Glutamate in dorsolateral prefrontal cortex and auditory verbal hallucinations in patients with schizophrenia

Curcic-Blake, Branisalava; Bais, Leonie; Sibeijn-Kuiper, Anita; Pijnenborg, Hendrika Maria; Knegtering, Henderikus; Liemburg, Edith; Aleman, André

Published in:
Progress in Neuro-Psychopharmacology & Biological Psychiatry

DOI:
10.1016/j.pnpbp.2017.05.020

Publication date:
2017

Progress in Neuropsychopharmacology & Biological Psychiatry 78 (2017) 132–139

Contents lists available at ScienceDirect

Progress in Neuropsychopharmacology & Biological Psychiatry

journal homepage: www.elsevier.com/locate/pnp

Glutamate in dorsolateral prefrontal cortex and auditory verbal hallucinations in patients with schizophrenia: A ^{1}H MRS study

Branislava Ćurčić-Blake[a,*], Leonie Bais[a,b], Anita Sibeijn-Kuiper[a], Hendrika Maria Pijnenborg[c,d], Henderikus Knegtering[b], Edith Liemburg[a], André Aleman[a]

[a] Department of Neuroscience, University of Groningen, University Medical Center Groningen, A. Deusinglaan 2, 9713 AW Groningen, The Netherlands
[b] Lentis Psychiatric Institute, Lentis Research, Hereweg 80, 9725 AG Groningen, The Netherlands
[c] Department of Clinical Psychology & Experimental Psychopathalogy, University of Groningen, Grote Kruisstraat 2/1, 9712 TS Groningen, The Netherlands
[d] Department of Psychotic Disorders, GGZ-Drenthe, Dennenweg 9, 9404 LA Assen, The Netherlands

ARTICLE INFO

Keywords:
Auditory verbal hallucinations
Glutamate
Schizophrenia
Magnetic resonance spectroscopy (MRS)
Glutamate with glutamine (Glx)

ABSTRACT

Purpose: Glutamatergic models of psychosis propose that dysfunction of *N*-methyl-D-aspartate (NMDA) receptors, and associated excess of glutamate, may underlie psychotic experiences in people with schizophrenia. However, little is known about the specific relation between glutamate and auditory verbal hallucinations (AVH) in patients with psychosis. In this study, levels of glutamate + glutamine (Glx) in the left lateral prefrontal lobe were determined using proton magnetic resonance spectroscopy (^{1}H MRS) to calculate their association with AVH.

Methods: Sixty-seven patients with schizophrenia and thirty healthy control participants (HC) underwent magnetic resonance spectroscopy (MRS) to estimate levels of Glx in the white matter of the left prefrontal lobe. The spectrum was estimated from an 8 mm^3 voxel placed in the left lateral prefrontal region, belonging to both the cingulum and forceps minor. Patients with lifetime AVH (AVH group; n = 45) and patients without lifetime AVH were compared (NoAVH group; n = 22) to control participants.

Results: Levels of Glx were significantly different between the groups ($F(2,94) = 5.27$, $p = 0.007$). Planned comparisons showed that higher Glx levels were found in control participants than in the total patient group ($p = 0.010$). However, patients with lifetime AVH had higher levels of Glx compared to patients without lifetime AVH ($p = 0.019$). Creatin levels were similar in all three groups. We found no association between Glx and the severity of symptoms (item P3 of the PANSS or PANSS positive subscale).

Conclusion: The higher Glx levels in patients with lifetime AVH as compared to patients without lifetime AVH suggest a mediating role for Glx in AVH. Our results are consistent with a previous study that found similar decreased levels of Glx in patients with schizophrenia, and increased levels in an AVH group as compared to a NoAVH group. The role of the glutamatergic system deserves further investigation, for example in different brain regions and in relation to clinical variables.

1. Introduction

Auditory verbal hallucinations (AVH), or hearing voices in the absence of corresponding external stimuli, are a frequently occurring symptom in people with schizophrenia. AVH have been consistently linked to abnormalities in fronto-temporal and inter-hemispheric connections (Allen et al., 2012; Geoffroy et al., 2014; Steinmann et al., 2014). These connections are engaged in a wide range of functions, most prominently language processing (Duffau, 2008; Mesulam, 1990), which is impaired in individuals with AVH (Dollfus et al., 2013). It is possible that abnormalities in these connections related to AVH are accompanied by irregularities in excitatory neurotransmitter levels such as glutamate. An MRI study with ketamine infusion published in 2015 found that when glutamate signalling is affected under MRI conditions, AVH are induced (Powers et al., 2015). Therefore, an investigation of the levels of glutamate present in white matter may

Abbreviations: ^{1}H MRS, Proton magnetic resonance spectroscopy; AVH, Auditory verbal hallucinations; ANOVA, analysis of variance; ANCOVA, analysis of covariance; CSF, cerebrospinal fluid; DLPFC, dorso-lateral prefrontal cortex; FWHM, full width half maximum; Glx, Glutamate with Glutamine; GM, grey matter; n.s., not significant; HC, healthy control subjects; NoAVH, schizophrenia patients with no history of AVH; PANSS, positive and negative syndrome scale; PRESS, Point Resolved Spectroscopy; SNR, Signal to noise ration

* Corresponding author at: Department of Neuroscience, University of Groningen, University Medical Center Groningen, Antonius Deusinglaan 2, 9713 AW Groningen, The Netherlands.
E-mail address: b.curcic@umcg.nl (B. Ćurčić-Blake).

http://dx.doi.org/10.1016/j.pnpbp.2017.05.020
Received 25 January 2017; Received in revised form 15 May 2017; Accepted 21 May 2017
Available online 22 May 2017
0278-5846/

Table 1
Demographic data of subjects. The left column lists the demographic variables. The second to fourth columns from the left show average values of the variables across the group; the standard deviations are in brackets. Education level was rated according to a six-point scale defined by Verhage, which ranges from primary school (1) to university level (6). Non-parametric tests were used to test the group differences for PANSS and Medication equivalent (Mann-Whitney test), and gender (Chi-square).

	Mean (+ SD)			Significance		
	Healthy (n = 30)	Schizophrenia patients NO AVH (n = 22)	Schizophrenia patients AVH (n = 45)	3 groups	HC vs patients p value	NoAVH vs AVH
Age in years	27.1 (10.6)	33.6 (9.0)	31.5 (10.8)	F(2,97) = 2.8 (0.066)	0.02	0.41
Gender males	21/9	18/4	34/11	χ(2,97) = 0.96 (0.62)	n.s	n.s
Education	5.7 (0.9)	5.0 (1.7)	4.7 (1.7)	F(2,92) = 4.2 (0.017)	0.010	n.s
Diagnosis						
Schizophrenia	–	21	40			
Schizophreniform disorder	–	0	1			
Psychosis not otherwise specified	–	1	2			
Substance-induced psychosis	–	0	1			
Delusional disorder	–	0	1			
PANSS positive	–	13.4 (4.8)	14.7 (4.4)			U = 373(0.27)
PANSS negative	–	17.0 (6.3)	15.6 (5.0)			U = 515 (0.35)
PANSS general	–	31.3 (7.6)	31.0.7 (6.8)			U = 450 (1.0)
PANSS total	–	61.8 (14.6)	61.3 (12.9)			U = 416 (0.9)
P3 hallucination item of PANSS		1.95 (1.43)	3.04 (1.48)			T(2,63) = 2.78 (0.007)
Duration of illness in years	–	8.8 (9.5)	8.3 (8.2)			T(2.65) = − 0.18 (0.9)
Medication [mg] haloperidol equivalent	–	5.8 (5.9)	5.6 (7.4)			U = 265 (0.9)

increase our understanding of the neural underpinnings of AVH.

Glutamate is a proteinogenic amino acid that is abundant in the human body. This excitatory neurotransmitter affects synaptic plasticity and is thought to be vital in cognitive functions such as learning and memory (Bliss and Collingridge, 1993). The dysfunction or blockade of glutamate receptors, specifically the *N*-methyl-D-aspartate (NMDA) receptors, by neurochemical compounds such as ketamine can cause schizophrenia-like symptoms including AVH (Coyle, 2006; Krystal et al., 1994; Lahti et al., 1995). Several reviews summarize investigations of glutamate levels in frontal and medial/temporal areas in chronic patients with schizophrenia (Marsman et al., 2013; Poels et al., 2014a; Poels et al., 2014b; Wijtenburg et al., 2015). Whereas studies in chronic patients consistently show decreased levels of glutamate in the ventro-medial prefrontal cortex (VMPFC), changes in the dorso-lateral prefrontal cortex (DLPFC) have been relatively little investigated and show inconsistencies (Merritt et al., 2016; Poels et al., 2014a).

Proton magnetic resonance spectroscopy (^{1}H MRS) is a technique that is used to non-invasively determine the chemical composition of brain tissue, using standard MRI equipment with special sequences. In short, this is a type of nuclear magnetic resonance spectroscopy, where the target is the nucleus of hydrogen atoms in its most common state ^{1}H (consisting of only one proton). This nucleus is excited by a radio-frequency pulse delivered at the resonance frequency of ^{1}H. After excitation, the nucleus will release absorbed energy and one can determine the concentration of ^{1}H in the given material by measuring the height of a peak in its relaxation spectrum. The resonance frequency (also known as the chemical shift) depends on the molecule (neuro-metabolite) to which ^{1}H is bonded, which consequently determines the frequency of the relaxation peak. This is why in ^{1}H MRS a range of frequencies that excite ^{1}H in different molecules is delivered to a particular voxel. From the measured spectral response one can estimate the concentrations of various metabolites in this particular voxel. However, with a 3 Tesla MRI scanner, it is difficult to separate the glutamate from the glutamine signals. Therefore, a combined measure of both metabolites is often measured, referred to as Glx.

Thus far, only one study has investigated AVH in association with levels of glutamate in several language regions (Hugdahl et al., 2015). As measured in both the temporal and frontal lobe, patients with schizophrenia as a group showed lower Glx levels than healthy controls. However, Glx levels were higher in patients with AVH relative to patients without AVH. This study included only twenty-three patients, which is relatively low for this type of investigation.

In the current study we aimed to investigate levels of glutamate in relation to AVH in the lateral prefrontal cortex in a specific region of white matter that is involved in both fronto-temporal and interhemispheric connectivity. For this purpose, we contrasted data obtained from two specifically defined groups of patients- those with past experience of AVH and those who had never experienced AVH. Based on the findings by Hugdahl et al. (2015), we hypothesized that patients with psychosis would demonstrate lower Glx levels than control participants, but that within this patient group, patients with the trait of experiencing AVH would demonstrate higher Glx levels than patients who had never experienced AVH.

2. Materials and methods

2.1. Participants

For this study ^{1}H MRS data of sixty-seven patients and thirty healthy controls (HC) were pooled from four studies run by our centre investigating schizophrenia and related psychotic disorders; all studies applied the same acquisition methods. The first study was a randomized controlled trial with rTMS for the treatment of negative symptoms of schizophrenia (Dutch Trial Registry: NTR1261; (Dlabac-de Lange et al., 2015)). The second study was a trial that compared the effects of aripiprazole versus risperidone on negative symptoms of schizophrenia and related psychotic disorders (EUDRA-CT: 2007-002748-79; (Liemburg et al., 2015)). The third study was a trial to investigate the effects of a cognitive-emotional intervention to improve insight in patients with schizophrenia (Dutch Trial Registry: NTR1799; (Pijnenborg et al., 2011). The fourth study investigated the neural basis of cognitive-emotional processing in individuals with an at-risk mental state (Current Controlled Trials: ISRCTN21353122).

AVH are closely related to the language network, which is in turn lateralized to the left hemisphere in the majority of right-handed people (e.g. the thickness of fibres (Parker et al., 2005)). Because lateralization is not consistent for left-handed people (Knecht et al., 2000), all selected subjects were right handed. In patients the diagnosis was confirmed either by the Mini-International Neuropsychiatric Interview (M.I.N.I.; (Sheehan et al., 1998)) or the Schedules for Clinical Assessment in Neuropsychiatry (SCAN) interview (Giel and Nienhuis, 1996). The majority of the patients had a diagnosis of schizophrenia, but several patients with other psychotic disorders were also included

(Table 1). The severity of current symptoms was assessed in all patients using the positive and negative syndrome scale (PANSS; (Kay et al., 1987)). In addition, special attention was paid to whether patients had ever experienced AVH (AVH group; $n = 45$) or had never experienced them (NoAVH group; $n = 22$). This was done by careful examination of interview recordings, interview notes and M.I.N.I. item M6a where applicable. Patients were only included in the study if it could be confirmed whether or not they had ever experienced AVH. Some patients in the NoAVH group had experienced tactile or olfactory hallucinations. Most patients fulfilled the DSM IV criteria of schizophrenia, whereas others fulfilled the criteria of related disorders such as schizoaffective disorder (for an overview of diagnoses, see Table1).

The control participants were recruited through local advertisements and through word of mouth, and reported to be healthy. The absence of psychiatric problems was confirmed using screening questions of the SCAN interview (Giel and Nienhuis, 1996).

Education was scored according to the Verhage system, with a scale ranging from 1 = primary school to 8 = university. Supplementary Table S1 summarizes antipsychotic medication taken by the participants. Antipsychotic medication was converted to haloperidol equivalent using primarily the conversion rules by Andreasen et al. (2010). Levels of paloperidone, flupentixol and primozide were first converted to olanzepine by the method of Gardner et al. (2010) and subsequently converted to haloperidol (Andreasen et al., 2010).

Exclusion criteria for all participants were having any/co-morbid neurological disorder, insufficient mastery of the Dutch language, and standard MRI exclusion criteria (such as claustrophobia, metal implants etc.). In addition, substance dependency within the previous six months was an exclusion criterion for three of the studies (Dlabac-de Lange et al., 2015; Liemburg et al., 2015; van der Velde et al., 2015). The participants provided written informed consent before the scanning session, after the procedure had been fully explained. All study protocols were fully approved by the medical ethical board of the University Medical Center Groningen (METC; UMCG), with exception of the study on at-risk mental state that was approved by the Mental Healthcare Research Ethics Committee (METiGG). All procedures were carried out according to the declaration of Helsinki.

2.2. MRS acquisition

All subjects underwent magnetic resonance spectroscopy (MRS) to estimate levels of glutamate + glutamine (Glx) in the left lateral prefrontal region. The images were acquired using a 3 T Philips Intera MRI scanner (Philips, Best, The Netherlands). The standard 8-channel SENSE head coil was used to acquire Point Resolved Spectroscopy (PRESS) images in a specific voxel (Fig. 1), over a duration of 5 min. PRESS images were acquired with one 90° and two 180° pulses, and water suppression used a selective 140 Hz radio frequency pulse and a subsequent radio frequency inversion pulse. This was the standard protocol when the data acquisition started. Automated first-order B0 shimming at the ROI was performed prior to MRS. Spectra were recorded according to the following parameters: TE = 144 ms, TR = 2000 ms, samples = 1024, bandwidth = 2000 Hz, VOI = 20 × 20 × 20 mm, signal averages (NSA) = 128. In addition, a T1-weighted image (160 slices; isotropic voxels of 1 mm; TR 25 ms; TE 4.6 ms; α 30°; FOV 256 mm) covering the whole brain was acquired. The spectrum was estimated from a 8 cm^3 voxel (2 × 2 × 2 cm) placed in the left lateral prefrontal region, belonging to both the cingulum and forceps minor. A T1 image was used as a reference for placing the MRS voxel such that it was in line with the genu of the corpus callosum on the anterior side and oriented on the same line as the corpus callosum and the falx cerebri, such that the inclusion of white matter was maximized.

2.3. Data analysis

The obtained spectra were processed using the LC model ((Provencher, 1993); version 6.2-2b) such that the peaks of the expected metabolites were fitted to the observed peak amplitudes of the measured spectra (Fig. 2). Estimated levels refer to joint levels of Glutamate and Glutamine (Glutamate + Glutamine = Glx). Absolute metabolite levels were determined by scaling based on the unsuppressed water peak. Data were excluded if the standard deviation of the estimated metabolite concentration was higher than 20% of the concentration (Cramer-Rao bounds) (Wijtenburg et al., 2015) or if the estimated concertation deviated by > 3 standard deviations (SDs) from the group mean. In the MRS voxel, the grey (GM) and white matter (WM) as well as cerebrospinal fluid (CSF) concentrations were estimated from an anatomical scan, using SPM8 (FIL Wellcome Department of Imaging Neuroscience, London, UK) for segmentation. Correct localization of the voxel on the segmented scans was confirmed by checking a picture of the voxel placement acquired during scanning. To correct for partial volume effects, the estimated percentages of GM and CSF were added to subsequent regression analyses.

Statistical analysis was performed with SPSS 22 (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.). Data were compared using appropriate statistical tests. Demographic data among groups were compared either with analysis of variance (ANOVA), or t-tests, when appropriate. As demographic and clinical data were not normally distributed, non-parametric tests were applied to test for group differences. Demographic differences between the three groups were compared using Kruskal-Wallis tests and subsequent post-hoc Mann-Whitney *U* tests in case of significant differences. Chi-square tests were applied to test for differences in group distributions of gender. Clinical differences between the two patient groups were evaluated using Mann-Whitney *U* tests.

To check for differences in data quality between the three different studies, and between the three subject groups, comparisons of grey matter, cerebrospinal fluid, full-width-half-maximum, and signal-to-

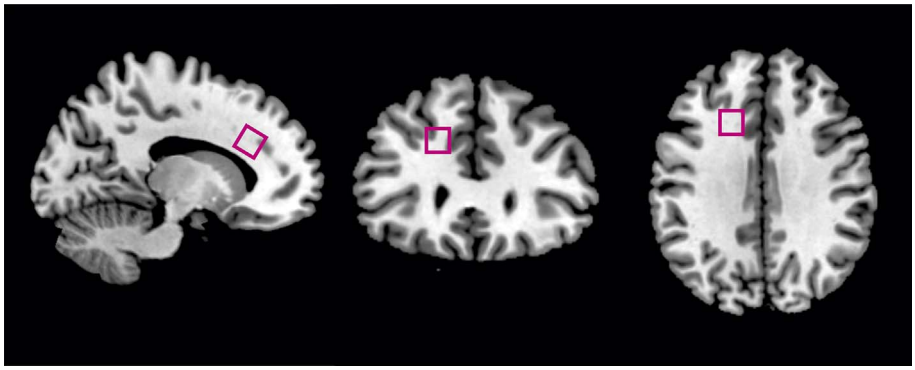

Fig. 1. Illustration of the position of the voxel from which the ^{1}H spectrum is obtained.

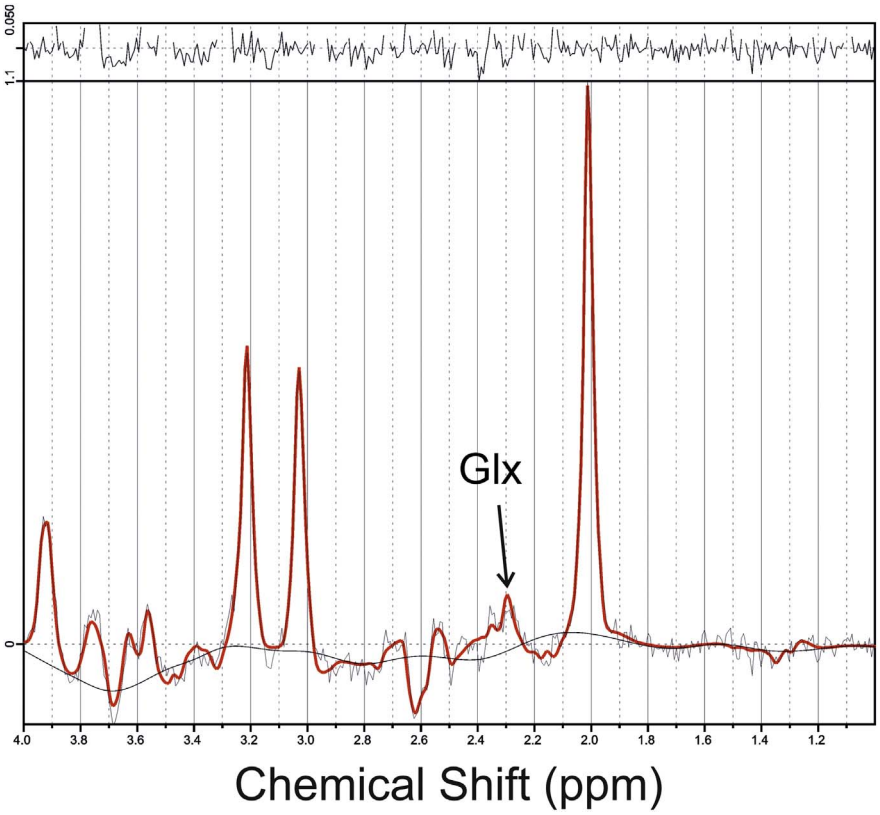

Fig. 2. Spectrum of ^{1}H MRS. A) Data taken directly from scanner (orange line) and peak fit (blue line). B) Estimation of metabolites by LC model. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

noise ratio values were performed. Next, the average Glx levels and their standard deviations were determined, as well as the 95% confidence interval (Cramer-Rao bounds) of the measurement precision. The Glx values were normally distributed.

Group differences in Glx levels were compared using univariate Analysis of Variance (ANOVA), with planned Helmert comparisons in which the levels of one variable are compared with the mean of the subsequent levels of the variable. Thus, the Glx levels of the healthy control group were tested against the Glx levels of the total patient group (AVH and NoAVH groups together), and then the Glx levels of the AVH group were tested against those of the NoAVH group. Next, univariate Analysis of Covariance (ANCOVA) was performed to calculate group differences in Glx levels with significant covariates (age, signal-to-noise ratio, and full-width-half-maximum), with planned comparisons similar to those used in the previous test. Spearman correlations were calculated to investigate possible correlations between hallucination severity and positive symptoms (measured with Hallucinations item P3 of the PANSS and Positive symptoms subscale of the PANSS) with Glx levels.

3. Results

3.1. Demographic details

The three groups (control participants, AVH and NoAVH) did not significantly differ in gender (details in Table 1). The two patient groups did not differ in medication (haloperidol equivalents), negative symptoms or general psychopathology (subscales of PANSS) (Table 1). There was a significant difference in education and a trend towards significance in age among groups, with the HC group having lower age and higher education then the combined patient groups. Therefore, we included age and education as covariates in the analysis of Glx levels (ANCOVA).

In addition, to ensure that the differences found were not driven by differences in age, we performed supplementary analysis. To match the three groups we excluded 3 healthy controls of age 18, two of whom had a higher level of education and one of whom was female. As a consequence, the HC and patient groups were matched for age (although planned post-hoc comparison still had a trend to significance between HC and patients; Supplementary Table S2).

In line with patient selection, out of all positive items of the PANSS questionnaire, only P3 (hallucinations) differed significantly between the two groups of patients ($U = 450$ $p = 0.006$; higher in AVH group).

3.2. Glutamate levels

ANOVA analysis of Glx revealed a significant effect of the group ($F(2,94) = 5.27$, $p = 0.007$). Planned comparisons showed that the control participants had significantly higher Glx levels than the total patient group ($p = 0.010$). However, within the total patient group, the AVH group had higher Glx levels than the NoAVH group ($p = 0.019$). Additionally, Glx levels did not differ between the AVH group and control participants (Table 2).

The main effect of the group was marginally significant after adding the significant covariates age, education, signal-to-noise ratio, and FWHM ($F(6,88) = 2.82$ $p = 0.065$). The difference between control participants and the patient group was no longer significant ($p = 0.104$), but Glx levels were significantly higher in the AVH group than in the NoAVH group ($p = 0.047$).

We found no significant difference in Glx levels between medicated and non-medicated patients, and the results for the comparison of Glx levels between the two patient groups did not change after adding medication as a covariate (Supplementary Table 4). In addition, our examination of Glx levels relative to creatine with phospocreatine (Glx/Cr) is summarized in Supplementary Table 5. The difference between control participants and the patient group was not significant, but the

Table 2
Quality check of MRS data and Glx levels per group.

Levels	Mean (SD)			ANOVA	HC vs patients	NoAVH vs AVH
	HC	AVH	NoAVH			
GM	0.36 (0.09)	0.35 (0.10)	0.36 (0.08)	F(2,94) = 0.26 (0.77)	n.s.	n.s.
CSF	0.04 (0.05)	0.05 (0.05)	0.04 (0.02)	F(2,94) = 0.34 (0.71)	n.s.	n.s.
Cr +	7.1 (0.9)	12.7 (1.6)	12.8 (1.3)	F(2,94) = 1.14 (0.32)	n.s.	n.s.
SNR	22.7 (2.9)	20.4 (4.0)	21.2 (3.2)	F(2,94) = 3.28 (0.04)	0.013	n.s.
FWHM	0.04 (0.01)	0.04 (0.01)	0.04 (0.01)	F(2,94) = 1.08 (0.34)	n.s.	n.s.
Glx	10.9 (1.2)	9.8 (1.1)	10.5 (1.4)	F(2,94) = 5.27 (0.007)	0.010	0.019

GM – grey matter; CSF – cerebrospinal fluid; SNR – signal-to-noise ratio; FWHM – full-width-half-maximum; Glx – glutamate with glutamine; n.s. – not significant. The two right-hand columns are results of Post-Hoc tests.

Glx levels were still significantly higher in the AVH group than in the NoAVH group ($p = 0.01$) also after correction for covariates ($p = 0.024$).

We found no evidence for an association between the severity of either hallucinations (P3) or positive symptoms with Glx levels ($\rho = 0.088$, $p = 0.443$; $\rho = 0.065$, $p = 0.573$, respectively). In a group matched for age, the results were almost the same (Supplementary Table S3).

3.3. Data quality

3.3.1. Comparison of data quality among different studies

Using ANOVA, we found no significant difference in GM, CSF, full-width-half-maximum (FWHM) or signal-to-noise-ratio (SNR) among different studies. This implies that all MRS data were collected with the same quality regardless of the study (see Section Methods, subsection Participants).

3.3.2. General data quality

When the three groups of participants were compared (HC, AVH and NoAVH) with respect to GM, CSF, FWHM and SNR values, only SNR differed between groups ($F(2,96) = 3.28$, $p = 0.04$). A post-hoc *t*-test revealed significant differences between patients and HC ($p = 0.01$), but not between the two patient groups ($p = 0.351$). Furthermore, both SNR and FWHM values were correlated with levels of Glx ($\rho = 0.363$, $p < 0.001$; $\rho = 0.305$, $p = 0.002$, respectively). For this reason, we included SNR and FWHM in the ANCOVA analysis of Glx levels.

Of the ninety-five subjects, none had a standard deviation of estimated Glx exceeding 20, leading to the conclusion that Glx was estimated with good precision.

The Cr + PCr level was estimated with good precision. No subject had a standard deviation of the estimated Cr level larger than 20.

We found no significant differences in Cr + PCr level either between healthy controls and patients or among patients (Table 1).

The voxel composition in terms of white matter (WM) and grey matter (GM) did not differ between groups (Table 1). The voxel mainly consisted of WM with the percentage of GM 35 ± 9% and that of CSF 4 ± 3%.

4. Discussion

We investigated the association of auditory verbal hallucinations in patients with schizophrenia and related psychotic disorders with levels of glutamate + glutamine (Glx) in the white matter of the left lateral prefrontal region at a position that encompasses fronto-temporal and inter-hemispheric white matter pathways. We found that the patient group showed lower levels of Glx than control participants. Among patients, those who had experienced AVH exhibited higher levels of Glx than those who had never experienced AVH. Interestingly, the level of Glx did not differ between patients with lifetime AVH and control participants. After controlling for relevant covariates, the main difference between the three groups became marginally significant, while the difference between the two patient groups remained.

The finding that Glx levels appear to be higher in patients with lifetime AVH than in patients without lifetime AVH is in line with the only previous study that related Glx to AVH (Hugdahl et al., 2015), and supports the idea that glutamatergic metabolites are a mediating factor in AVH. However, contrary to the findings of Hugdahl et al. (2015), we did not observe a significant correlation between Glx level and the severity of AVH. Methodological differences between the study of Hugdahl et al. and the present study complicate direct comparison of the results. In the former study, 23 patients with schizophrenia were divided in two relatively small groups: 7 patients in a high-symptom group and 16 in a low symptom load group, based on their PANSS Hallucination score (P3). The PANSS scores reflect the severity of a patient's symptoms during the previous week. In contrast, in the present study Glx levels were assessed in two groups of patients with schizophrenia (or a related psychotic disorder), which were defined based on the presence or absence of lifetime AVH. Moreover, whereas we measured Glx levels in one voxel in the left lateral prefrontal region, Hugdahl et al. collected data from four voxels, two in the bilateral superior posterior temporal lobe, and two voxels in the bilateral inferior frontal lobe. These two brain regions demonstrated similar Glx patterns within the three groups. In addition, the patients in the study of Hugdahl et al. appeared to have suffered from schizophrenia for longer than the patients in our study (12.25 vs. 8.4 years). Despite the differences between the two studies, a similar pattern of Glx levels was observed, which encourages further exploration of possible associations between glutamatergic metabolites and AVH in future studies.

Importantly, although the two patient groups in the present study differed in Glx level, the combined patient group demonstrated a decreased level of Glx in comparison with the control group. One recent meta-analysis reports that patients with schizophrenia have generally higher glutamatergic metabolite levels in the brain than control participants (Merritt et al., 2016), although local deviations were described. In another meta-analysis, lower frontal glutamate concentrations in patients with schizophrenia compared to healthy individuals were found (Marsman et al., 2013). Moreover, there is some evidence that a decrease in frontal glutamatergic metabolites is particularly present in chronically ill patients (Liemburg et al., 2016; Ohrmann et al., 2007). It should be noted though that both increases and decreases of glutamate levels in the DLPFC have been reported in schizophrenia patients (Merritt et al., 2016; Poels et al., 2014a).

The mean duration of illness in our study was 8.2 years for the AVH group and 9.5 years for the NoAVH group. Therefore, the patient group can be characterized as chronically ill. Our results thus seem to support the findings that chronicity is associated with lower Glx levels.

Our findings that Glx levels are higher in patients who experience AVH than in patients who have never experienced them are interesting, yet difficult to explain. An explanation may be that the increased levels of glutamate compensate for a blockade or hypofunction of the *N*-

methyl-D-aspartate receptor (NMDAR) (Poels et al., 2014a). This explanation is in line with the dopamine-glutamate dysfunction hypothesis of psychosis in general. The glutamate hypothesis of schizophrenia is based on the finding that the administration of NMDAR-antagonists, such as ketamine and phencyclidine (PCP or angel dust), results in schizophrenia-like symptoms (Javitt and Zukin, 1991). It is therefore assumed that schizophrenia is associated with hypofunctioning of the NMDAR. However, this is not specific to hallucinations and chronic blockage of the NMDAR can also cause negative symptoms and cognitive impairment (Poels et al., 2014c). Thus, the pure blockage of one glutamate receptor is not sufficient to explain the differences that we observe in our study, namely increased levels of Glx in the white matter of the prefrontal cortex.

The exact relationship between the hypothesized NMDAR hypofunction and symptoms of schizophrenia remains to be elucidated. Several underlying mechanisms have been proposed, which can be broadly divided into pre- and postsynaptic hypotheses. Presynaptic hypotheses assume that NMDAR hypofunction leads to an increase in presynaptic glutamate levels. This glutamate may then bind to non-NMDA receptors, possibly causing disturbances on a cognitive and motor level. Moreover, NMDAR dysfunction may lead to a reduced input towards inhibitory GABA neurons, factors which combine to cause an imbalance in glutamate transmission (Moghaddam and Javitt, 2012). It is also proposed that an abundance of glutamate can contribute to structural changes, as it may have a toxic effect on neurons, ultimately resulting in lower glutamate levels (Plitman et al., 2014). Among the postsynaptic hypotheses is the ascription of NMDAR dysfunction to alterations of NMDAR subunit compositions in patients with schizophrenia (Moghaddam and Javitt, 2012). Both pre- and postsynaptic hypotheses remain inconclusive, which complicates the drawing of conclusions regarding causal relations between altered Glx values and symptom severity. Furthermore, Veerman et al. (2014) reviewed and meta-analysed studies that investigated the effects of adding glutamate modulators to existing clozapine treatment in patients with schizophrenia. Intriguingly, they found that treatment with the glutamate agonist glycine specifically worsened positive symptoms. An opposite but weaker effect was observed when lamotrigine, an antagonist of postsynaptic voltage-gated Na^{2+} channels that decreases the presynaptic release of glutamate, was added to existing clozapine treatment. Patients in active groups showed a trend towards the improvement of positive symptoms (Veerman et al., 2014). The glutamate antagonist topiramate, which affects both GABAergic and postsynaptic glutamate receptors (kainite and AMPA), had no effect on positive, negative or composite symptoms. Finally, the NMDAR antagonist memantine had beneficial effects on all symptoms, yielding strong improvement in positive, negative and composite symptoms, as well as cognitive functions of patients. These findings suggest that whereas the NMDAR is probably dysfunctional in schizophrenia patients, the presynaptic release of glutamate is probably even more affected in patients with hallucinations. These studies, together with our results, suggest that an excess of glutamate is released in the synaptic cleft in patients with hallucinations, which is reflected in the increased Glx level that we find. However, we should note that at this point we have no direct proof of this scenario because MRS (at least with the protocols currently used) cannot distinguish whether the measured levels of glutamate come from extra- or intra-cellular compartments (see Limitations Section).

One may ask how an increased level of glutamate correlates with studies that have found altered brain connectivity related to the involved pathways. The voxel from which we obtained glutamate levels belongs to the cingulum and forceps minor. The forceps minor is the frontal part of the corpus callosum and connects frontal parts of the left and right hemispheres. The forceps minor and genu of the corpus callosum are considered to be important for emotional connotations of hallucinations (Karbe et al., 1998) as they connect Broca with its right counterpart, which is suggested to be involved in emotional aspects of speech (Allen et al., 2008; Wildgruber et al., 2006). Indeed, several DTI studies report abnormalities along this pathway in chronic patients with AVH (Ćurčić-Blake et al., 2015; Knochel et al., 2012).

The cingulum is the long-range tract that connects the frontal lobe with the posterior cingulate cortex (involved in self-related processing) and the parahippocampal gyrus (involved in memory). Previous studies have found decreased fractional anisotropy (FA) of this tract in patients compared to control participants (Hubl et al., 2004; Shergill et al., 2007) and alterations in white matter integrity in association with AVH (Seok et al., 2007; Shergill et al., 2007; Whitford et al., 2014). In line with our finding, in previous studies decreases in FA were found in patients with positive symptoms (AVH and delusions), specifically in the rostral part of the cingulum which is approximately where our voxel was placed (Ćurčić-Blake et al., 2015; Whitford et al., 2014). This part of the cingulum is involved in emotional processes and error monitoring.

We found no significant correlation of the hallucination item of PANSS (P3) with levels of Glx despite clear differentiation of the levels of Glx among groups. The reason for this observation might be the fact that P3 is not specifically selective with respect to AVH, even though it will be biased towards picking up auditory hallucinations. However, our participants were selected on the basis of lifetime experience of hallucinations, which may explain why P3 is not very high in the AVH group and it may refer to other types of hallucinations such as olfactory or tactile, which were present in both groups. Furthermore, PANSS rates psychotic experiences that occurred during the week preceding the interview. In contrast, our group comparison involved groups that differed according to the lifetime presence or absence of AVH.

4.1. Limitations

The ^{1}H MRS method provides a total tissue estimate of Glx but does not distinguish between intracellular or extracellular compartments, nor is it specific for synapses. Thus, we cannot discern for example whether the levels of Glx are related to increased synaptic firing or postsynaptic signalling. This imposes a limitation on possible conclusions about on-going processes and specific deficiencies in signalling that can arise from increased levels of glutamate even in a specific brain region such as the left lateral prefrontal region. Moreover, with the sequence that we applied, we were not able to discriminate between glutamate and glutamine, so the estimate may be an under- or overrepresentation of the actual glutamate level. Finally, the distinction between the AVH and noAVH groups was made based on lifetime prevalence of AVH. It may thus be possible that patients in the AVH group were not experiencing AVH at the time of scanning, a notion supported by the low average Hallucination score (P3 of the PANSS). Hence, the findings of this study are indicative for trait differences rather than state differences with respect to AVH. An additional confound to the MRS measurements could be antipsychotic medication. For example, studies in rats suggested that clozapine or haloperidol treatment can modulate glutamatergic neurotransmission in prefrontal cortex (Lopez-Gil et al., 2007) and that antipsychotic medication down-regulates the Glx levels in medial prefrontal cortex (Kegeles et al., 2012). Kraguljac et al. (2015) found that the functional brain connectivity changes six weeks after patients started to take risperidone. In our study, the medication intake was balanced between groups. In both groups there were a number of non-medicated patients. We found no significant differences in Glx levels when medicated patients were compared to non-medicated patients, and our results remained the same after medication levels were entered as a covariate (Supplementary materials).

5. Conclusion

This study aimed to investigate levels of glutamate in the white matter tracts belonging to the cingulum and forceps minor in associa-

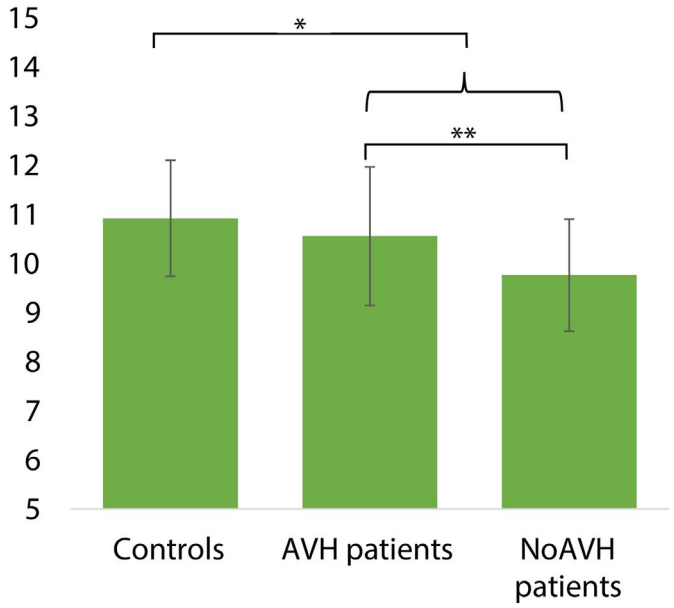

Fig. 3. Levels of Glx in control participants, patients with lifetime AVH, and patients without lifetime AVH, obtained from a voxel in the white matter of the left lateral prefrontal cortex. Error bars represent standard deviations. Planned comparisons with Helmert contrast (without significant covariates) showed a significant difference between control participants and total patient group (*$p = 0.010$), and a significant difference between AVH patients and noAVH patients (**$p = 0.019$).

tion with AVH in patients with schizophrenia. We found lower levels of Glx in patients with schizophrenia compared to control participants. However, within the patient group, patients with lifetime AVH demonstrated higher Glx levels than patients without lifetime AVH, suggesting a mediating role for Glx in AVH. This finding is in line with an earlier study, and may support the dopamine-glutamate dysfunction hypothesis of psychosis, suggesting that increased levels of glutamate may compensate for acute NMDAR blockage or hypofunction, contributing to the development of psychotic symptoms. Future studies in this field may aim to distinguish how the glutamate levels vary in different parts of the brain and whether this is associated with specific alterations in brain functioning and symptomatology. Here more sensitive sequences might be applied, and such studies may longitudinally study glutamate levels in relationship to various clinical parameters (Fig. 3).

Ethical statement

All study protocols were fully approved by the medical ethical board of the University Medical Center Groningen (METC; UMCG), with exception of the study on at risk mental state that was approved by the Mental Healthcare Research Ethics Committee (METiGG). All procedures were carried out according to the declaration of Helsinki.

Conflict of interest

All authors declare no conflict of interest. All authors approved the article.

Acknowledgment

The authors thank G.R. Blake for his comments on earlier versions of the manuscript. This work was supported by an ERC consolidator grant, project no. 312787 awarded to A. Aleman.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.pnpbp.2017.05.020.

References

Allen, P., Laroi, F., McGuire, P.K., Aleman, A., 2008. The hallucinating brain: a review of structural and functional neuroimaging studies of hallucinations. Neurosci. Biobehav. Rev. 32, 175–191.
Allen, P., Modinos, G., Hubl, D., Shields, G., Cachia, A., Jardri, R., Thomas, P., Woodward, T., Shotbolt, P., Plaze, M., Hoffman, R., 2012. Neuroimaging auditory hallucinations in schizophrenia: from neuroanatomy to neurochemistry and beyond. Schizophr. Bull. 38, 695–703.
Andreasen, N.C., Pressler, M., Nopoulos, P., Miller, D., Ho, B.C., 2010. Antipsychotic dose equivalents and dose-years: a standardized method for comparing exposure to different drugs. Biol. Psychiatry 67, 255–262.
Bliss, T.V., Collingridge, G.L., 1993. A synaptic model of memory: long-term potentiation in the hippocampus. Nature 361, 31–39.
Coyle, J.T., 2006. Glutamate and schizophrenia: beyond the dopamine hypothesis. Cell. Mol. Neurobiol. 26, 365–384.
Ćurčić-Blake, B., Nanetti, L., van der Meer, L., Cerliani, L., Renken, R., Pijnenborg, G.H., Aleman, A., 2015. Not on speaking terms: hallucinations and structural network disconnectivity in schizophrenia. Brain Struct. Funct. 220, 407–418.
Dlabac-de Lange, J.J., Bais, L., van Es, F.D., Visser, B.G., Reinink, E., Bakker, B., van den Heuvel, E.R., Aleman, A., Knegtering, H., 2015. Efficacy of bilateral repetitive transcranial magnetic stimulation for negative symptoms of schizophrenia: results of a multicenter double-blind randomized controlled trial. Psychol. Med. 45, 1263–1275.
Dollfus, S., Alary, M., Razafimandimby, A., 2013. Speech processing and anuditory hallucinations. In: Jardri, R., Cachia, A., Thomas, P., Pins, D. (Eds.), The Neuroscience of Hallucinations. Springer, pp. 123.
Duffau, H., 2008. The anatomo-functional connectivity of language revisited. New insights provided by electrostimulation and tractography. Neuropsychologia 46, 927–934.
Gardner, D.M., Murphy, A.L., O'Donnell, H., Centorrino, F., Baldessarini, R.J., 2010. International consensus study of antipsychotic dosing. Am. J. Psychiatry 167, 686–693.
Geoffroy, P.A., Houenou, J., Duhamel, A., Amad, A., De Weijer, A.D., Curcic-Blake, B., Linden, D.E., Thomas, P., Jardri, R., 2014. The arcuate fasciculus in auditory-verbal hallucinations: a meta-analysis of diffusion-tensor-imaging studies. Schizophr. Res. 159, 234.
Giel, R., Nienhuis, F.J., 1996. Schedules for Clinical Assessment in Neuropsychiatry (in Dutch). World Health Organization, Geneva/Groningen.
Hubl, D., Koenig, T., Strik, W., Federspiel, A., Kreis, R., Boesch, C., Maier, S.E., Schroth, G., Lovblad, K., Dierks, T., 2004. Pathways that make voices: white matter changes in auditory hallucinations. Arch. Gen. Psychiatry 61, 658–668.
Hugdahl, K., Craven, A.R., Nygård, M., Løberg, E., Berle, J.Ø., Johnsen, E., Kroken, R., Specht, K., Andreassen, O.A., Ersland, L., 2015. Glutamate as a mediating transmitter for auditory hallucinations in schizophrenia: a 1H MRS study. Schizophr. Res. 161, 252–260.
Javitt, D.C., Zukin, S.R., 1991. Recent advances in the phencyclidine model of schizophrenia. Am. J. Psychiatry 148, 1301–1308.
Karbe, H., Herholz, K., Halber, M., Heiss, W.D., 1998. Collateral inhibition of transcallosal activity facilitates functional brain asymmetry. J. Cereb. Blood Flow Metab. 18, 1157–1161.
Kay, S.R., Fiszbein, A., Opler, L.A., 1987. The positive and negative syndrome scale (PANSS) for schizophrenia. Schizophr. Bull. 13, 261–276.
Kegeles, L.S., Mao, X., Stanford, A.D., Girgis, R., Ojeil, N., Xu, X., Gil, R., Slifstein, M., Abi-Dargham, A., Lisanby, S.H., Shungu, D.C., 2012. Elevated prefrontal cortex gamma-aminobutyric acid and glutamate-glutamine levels in schizophrenia measured in vivo with proton magnetic resonance spectroscopy. Arch. Gen. Psychiatry 69, 449–459.
Knecht, S., Drager, B., Deppe, M., Bobe, L., Lohmann, H., Floel, A., Ringelstein, E.B., Henningsen, H., 2000. Handedness and hemispheric language dominance in healthy humans. Brain 123 (Pt 12), 2512–2518.
Knochel, C., Oertel-Knochel, V., Schonmeyer, R., Rotarska-Jagiela, A., van de Ven, V., Prvulovic, D., Haenschel, C., Uhlhaas, P., Pantel, J., Hampel, H., Linden, D.E., 2012. Interhemispheric hypoconnectivity in schizophrenia: Fiber integrity and volume differences of the corpus callosum in patients and unaffected relatives. NeuroImage 59, 926–934.
Kraguljac, N.V., White, D.M., Hadley, J.A., Visscher, K., Knight, D., ver Hoef, L., Falola, B., Lahti, A.C., 2015. Abnormalities in large scale functional networks in unmedicated patients with schizophrenia and effects of risperidone. Neuroimage Clin. 10, 146–158.
Krystal, J.H., Karper, L.P., Seibyl, J.P., Freeman, G.K., Delaney, R., Bremner, J.D., Heninger, G.R., Bowers Jr., M.B., Charney, D.S., 1994. Subanesthetic effects of the noncompetitive NMDA antagonist, ketamine, in humans. Psychotomimetic, perceptual, cognitive, and neuroendocrine responses. Arch. Gen. Psychiatry 51, 199–214.
Lahti, A.C., Koffel, B., LaPorte, D., Tamminga, C.A., 1995. Subanesthetic doses of ketamine stimulate psychosis in schizophrenia. Neuropsychopharmacology 13, 9–19.
Liemburg, E.J., Dlabac-De Lange, J.J., Bais, L., Knegtering, H., van Osch, M.J., Renken, R.J., Aleman, A., 2015. Neural correlates of planning performance in patients with schizophrenia—relationship with apathy. Schizophr. Res. 161, 367–375.
Liemburg, E., Sibeijn-Kuiper, A., Bais, L., Pijnenborg, G., Knegtering, H., van der Velde, J., Opmeer, E., de Vos, A., Dlabac-De Lange, J., Wunderink, L., Aleman, A., 2016. Prefrontal NAA and glx levels in different stages of psychotic disorders: A 3T 1H-MRS study. Sci. Rep. 6, 21873.

Lopez-Gil, X., Babot, Z., Amargos-Bosch, M., Sunol, C., Artigas, F., Adell, A., 2007. Clozapine and haloperidol differently suppress the MK-801-increased glutamatergic and serotonergic transmission in the medial prefrontal cortex of the rat. Neuropsychopharmacology 32, 2087–2097.

Marsman, A., van den Heuvel, M.P., Klomp, D.W., Kahn, R.S., Luijten, P.R., Hulshoff Pol, H.E., 2013. Glutamate in schizophrenia: a focused review and meta-analysis of (1)H-MRS studies. Schizophr. Bull. 39, 120–129.

Merritt, K., Egerton, A., Kempton, M.J., Taylor, M.J., McGuire, P.K., 2016. Nature of glutamate alterations in schizophrenia: a meta-analysis of proton magnetic resonance spectroscopy studies. JAMA Psychiatry. 73, 665–674.

Mesulam, M., 1990. Large-scale neurocognitive networks and distributed processing for attention, language, and memory. Ann. Neurol. 28, 597–613.

Moghaddam, B., Javitt, D., 2012. From revolution to evolution: the glutamate hypothesis of schizophrenia and its implication for treatment. Neuropsychopharmacology 37, 4–15.

Ohrmann, P., Siegmund, A., Suslow, T., Pedersen, A., Spitzberg, K., Kersting, A., Rothermundt, M., Arolt, V., Heindel, W., Pfleiderer, B., 2007. Cognitive impairment and in vivo metabolites in first-episode neuroleptic-naive and chronic medicated schizophrenic patients: a proton magnetic resonance spectroscopy study. J. Psychiatr. Res. 41, 625–634.

Parker, G.J., Luzzi, S., Alexander, D.C., Wheeler-Kingshott, C.A., Ciccarelli, O., Lambon Ralph, M.A., 2005. Lateralization of ventral and dorsal auditory-language pathways in the human brain. NeuroImage 24, 656–666.

Pijnenborg, G.H., Van der Gaag, M., Bockting, C.L., Van der Meer, L., Aleman, A., 2011. REFLEX, a social-cognitive group treatment to improve insight in schizophrenia: study protocol of a multi-center RCT. BMC Psychiatry 11 (161-244X-11-161).

Plitman, E., Nakajima, S., de la Fuente-Sandoval, C., Gerretsen, P., Chakravarty, M.M., Kobylianskii, J., Chung, J.K., Caravaggio, F., Iwata, Y., Remington, G., Graff-Guerrero, A., 2014. Glutamate-mediated excitotoxicity in schizophrenia: a review. Eur. Neuropsychopharmacol. 24, 1591–1605.

Poels, E.M.P., Kegeles, L.S., Kantrowitz, J.T., Slifstein, M., Javitt, D.C., Lieberman, J.A., Abi-Dargham, A., Girgis, R.R., 2014a. Imaging glutamate in schizophrenia: review of findings and implications for drug discovery. Mol. Psychiatry 19, 20–29.

Poels, E.M., Kegeles, L.S., Kantrowitz, J.T., Slifstein, M., Javitt, D.C., Lieberman, J.A., Abi-Dargham, A., Girgis, R.R., 2014b. Imaging glutamate in schizophrenia: review of findings and implications for drug discovery. Mol. Psychiatry 19, 20–29.

Poels, E.M., Kegeles, L.S., Kantrowitz, J.T., Javitt, D.C., Lieberman, J.A., Abi-Dargham, A., Girgis, R.R., 2014c. Glutamatergic abnormalities in schizophrenia: a review of proton MRS findings. Schizophr. Res. 152, 325–332.

Powers III, A.R., Gancsos, M.G., Finn, E.S., Morgan, P.T., Corlett, P.R., 2015. Ketamine-induced hallucinations. Psychopathology 48, 376–385.

Provencher, S.W., 1993. Estimation of metabolite concentrations from localized in vivo proton NMR spectra. Magn. Reson. Med. 30, 672–679.

Seok, J.H., Park, H.J., Chun, J.W., Lee, S.K., Cho, H.S., Kwon, J.S., Kim, J.J., 2007. White matter abnormalities associated with auditory hallucinations in schizophrenia: a combined study of voxel-based analyses of diffusion tensor imaging and structural magnetic resonance imaging. Psychiatry Res. 156, 93–104.

Sheehan, D.V., Lecrubier, Y., Sheehan, K.H., Amorim, P., Janavs, J., Weiller, E., Hergueta, T., Baker, R., Dunbar, G.C., 1998. The mini-international neuropsychiatric interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. J. Clin. Psychiatry 59 (Suppl. 20), 22–33.

Shergill, S.S., Kanaan, R.A., Chitnis, X.A., O'Daly, O., Jones, D.K., Frangou, S., Williams, S.C., Howard, R.J., Barker, G.J., Murray, R.M., McGuire, P., 2007. A diffusion tensor imaging study of fasciculi in schizophrenia. Am. J. Psychiatry 164, 467–473.

Steinmann, S., Leicht, G., Mulert, C., 2014. Interhemispheric auditory connectivity: Structure and function related to auditory verbal hallucinations. Front. Human Neurosci. 8.

Veerman, S.R., Schulte, P.F., Begemann, M.J., Engelsbel, F., de Haan, L., 2014. Clozapine augmented with glutamate modulators in refractory schizophrenia: a review and metaanalysis. Pharmacopsychiatry 47, 185–194.

van der Velde, J., Opmeer, E.M., Liemburg, E.J., Bruggeman, R., Nieboer, R., Wunderink, L., Aleman, A., 2015. Lower prefrontal activation during emotion regulation in subjects at ultrahigh risk for psychosis: an fMRI-study. NPJ Schizophrenia 1, 15026.

Whitford, T.J., Lee, S.W., Oh, J.S., de Luis-Garcia, R., Savadjiev, P., Alvarado, J.L., Westin, C.F., Niznikiewicz, M., Nestor, P.G., McCarley, R.W., Kubicki, M., Shenton, M.E., 2014. Localized abnormalities in the cingulum bundle in patients with schizophrenia: a diffusion tensor tractography study. Neuroimage Clin. 5, 93–99.

Wijtenburg, S.A., Yang, S., Fischer, B.A., Rowland, L.M., 2015. In vivo assessment of neurotransmitters and modulators with magnetic resonance spectroscopy: application to schizophrenia. Neurosci. Biobehav. Rev. 51, 276–295.

Wildgruber, D., Ackermann, H., Kreifelts, B., Ethofer, T., 2006. Cerebral processing of linguistic and emotional prosody: fMRI studies. Prog. Brain Res. 156, 249–268.