was born on September 21st, 1978 in Volendam, the Netherlands. She attended secondary school in Amsterdam and graduated from Gymnasium in 1996. From 1996 till 2001 she studied medicine at the Academic Medical Center, the medical faculty of the University of Amsterdam.

In 2001 she temporarily ceased her medical studies when her son Aron was born. In 2003 she continued her medical internship in several general hospitals in Amsterdam. She performed medical research on the effect of hyperbaric oxygenation on late sequelae of radiation therapy in the head and neck region. After obtaining her medical degree in 2005 she worked as a psychiatric resident in Amsterdam. Between 2007 and 2011 she completed her specialist training in psychiatry at Mental Health Services in Geest at various facilities in Zaandam, Haarlem, Amstelveen, Alkmaar and Hoofddorp.

Since July 2011 she has combined her clinical work as a psychiatrist in a Flexible Assertive Community Treatment (FACT) facility in Alkmaar with the research presented in this thesis. Since then she has actively participated in several committees of Mental Health Service Noord-Holland Noord, including the psychosis expertise platform, the taskforce for early-onset psychosis, the steering group against solitary confinement of patients, and the pharmaceutical and scientific committee.

Since 2017 she has been Chair of the expert network psychosis of Mental Health Service Noord-Holland Noord. She also participates in the training of medical students and residents in psychiatry. She is a local researcher for several clinical studies on psychosis and she intends to continue conducting research on psychosis.

Since 2015 she has been a member of the Representative Advisory Board of Tabor College, which consists of three secondary schools for talented children in Hoorn. Selene lives with her husband Michel Bond and her son Aron. She enjoys playing the piano, reading and swimming.
List of publications
List of publications

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Abstracts


This thesis focuses on treatment challenges such as severe cognitive impairments, prominent persistent negative symptoms and treatment-resistant positive symptoms. New treatment approaches to overcome glutamatergic deficits in schizophrenia seem promising in treatment-resistant schizophrenia. The glutamate hypothesis proposes that the specific combination of the glutamate agonist clozapine and the voltage-dependent N-methyl-D-aspartate (NMDA) receptor antagonist memantine results in upregulation of the NMDA receptor. The encouraging results of a first proof-of-concept study with large effect size for all symptom domains of schizophrenia impelled me to further investigate this combination therapy. My research team and I have provided additional encouraging clinical results for memantine as an adjunctive to clozapine in refractory schizophrenia. Memantine slightly improved memory and negative symptoms after twelve weeks of treatment in a double-blind, placebo-controlled study. In an open-label, one-year extension study the small improvement of memory was sustained and a large improvement in negative and positive symptoms and impaired psychosocial functioning was found in the first 26 weeks and second 26 weeks. In conclusion, further research on memantine as clozapine augmentation strategy in patients suffering from treatment-resistant schizophrenia is justified.