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# Impact of glutamate levels on neuronal response and cognitive abilities in schizophrenia

Liv E. Falkenberg[a,*], René Westerhausen[a,b], Alexander R. Craven[a], Erik Johnsen[b,c], Rune A. Kroken[b], Else-Marie Løberg[a,b], Karsten Specht[a,d], Kenneth Hugdahl[a,b,e,f]

[a]Department of Biological and Medical Psychology, University of Bergen, Haukeland University Hospital, Bergen, Norway
[b]Division of Psychiatry, University of Bergen, Haukeland University Hospital, Bergen, Norway
[c]Department of Clinical Medicine, Psychiatry Section, University of Bergen, Haukeland University Hospital, Bergen, Norway
[d]Department of Clinical Engineering, University of Bergen, Haukeland University Hospital, Bergen, Norway
[e]Department of Radiology, Haukeland University Hospital, Bergen, Norway
[f]NORMENT Senter for Fremragende Forskning, Oslo, Norway

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ABSTRACT

Schizophrenia is characterized by impaired cognitive functioning, and brain regions involved in cognitive control processes show marked glutamatergic abnormalities. However, it is presently unclear whether aberrant neuronal response is directly related to the observed deficits at the metabolite level in schizophrenia. Here, 17 medicated schizophrenia patients and 17 matched healthy participants underwent functional magnetic resonance imaging (fMRI) when performing an auditory cognitive control task, as well as proton magnetic resonance spectroscopy ($^1$H-MRS) in order to assess resting-state glutamate in the anterior cingulate cortex. The combined fMRI–$^1$H-MRS analysis revealed that glutamate differentially predicted cortical blood-oxygen level-dependent (BOLD) response in patients and controls. While we found a positive correlation between glutamate and BOLD response bilaterally in the inferior parietal lobes in the patients, the corresponding correlation was negative in the healthy control participants. Further, glutamate levels predicted task performance in patients, such that lower glutamate levels were related to impaired cognitive control functioning. This was not seen for the healthy controls. These findings suggest that schizophrenia patients have a glutamate-related dysregulation of the brain network supporting cognitive control functioning. This could be targeted in future research on glutamatergic treatment of cognitive symptoms in schizophrenia.



## 1. Introduction

Cognitive control dysfunction is one of the core deficits in schizophrenia. It is often manifested as problems in relating daily activities to internal goals and intentions (Miller, 2000), and effective pharmacological treatment for these symptoms is still lacking (Lesh et al., 2011). A fronto-parietal brain network assembling around the dorsal anterior cingulate cortex (ACC) is considered the anatomical substrate of cognitive control processes (Gruber and Goschke, 2004; Ridderinkhof et al., 2004; Vincent et al., 2008). Accordingly, anatomical (Benes et al., 1992; Fornito et al., 2009) as well as functional (Minzenberg et al., 2009) alterations in this brain network have been reported in schizophrenia. These alterations are also reflected on the biochemical level, as revealed in studies using proton magnetic resonance spectroscopy ($^1$H-MRS; Port and Agarwal, 2011). More specifically, studies on ACC and medial prefrontal cortex indicate that schizophrenia patients have alterations in the glutamate system (Benes et al., 1992), often presenting as elevated glutamate levels as measured by $^1$H-MRS early in the disease, with a decrease or normalization with time and use of medication (Marsman et al., 2011; Poels et al., 2014). Glutamate is the most widely distributed excitatory neurotransmitter in the brain and also acts as an intermediate in cerebral energy metabolism (Rothman et al., 2003). The glutamatergic hypothesis of schizophrenia is currently well recognized, claiming a crucial role of the glutamate system in the genesis of schizophrenia (see Coyle et al., 2012 for a review), although presently the underlying abnormalities are not fully understood. The glutamatergic abnormalities have been linked to a possible excitatory/inhibitory imbalance related to N-methyl-D-aspartate (NMDA) and metabotropic receptors (Coyle et al., 2012; Woo et al., 2008), in addition to a more indirect imbalance through $\gamma$-aminobutyric acid (GABA)ergic dysregulation (Lewis et al., 2005).

The combination of functional magnetic-resonance imaging (fMRI) and $^1$H-MRS constitutes a promising method for probing the

* Corresponding author at: Department of Biological and Medical Psychology, University of Bergen, Jonas Lies vei 91, Bergen 5009, Norway.
*E-mail address:* liv.falkenberg@psybp.uib.no (L.E. Falkenberg).

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relationship between glutamate and neuronal response during tasks and resting-state (Duncan et al., 2011; Enzi et al., 2012; Falkenberg et al., 2012; Horn et al., 2010; Kapogiannis et al., 2013; Schmaal et al., 2012). Using this combined approach, we previously found that resting-state glutamate (rsGlu) levels in the ACC predicted the blood-oxygen level-dependent (BOLD) response in several brain regions when healthy individuals performed a cognitive control task (Falkenberg et al., 2012). Considering schizophrenia patients' problems on tasks assessing cognitive control functions, together with the known glutamatergic pathology, we now suggest that glutamate differentially affects the BOLD response in schizophrenia patients and healthy individuals and that this is a mediating factor behind cognitive impairment in schizophrenia. Such a result would allow us to link glutamatergic mediation of neuronal activity at the metabolite-level to cognitive abnormalities found in schizophrenia. Moreover, we expected that patients with schizophrenia would show impaired performance on a cognitive control task and that the level of task performance would be moderated by glutamate levels.

## 2. Materials and methods

### 2.1. Participants

Seventeen patients with schizophrenia (DSM-IV/ICD-10) and 17 healthy participants matched for age, sex, and handedness participated in the study. Exclusion criteria were a history of neurological disorders, traumatic brain injuries, and metallic implants. Patients were assessed with the Positive and Negative Syndrome Scale (PANSS; Table 1; Kay et al., 1987) and obtained a score of four or higher on the P3 Hallucinations-item (moderate to severe hallucinations). The patients were part of a general study on schizophrenia patients with auditory verbal hallucinations, and all were on atypical antipsychotic medication (olanzapine, clozapine, aripiprazole, quetiapine, paliperidone, or amisulpride). Due to the verbal nature of the cognitive control task (see below), hearing threshold was assessed with the Hughson–Westlake audiometric test (Oscilla USB-300, Inmedico, Lystrup, Denmark). Participants with an averaged inter-aural acuity difference of more than 10 dB were excluded. Eleven of the healthy participants were also part of a previous study (Falkenberg et al., 2012) and were included as control subjects for the schizophrenia patients based on the best match according to the above criteria. The study was approved by the Regional Committee for Medical Research Ethics in Western Norway (REK-Vest), and informed consent was obtained from all participants before the study.

### 2.2. Auditory cognitive control task

The experimental paradigm was a version of the Bergen dichotic listening task, which is an auditory speech perception task with simultaneous pair-wise presentations of consonant–vowel syllables (/ba/, /da/, /ga/, /ka/, /pa/, /ta/; see Hugdahl, 2003 and Falkenberg et al., 2011 for details). A stimulus-driven (bottom-up) and an instruction-driven (top-down) component were included in the paradigm so as to achieve systematic variation in the need for cognitive control. The bottom-up component was implemented by varying the stimulus salience through different levels of inter-aural sound intensity (Hugdahl et al., 2008; Westerhausen and Hugdahl, 2010). Five levels of interaural intensity differences (IIDs) were used: 18 dB in favor of the left ear, 9 dB in favor of the left ear, no intensity difference, 9 dB in favor of the right ear and 18 dB in favor of the right ear. The stimulus was presented at a 70 dB sound pressure level (SPL) at both ears in the condition with no intensity difference. The other four conditions were presented with 70 dB SPL for the louder stimulus, while the weaker was reduced to either 61 or 52 dB SPL. The instruction-driven top-down component was applied by selectively directing the attention of the subject. The subjects were instructed to specifically focus and report from the right- (forced right condition, FR) or the left-ear stimulus (forced left condition, FL; Hugdahl, 2003; Hugdahl and Andersson, 1986). The interaction between the bottom-up and top-down conditions thereby determines the demand for cognitive control, that is when the IID and attention instruction (ATT) are incongruent (e.g., attention focused on the left ear while the right ear is louder) there is higher need for cognitive control mechanisms than when IID and ATT are congruent (Falkenberg et al., 2011; Westerhausen et al., 2010).

The two attentional instructions (FR, FL) combined with the five levels of IID resulted in 10 experimental conditions. Each condition consisted of 18 dichotic presentations, resulting in a total of 180 stimulus presentations pseudo-randomly intermixed with 90 silent null-events. This created a stochastic event-related design for fMRI acquisition (Friston et al., 1999), recorded in a single session. Attention instructions were randomly intermixed, preceded the stimuli by 1.5 s, and were given in writing through goggles mounted on the head coil (NordicNeuroLab Inc., Bergen, Norway). The dichotic syllables were presented at the beginning of the silent gaps of the sparse sampling protocol for the fMRI acquisition (van den Noort et al., 2008) using headphones (NordicNeuroLab Inc.). The participants responded orally immediately after the stimulus by naming the syllable they heard, thereby avoiding movement artifacts during fMRI-acquisition. Stimulus administration and synchronization were performed using E-Prime software (version 2.0, Psychology Software Tools Inc., Pittsburgh, PA, USA).

### 2.3. Functional magnetic resonance imaging (fMRI) acquisition and analysis

Imaging was performed on a 3.0 T GE Signa HDx scanner, using an eight-channel head coil. A short scout sequence and structural image were acquired first, followed by the fMRI and the $^{1}$H-MRS. Structural imaging was performed with a T1-weighted pulse sequence (Fast Spoiled Gradient, FSPGR; TR = 7.9 ms; TE = 3.1 ms; 11° flip angle) measuring 180 sagittal slices of 1 mm thickness (field of view, FOV (mm) = 256 × 256; 256 × 256 scan matrix).

fMRI was performed using an echo-planar imaging (EPI) sequence (TE = 30 ms; 90° flip angle) and was oriented to the structural image. A sparse sampling protocol was used (TR = 3.5 s, TA = 1.5 s) leaving a silent gap of 2.0 s between consecutive scans for task implementation, thereby reducing interference from scanner noise and avoiding movement artifacts during scanning. EPI volumes covered the cerebrum and most of the cerebellum, and contained 25 axial slices of 5 mm thickness (0.5 mm inter-slice gap; FOV 220 × 220 mm, 64 × 64 scan matrix), resulting in a voxel size of 3.44 × 3.44 × 5.0 mm. Image preprocessing and statistical analysis of the data were performed using Statistical Parametrical Mapping (SPM8) analysis software package (Wellcome Department of Cognitive Neurology, London, UK). The EPI images were realigned intra-individually to the first image in each time series and unwarped for correction of head movements and related image distortions. The images were then normalized to standard stereotactic space using the MNI-template and re-sampled to a cubic voxel size of 3 mm as well as smoothed using a 6 mm FWHM Gaussian filter. First-level individual statistical analysis of the fMRI data was set up as a model including a predictor for each of the 10 experimental conditions, and movement parameters were added as regressors. The predictors were convolved with the canonical hemodynamic response function (hrf) and a temporal high pass filter (cutoff at 128 s) was applied. The resulting individual beta-maps were used for the second-level group analysis.

For the fMRI group analysis, only four of the 10 conditions were included in order to investigate the BOLD response during cognitive control. This involved two conditions where the IID and attention were incongruent (FR with 18 dB in favor of the left ear, and FL with 18 dB in favor of the right), and two conditions with congruent IID and attention (FL with 18 dB in favor of the left ear, and FR with