Psychosis, social cognition and glutamatergic transmission - characteristics of deficits and opportunities for treatment

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List of abbreviations

BOLD	Blood oxygen level-dependent
CHR-P	Clinical High Risk for Psychosis
MRS	Magnetic Resonance Spectroscopy
NAC	N-Acetyl-Cysteine
NMDA	N-Methyl-D-aspartate
pSTS	Posterior Superior Temporal Sulcus
ROI	Region of Interest
SC	Social Cognition

1. Abstract

People affected by psychotic disorders often experience severe impairments in social cognition (SC) that correlate highly with deficits in community functioning and are thus an important potential treatment target. Because deterioration of social cognitive capabilities often persists throughout treatment with dopamine-receptor affine antipsychotics, altered glutamatergic transmission, a second possible pathway in the pathophysiology of schizophrenia, might partly explain these deficits. To test this hypothesis, studies 1 + 2 of this thesis investigated whether SC deficits can be temporarily induced by altering glutamatergic transmission. To this end, healthy participants received placebo or ketamine, which non-competitively antagonizes the N-methyl-D-aspartate receptor, thus altering glutamatergic transmission. In study 1, participants completed a video-based mentalizing task during functional magnetic resonance imaging. Behavioral data showed more answers not pertaining to any mentalizing capacity in the ketamine group. Functional imaging data showed hyperactivity of a cluster in the right posterior superior temporal sulcus in these participants. This cluster showed increased functional task-based connectivity with precuneus, potentially indicating that a dysfunctional shift of attention might partly be responsible for SC deficits. In study 2, participants were asked to encode and retrieve words in an episodic memory task and to make metacognitive confidence judgments about their performance. While showing no changes in episodic memory, participants receiving ketamine tended to be overconfident about their performance during the task. In the brain, this was accompanied by higher activity in the right superior-posterior parietal cortex during metacognitive judgments. This suggests that metacognition of memory, a process relevant in SC, might also be affected by disturbances of the glutamatergic pathway. In study 3, an experimental therapy targeting the glutamate pathway was given to patients at high risk for psychosis to reduce transition to schizophrenia and improve SC. They received either N-Acetyl-Cysteine or a placebo for 26 weeks. Additionally, they received either a specialized psychological intervention or stress-management training. Even though this multicentric clinical trial ultimately failed to sufficiently recruit patients, a tendency towards less transitions and improved SC in the treatment groups was apparent. In conclusion, the presented results indicate an important relationship between glutamatergic transmission and SC and their role as potential treatment target in early schizophrenia or clinical high risk for psychosis.

2. Introduction and research questions

“To see ourselves as others see us is a most salutary gift. Hardly less important is the capacity to see others as they see themselves.”

Aldous Huxley, The Doors of Perception

In his autobiographic work “The Doors of Perception” (1954) the famous author explored consciousness while pondering impressions of his psychedelic experiences. He encountered the strong connection between psychoactive compounds and their effects on perception of our social world and its phenomena. Several of these effects are reminiscent of schizophrenia, a disorder strongly associated with altered perception (Carhart-Harris et al., 2014).

Positive and negative symptoms define the phenotype of schizophrenia. Positive symptoms are characterized by hallucinations, paranoia, illusions of grandeur or difficulties with formal thinking and can be treated reliably with antipsychotic medication (Haddad and Correll, 2018). Negative symptoms are outlined by affective flattening, alogia, anhedonia, asociality, avolition or apathy and often remain unaffected by treatment in comparison (Galderisi et al., 2018). By extension, difficulties with social cognition can be named among the apathy dimension of negative symptoms (Bègue et al., 2020). In the context of schizophrenia, social cognition is especially relevant for community functioning (Fett et al., 2011). Because its deficits often persist throughout the course of treatment, it is important to explore possible pathways to ultimately improve social cognition within the disorder (Riccardi et al., 2021).

2.1 Glutamate and schizophrenia

The dopamine-hypothesis has been employed to explain pathogenesis of schizophrenia since well over 50 years (Howes et al., 2015). It is best defined by an imbalance in both, mesolimbic and mesocortical dopamine, which also serves as main target for dopaminergic antipsychotic compounds (McCutcheon et al., 2020). While the current literature does not clearly support a second important pathway (Kruse and Bustillo, 2022), changes in glutamatergic neurotransmission in schizophrenia have consistently been

traced back to genetic, post-mortem and imaging studies (Howes et al., 2015). The main mode of action by which glutamate is believed to contribute to the development of schizophrenia is by hypofunctioning of the N-Methyl-D-aspartate (NMDA) receptor on the gamma-aminobutyric acid interneurons that normally inhibit glutamatergic pyramidal neurons in the cortex (Moghaddam and Jackson, 2003; Zhang et al., 2021). The resulting hyperexcitability is often evident during first manifestation of pre-psychotic symptoms in adolescence and an indicator of an imbalance of excitatory-to-inhibitory neurotransmitters (Rapoport et al., 2012). This hints at glutamatergic transmission being a possible treatment target particularly in early schizophrenia (Fusar-Poli et al., 2020).

Phencyclidin and its derivative, ketamine, are non-competitive NMDA-receptor antagonists that block the influx of Ca^{2+} through ion channels and have been used in animal and human challenges to evoke psychotomimetic symptoms by emulating hypofunctioning of the NMDA-receptor (Zanos et al., 2018). In humans, ketamine can cause hallucinations and delusions. However, manifestations that are reminiscent of negative symptoms in schizophrenia like dissociation or blunting of affect occur more frequently (Curic et al., 2019; Tyler et al., 2017). Therefore, the persistence of negative and cognitive symptoms throughout the lifespan of patients with chronic schizophrenia might partly be explained by the fact that current antipsychotic treatment regimens do not influence or alter glutamatergic signaling.

As a result, ketamine has been used to model psychosis and schizophrenia in many instances (Krystal et al., 1994; Haaf et al., 2018), and studies have explored how ketamine influences various cognitive functions, such as memory, perception and response inhibition (Umbricht et al., 2000; Pomarol-Clotet et al., 2006; Scheidegger et al., 2016). Additionally, glutamatergic transmission has been a treatment target in various early clinical trials that target the NMDA receptor in patients with schizophrenia. Possible mechanisms involve targeting the glycine binding-site of the NMDA receptor as co-agonist or inhibiting the glycine transporter (Kruse and Bustillo, 2022). Results of these trials showed small to no effects on cognitive symptoms, but moderate effects on negative symptoms (Iwata et al., 2015; Tuominen et al., 2006).

2.2 Schizophrenia, social cognition and metacognition

To establish whether social functioning might correlate with altered glutamatergic transmission, it is important to characterize these deficits in detail. Social cognition can be divided into different processes and domains: An extensive review by Green et al. (2015) inspected four different processes and whether they are generally impaired in schizophrenia. While experience sharing (e.g. motor resonance or affect sharing) seems to remain unaltered, mentalizing and social cue perception are often impaired within the context of the disorder. Both are important for successful social interaction. As Frith (2004) puts it: “A person that does not have Theory of Mind takes no account of the beliefs and desires of other people when trying to understand their behavior”. This statement not only explains the meaning of Theory of Mind or mentalizing, but also gives an outlook on the characterization of its deficits in schizophrenia: For example, a man romantically interested in a woman might tell her a story about how he helped an old woman to cross the street. A person experiencing strong negative symptoms might conclude the man tells the story, because he deems it interesting. This interpretation, devoid of most social layers, would constitute “undermentalizing” (Montag et al., 2011; Peyroux et al., 2019). Conversely, paranoid thoughts often consist of overinterpretation of these social situations (Frith, 2004). In our example a person that “overmentalizes” might think the man harbors the intention to prove to the woman there may be no alternative to get romantically involved with him while maybe also appearing threatening in his intention. Overmentalizing has been shown to be correlated with the presence of positive symptoms (Fretland et al., 2015). In the brain, these mentalizing deficits can often be linked to areas in the posterior superior temporal sulcus (pSTS), precuneus, temporo-parietal-junction and the medial prefrontal cortex. Together these brain regions are also known as the mentalizing network (Frith and Frith, 1999).

Metacognition is closely intertwined with social cognition and can be defined as “thinking about thinking” (Flavell, 1979). It is assumed to have a major role in enhancing social interactions by establishing confidence in collaboration with others to achieve joint goals (Frith, 2012). Unlike social cognition, which often concerns itself with interpretations of

external stimuli like faces or intentions of others, metacognition is better characterized by evaluation of personal thinking behavior. However, the relationship between both constructs can be described as an iterative computational approach of recursive loops between our own and others' mental states (Vaccaro and Fleming, 2018). Metacognition is a broad construct and can be separated between "online" and "offline" metacognition (Fitzgerald et al., 2017). While offline metacognition can be assessed with questionnaires and concerns itself with overall thinking patterns in everyday life, online metacognition can be quantified by asking participants about their confidence in regard to their own task performance. The differentiation is important, because these constructs do not necessarily align (Lehmann et al., 2022). Generally, neural correlates of online metacognition are located in a network consisting of medial and lateral prefrontal cortex, precuneus and insula (Jia, 2020; Vaccaro and Fleming, 2018) The close conjunction between metacognition and mentalizing is reflected by activations in similar brain regions, like the ventromedial and dorsomedial prefrontal cortex (Motut et al., 2023; Vaccaro and Fleming, 2018).

2.3 Social cognition and glutamate

Arguably, intact metacognition and social cognition are both needed in order to have adequate agency in encounters of everyday life. However, since it has been established that these domains show only moderate improvement with antipsychotic treatment of psychotic disorders, it stands to question whether these deficits might be connected to altered glutamatergic dysfunction. In the animal model it has been shown that mice that had been treated perinatally with ketamine showed deficits in social situations with conspecifics (Phensy et al., 2017). In the human model, no such study has investigated these specific deficits under the influence of any NMDA-receptor antagonist.

Interestingly, the paper by Phensy et al. (2017) did not only present a way to create, but also to prevent these deficits in mice that had received ketamine perinatally. They received N-Acetyl-Cysteine (NAC) or a placebo perinatally as injections and as supplementation to their drinking water during the later course of the life cycle. The authors were able to show that mice that had received NAC showed more contacts during the social challenge with another conspecific.

NAC employs two distinct mechanisms that indirectly influence glutamatergic neurotransmission: First, it mitigates oxidative stress by activating the NMDA-receptor through cysteine donation, reversing its hypofunctioning. Second, it modulates glutamatergic signaling by activating the cysteine-glutamate antiporter through cysteine donation. This, in turn facilitates uptake of excessive extracellular glutamate (Berk et al., 2013; Egerton et al., 2020).

2.4 Research Questions

The primary aim of this thesis is the investigation of the relationship between glutamatergic transmission and social cognition or metacognition in healthy participants and in patients with clinical high risk for psychosis (CHR-P). To this end, studies 1 + 2 investigated whether a subanesthetic dose of ketamine altered social cognitive processes and their neurobiological correlates. In study 3, a multicentric clinical trial, patients received a psychological intervention targeted at improving social cognitive processes and/or NAC or a corresponding placebo to find out whether these deficits can be mitigated. The following are the main research questions:

Q1: Does ketamine have an influence on mentalizing, and is it connected to more or less mentalizing?

Q2: Does ketamine influence metacognition?

Q3: Can transition to psychosis be prevented with NAC and a specialized psychological intervention and does it influence social cognition?

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3. Publications

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Wasserthal, S., Lehmann, M., Neumann, C., Delis, A., Philipsen, A., Hurlemann, R., Ettinger, U., & Schultz, J. (2023). Effects of NMDA-receptor blockade by ketamine on mentalizing and its neural correlates in humans: A randomized control trial. Scientific Reports, 13(1), 17184. https://doi.org/10.1038/s41598-023-44443-6

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3.1. Publication 1: Effects of NMDA-receptor blockade by ketamine on mentalizing and its neural correlates in humans: a randomized control trial

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Effects of NMDA-receptor blockade by ketamine on mentalizing and its neural correlates in humans: a randomized control trial

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Schizophrenia is associated with various deficits in social cognition that remain relatively unaltered by antipsychotic treatment. While faulty glutamate signaling has been associated with general cognitive deficits as well as negative symptoms of schizophrenia, no direct link between manipulation of glutamate signaling and deficits in mentalizing has been demonstrated thus far. Here, we experimentally investigated whether ketamine, an uncompetitive N-methyl-D-aspartate receptor antagonist known to induce psychotomimetic effects, influences mentalizing and its neural correlates. In a randomized, placebo-controlled between-subjects experiment, we intravenously administered ketamine or placebo to healthy participants performing a video-based social cognition task during functional magnetic resonance imaging. Psychotomimetic effects of ketamine were assessed using the Positive and Negative Syndrome Scale. Compared to placebo, ketamine led to significantly more psychotic symptoms and reduced mentalizing performance (more "no mentalizing" errors). Ketamine also influenced blood oxygen level dependent (BOLD) response during mentalizing compared to placebo. Specifically, ketamine increased BOLD in right posterior superior temporal sulcus (pSTS) and increased connectivity between pSTS and anterior precuneus. These increases may reflect a dysfunctional shift of attention induced by ketamine that leads to mentalizing deficits. Our findings show that a psychotomimetic dose of ketamine impairs mentalizing and influences its neural correlates, a result compatible with the notion that deficient glutamate signaling may contribute to deficits in mentalizing in schizophrenia. The results also support efforts to seek novel psychopharmacological treatments for psychosis and schizophrenia targeting glutamatergic transmission.

People with schizophrenia frequently show deficits in social cognition, a cognitive domain comprising multiple processes including social cue perception, mentalizing, regulation of emotion and experience sharing[1,2]. These impairments affect community functioning more than impairments in other cognitive domains[3,4]. Deficits in mentalizing—the ability to infer mental states in others or oneself—can be observed across all stages of the disorder[5–7] and can consist of over- or undermentalizing, corresponding to excessive or insufficient attribution of mental states to other agents[8]. While overmentalizing is more frequently associated with positive symptoms—paranoid thoughts often consist of attributing more intentions to social situations than are actually present—undermentalizing has been associated with negative or disorganized symptoms that numb experiences of the surrounding world, leading to diminished attribution of intentions to others[8–10]. Neural correlates of mentalizing deficits in schizophrenia include abnormal activation of the superior temporal sulcus (STS), anterior cingulate

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cortex (AC), bilateral temporo-parietal junctions (TPJ), medial prefrontal cortex (mPFC) and precuneus[11–14]. Both hypo- and hyperactivation of STS and precuneus have been reported during mentalizing[15,16].

While in some instances social cognition or mentalizing were shown to improve upon treatment with atypical antipsychotics[17,18], deficits in these domains remain largely unchanged during the course of the illness[19]. At present, evidence about the use of antipsychotics targeting faulty dopamine-signaling via D2-receptor-binding to treat social cognition deficits appears inconclusive[20]. However, various studies point to a significant role of the glutamatergic *N*-methyl-D-aspartate (NMDA) receptor in the development of negative and cognitive symptoms. The mechanism by which NMDA-receptors are believed to contribute to the provenance of cognitive symptoms involves hypofunctioning of these receptors on gamma-aminobutyric acid (GABA) interneurons. In this hypothesis, impairment of these interneurons then leads to disinhibition of pyramidal cells in corticolimbic circuits. To test theories about deficient glutamate signaling and its connection to schizophrenia in a behavioural design, multiple studies have used NMDA-receptor antagonists to evoke psychosis-like states in healthy subjects or animals[21]. One such uncompetitive NMDA-receptor antagonist is ketamine. Mainly used to induce a narcotic state, it blocks the NMDA-receptor ion channels and thus the influx of Ca^{2+}, partly explaining hypofunction on GABAergic interneurons and leading to locally specific hyper- or hypoactivity in the brain[22].

Since discovery of its psychotomimetic qualities, subanesthetic ketamine has been used as a model for psychosis in humans and rodents[23]. In a study of Phensy and colleagues, male mice postnatally injected with ketamine showed disrupted social investigation patterns when introduced to unfamiliar conspecifics[24]. Disruption of these patterns has repeatedly been shown in similar experiments with ketamine in the animal model[25,26]. However, mentalizing, a key social cognitive skill deficient in schizophrenia, is arguably best tested in humans. And while studies with human subjects have explored ketamine effects with respect to memory[27,28], modulation of emotion-cognition interaction[29], response inhibition[30], probabilistic inference[31], metacognition[32] and processing of emotional faces[33], to our knowledge, no study has investigated the influence of ketamine on mentalizing.

The aim of this study was to find out whether manipulating NMDA-receptor activation via ketamine would influence mentalizing and its neural correlates in healthy volunteers. We used a modified version of the Movies for the Assessment of Social Cognition (MASC) task by Dziobek and colleagues to assess the level of mentalizing (normal, over-, under- or no mentalizing)[34], a task on which patients with schizophrenia were shown to exhibit different mentalizing patterns than controls[8,34,35]. We expected volunteers subjected to ketamine to display more undermentalizing or no-mentalizing than individuals receiving placebo. We also predicted abnormal activation of brain regions associated with mentalizing.

Methods

Participants

387 participants were recruited via online message boards at the University of Bonn and screened for eligibility via an online questionnaire between June 2019 and September 2020. Among 85 eligible participants, 70 participants (mean age = 24.18, SD = 4.17, range = 18–34, 37 female) took part in the MRI study. Randomization algorithms were created by U.E. and all personnel except anesthesiologists remained blinded until preprocessing of fMRI data had been conducted. There were no significant differences between ketamine and placebo groups in terms of gender [X^2 (1, N = 63) = 0.15, p = 0.701] or age [t(61) = 0.80, p = 0.427; independent samples t-test].

If participants showed a dominance for the right hand[36], were non-smoking[37], considered themselves non-claustrophobic and had never taken or received ketamine, they were invited for an on-site screening. The on-site screening consisted of a short structured clinical interview (Mini-International Neuropsychiatric Interview v. 5.0), items assessing positive symptom load indicative of psychosis risk from the Structured Interview for Prodromal Symptoms (SIPS)[38], a urine drug-screen (SureStep Urine Multi Drug, Innovacon Inc.) and—for female participants—a pregnancy test (hCG cassette, Alere). Details on inclusion criteria can be found in Table 1. One participant cancelled an appointment for the MRI experiment after randomization and will thus be treated as dropout.

Ethical approval

The randomized and placebo-controlled study was performed in accordance with the declaration of Helsinki after being approved by the local ethics committee at the Department of Psychology at the University of Bonn,

Exclusion criterion	Instrument
Tobacco use	Fagerström test for nicotine dependence
Left-handedness	Edinburgh Inventory of Handedness
Current or past psychiatric diagnosis	MINI v5 (excluding "K"-scale, only filter-items)
Clinical high risk for psychosis	SIPS items P1-P5, cutoff value ≥ 3
Current (or past) drug abuse	Urine drug screening (MINI)
Current pregnancy	Urine pregnancy test
Other relevant medical conditions	Unstructured Interview
No concomitant medication	Unstructured Interview

Table 1. List of exclusion criteria with instruments. *MINI* mini-international neuropsychiatric interview, *SIPS* structured interview for prodromal symptoms.

Germany. Data was collected in an MRI facility at the University Hospital of Bonn between June 2019 and September 2020.

General procedure

On the day of the experiment, participants were required to arrive with an empty stomach, having fasted food at least 6 h and clear fluids 2 h prior to examination. Participants gave written informed consent and received written instructions. After medical examination by an anesthesiologist, an intravenous access was applied to one arm and participants were led into the MRI scanner. After participants were placed inside the MRI scanner and preparations were complete, the infusion was initialized. The anesthesiologist ensured there were no adverse events and participants were able to familiarize themselves with the substance prior to scanning to avoid anxiety-related adverse effects. The experimental task and MRI data acquisition were then initiated under continuous heart rate and oxygen level monitoring throughout the infusion. After completion of MRI data acquisition, the infusion was stopped, and participants completed the Positive and Negative Syndrome Scale (PANSS) interview with a trained blinded rater.

Drug administration

A subanesthetic dose of ketamine was delivered via a Graseby 3500 intravenous infusion pump controlled by the STANPUMP software (Steven Shafer, M.D., Anesthesiology Service, PAVAMC 3801 Miranda Ave., Palo Alto, USA). Target plasma levels were 100 ng/ml with an initial bolus administered as a 2 mg/ml solution. The plasma level of 100ng/ml was chosen in accordance with a study of Krystal et al.[23], who showed that a dose of 0.5 mg/kg (≈ 100–250 ng/ml) ketamine reliably evoked psychotomimetic symptoms. As earlier work has shown that blood-plasma-levels using this equipment were close to the targeted plasma-levels, no blood samples were drawn[30]. A saline solution (0.9% sodium chloride) was used as placebo. The ketamine/saline solution was prepared by an unblinded anesthesiologist. Thirty-four participants received an infusion with racemic ketamine (Ketamin Inresa, 50mg/ml, 10ml solution). Adverse effects were observed in two subjects, who suffered from nausea and low blood pressure. Both subjects were treated accordingly, released home after monitoring, and checked on the day after the experiment by telephone—no subsequent symptoms were reported. Thirty-five participants received a placebo saline infusion using the same setup; none reported adverse effects.

Experimental task

We employed a modified version of the MASC task, which assesses mentalizing performance using a set of videos depicting subsequent stages of social interactions between four human agents. The four agents spend an evening together, take part in various activities and interact in friendly, hostile, or romantic ways. The movie is interrupted at key timepoints by four-alternative-forced-choice questions about the currently ongoing social situation (e.g., "what is Michael feeling?"). The four response options represent four possible levels of mentalizing: overmentalizing, "normal" mentalizing, undermentalizing or no mentalizing. The "normal" mentalizing response is the answer that most healthy participants would give and is considered the "correct" answer to the question. Our version of the MASC task was structured as follows (Fig. 1): First, the question to be answered was shown and a button press by the participant initiated the presentation of the video (question phase); then the video sequence was shown (video phase); after the video, the question was presented again for four seconds and participants were instructed to think of an answer during that time. Subsequently, the four possible answers were presented until participants selected their preferred answer (response phase). Finally, they indicated their confidence in their response using a 6-point Likert scale (confidence rating phase; confidence ratings will not be further considered in the present report). To allow contrasting of the neural activation evoked during mentalizing, participants answered an equal number of non-social control questions pertaining to the physical surrounding of the actors. The adaption of the MASC for this study is described in detail in the supplementary materials S1.

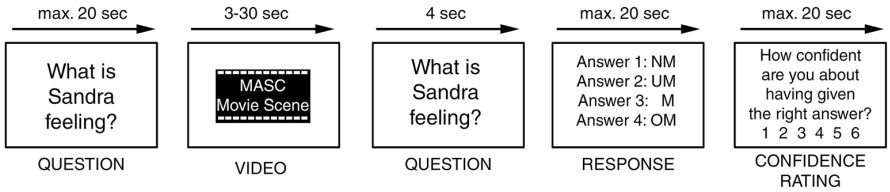

Figure 1. Depiction of one trial of the experimental task, a modified version of the MASC experiment by Dziobek et al.[34]. First, participants were presented with a question about the depicted social situation or the physical surroundings in which the agents interact. Next, a short video clip (max. length = 30s, average = 10s) was presented in which two to four agents interact socially. Afterwards, the question was shown again for 4000 ms and participants were instructed to think about their response. Next, they selected the best fitting response via a button press. Lastly, participants indicated their confidence in the given response. Stimuli are not to scale abbreviations: *NM* no mentalizing, *UM* undermentalizing, *M* mentalizing, *MASC* movie for the assessment of social cognition, *OM* overmentalizing.

PANSS

The Positive and Negative Syndrome Scale (PANSS) is a semi-standardized medical interview used for measuring symptom severity in schizophrenia. Symptoms are by default subdivided into positive symptoms, negative symptoms and general symptoms[39]. While the scale for positive and negative symptoms are assessed by seven Items each, the general scale features 16 items, each with a scale ranging from 1 to 7. In this study we used the five-factor structure proposed by Lehoux and colleagues to model the effects of ketamine in more detail (positive, cognitive/disorganization, hostility, negative, depression/anxiety)[40]. The PANSS has previously been used in studies analyzing the psychotomimetic effects of ketamine, showing that symptoms of most subscales were significantly increased when participants had received a dose of ketamine[41,42]. PANSS data were collected by a trained and blinded rater not involved in preparation and conduction of the MRI experiment. PANSS data is only available for n = 58 subjects, as the interviewer was absent on one occasion.

fMRI image acquisition and data analysis

Images were acquired using a 3T Siemens TrioTim MRI scanner (Siemens, Erlangen, Germany). Because participants wore pneumatic headphones, we used a 12- instead of a 32-channel headcoil as the latter was too small to fit participants' heads with headphones. Using a 12-channel headcoil does not significantly decrease blood-oxygen-level-dependent (BOLD) signal in cortical areas[43].

Functional images were acquired using a T2*-weighted echo-planar imaging sequence (echo time (TE) = 30 ms, repetition time (TR) = 2500 ms, flip angle = 90°, voxel-size = 2 × 2 × 3 mm, slice thickness = 3mm, field of view (FOV) = 192 mm, 37 slices). For purposes of normalization, a T1-weighted structural scan was acquired (TE = 2.54 ms, TR = 1660 ms, inversion time = 800 ms, slice thickness = 0.8 mm, matrix size = 320 × 320, FOV = 256 mm, flip angle = 9°, voxel-size = 0.8 × 0.8 × 0.8 mm, 208 sagittal slices).

First, fMRI images were checked manually for abnormalities upon which three participants had to be excluded due to artifacts in functional images. Functional EPI images were analyzed using SPM12 (Wellcome Center for Neuroimaging, London, UK, https://www.fil.ion.ucl.ac.uk/spm/software/spm12/) running in MATLAB 2019a (The Math Works, Natick, USA). Head motion was evaluated for every participant using the ART toolbox (https://www.nitrc.org/projects/artifact_detect). Any participant showing a shift in the z-dimension greater than 3mm or exceeding a volume-to-volume movement threshold greater than 1.5mm in 20% of the scans was excluded[44]. Following this procedure, two additional participants were excluded, leaving a final sample of 64 participants. The first six images of every participant were excluded as dummy scans to account for T1 equilibration. Functional images were realigned to the first image in that series using rigid-body transformation. These images were then coregistered to T1 structural scans and normalized to a standard space EPI template volume of the Montreal Neurological Institute (MNI) as provided with SPM12. Normalization failed with one participant, who had to be excluded from further analysis. In a final preparation step, images were smoothed with a Gaussian kernel of 6 mm.

fMRI data analysis

For each participant, a fixed-effects general linear model (GLM) was fitted to the preprocessed BOLD signal data using SPM12. The GLM contained separate sets of four regressors for the social and physical trials, modeling the following trial parts: presentation of the question before and after the video, presentation of the video, response, and confidence rating. The model further included six movement regressors representing estimates of rotation and translation created during the realignment step of data preprocessing, and a constant term. While the task was not designed to separate neural activation evoked during the subsequent trial parts, modeling these trial parts separately increased the flexibility of the GLM at small costs on degrees of freedom. The response phase was modelled from offset of the second question presentation to participants' answer to the mentalizing question, and the confidence rating phase spanned the time between answer to the mentalizing question and locking of the confidence rating. Regression coefficients (parameter estimates) for these regressors were estimated for each voxel of each participant's brain. Linear contrasts were applied to the individual parameter estimates of the response to the experimental conditions in order to contrast each of these four trial parts of the social and physical trials.

To determine the effect of ketamine on BOLD signal during mentalizing, we first verified that the social cognition task evoked activation in brain regions known to be involved in social cognition under placebo. To this end, we constructed a second-level (random effects) model in SPM that allowed us to contrast neural correlates of social vs non-social cognition under placebo and ketamine in the four trial parts (see Fig. 1). In each participant's fixed-effects GLM (see above), we calculated the social(SOC) > physical (PHY) contrast for each trial part and imported those contrasts into a second-level full factorial model with the within-subject factor "trial part" and the between-subject factor "substance". We then used this model to obtain effects of ketamine on regions sensitive to the social task. We thus tested the following eight contrasts: (1) ketamine < placebo, trial part (TP): question; (2) ketamine > placebo, TP: question; (3) ketamine < placebo, TP: video; (4) ketamine > placebo, TP: video; (5) ketamine < placebo, TP: response; (6) ketamine > placebo, TP: response; (7) ketamine < placebo, TP: confidence rating; (8) ketamine > placebo, TP: confidence rating.

Parameter estimates from a region of interest (ROI) in the right pSTS region, identified in one of the t-tests (see Results), were exported using MarsBaR in SPM12[45].

Effects of ketamine on functional connectivity between the pSTS ROI and other areas of the brain were assessed using a generalized psychophysiological interactions (gPPI) analysis with the CONN-toolbox in SPM 12[46]. For each participant, eigenvariates of the BOLD signal in the pSTS ROI were extracted and used to create a set of psychophysiological interaction (PPI) factors, which are an interaction of the deconvolved pSTS BOLD signal[47] and the psychological factors of interest, namely the "question" part of the social and physical trials. The

gPPI toolbox then inserted these PPI regressors into the individual GLMs described above and fitted these new GLMs to the BOLD data to yield functional connectivity parameter estimate maps.

Contrast images between functional connectivity during the social and physical question part of the trial (SOC > PHY) were then separately assessed in the participants of the placebo and the ketamine group using random effects one-sample t-tests in SPM 12; the effect of ketamine was assessed by comparing these contrast maps across groups using an independent samples t-test. All results of the fMRI analyses were considered significant if they exceeded the threshold of $p < 0.05$ after family-wise error correction for multiple comparisons at the cluster level across all the voxels of the brain, based on an uncorrected threshold (= cluster-forming threshold) of $p < 0.001$ at the voxel level. These results are indicated as $p_{(corr)}$ in the text.

Analysis of the behavioural data

Behavioural data were analyzed using SPSS 27 (IBM Corp., Armonk, USA). Effects of substance on PANSS were tested using independent samples *t*-tests for each of the five PANSS factors. Responses in the modified MASC were assessed in two ways: (1) in terms of accuracy (% correct responses in the social and physical trials) using a two-way, mixed analysis of variance (ANOVA) (between-subjects factor: placebo vs. ketamine ; within-subject factor: social vs. physical); and (2) subsequently, only for the social trials, by comparing the number of times each type of response (mentalizing, over-mentalizing, under-mentalizing, no mentalizing) was given by participants of the two groups using a one-factorial ANOVA.

Results

Effects of ketamine on symptoms of schizophrenia

Participants under ketamine showed significantly more schizophrenia-related symptoms than controls in four of the five factors used to assess the PANSS data: positive symptoms ($t(33.52) = -5.42$, $p < 0.001$, $d = -1.46$), cognitive disorganization ($t(51.57) = -4.21$, $p < 0.001$, $d = -1.12$), negative symptoms ($t(47.49) = -3.74$, $p < 0.001$, $d = -1.05$) and depression/anxiety symptoms ($t(33.26) = -3.48$, $p = 0.001$, $d = -0.94$); no significant change in hostility symptoms was found ($t(47,49) = -1.13$, $p = 0.264$). In response to a reviewer's suggestion, we calculated correlations between PANSS subscales and mentalizing performance in the ketamine group, but found no significant associations. The results are reported in Supplementary Table S1.

Effects of ketamine on mentalizing

Participants made more errors in the social than in the physical trials of the modified MASC task ($F(1, 61) = 5.84$, $p = 0.019$, $\eta^2 = 0.087$), and participants in the ketamine group made more errors than participants in the placebo group ($F(1, 61) = 6.52$, $p = 0.013$, $\eta^2 = 0.097$). The interaction between the factors trial type and participant group was not significant ($F(1, 61) = 0.93$, $p = 0.34$). The physical trials served only as control condition for the assessment of the BOLD response during social trials, in which participants were engaged in mentalizing, the main focus of our experiment. To assess if ketamine influenced the level of mentalizing that participants engaged in, we compared the frequency with which participants of both groups chose each possible type of answer. We found that ketamine affected the pattern of responses (Fig. 2; one-way ANOVA: "no mentalizing": $F(1,61) = 4.78$, $p = 0.033$, $\eta^2 = 0.073$; "undermentalizing": $F(1,61) = 0.18$, $p = 0.672$, $\eta^2 = 0.003$; "mentalizing": $F(1,61) = 2.45$, $p = 0.123$, $\eta^2 = 0.039$; "overmentalizing": $F(1,61) = 0.45$, $p = 0.505$, $\eta^2 = 0.007$). Thus, ketamine led to an increase in "no mentalizing" responses.

Neural correlates of mentalizing under ketamine

We expected social trials to evoke BOLD signal increases in social brain regions when compared to physical trials (control). This manipulation check was performed on the data of the placebo participant group creating a second-level model, using the SOC > PHY contrast images for the question part of the trial. Results (Fig. 3A)

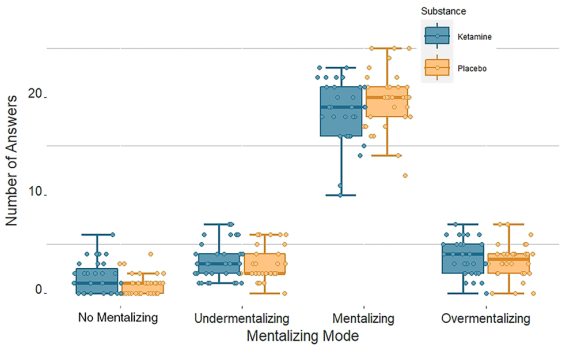

Figure 2. Effects of ketamine on mentalizing: participants under ketamine gave more "no mentalizing" answers in our movie-based theory-of-mind task (modified MASC) than controls; *MASC* movie for the assessment of social cognition.

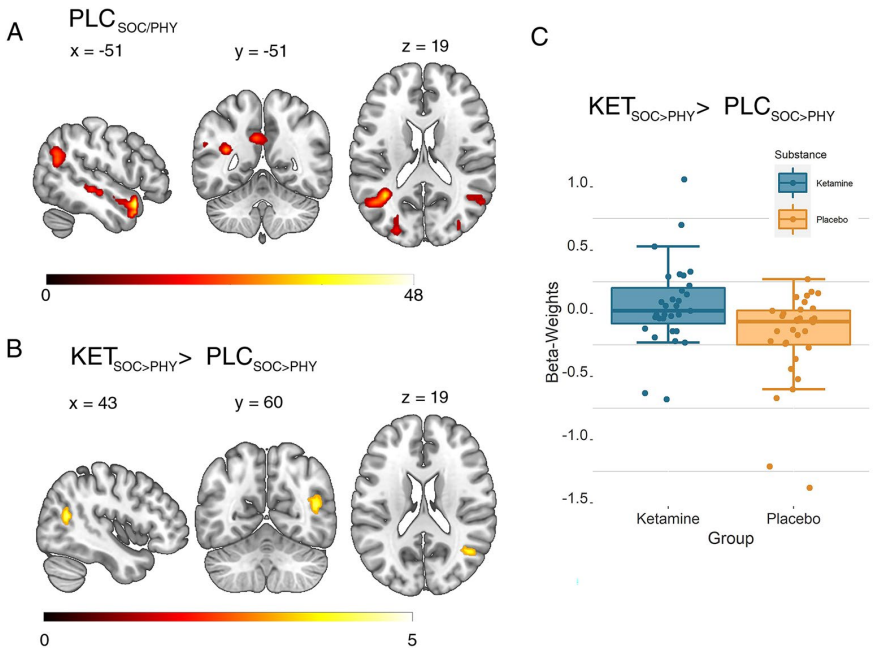

Figure 3. Neural correlates of social cognition and effects of ketamine during the modified MASC task. Threshold at p(FWE-cluster level) = 0.05, based on a voxelwise uncorrected threshold of p < 0.001. (**A**) Activation changes in left pSTS and precuneus in the placebo participant group during the question phase of the trial. (**B**) Increased activation in the right pSTS during social cognition under ketamine in the question condition (independent-samples t-test comparing the social > physical contrast images across participant groups). (**C**) Contrast of parameter estimates in the right pSTS cluster depicted in (**B**). *BOLD* blood-oxygen-level-dependent, *FEW* correction for family-wise errors, *KET* ketamine, *MASC* movie for the assessment of social cognition, *PLC* placebo, *PHY* physical, *SOC* social, *STS* superior temporal sulcus.

revealed changes in activation in the social compared to the physical trials in left pSTS [MNI-coordinates of activation peak: x = − 48, y = 17, z = − 19, k_e = 193, $F(1,244) = 47.66$, $p_{(Corr)} < 0.001$; x = − 36, y = − 55, z = 20, k_e = 175, $F(1,244) = 38.02$, $p_{(Corr)} < 0.001$], cuneus [x = − 21, y = − 97, z = 5, k_e = 317, $F(2,244) = 33.34$, $p_{(Corr)} < 0.001$], middle occipital gyrus [x = 30, y = − 94, z = 11, k_e = 222, $F(1,244) = 31.63$, $p_{(Corr)} = 0.019$], precuneus [x = − 6, y = − 52, z = 32, k_e = 47, $F(1,244) = 25.72$, $p_{(Corr)} = 0.019$] and right pSTS [x = 54, y = − 61, z = 20, k_e = 36, $F(1,244) = 25.72$, $p_{(Corr)} = 0.054$]. This pattern of findings is compatible with involvement of brain regions associated with social cognition during the social trials[48].

We proceeded to evaluate effects of ketamine on social cognition during the MASC by comparing, for each of the four trial parts, the social with the physical trials (SOC > PHY) between groups. We found a significant increase in BOLD signal in the ketamine group during presentation of the questions in a cluster located in the right posterior superior temporal sulcus region [pSTS; x = 45, y = − 58, z = 17, k_e = 52, $t(244) = 4.42$, $p_{(Corr)} = 0.02$; Fig. 3B,C]. No other differences between participant groups were found. The BOLD-signal from this pSTS-cluster was used as seed for a functional connectivity analysis (see next section).

Clusters of voxels that exhibited increased functional connectivity with the right pSTS in the placebo group during the question phase of the social compared to the physical trials were found (see Fig. 4A, warm colours) in left superior temporal gyrus [x = − 56, y = − 40, z = 10, k_e = 162, $t(31) = 5.80$, $p_{(Corr)} < 0.001$] and posterior cingulate gyrus [x = 0, y = − 62, z = 4, k_e = 110, $t(31) = 5.22$, $p_{(Corr)} = 0.003$]. Decreases in connectivity with right pSTS during the social compared to the physical trials were found (see Fig. 4A, cold colours) in cuneus [x = 16, y = − 92, z = 4 k_e = 122, $t(31) = -5.14$, $p_{(Corr)} = 0.002$] as well as in middle occipital gyrus [MNI peak coordinates: x, y, z: − 24, − 94, − 2, k_e = 111, $t(31) = -4.97$, $p_{(Corr)} = 0.003$]. In the ketamine group, clusters of voxels showing higher connectivity with the right pSTS during the question phase of the social compared to the physical trials were found in anterior precuneus (x = − 2, y = − 68, z = 28, k_e = 212, $t(30) = 4.98$, $p_{(Corr)} < 0.001$) and left middle temporal gyrus [x = − 50, y = − 46, z = 4, k_e = 164, $t(30) = 5.92$, $p_{(Corr)} < 0.001$; x = − 56, y = − 10, z = − 4, k_e = 127, $t(30) = 5.87$, $p_{(Corr)} = 0.001$]; clusters showing lower connectivity with the right pSTS in the social compared to the physical trials were identified in posterior precuneus [x = 24, y = − 76, z = 40, k_e = 244, $t(30) = -5.81$, $p_{(Corr)} < 0.001$] and superior occipital gyrus [x = 32, y = − 86, z = 22, k_e = 107, $t(30) = -6.51$, $p_{(Corr)} = 0.003$]. Of particular importance for our study, a t-test for independent samples across participant groups revealed one cluster showing a larger difference in connectivity with the right pSTS between social and physical trials under ketamine compared to placebo, in the anterior precuneus [see Fig. 4C; x = 6, y = -58, z = 34, k_e = 130, $t(61) = 4.77$, $p_{(Corr)} = 0.042$].

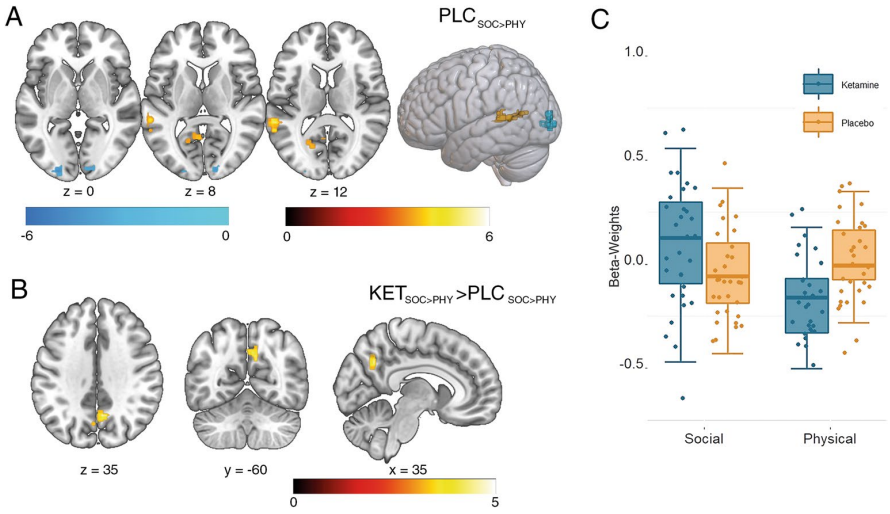

Figure 4. Functional connectivity during social cognition and effects of ketamine. Threshold at p(FWE-cluster level) = 0.05, based on a voxelwise uncorrected threshold of $p < 0.001$. (**A**) Under placebo, several regions showed changes in functional connectivity with the right pSTS cluster (Fig. 3B) during the question phase of the social compared to the physical trials: connectivity with STG and posterior cingulate increased, while connectivity with the cuneus and middle occipital gyrus decreased. (**B**) Under ketamine, one cluster in the anterior precuneus showed a larger difference in connectivity with the right pSTS cluster between social and physical trials. (**C**) Functional connectivity beta-weights in the cluster shown in (**B**). *KET* ketamine, *MTG* middle temporal gyrus, *PHY* physical, *PLC* placebo, *SOC* social.

Discussion

The present study indicates that a dose of ketamine inducing positive and negative symptoms associated with schizophrenia impairs mentalizing during the observation of social interactions and increases neural activity in right pSTS as well as connectivity between right pSTS and anterior precuneus during this task. These findings suggest that intravenous subanesthetic ketamine impairs social cognition and affects its neural correlates, supporting a link between a proposed psychopathological mechanism in schizophrenia and deficits in social cognition associated with that disorder. However, it should be mentioned that these deficits were not limited to social cognition, as participants were also presenting with reduced neurocognitive functioning in the control-condition.

The MASC task used here allows to characterize mentalizing deficits specifically by differentiating over- from undermentalizing[34]. It has revealed undermentalizing in individuals with schizophrenia and their unaffected relatives[8,35], as well as in individuals with autism spectrum disorder (ASD)[49]. In the present study, participants under ketamine also mentalized less (i.e., gave significantly more "no mentalizing" answers). These findings lead to the question of how NMDA-receptor blockade by an agent like ketamine might cause a reduction in mentalizing, and whether such mechanisms might be occurring in schizophrenia or ASD. As previously mentioned, changes in glutamate signaling lead to dysfunctions in social cognition in animal models, in particular to a disruption of social preference (shown by relative preference for social over non-social stimuli)[24], a key factor for the development of healthy social cognition that is deficient in schizophrenia[50].

While no study has investigated the causal effects of acute blockade of NMDA-receptors on mentalizing in healthy volunteers, several correlational studies have linked high glutamate concentration, or high glutamate to GABA ratios, with social cognitive dysfunction. For example, glutamate concentration in dorsolateral prefrontal cortex of healthy participants measured with magnetic resonance spectroscopy (MRS) was negatively correlated with perspective taking scores in a task designed to assess empathy in people with schizophrenia and ASD[51]. Another MRS study revealed that healthy people with higher scores in an ASD/schizotypy-questionnaire, which quantifies social dysfunction, exhibit a higher glutamate concentration in the superior temporal region (ST) bilaterally and increased glutamate to GABA ratio in the right ST[52]. Links between increased glutamate and dysfunctions of mentalizing have also been reported in schizophrenia and ASD[1,11,53]. For example, a negative correlation between total glutamatergic metabolites in the left thalamus and social functioning has been found in people with schizophrenia[54]. In ASD, GABA receptor down-regulation is thought to lead to cortical disinhibition, which also leads to an increased excitation/inhibition ratio[52]. One study reported that higher glutamate levels in the right superior temporal cortex were related to poorer social and interpersonal skills in people with ASD, and that this relationship was increasingly strong when GABA was reduced[55]. Overall, there seems to be a functional link between altered glutamate signaling and complex social cognition in ASD and schizophrenia, the underlying mechanisms of which are not yet fully understood[56].

Our results support this hypothesis by demonstrating causal alteration of mentalizing in participants undergoing NMDA-receptor blockade by ketamine. We note that we cannot make conclusions specific to mentalizing, as performance in the non-social condition of our task was also reduced under ketamine. The lower performance

observed in both the control and social cognition conditions within the ketamine group can possibly be partly attributed to the dissociative effects of ketamine[57]. Additionally, we cannot rule out the possibility that stress might be a mediator driving changes in behavior. A study found that ketamine evoked stress-like alertness in healthy participants[58] correlating with hyperconnectivity of hippocampus and precuneus at rest. As such, an alternative explanation to our findings might be that participants experience stress as result of changes to their perception, which in turn leads to a well-established, stress-induced decrease of memory function, resulting in worse performance in a variety of tasks. This possibility should also be addressed in future studies when looking at effects of ketamine on task performance.

At the neural level, we found activation in left pSTS, cuneus, middle occipital gyrus, precuneus and right pSTS during the question phase of the MASC in the placebo group during our manipulation check. Some of these regions (pSTS and precuneus) are part of the mentalizing network and are thus typical of activations expected in a social cognition task[14]. However, activations in cuneus and middle occipital gyrus are not typically linked to the mentalizing network, and may reflect the complexity of the task structure, in which multi-part trials build sequentially on each other in the social condition. Ketamine increased differences in the right posterior STS BOLD signal evoked by answering a question related to emotions or intentions of others (mentalizing condition), compared to a non-social control question. Activation in the pSTS, particularly in the right hemisphere, is frequently observed during mentalizing[59]. For instance, a meta-analysis by Schurz and colleagues revealed that mentalizing evoked by different sets of social animation tasks was associated with a high probability of activation in the right pSTS[60]. Our results are also compatible with findings in participants with schizophrenia: for example, one study using comic strips reported increased activity during mentalizing compared to a non-social control task in a very closely located pSTS-cluster[13], while a different study using static face stimuli reported higher activation in another right pSTS location during mentalizing compared to emotion recognition in patients compared to controls[16]. Repeatedly observed hyperactivity of pSTS in schizophrenia during social tasks led the authors to state increased BOLD activity in pSTS might constitute an endophenotype of schizophrenia[61]. Severity of positive symptoms was positively correlated to observed disinhibition of pSTS during social cognition in another recent study[62]. The authors used dynamic causal modeling to estimate connectivity between two brain regions active during motion perception (both, social and non-social) and found that connectivity increased between V5 and pSTS in patients, concluding that this increased connectivity might contribute to wrongful attribution of social states to agents.

Several regions showed changes in functional connectivity with the seed cluster under placebo during the question phase (social > physical): connectivity with STG and posterior cingulate increased, possibly reflecting reasoning about mental states of others and oneself[63,64]. The fact that, compared to other studies, we found decreased connectivity with cuneus and middle occipital gyrus could potentially be attributed to the complexity of the naturalistic mentalizing task performed by our participants or the use of PPI to investigate functional connectivity in our study. Decreased connectivity between these regions might reflect the participants' attempt to focus more on social situations and less on objects[65]. When compared to ketamine, one cluster in the anterior precuneus showed a larger difference in connectivity between the social compared to the physical condition under ketamine. Generally, activation in anterior precuneus is frequently observed during mentalizing[66] and self-related consciousness in healthy participants[67]. One study in healthy participants reported increased task-based connectivity in the ("mentalizing"-)network of the dmPFC, pSTS and precuneus during a task evoking spontaneous mentalizing[68]. The authors concluded that the integrative functions of the precuneus, along with its association with self-awareness might explain why social cognition tasks lead to an increase in connectivity between these regions. Findings from two studies in patients with schizophrenia support this idea: increased perfusion of the precuneus, measured with single photon emission computed tomography, was correlated to better insight into one's own mental disorder[67], while functional connectivity of precuneus negatively correlated with apathy in another study[69].

Coactivation of pSTS and precuneus in healthy participants has been observed during naturalistic social tasks involving reasoning about emotional and mental states of others[66,70]. However, also more abstract tasks that involve animacy and biological motion have been linked to connectivity between right pSTS and precuneus in healthy participants[71]. This is unsurprising, as both precuneus and pSTS play an important role in the mentalizing network. Increased functional connectivity between pSTS and precuneus in patients with schizophrenia compared to controls was found during processing of ambiguous word pairs that could be understood either literally (e.g., "birth weight") or as part of a metaphor (e.g. "sealed lips")[72]. These connectivity findings may be related to abnormal glutamate and GABA signalling in schizophrenia, as these neurotransmitters are known to modulate functional connectivity in the healthy brain[73]. What might our functional connectivity findings represent? The authors of the above-mentioned study proposed that increased precuneus-pSTS connectivity reflects their patients' attempt to compensate for their impaired metaphor comprehension[72]. Thus, both in schizophrenia and in our ketamine study, the increases in precuneus-pSTS functional connectivity may represent neural correlates of an attempt to compensate for abnormal neurotransmission. In this context, functional connectivity with precuneus might reflect a shift of attention, as observed e.g. during attentional deployment for emotion regulation when the attentional focus is moved away from negative emotional stimuli such as a car accident and towards something else. One study reported increased precuneus-to-amygdala functional connectivity when participants could freely view unpleasant images and focused their gaze on a non-arousing region[74]. Following this idea, we could propose that our connectivity finding represents a dysfunctional shift of attention away from a mentalizing perspective (needed to correctly identify intentions or emotions of others) and towards a more concretistic layer of attention (focused on superficial details of a situation). For patients with schizophrenia, this finding might imply that they miss social cues due to their heightened attention towards non-social attributes of their surrounding environment. This in turn might lead to difficulties with social interactions and thus, finally,

to social avoidance, leading to diminished social functioning[3]. The scarcity of pSTS-related connectivity findings in schizophrenia, however, constrains our interpretation attempts and calls for additional research.

Limitations

Limitations in our study concern the behavioral task, the ketamine-model for psychosis and whether changes evoked by ketamine reflect changes in glutamate-signalling. In our task, participants relied both on visual and acoustic features of the video clips to perform the social task, while solely visual information mattered in the physical condition, which yields asymmetry between the conditions.

A limitation of the ketamine-model for psychosis comes from the smaller impact on PANSS levels of ketamine (mean of 40 in our and similar studies[41,75]) compared to the PANSS levels observed during clinically relevant psychotic symptoms in schizophrenia (values of > 75 are frequently observed, e.g. Leucht et al.[76]).Therefore, comparisons between the effect of our manipulation and the effect of psychosis in schizophrenia on mentalizing should be made with caution. In addition, blinding is difficult in ketamine studies[77], partly due to nausea and disorientation at the start of the ketamine infusion[57]. To minimize these effects, we only recruited ketamine-naïve participants who were unlikely to be able to tell whether their experience was related to ketamine or to undergoing MRI. As using a control substance such as midazolam might result in unwanted influences of neural activation[78], we decided against this strategy. Conclusively, blinding in our study may have been single- rather than double-blind, in particular for the ketamine-receiving group.

Finally, since we did not measure glutamate levels after administering ketamine, conclusions about whether the fMRI results were caused by changes in glutamate transmission cannot be drawn. Even though most MRS studies showed that ketamine causes changes in glutamatergic transmission in anterior cingulate, not all studies were able to replicate these effects[75,79,80]. An early study reported that the direction of these changes in the prefrontal cortex varied as a function of the amount of ketamine administered[81]. Unfortunately, as of today, no studies have explored direct manipulation of the glutamatergic system within our regions of interest, i.e., pSTS or precuneus. Future studies could therefore explore the direction of change in glutamatergic transmission following administration of subanesthetic doses of ketamine in mentalizing regions.

Taken together, our findings show that people who received ketamine were exhibiting neurocognitive and social cognitive deficits in a naturalistic mentalizing-task. They mentalized less and demonstrated changes in BOLD-activity and task-related connectivity in right pSTS and precuneus, hinting to how glutamatergic neurotransmission might be tied to emergence of these deficits in disorders like schizophrenia.

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Author contributions

S.W.: conceptualization, resources, data curation, software, formal analysis, validation, investigation, visualization, methodology, writing—original draft, project administration, writing—review and editing; J.S.: conceptualization, resources, data curation, software, formal analysis, supervision, validation, visualization, methodology, writing—original draft, writing—review and editing; M.L.: conceptualization, data curation, software, investigation, methodology, writing—original draft, writing—review and editing. C.N., A.D.: investigation, project administration, methodology, resources, investigation, writing—review and editing; U.E., R.H., A.P.: conceptualization, funding acquisition, resources, writing—review and editing.

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Competing interests

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3.2. Publication 2: Effects of Ketamine on Brain Function during Metacognition of Episodic Memory

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Research Article

Effects of ketamine on brain function during metacognition of episodic memory

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Abstract

Only little research has been conducted on the pharmacological underpinnings of metacognition. Here, we tested the modulatory effects of a single intravenous dose (100 ng/ml) of the *N*-methyl-D-aspartate-glutamate-receptor antagonist ketamine, a compound known to induce altered states of consciousness, on metacognition and its neural correlates. Fifty-three young, healthy adults completed two study phases of an episodic memory task involving both encoding and retrieval in a double-blind, placebo-controlled fMRI study. Trial-by-trial confidence ratings were collected during retrieval. Effects on the subjective state of consciousness were assessed using the 5D-ASC questionnaire. Confirming that the drug elicited a psychedelic state, there were effects of ketamine on all 5D-ASC scales. Acute ketamine administration during retrieval had deleterious effects on metacognitive sensitivity (meta-d′) and led to larger metacognitive bias, with retrieval performance (d′) and reaction times remaining unaffected. However, there was no ketamine effect on metacognitive efficiency (meta-d′/d′). Measures of the BOLD signal revealed that ketamine compared to placebo elicited higher activation of posterior cortical brain areas, including superior and inferior parietal lobe, calcarine gyrus, and lingual gyrus, albeit not specific to metacognitive confidence ratings. Ketamine administered during encoding did not significantly affect performance or brain activation. Overall, our findings suggest that ketamine impacts metacognition, leading to significantly larger metacognitive bias and deterioration of metacognitive sensitivity as well as unspecific activation increases in posterior hot zone areas of the neural correlates of consciousness.

Keywords: metacognition; confidence; ketamine; episodic memory; glutamate

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Introduction

Many of our thoughts in everyday life revolve around other thoughts, about something we said or a decision we made. It has been postulated that these *meta*-thoughts constitute a distinct feature of consciousness. According to Block (1995), consciousness can be divided into phenomenal consciousness, access consciousness, self-consciousness, and monitoring consciousness. The latter concerns metacognition, i.e., the ability to reflect upon our own thoughts and knowledge and to monitor the quality of ongoing cognitive or perceptual processes (Grimaldi *et al.* 2015). The link between metacognition and consciousness is based on the intuition that, if an individual is unable to reflect on a particular mental state, this state cannot be conscious and consequently, some kind of metacognition should accompany all conscious representations (Shea and Frith 2019).

Metacognition is frequently measured on a trial-by-trial-basis as participants indicate their level of confidence about the accuracy of a perceptual or mnestic judgment (Grimaldi *et al.* 2015). A second-order confidence rating (Type 2 response) is therefore based on a first-order judgment (Type 1 response). Measures of metacognitive sensitivity tap how well participants introspectively assess or monitor their own cognitive processes (Fleming and Lau 2014). By applying signal-detection-theory (SDT) methodology, metacognitive *sensitivity* (as meta-d′) can be quantified independently of interindividual differences in response tendencies (Maniscalco and Lau 2012). The meta-d′-framework also allows to control for the influence of primary task performance on metacognitive sensitivity (Maniscalco and Lau 2014): metacognitive *efficiency* (meta-d′/d′) represents the amount of signal strength available for the metacognitive process, expressed as a fraction of the amount of signal strength available for the Type 1 task (McCurdy *et al.* 2013). Finally, it is important to consider the general tendency for higher or lower confidence ratings, the so-called metacognitive *bias* (Fleming and Lau 2014).

But what is the neural basis of metacognition? By drawing on evidence from no-report paradigms, Koch *et al.* (2016) argue that the neural correlates of consciousness are primarily localized in a posterior cortical network labeled a “hot zone” for conscious functions. However, neuroimaging and lesion studies suggest that higher-order conscious functions such as metacognition may also engage a frontoparietal network (Rouault *et al.* 2018; Vaccaro and Fleming 2018).

A more complete understanding of the neural mechanisms of metacognition also requires insight into the underlying neurotransmitter systems. To date, very little is known about the pharmacology of metacognition. Recently, Hauser *et al.* (2017) revealed that blockade of noradrenaline led to increased metacognitive sensitivity with unchanged perceptual decision-making performance, whereas both perceptual discrimination and metacognition remained unaffected by dopamine blockade.

One neurotransmitter likely to mediate aspects of consciousness is the glutamatergic system. Antagonists at the N-methyl-D-aspartate (NMDA) glutamate receptor, such as phencyclidine or ketamine, provoke psychedelic states which are clearly distinct from a normal waking state of consciousness (Anis *et al.* 1983; Umbricht *et al.* 2002; Morris and Wallach 2014), characterized by dissociative experiencing including vigilance reduction, ego transcendence, disembodiment, and visual and sensory disturbances (Vlisides *et al.* 2018). The noncompetitive NMDA-receptor antagonist ketamine is dose-dependently used for the treatment of depression (Murrough *et al.* 2013) and general anesthesia (Kurdi *et al.* 2014; Sarasso *et al.* 2015); in addition, it is a well-established research tool with an excellent safety record in both clinical and experimental applications (Javitt *et al.* 2012; Doyle *et al.* 2013). Ketamine-induced psychotropic effects such as distorted sense of space and time, euphoria and out-of-body experiences have contributed to its abuse as a recreational drug (Schifano *et al.* 2008; Giorgetti *et al.* 2015). Based on findings that acute ketamine administration temporarily and reversibly induces a range of both positive (hallucinations, thought disorder, delusions) and negative (social withdrawal, emotional blunting) psychosis-like symptoms in otherwise healthy volunteers, the compound is also a widely used pharmacological model of schizophrenia (Krystal *et al.* 1994; Malhotra *et al.* 1996).

Ketamine effects on cognition include a selective degradation of episodic memory (Hetem *et al.* 2000; Morgan *et al.* 2004). In episodic memory tasks, participants typically encode word items, and later retrieve those items by writing down as many words as they can remember (*recall*) or indicate whether a given item had previously been encoded or not (*recognition*) (Honey *et al.* 2005b). Previous findings suggest that retrieval performance is disturbed when ketamine is administered during encoding but remains unimpaired when only recognition, but not encoding, takes place under the influence of ketamine (Oye *et al.* 1992; Hetem *et al.* 2000; Honey *et al.* 2005b). This effect may, however, also depend on the depth of semantic processing of the encoded items: Honey *et al.* (2005b) found that ketamine reduced retrieval performance only when items were encoded at an intermediate level of processing (LoP), not on deep or shallow levels. A functional magnetic resonance imaging (fMRI) study by Honey *et al.* (2005a) reported that ketamine affects brain function during retrieval even if encoding occurred prior to ketamine administration: ketamine was associated with attenuated left prefrontal cortical response to deeply encoded items, whereas anterior cingulate activation was reduced for incorrect compared to correct responses.

Even though growing research effort is directed towards identifying the neural underpinnings of metacognition, and previous studies have aimed at specifying the role of glutamate in various cognitive functions, the involvement of this neurotransmitter system in metacognition has not yet been examined. In this double-blind, placebo-controlled fMRI study, the primary aim was to investigate the role of the glutamate system in metacognition and its underlying neural activity by applying a psychotomimetic dose of ketamine. Confidence ratings were collected in an episodic memory framework, based on the dissociation of ketamine effects on encoding and retrieval as operationalized by Honey *et al.* (2005a).

Specifically, we applied a task in which differences in Type 2 responses should not be due to altered Type 1 performance, since ketamine was previously shown to leave episodic memory performance in deep and shallow encoding conditions unaffected (Honey *et al.* 2005b). Metacognitive sensitivity was quantified using the meta-d′-framework, which was previously shown to be sensitive to the effects of pharmacological challenges (Clos *et al.* 2019) and drug consumption (Sadeghi *et al.* 2017). We expected metacognitive sensitivity to be altered by ketamine in both study phases and further predicted ketamine to affect neural activity during both metacognitive confidence ratings and encoding. The secondary study aims included investigation of LoP effects on retrieval performance and metacognitive accuracy as well as confirmation of the subjective, phenomenological effects of ketamine by including a self-report measure of altered states of consciousness.

Materials and Methods

Participants

Fifty-three healthy, non-smoking, right-handed volunteers (aged 18–34, $M = 23.47$, $SD = 3.24$; 29 female) with normal or corrected to normal vision and native speaker level command of German language were recruited for this study. Exclusion criteria were as follows: prior experience with ketamine, history of psychiatric or neurological disorder, claustrophobia, metalliferous implants, pregnancy, positive drug test, under- or overweight (Body Mass Index: <17; ≥ 30), or consumption of any medication. Further medical contraindications for the administration of ketamine included hypertension and hyperthyroidism.

The study was approved by the Research Ethics Committee at the Department of Psychology, University of Bonn (approval number: 18-03-28). In accordance with this approval, data of the study are not stored on public repositories, but behavioral data are available as Supplementary materials, and fMRI data will be made available upon request. Materials, analysis scripts, and preregistration of the study are available in Open Science Framework (https://osf.io/numxs/).

Screening procedure

An online prescreening interview was conducted with individuals who responded to study advertisements. Those who met all inclusion criteria were invited for a screening visit in the laboratory, where the German version of the 5.0.0 MINI-International Neuropsychiatric Interview (Ackenheil *et al.* 1999), a urine drug screen (Drug-Screen Multi-5T, nal von minden GmbH) and, for females, a pregnancy test (NADAL hCG Pregnancy Test, nal von minden GmbH) were carried out to screen for exclusion criteria of psychiatric illness, drug abuse, and pregnancy. Measurements of height, bodyweight, and blood pressure were obtained. A medical questionnaire was used to exclude any current or past medical conditions, or any diagnosis of psychotic disorders among first-degree relatives. Additionally, the first five questions of the Structured Instrument for Prodromal Syndromes (SIPS 5.0) were included to rule out prodromal symptoms of schizophrenia (McGlashan *et al.* 2001). Suitable individuals were invited for assessment visits.

Study design

A double-blind, randomized, placebo-controlled between-subjects design was employed. Randomization lists were created independently for females and males. The study team carrying out the assessments was not involved in the process of randomization. An unblinded study anesthesiologist prepared the infusion solution and constantly monitored oxygen saturation and heart rate of the participants during the infusion. Twenty-four participants were administered a subanesthetic dose of racemic ketamine (Ketamin-Ratiopharm 500 injection solution, Ratiopharm, Ulm, Germany), 29 participants received a saline solution (0.9% sodium chloride).

Ketamine was administered as a 2 mg/ml solution with a constant target plasma level of 100 ng/ml by a bolus and continuous infusion using a computerized infusion pump (Graseby 3500, Smith Medical Int. Ltd, Luton, UK). The solutions were administered using the STANPUMP program (Steven Shafer, M.D., Anesthesiology Service, PAVAMC 3801 Miranda Ave., Palo Alto, USA) based on the three-compartment model described by Domino *et al.* (1982). Previous studies of our group (Steffens *et al.* 2016, 2018) using the same infusion equipment and procedure confirmed that ketamine concentrations were close to the targeted plasma level and no residual traces of ketamine solution from the infusion site contaminated the results; therefore, no blood samples were drawn in this study.

General procedure

On assessment days, participants were required to refrain from solid food for 6 h and clear fluids for 2 h before the infusion. Within 24 h before, participants were also instructed to take no medication and to stay abstinent from alcohol. Female participants took another pregnancy test on the day of assessment. After participants arrived, they completed the first study task (see below) before an additional medical screening was performed by the study anesthesiologist. Participants were then fitted with intravenous access into the nondominant arm and positioned in the MRI scanner. Following an individual adjustment of the field of view and an initial high-resolution structural imaging scan, the infusion was started.

Ketamine effects on metacognition, encoding, and retrieval in an episodic memory task were assessed in two separate study-test phases. Stimuli were selected from the Berlin Affective Word List (Võ *et al.* 2009); word class, frequency, emotionality, arousal level, number of syllables, and vividness were counterbalanced between conditions.

In Study Phase I, items were presented on a computer screen outside the MRI scanner, prior to drug infusion. Retrieval was tested ~60 min after the end of the first encoding task, while BOLD data were acquired during infusion. In this first retrieval task, participants responded to stimuli by categorizing them either as "old items", if they had previously been presented in the encoding task, or "new items", if they had not been presented, and afterwards reported their metacognitive confidence (Type 2 response). Subsequently, in Study Phase II, another word list consisting entirely of novel items was encoded, as participants were still undergoing infusion in the MRI scanner. Retrieval of these items was tested ~60 min after the infusion was terminated and participants had left the scanner. Immediately upon leaving the scanner, participants completed the 5D-ASC questionnaire to assess altered states of consciousness (Dittrich 1998). In the second retrieval task, items encoded in the second encoding task ("old items") were again presented on a computer screen alongside "new items", again requiring participants to state their confidence after each Type 1 response. Figure 1 provides an overview of the general procedure of assessment days.

Task design

Study Phase I

Participants were presented with a total of 120 word items displayed in the center of a computer screen and were instructed to make one of two types of judgments about these items, which served as a manipulation of the depth of processing. We aimed for two levels of processing (deep/shallow) and selected a manipulation that could be expected to yield a pronounced LoP effect (Honey *et al.* 2005b). For each of 60 word items, participants indicated their subjective judgment of the pleasantness (pleasant/unpleasant) of the word (leading to deep encoding), whereas the other 60 items were encoded in a shallow manner, by participants reporting the number of syllables of each word (even/odd). Participants were not told that the retrieval of these items would be tested afterwards. These encoding tasks alternated blockwise, with each of four blocks comprising 30 items;

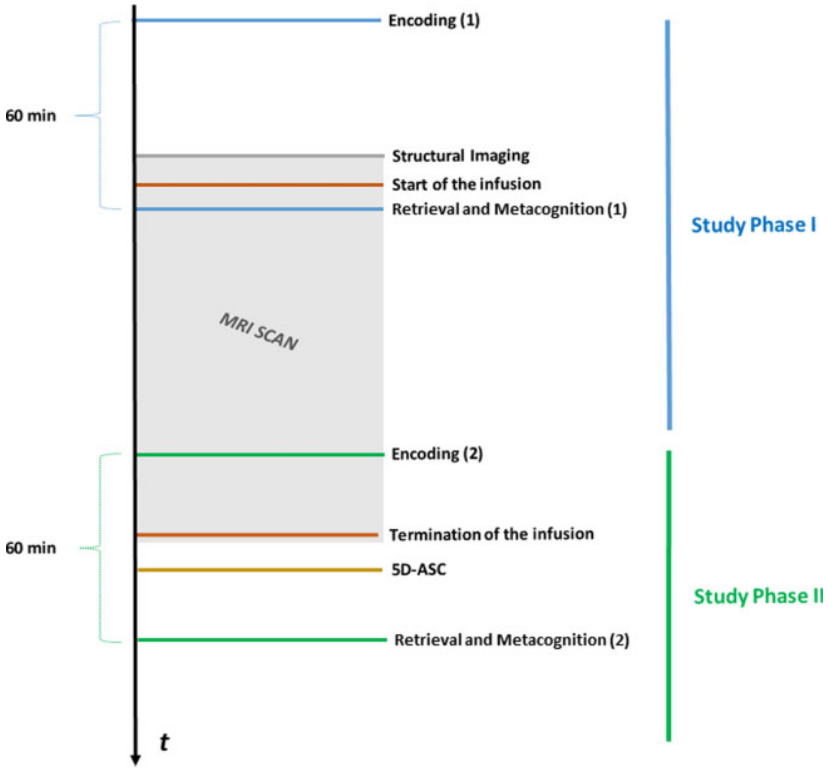

Figure 1. Study protocol. In Study Phase I (shown in blue), participants first encoded word items in the absence of infusion and before entering the MRI scanner. After a medical screening (in purple), participants completed a structural scan (in gray). Following the start of the infusion (in brown), retrieval of encoded items and corresponding metacognitive confidence was tested. As participants were still undergoing infusion in the MRI scanner, in Study Phase II (in green), participants encoded a second word list which was later retrieved outside the scanner, after termination of the infusion. Questionnaire data were collected using the 5D-ASC (in yellow). The MRI scanning period is represented by the grey box.

the starting condition was determined randomly. Items were presented until keypress for a maximum of 3 s, with an interstimulus interval (ISI) of 0.5 s.

The fMRI retrieval task was implemented in an event-related design. Participants responded to items presented on the center of a monitor behind the MRI scanner via a mirror by predefined button presses. A total of 180 word items were used, including the 120 items that had been encoded in the previous task as well as 60 new items. The 2:1 ratio of old to new items was based on previous studies (Honey *et al.* 2005a). Items were presented in randomized order for a duration of 2.5 s followed by an ISI that varied randomly between 2 s and 6 s; participants were instructed to respond to items which they considered to be old, i.e., having previously been presented, with a left index finger button press and to items which they labeled as new with a right index finger button press.

There were two types of second-order ratings: subsequent to 120 of these Type 1 responses, participants rated their subjective confidence regarding the judgment on a 6-point Likert scale (1 = "not confident at all", 6 = "very confident"). In this "Report" condition, designed to tap metacognitive processes, participants moved a cursor along the scale, using their index fingers, until they reached the position on the scale that most accurately matched their subjective confidence, which they were instructed to confirm by a left or right thumb press. During the 60 "Follow" trials which served as a control condition not involving the actual process of confidence formation (Yokoyama *et al.* 2010; Fleming *et al.* 2012), participants were instructed to navigate the cursor towards a predefined number on the scale, highlighted in blue. The initial position of the cursor was random in each condition; there were no written labels to either point of the scale to avoid extreme responding bias (Overgaard *et al.* 2006). "Report" and "Follow" trials alternated in randomized order; exactly two-third of each of the episodic memory condition trials (deep/shallow/new) were followed by the "Report" condition. The duration of the decision window for this second-order response was 3.5 s, followed by a 0.5 s screen where a change in cursor color from white to red highlighted the participant's response. Another variable ISI (2–6 s) preceded the onset of the next trial. In order to minimize exhaustion, the experiment was paused halfway through the task and a separate scan was started for the second half of the experiment. Figure 2 provides an illustration of the task.

Study Phase II

Following the completion of this first retrieval task, participants remained in the scanner and performed a second encoding task. Here, they were presented 100 novel word items in a block design; again, 50% of the items were encoded deeply by rating the subjective pleasantness of each word, whereas 50% of the items were encoded in a shallow manner by reporting the number of syllables. Again, encoding tasks alternated blockwise, with 10 blocks each comprising 10 items. At the beginning of each block, an instruction about the upcoming task was shown for 2 s. Participants responded via left or right button presses within a 3 s window (ISI = 0.5 s) for each item.

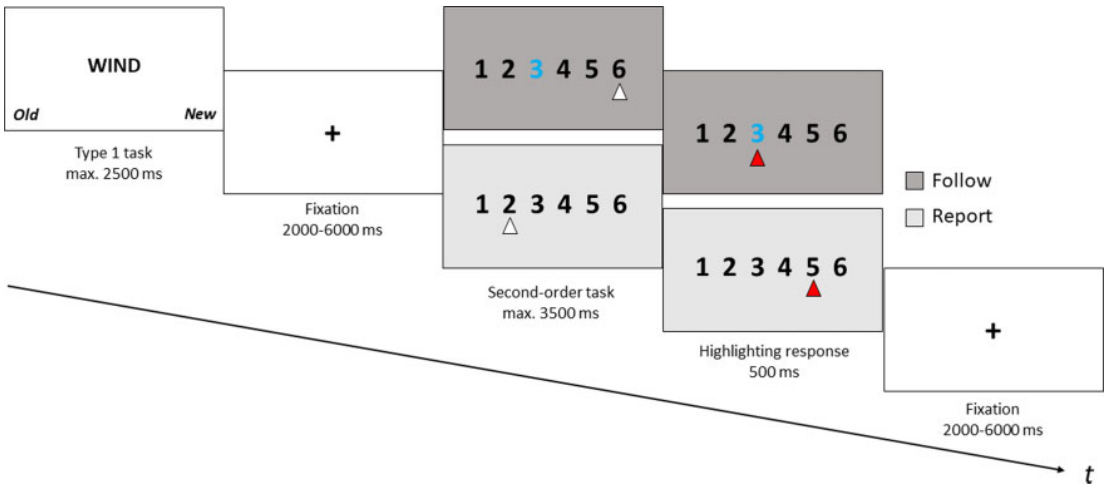

Figure 2. Schematic trial representation for the first retrieval task (stimuli are not to scale). Each trial consisted of two parts: first, participants categorized a presented word stimulus either as old (presented in the previous encoding task) or new (not having been presented before) (Type 1 task). Subsequently, they either indicated their subjective confidence ("Report" condition, shown in white) or placed the cursor at a color-coded position on the scale ("Follow" condition, grey) (Second-order task). The second retrieval task was similar, only here, the second-order task consisted entirely of "Report" trials, and the fixation period between task screens was shorter (1000 ms).

After termination of infusion and leaving the scanner, participants filled in the 5D-ASC, marking their extent of agreement with statements regarding various phenomenal experiences (Dittrich 1998). The 5D-ASC is a self-report questionnaire to retrospectively assess five dimensions of altered states of consciousness. These include three primary, etiology-independent scales, "Oceanic Boundlessness", "Dread of Ego Dissolution", and "Visionary Restructuralization", which can be conflated to a global measure of altered consciousness, and two secondary, etiology-specific scales comprising further aspects of altered experiences, "Auditory Alterations" and "Vigilance Reduction". 5D-ASC scale scores were formed following guidelines by Dittrich *et al.* (2006).

One hour after completion of the second encoding task, retrieval of those items was tested in a second retrieval task, without infusion at a time when plasma levels of ketamine are significantly reduced (Honey *et al.* 2005b). The design of the second retrieval task was almost identical to the first one, with two exceptions: ISI was constant (1 s), and there was no "Follow" condition, so participants had to report their confidence on each of the 150 trials (100 old, 50 new).

fMRI data acquisition and analysis

Imaging was conducted using a 1.5 T Avanto MRI scanner (Siemens, Erlangen, Germany). High-resolution structural images were acquired to optimize normalization of functional imaging data using a T1-weighted 3D MPRAGE sequence [Repetition time (TR) = 1660 ms, echo time (TE) = 3.09, inversion time = 800 ms, matrix size = 256 × 256, slice thickness = 1.0 mm, FoV = 256 mm, flip angle = 15°, voxel size = 1 × 1 × 1 mm^2, 160 sagittal slices]. Task-related BOLD fMRI data were acquired using a T2*-weighted echo-planar imaging sequence (TR = 2500 ms; TE = 45 ms, matrix size = 64 × 64, slice thickness = 3.0 mm, FoV = 192 mm, flip angle = 90°, voxel size = 3 × 3 × 3 mm, 31 slices). A standard 12-channel head coil was used for radio frequency transmission and reception.

fMRI data were analyzed using Statistical Parametric Mapping 12 software (Wellcome Centre for Neuroimaging, London, UK; http://www.fil.ion.ucl.ac.uk/spm) implemented in Matlab R2014a (The MathWorks Inc., Natick, USA). To allow for T1 equilibration, the first five volumes of each functional time series were discarded. Each participant's structural image was segmented into gray matter, white matter, and cerebro-spinal fluid using a forward deformation field to map it onto template tissue probability maps (Ashburner and Friston 2005). Functional images were realigned to the first image of each time series to correct for head movement, using a six-parameter rigid body transformation. The realigned functional images were then coregistered to the anatomical image. For spatial normalization, functional scans were transformed into standard stereotaxic space of the Montreal Neurological Institute (MNI) template (Evans *et al.* 1992; Holmes *et al.* 1998) and resampled at 2 × 2 × 2 mm voxel size. Finally, images were spatially smoothed using an 8 mm full-width-at-half-maximum Gaussian kernel.

Following pre-processing, at the first (single-subject) level for Study Phase I, the onset of each stimulus was defined as the onset of the event; for Type 1 responses, the duration was set to be the reaction time from stimulus presentation to button press. For second-order responses, the function spanned the time from onset of scale presentation to the first movement participants made on the scale. This was done as the decisive metacognitive processes during Report trials were expected to take place during that time, and to eliminate motion-related activation. The realignment parameters were added to the model as covariates of no interest. Correctly retrieved deep, shallow and new items were included as Type 1 regressors; since there were too few cases of incorrect answers in the majority of participants, an overall residual regressor of no interest was formed for incorrect answers, thereby departing from our preregistered analysis plan.

Overall, there were four Type 1 regressors: "Deep" (*mean number of trials across participants*: 49, SD = 8.6); "Shallow" (M = 27.19, SD = 11.34); "New" (M = 47.02, SD = 11.65); and "Incorrect" (M = 41.85, SD = 9.33). For each of these four regressors, two separate regressors were included for second-order ratings, resulting in a total of eight second-order regressors: "DeepReport" (M = 29.92, SD = 8.05); "DeepFollow" (M = 17.68,

SD = 7.58); "ShallowReport" (M = 18.96, SD = 8.95); "ShallowFollow" (M = 7.53, SD = 4.7); "NewReport" (M = 26.72, SD = 6.69); "NewFollow" (M = 13.17, SD = 3.53); "IncorrectReport" (M = 31.02, SD = 9.04); "IncorrectFollow" (M = 13.94, SD = 5.03). All contrasts were estimated by comparing specific effects against the baseline of the respective first-level-model; consequently, the two separate runs were conflated in this step. Additionally, we set up an exploratory first-level-model, in which "Report" regressors were parametrically modulated by the selected confidence rating in each trial, all other regressors remaining unmodified, as only "Report" ratings were expected to require the engagement of metacognitive Type 2 evaluations.

For Study Phase II, the function spanned the time from onset of word presentation to button press. Here, a simpler model with conditions "Deep" and "Shallow" was specified. Also departing from preregistration, the factor "Retrieval Performance" (later correctly/incorrectly retrieved) could not be applied, as there was an insufficient amount of incorrect answers.

On the second level, a full factorial analysis was carried out on Study Phase I data using between-subjects factor "Drug" (ketamine/placebo) and within-subjects factor "Word Type" (deep/shallow/new) for Type 1 contrasts with an additional within-subjects-factor "Rating Type" (report/follow) for second-order contrasts. A separate full factorial analysis was conducted on Study Phase II data, using between-subjects-factor "Drug" (ketamine/placebo) and within-subjects-factor "Encoding Level" (deep/shallow).

All second-level analyses were conducted at the whole-brain-level. The statistical height threshold was $P < 0.001$, and significant clusters were inferred if the peak voxel of the cluster survived a statistical threshold of $P < 0.05$ family-wise-error (FWE) corrected (cluster-level). In order to assign anatomical labels, the anatomy toolbox was utilized (Eickhoff *et al.* 2005). To determine whether significant clusters of each contrast represented activations or deactivations, mean summary functions were created using MarsBaR (https://sourceforge.net/projects/marsbar).

BOLD data of four participants during Study Phase I and of three participants during Study Phase II had to be excluded from fMRI analysis because normalization failed. Consequently, fMRI data analysis was performed on 49 participants (23 ketamine, 26 placebo) for Study Phase I and on 50 participants (23 ketamine, 27 placebo) for Study Phase II. Behavioral data analysis was carried out on all 53 participants who completed the assessment.

Behavioral data analysis

Following our preregistration, Type 1 (retrieval) and Type 2 (metacognitive) performance was assessed in an SDT framework (Green and Swets 1966; Barrett *et al.* 2013). We applied meta-d′ analysis (Maniscalco and Lau 2012) to quantify metacognitive *sensitivity*—i.e., the individual ability to discriminate between correct and incorrect retrieval judgments. Meta-d′ represents a response-bias free measure of how well confidence ratings track task accuracy and is on the same scale as the Type 1 sensitivity measure d′ (Maniscalco and Lau 2014). Meta-d′ was estimated in a maximum-likelihood-estimation model using code by Maniscalco (http://www.columbia.edu/~bsm2105/type2sdt) in Matlab R2016a (The MathWorks Inc., Natick, USA); only "Report" trials in which participants provided button presses on both retrieval and confidence rating were used for calculation. Additionally, metacognitive *efficiency* was calculated by dividing meta-d′ by d′ to provide an index of Type 2 performance that takes into account differences in Type 1 performance (Fleming and Lau 2014). To evaluate Type 2 performance, we therefore considered both absolute Type 2 sensitivity (meta-d′) and Type 2 efficiency relative to Type 1 performance (meta-d′/d′).

In addition to our preregistered analyses, we also conducted various exploratory analyses to facilitate mechanistic understanding of the outcomes. For example, we decided to expand our analysis to investigate ketamine effects on performance-corrected metacognitive bias (quantified as *mean judgment minus mean performance*) to test for differences in the selected confidence ratings between the two groups while controlling for the confounding influence of performance on confidence levels (Fleming and Lau 2014). Moreover, we explored Pearson's correlations between Type 1 and both Type 2 performance measures as well as metacognitive bias in both study phases with the 5D-ASC global measure of altered consciousness; alpha-level was Bonferroni-corrected ($\alpha = .05/8 = .006$). Finally, we applied an extension of the HMeta-d toolbox (Fleming 2017), a hierarchical Bayesian estimation of metacognitive efficiency (https://github.com/metacoglab/HMeta-d) in Matlab R2016a, which estimates group-level parameters over log(meta-d′/d′) while taking into account uncertainty in model fits at the single-subject level. To test for a true group difference in metacognitive efficiency, we fitted separate models for the ketamine and placebo group and calculated the 95% highest-density intervals (HDIs; the interval containing 95% of the Markov chain Monte Carlo posterior samples) on the difference between the group posterior densities and evaluated their potential overlaps with zero (Kruschke 2014). We ran three chains for estimation and ensured chain convergence (Fleming 2017).

All other behavioral data analyses were conducted using SPSS 22 (IBM Corp., Armonk, USA). Data were tested for violation of statistical assumptions; Kolmogorov–Smirnov tests were applied to test for normality of distribution, Mauchly's tests checked for sphericity, Levene's statistics tested for homogeneity of variances and Box-M-tests for homogeneity of covariances. When normality was violated in only one variable of a group, none of the variables were transformed. Drug effects on 5D-ASC scales, Type 1 and Type 2 reaction times and metacognitive bias were tested via independent samples *t*-tests. Paired *t*-tests were employed to compare Type 1 and Type 2 reaction times and metacognitive bias between deeply vs. shallowly encoded items. Separate mixed-design ANOVAs were employed with factors "Encoding Level" and the "Drug" for Type 1 and Type 2 sensitivity and Type 2 efficiency. Effect sizes for *t*-tests are given in Cohen's *d* (Cohen and Maydeu-Olivares 1992), effect sizes for ANOVAs in partial eta-squared (Cohen 1973).

Results

5D-ASC

There was a significant ketamine effect on the 5D-ASC global measure of altered consciousness [$t(23.7) = 4.69$, $P < 0.001$, $d = 1.35$] and on all scales. Participants who had received ketamine scored significantly higher on the three primary dimensions "Oceanic Boundlessness" [$t(23.23) = 4.04$, $P < 0.001$, $d = 1.17$], "Dread of Ego Dissolution" [$t(25.73) = 4.56$, $P < 0.001$, $d = 1.31$], and "Visionary Restructuralization" [$t(23.43) = 3.48$, $P = 0.002$, $d = 1.01$]. They also achieved significantly higher values on the "Auditory Alterations" [$t(28.17) = 4.55$, $P < 0.001$, $d = 1.29$]

Table 1. Descriptive statistics of 5D-ASC questionnaire scores by drug.

Scale	Placebo ($n = 29$)		Ketamine ($n = 24$)	
	M	SD	M	SD
[Global Index of Altered States]	1.08	1.87	14.51	13.91
Oceanic Boundlessness	0.71	1.48	16.63	19.25
Dread of Ego Dissolution	2.05	3.26	13.71	12.18
Visionary Restructuralization	0.52	1.74	12.27	16.45
Auditory Alterations	1.85	4.63	14.14	12.56
Vigilance Reduction	12.58	14.04	47.71	25.75

Note: Scale values are given in percent. M, mean; SD, standard deviation.

and "Vigilance Reduction" scales [$t(34.01) = 5.99$, $P < 0.001$, $d = 1.69$]. Descriptive statistics are provided in Table 1.

Exploratory analyses

There were no significant correlations of the 5D-ASC global measure of altered consciousness with Type 1 and Type 2 outcomes in either study phase (all $P > 0.006$).

Study Phase I

Descriptive statistics of Type 1 and Type 2 measures for Study Phase I are provided in Table 2. Distribution plots of raw data for all relevant dependent variables can be found in the Supplementary materials.

Type 1 behavioral analyses

The LoP manipulation was successful: participants showed significantly enhanced retrieval performance for deeply compared to shallowly encoded items [main effect of "Encoding Level": $F(1,51) = 241.44$, $P < 0.001$, $\eta_p^2 = 0.83$]. However, there was no main effect of "Drug" [$F(1,51) = 1.78$, $P = 0.188$, $\eta_p^2 = 0.03$]; ketamine did not significantly alter retrieval performance. Type 1 reaction times were significantly shorter for deeply than shallowly encoded items [$t(52) = 9.17$, $P < 0.001$, $d = 0.71$] but were unaffected by ketamine [$t(51) = 0.04$, $P = 0.972$, $d < 0.01$]. There were no significant interactions ($P > 0.05$).

Type 1 fMRI analyses

For BOLD data during retrieval, there was no significant difference between ketamine and placebo ($P > 0.05$). For a detailed summary of LoP and Old vs. New effects, see Supplementary materials.

Type 2 behavioral analyses

Participants showed enhanced metacognitive sensitivity for deeply compared to shallowly encoded items [$F(1,51) = 186.36$, $P < 0.001$, $\eta_p^2 = 0.79$]. Importantly, there was a significant main effect of "Drug" [$F(1,51) = 4.64$, $P = 0.036$, $\eta_p^2 = 0.08$]: metacognitive sensitivity deteriorated under ketamine. However, there was no significant main effect of either "Drug" [$F(1,50) = 1.03$, $P = 0.315$, $\eta_p^2 = 0.02$) or "Encoding Level" [$F(1,50) = 2.17$, $P = 0.147$, $\eta_p^2 = 0.04$] on metacognitive efficiency. Type 2 reaction times were faster for deeply encoded items [$t(52) = 4.25$, $P < 0.001$, $d = 0.41$] but were found to be unaltered by "Drug" [$t(51) = 0.03$, $P = 0.98$, $d < 0.01$]. There were no significant interactions ($P > 0.05$).

Table 2. Descriptive statistics of study phase I sensitivity measures (type 1 and type 2) and reaction times (type 1 and type 2) by drug and encoding level.

Measure	Placebo ($n = 29$)		Ketamine ($n = 24$)	
	M	SD	M	SD
Type 1 performance (d')[a]				
Deep vs. new	2.11	0.63	1.94	0.49
Shallow vs. new	0.85	0.38	0.74	0.37
Type 2 sensitivity (meta-d')[a,b]				
Deep vs. new	2.41	0.95	2.06	0.72
Shallow vs. new	0.89	0.49	0.58	0.39
Type 2 efficiency (meta-d'/d')				
Deep vs. new	1.17	0.38	1.13	0.48
Shallow vs. new	1.15	0.61	0.92	0.69
Type 1 reaction times (in ms)[a]				
Deep	1415.66	216.69	1458.75	189.0
Shallow	1572.63	197.31	1576.0	174.66
New	1600.11	227.04	1549.02	152.33
Type 2 reaction times (in ms)[a]				
Deep	1592.22	210.58	1578.45	221.79
Shallow	1670.7	229.7	1698.43	297.13
New	1755.37	251.38	1731.03	284.39

M, mean; ms, milliseconds; SD, standard deviation.
[a]Significant effects of encoding level.
[b]Significant effects of drug.

Exploratory analyses. Hierarchical Bayesian estimation of group-level meta-d′/d′ confirmed that we cannot be certain that there is a true difference in metacognitive efficiency between the two groups, even though the estimated difference between groups was relatively high [mean: 0.23 (highest-density interval: −0.04 to 0.58)]. Figure 3 provides an illustration of the estimated group-level parameters of metacognitive efficiency.

There was also a significant effect of "Drug" on metacognitive bias scores [$t(51) = 2.15$, $P = 0.037$, $d = 0.59$), with participants under ketamine being overconfident. In addition, there was a significant effect of "Encoding Level" on metacognitive bias, with ratings for shallowly encoded items reflecting overconfidence [$t(48) = 7.25$, $P < 0.001$, $d = 1.24$].

Second-order fMRI analyses

Report vs. follow effects. Higher BOLD responses during Report than Follow were found in a right visual cluster of right calcarine and lingual gyrus (Figure 4, Table 3). The cluster furthermore encompassed left and right cuneus, as well as bilateral superior occipital gyrus. A second, left-hemispheric, cluster was located in the posterior medial frontal cortex (pMFC).

The reverse effect (Follow>Report, indicating BOLD responses that were higher when participants had to select a predefined specification on the scale) revealed a total of 11 clusters (Figure 4, Table 4). These correspond to the default-mode network (DMN) that is active in the absence of task demands (Andrews-Hanna 2012), which encompasses angular gyrus, precuneus, posterior cingulate cortex (PCC), superior frontal areas, and parahippocampal gyrus, all of which were activated in the contrast.

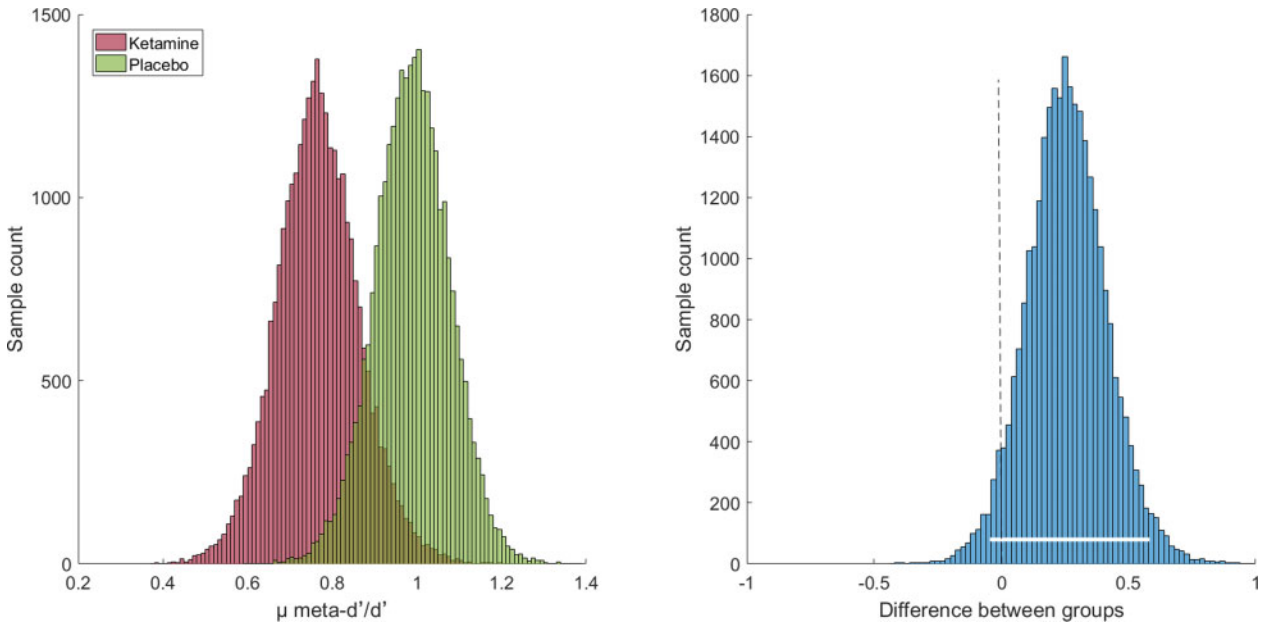

Figure 3. Hierarchical Bayesian estimation of metacognitive efficiency ($\mu_{meta\text{-}d'/d'}$) in Study Phase I. Left panel: Group-level values for the ketamine group (red histogram) and the placebo group (green histogram). Right panel: Difference in group posteriors (in log units). The white bar indicates the 95% highest-density interval which narrowly overlaps with zero.

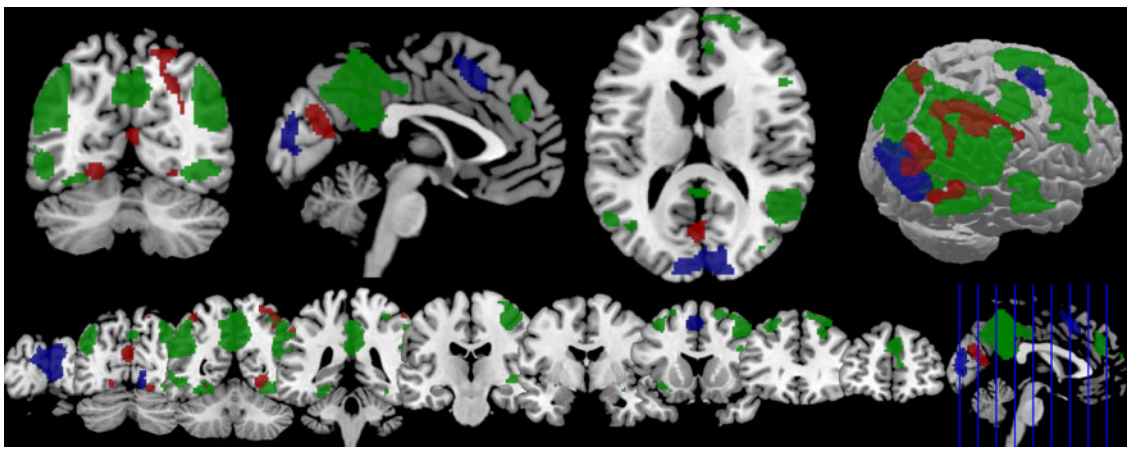

Figure 4. Second-order fMRI results. Significant activation in the contrasts Ketamine>Placebo (red), Report>Follow (blue) and Follow>Report (green) at significance level $P < 0.001$ (uncorrected).

Table 3. Summary of significant clusters for the report > follow contrast.

Anatomical label	Laterality	Cluster size [k]	T-value	Peak voxel MNI coordinates		
Calcarine gyrus	R	1599	5.33	8	−86	4
Lingual gyrus	R		5.24	12	−80	−8
Cuneus	R		4.88	8	−86	26
Cuneus	L		4.61	−6	−94	22
Superior occipital gyrus	L		4.58	−10	−96	20
Superior occipital gyrus	R		3.81	18	−96	18
pMFC	L	352	6.08	−4	16	48

Combined sample. Only unique anatomical labels are reported for each cluster at one laterality. Whole-brain cluster-level FWE corrected ($P < 0.001$ uncorrected).
FWE, family-wise error; L, left; MNI, Montreal Neurological Institute; pMFC, posterior medial frontal cortex; R, right.

Table 4. Summary of significant clusters for the follow > report contrast.

Anatomical label	Laterality	Cluster size [k]	*T*-value	Peak voxel MNI coordinates		
Angular gyrus	R	3662	9.36	56	−52	36
Superior parietal lobule	R		4.8	36	−44	58
Middle occipital gyrus	R		4.53	36	−80	10
Postcentral gyrus	R		3.36	24	−44	66
Precuneus	R	3384	7.18	10	−50	38
PCC	R		6.96	4	−48	28
MCC	R		6.85	10	−44	32
Precuneus	L		5.46	−6	−52	44
MCC	L		5.11	−4	−46	48
Superior frontal gyrus	R	2630	7.22	4	46	30
Middle frontal gyrus	R		5.54	30	24	54
IFG (p. Triangularis)	R		4.53	48	24	24
Inferior parietal lobule	L	2548	7.14	−54	−54	36
Angular gyrus	L		5.97	−40	−72	38
Supramarginal gyrus	L		4.37	−62	−36	38
Middle occipital gyrus	L		4.05	−36	−80	28
Fusiform gyrus	L	1094	7.34	−30	−52	−16
Inferior temporal gyrus	L		4.61	−54	−54	−8
Middle temporal gyrus	L		4.07	−60	−50	−2
Parahippocampal gyrus	L		3.32	−22	−28	−18
Fusiform gyrus	R	807	7.5	30	−52	−16
Inferior occipital gyrus	R		5.35	36	−72	−10
Inferior temporal gyrus	R		3.88	52	−64	−8
Precentral gyrus	R	753	5.67	38	−22	54
Middle temporal gyrus	R	682	5.5	60	−20	−10
Posterior insula	R		4.49	34	−6	−12
Insula lobe	R		4.41	40	−18	−2
Superior temporal gyrus	R		3.45	50	−12	−10
Middle frontal gyrus	L	674	5.57	−32	24	50
Superior frontal gyrus	L		4.3	−22	22	56
Superior frontal gyrus	R	203	4.48	14	66	16
Anterior insula	L	162	4.99	−28	6	−14
Insula lobe	L		4.49	−32	16	−12

Combined sample. Only unique anatomical labels are reported for each cluster at one laterality. Whole-brain cluster-level FWE corrected ($P < 0.001$ uncorrected).
FWE, family-wise error; IFG, inferior frontal gyrus; L, left; MCC, midcingulate cortex; MNI, Montreal Neurological Institute; PCC, posterior cingulate cortex; R, right.

Table 5. Summary of significant clusters for the ketamine > placebo contrast.

Anatomical label	Laterality	Cluster size [k]	*T*-value	Peak voxel MNI coordinates		
Superior parietal lobule	R	642	5.51	36	−52	64
Supramarginal gyrus	R		3.56	60	−28	48
Middle occipital gyrus	R		3.32	30	−64	33
Inferior parietal lobule	R		3.26	40	−54	48
Angular gyrus	R		3.23	36	−56	48
Calcarine gyrus	L	257	4.59	−2	−72	18
Lingual gyrus	R	212	4.42	18	−70	−10
Inferior parietal lobule	L	188	4.24	−40	−52	60
Lingual gyrus	L	172	5.21	−18	−68	−8
Fusiform gyrus	L		3.98	−28	−52	−12

Only unique anatomical labels are reported for each cluster at one laterality. Whole-brain cluster-level FWE corrected ($P < 0.001$ uncorrected).
FWE, family-wise error; L, left; MNI, Montreal Neurological Institute; R, right.

Table 6. Descriptive statistics of study phase II sensitivity measures (type 1 and type 2) and reaction times (type 1 and type 2) by drug and encoding level.

Measure	Placebo ($n = 29$)		Ketamine ($n = 24$)	
	M	SD	M	SD
Type 1 performance (d')[a]				
Deep vs. new	1.79	0.58	1.59	0.54
Shallow vs. new	0.68	0.41	0.57	0.31
Type 2 sensitivity (meta-d')[a]				
Deep vs. new	1.97	0.78	1.77	0.68
Shallow vs. new	0.44	0.41	0.4	0.58
Type 2 efficiency (meta-d'/d')[a]				
Deep vs. new	1.08	0.35	1.2	0.6
Shallow vs. new	0.69	0.65	0.71	1.19
Type 1 reaction times (in ms)[a]				
Deep	1267.99	235.79	1233.3	187.04
Shallow	1368.44	245.62	1306.19	186.04
New	1416.41	268.68	1286.1	187.95
Type 2 reaction times (in ms)[a]				
Deep	1132.52	213.1	1077.98	300.41
Shallow	1194.43	253.64	1112.9	301.18
New	1228.7	290.89	1117.47	272.8

M, mean; ms, milliseconds; SD, standard deviation.
[a]Significant effects of encoding level.

Drug effects. During second-order ratings (both Report and Follow), there was larger BOLD with ketamine than placebo in five clusters (Figure 4, Table 5): The first, right-hemispheric, cluster included superior parietal lobule (SPL), supramarginal gyrus, inferior parietal lobule (IPL), and angular gyrus. A second cluster was located in left calcarine gyrus, a third cluster in right lingual gyrus. The fourth cluster included left IPL, whereas a fifth, left-hemispheric cluster encompassed lingual gyrus and fusiform gyrus. There were no significant effects for the reverse contrast and no significant interactions ($P > 0.05$).

Exploratory analyses. Parametric modulation analysis ("Report" trials parametrically modulated by the selected confidence rating) revealed very similar results, i.e., higher BOLD response for ketamine than placebo in bilateral lingual, fusiform, and calcarine gyrus and right SPL (see Supplementary Table 6). There were no significant effects for the reverse contrast and no significant interactions ($P > 0.05$).

Study Phase II

Encoding: fMRI analyses

There were no significant ketamine effects on BOLD during encoding ($P > 0.05$). For LoP effects, see Supplementary materials.

Type 1 behavioral analyses

Descriptive statistics of Type 1 and Type 2 measures for Study Phase II are provided in Table 6. Distribution plots of raw data for all relevant dependent variables can be found in the Supplementary materials.

Items that had been encoded deeply were recognized more often than shallowly encoded items [significant main effect of "Encoding Level": $F(1,51) = 273.94$, $P < 0.001$, $\eta_p^2 = 0.85$]. There was no significant effect of "Drug" on d′ [$F(1,51) = 1.8$, $P = 0.185$, $\eta_p^2 = 0.04$]. "Drug" also had no effect on Type 1 reaction times [$t(51) = 1.29$, $P = 0.203$, $d = 0.36$]; when deeply encoded items were presented, participants made significantly quicker button presses [$t(52) = 5.7$, $P < 0.001$, $d = 0.4$]. There were no significant interactions ($P > 0.05$).

Type 2 behavioral analyses

There were significant main effects of "Encoding Level" on metacognitive sensitivity [$F(1,50) = 263.38$, $P < 0.001$, $\eta_p^2 = 0.84$] and metacognitive efficiency [$F(1,49) = 18.01$, $P < 0.001$, $\eta_p^2 = 0.27$), but no effects of "Drug" on either meta-d′ [$F(1,50) = 0.655$, $P = 0.422$, $\eta_p^2 = 0.01$] or metacognitive efficiency [$F(1,49) = 0.16$, $P = 0.691$, $\eta_p^2 < 0.01$]. Type 2 reaction times were also significantly shorter for deeply encoded items [$t(51) = 2.68$, $P = 0.01$, $d = 0.19$], but there was no effect of "Drug" [$t(50) = 1.13$, $P = 0.264$, $d = 0.34$). There were no significant interactions ($P > 0.05$).

Exploratory analyses. Corresponding to overlaps of 95% HDIs with zero, we found no significant group difference in metacognitive efficiency between ketamine and placebo [0.03 (−0.35 to 0.043)]. Thus, there was no significant ketamine effect on any measure of Type 2 performance when retrieval took place after termination of the infusion. We did, however, observe a significant effect of "Drug" on metacognitive bias [$t(50) = 2.75$, $P = 0.008$, $d = 0.76$], with participants under ketamine displaying overconfidence. There was also significantly larger metacognitive bias for shallowly than for deeply encoded words [$t(50) = 9.31$, $P < .001$, $d = 1.33$].

Discussion

This study investigated the role of the glutamate system in metacognition and associated brain activity using a ketamine challenge during episodic memory tasks in two study phases.

Study Phase I

Task effects

For a detailed discussion on LoP effects both at the behavioral and the brain functional level, see Supplementary materials.

Two clusters were significantly more active during Report than Follow; the first includes right calcarine gyrus, bilateral cuneus, and right lingual gyrus. The latter structure has been demonstrated to display increased functional connectivity with prefrontal cortex (PFC) in Report compared to Follow trials (Fleming *et al.* 2012). The second cluster in left pMFC provides further evidence for its role in metacognition and resembles the anatomically adjacent dorsal anterior cingulate cortex cluster which Fleming *et al.* (2012) found to be involved in reporting confidence in a similar task design. A recent meta-analysis (Vaccaro and Fleming 2018) identified bilateral pMFC as one of the prime neural correlates of metacognitive judgments, representing the biggest cluster in the composite meta-analysis of all metacognition-related activity and the second-biggest cluster associated with metacognitive ratings following memory judgments.

In the reverse contrast (Follow>Report), increased activation was found in brain regions involved in the DMN, which has been linked to introspective mental activities such as mind

wandering (Andrews-Hanna 2012). Again, this confirms Fleming *et al.* (2012), who reported similar patterns in this contrast.

Drug effects

As expected, subjective measures (5D-ASC) revealed that ketamine caused phenomenological experiences significantly deviating from a normal state of consciousness on all scales of the questionnaire. This finding confirms the known psychotomimetic effects of ketamine (Anis *et al.* 1983; Vlisides *et al.* 2018) and validates the rationale for using this pharmacological challenge to investigate the glutamatergic basis of metacognition.

Our study is one of only very few to indicate a potential pharmacological modulation of metacognitive performance (Lou *et al.* 2011; Hauser *et al.* 2017) and the first to investigate ketamine effects on metacognition. We show that disrupting the glutamatergic system by means of ketamine administration may challenge introspective monitoring processes: at the behavioral level, ketamine application during retrieval resulted in deterioration of metacognitive sensitivity (meta-d′) and overconfidence (larger metacognitive bias). Differences in metacognitive bias have been suggested to reflect genuine differences in awareness (Schwiedrzik *et al.* 2011), suggesting a role of various conscious processes giving rise to this ketamine effect on metacognitive bias. Furthermore, as overconfidence has been reported in patients with schizophrenia (Moritz *et al.* 2014), this finding provides another piece of evidence for use of ketamine as a model system of schizophrenia. Importantly, ketamine did not affect retrieval (Type 1) performance, in line with previous reports (Honey *et al.* 2005b), even though some group-heterogeneity has to be considered in Type 1 performance. Additionally, both Type 1 and Type 2 reaction times were unaffected by ketamine, which also indicates that the drug did not lead to a general deterioration of cognitive performance.

However, when controlling for the influence of Type 1 performance (d′) on metacognitive sensitivity (meta-d′) by calculating metacognitive efficiency (meta-d′/d′), there was no significant group difference. It is advised to apply metacognitive efficiency measures when comparing different groups (Fleming and Lau 2014; Vaccaro and Fleming 2018) although the theoretical assumption of the relationship of Type 1 and Type 2 performance measures (Galvin *et al.* 2003; Maniscalco and Lau 2012) is frequently violated in cases of "hyper"-metacognitive efficiency (meta-d′/d′ $>$ 1), potentially arising as a consequence of post-decisional and/or second-order computation (Fleming and Daw 2017) as evidence continues to be accumulated after the Type 1 response (Murphy *et al.* 2015; Rausch and Zehetleitner 2016). In general, meta-d′ represents a measure of an individual's ability to discriminate between their own correct and incorrect responses independently of differences in response bias (Fleming and Lau 2014) and prior studies have reported meta-d′ either as the only measure of metacognitive sensitivity (Rausch *et al.* 2015) or alongside the meta-d′/d′ ratio (Beck *et al.* 2019).

While it is necessary to keep in mind that the ketamine-associated deterioration of Type 2 sensitivity might be influenced by non-significant group-heterogeneity in Type 1 performance, rather than reflecting a general deficit in the underlying metacognitive processes (Maniscalco and Lau 2012), it is still important to understand ketamine effects on meta-d′ in Study Phase I. This is based on the absence of group effects on Type 1 performance in our study but also on the fact that 95% HDIs only narrowly overlapped with zero in two-sided testing for group differences in metacognitive efficiency. The group-level estimation in a hierarchical Bayesian framework offers several methodological advantages over previous estimation methods for metacognitive efficiency (Fleming 2017). As illustrated in Figure 3, there was an almost perfect fit of the ideal observer model in the placebo group (group-level meta-d′/d′: 0.99), whereas the ketamine group (0.76) substantially deviated from the ideal observer model implied in the meta-d′-framework (Fleming 2017).

Furthermore, we observed a pronounced up-regulation of activity in posterior brain regions with ketamine. This effect was observed only during second-order ratings (including both metacognitive reports and the control condition), whereas Type 1 BOLD showed no difference in activation between the groups. Specifically, there was increased activity in the right-hemispheric superior-posterior cortex compared to placebo. The superior parietal lobe is mainly associated with spatial attention and plays a pivotal role in somatosensory and visuomotor integration (Culham and Valyear 2006; Iacoboni 2006), motor learning (Weiss *et al.* 2003; Wenderoth *et al.* 2004), mental rotation (Wolbers *et al.* 2003; Gogos *et al.* 2010), with a mosaic of specialized subregions (Wang *et al.* 2015). Increased BOLD with ketamine also occurred in left calcarine gyrus, where the primary visual cortex is concentrated (Goebel *et al.* 1998; Seghier *et al.* 2000); bilateral lingual gyrus, which has been linked to processing vision (especially letter-reading) and encoding visual memories (Mechelli *et al.* 2000); and left IPL, which is involved in language processing, mathematical operations and body image (Radua *et al.* 2010), agency (Chaminade and Decety 2002), and working memory (Ravizza *et al.* 2004). Importantly, these ketamine effects on BOLD were observed for both second-order rating types (Report/Follow) and are therefore not specific to genuine metacognitive processes. It should be noted, however, that Report trials were overall more frequent (2:3) than Follow trials and thus had a greater overall contribution to the ketamine effects on second-order BOLD.

Overall, it appears that ketamine affects brain function during second-order ratings by means of an up-regulation of posterior visuospatial cortical brain areas. The visual, affective word stimuli employed in this study may have evoked vivid, imaginative processes in all participants, irrespective of drug, during retrieval. In participants experiencing the altered state of consciousness induced by ketamine, these imaginative processes may yet have persisted well beyond the retrieval process and consequently perturbed the signal available for the second-order task, irrespective of its specific demands, which could account for both the deterioration in metacognitive sensitivity as well as the increased activation in visuospatial areas during second-order ratings. However, it should be reiterated that it is uncertain to what extent the observed effects are related to metacognition, or whether they do not simply reflect neural responses to the presentation of the rating scale.

It is intriguing, however, that the anatomical location of our results is of interest with regards to the "hot zone" for conscious functions proposed by Koch *et al.* (2016): As this hot zone primarily encompasses sensory areas, it is mainly associated with phenomenal qualities of conscious experiences, which self-reported 5D-ASC measures confirmed to be altered by ketamine. Thus, as individuals under the influence of ketamine processed the demands of the second-order task (including introspective assessments of their internal mental world), phenomenal qualities of their normal waking-state experience may be distinctly altered. The posterior parietal cortical areas found in this study have been proposed to encode decision confidence (Kiani and Shadlen 2009), but recent studies suggest that activity in these areas tracks reliability of the sensory input rather than the core process of confidence formation (Bang and Fleming 2018).

Accordingly, our findings suggest that not confidence formation itself, but early aspects of the metacognitive process could be impacted by ketamine as individuals struggle to make sense of a distorted input signal which results in an up-regulation of neural activity, whereas episodic memory or processing speed remain largely unaffected.

This interpretation is supported by evidence that ketamine increases bilateral temporoparietal functional connectivity (Höflich *et al.* 2015) and causes a significant alpha current reduction in posterior cortical areas such as precuneus and temporoparietal junction, which may reflect efforts to maintain ego integrity (Carhart-Harris *et al.* 2014; Vlisides *et al.* 2018). The ketamine-induced psychedelic state is characterized by elevated entropy in certain aspects of brain function, thereby collapsing the highly organized, low-entropy activity within the DMN (Carhart-Harris *et al.* 2014). This is in line with the notion by Carhart-Harris *et al.* (2014) that DMN integrity is a key foundation for accurate metacognition: upon perturbing DMN activity by inducing a psychedelic state, the functionality of metacognitive processes should hence be reduced, whereas the retrieval process may in many cases be based on a notion of familiarity with the word item, and therefore depend less on DMN integrity.

To achieve a comprehensive understanding of the findings, there are additional aspects to be considered. First, the lack of correlation between the 5D-ASC index of altered consciousness and ketamine effects on metacognitive sensitivity makes it difficult to draw a direct connection between the ketamine-induced altered subjective state and the observed objective effects on metacognition—although it may not be adequate to assume both effects to take place on the same conscious level, since the impairment of metacognition represents unconscious effects on conscious decisions (such as ratings given on the 5D-ASC). Second, it has to be considered that different causes might result in a deterioration of metacognitive sensitivity. Both a reduction in the sensory reliability of the input to the metacognitive process (i.e. increased noise in the evidence on which confidence formation is based) as well as trial-to-trial variability in the placement of confidence criteria might account for this effect. A clear interpretation remains difficult, but exploratory analysis of metacognitive bias, which revealed significantly higher bias (i.e. overconfidence) for the ketamine group, offers potential insights into the underlying mechanisms: fluctuations across individual trials in participants' confidence indicate that participants under the influence of ketamine based their confidence ratings on certain conscious experiences, which could be due to changes in conscious access as well as altered, hallucinatory-like experiences, and which are ultimately unknown to the experimenter (Fleming and Lau 2014). Ultimately, it is possible that the unspecific up-regulation of the posterior parietal areas during second-order ratings reflect either the disturbances in signal input or alterations in conscious experience, or even both.

Study Phase II

Drug effects

There were no ketamine effects on Type 1 sensitivity or Type 2 sensitivity and efficiency of items encoded during maintained drug infusion. This was confirmed by exploratory hierarchical Bayesian estimation of group-level metacognitive efficiency; unexpectedly, there was no group difference in metacognitive performance for Study Phase II. The absence of ketamine effects on retrieval is in accordance with previous studies (Honey *et al.* 2005a,b) using a very similar LoP manipulation. We found no drug-related group differences in functional activity during encoding in the continued presence of drug infusion and were thus unable to reproduce the increased activation for deeply encoded items in left PFC with ketamine reported by Honey *et al.* (2005a). Moreover, there were no effects of ketamine on either Type 1 or Type 2 reaction times, again indicating that ketamine did not affect reaction speed. However, metacognitive bias (overconfidence) was again significantly higher in the ketamine group, as was the case in Study Phase I. Even when ketamine was absent at retrieval, ketamine participants were overconfident about their mnestic judgments, suggesting that ketamine evokes substantial distortions in the placement of confidence criteria, irrespective of whether encoding or retrieval took place under the influence of ketamine. While it not possible to retroactively rule out a baseline difference in confidence level between the groups, an overall diffuse memory trace might account for the observed overconfidence, as ketamine affects source memory (Honey *et al.* 2005b). Therefore, ketamine effects on metacognitive bias could be driven by shared and distinct mechanisms for the two study phases.

Limitations

The employment of a between-subjects-design might be a potential shortcoming, as homogeneity in all relevant individual factors can never be achieved across the groups. However, the advantage of this design is that expectancy biases based on experience with the first of two assessments in a within-subjects-design are eliminated.

Whilst the infusion protocol served to keep plasma levels of ketamine constant, it cannot be ruled out that participants became accustomed to the ketamine-induced state of consciousness and developed mechanisms to stabilize higher-order cognitive functions over the course of the infusion. This potential habituation effect may account for the observation that encoding processes in Study Phase II were less affected by ketamine than previously observed (Honey *et al.* 2005a,b).

As participants were not informed about the subsequent retrieval task at encoding in either study phase, it is important to point out that during the encoding task in Study Phase II, participants might have been more likely to infer the subsequent memory testing, which could have altered their encoding strategy. This introduces an additional difference between the two study phases, which complicates a direct comparison of ketamine effects between the phases.

Another limitation is that only trials with correctly retrieved items could be included in the fMRI analyses, due to the fact that the majority of participants produced an insufficient amount of incorrect answers in the Type 1 task. Finally, even though the combined sample size of both groups corresponded to sample sizes of previous within-subject designs (Steffens *et al.* 2016, 2018; Van Loon *et al.* 2016), it is possible that the study lacked sufficient power to detect a statistically significant difference between groups not only on metacognitive sensitivity but also on efficiency.

Generally, additional research is required to gain further understanding of ketamine effects on metacognition. Such potential future research efforts could encompass the application of advanced modeling capable of contrasting theories, such as the Stochastic Detection and Retrieval Model (Jang *et al.* 2012), which could help disentangle the underlying mechanisms of the observed effects and allow to discriminate between increased noise in the sensory evidence accumulation and trial-

by-trial variability in the placement of confidence criteria. Furthermore, dynamic causal modeling of fMRI results could also help to clarify the extent to which the vivid, imaginative processes affect brain activity during second-order ratings.

Conclusions

In summary, we present evidence for a role of the NMDA-glutamate-receptor antagonist ketamine in metacognition, including significantly larger metacognitive bias and deterioration of metacognitive sensitivity with ketamine. We also observed unspecific up-regulation of activity in posterior brain areas during second-order ratings compared to placebo. Importantly, ketamine did not affect metacognitive efficiency as estimated in a hierarchical Bayesian framework. The reported effects are neither sufficiently strong nor specific enough to attribute metacognition solely to the function of the glutamatergic system. Our results do, however, suggest that ketamine impacts on metacognition, which could be due to a reduction in the sensory reliability of the input to the metacognitive process as well as alterations in conscious experience. Further research is required in order to expand our understanding of the neural and pharmacological underpinnings of metacognition.

Supplementary data

Supplementary data is available at *NCONSC Journal* online.

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Data Availability Statement

Behavioral data available in supplementary material; fMRI data will be made available upon request.

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3.3. Publication 3: N-Acetylcysteine and a Specialized Preventive Intervention for Individuals at High Risk for Psychosis: A Randomized Double-Blind Multicenter Trial

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N-Acetylcysteine and a Specialized Preventive Intervention for Individuals at High Risk for Psychosis: A Randomized Double-Blind Multicenter Trial

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***Background and Hypothesis*: Clinical high risk for psychosis (CHR-P) offers a window of opportunity for early intervention and recent trials have shown promising results for the use of *N*-acetylcysteine (NAC) in schizophrenia. Moreover, integrated preventive psychological intervention (IPPI), applies social-cognitive remediation to aid in preventing the transition to the psychosis of CHR-P patients. *Study Design*: In this double-blind, randomized, controlled multicenter trial, a 2 × 2 factorial design was applied to investigate the effects of NAC compared to placebo (PLC) and IPPI compared to psychological stress management (PSM). The primary endpoint was the transition to psychosis or deterioration of CHR-P symptoms after 18 months. *Study Results*: While insufficient recruitment led to early trial termination, a total of 48 participants were included in the study. Patients receiving NAC showed numerically higher estimates of event-free survival probability (IPPI + NAC: 72.7 ± 13.4%, PSM + NAC: 72.7 ± 13.4%) as compared to patients receiving PLC (IPPI + PLC: 56.1 ± 15.3%, PSM + PLC: 39.0 ± 17.4%). However, a log-rank chi-square test in Kaplan–Meier analysis revealed no significant difference of survival probability for NAC vs control (point hazard ratio: 0.879, 95% CI 0.281–2.756) or IPPI vs control (point hazard ratio: 0.827, 95% CI 0.295–2.314). The number of adverse events (AE) did not differ significantly between the four groups. *Conclusions*: The superiority of NAC or IPPI in preventing psychosis in patients with CHR-P compared to controls could not be statistically validated in this trial. However, results indicate a consistent pattern that warrants further testing of NAC as a promising and well-tolerated intervention for CHR patients in future trials with adequate statistical power.**

Key words: *N*-acetylcysteine/clinical high risk/integrated intervention/social functioning

Introduction

Psychotic disorders rank high on the global burden of disease statistic[1] and are often associated with a considerable loss of psychosocial function and quality of life.[2] Early detection and prevention aim to delay or even prevent transition to psychosis and functional decline. While clinical criteria for the detection of high risk for psychosis are well established[3] and offer a window of opportunity for early intervention almost unique in psychiatry,[3,4] there is an urgent need for the development of effective and tolerable interventions that facilitate the implementation of early intervention approaches.

The administration of second-generation antipsychotic substances in patients with clinical high risk for psychosis (CHR-P) has been shown to reduce symptom load in clinical trials.[5] However, antipsychotics have a significant risk of causing unfavorable side effects. Furthermore, over the last 10 years, a steady overall decline in transition rates of CHR-P patients has been observed in various studies[6] and only about one-fifth of CHR-P patients experience transition to psychosis within 2 years.[6] Even though mixed results on the efficacy of neuroprotective and anti-inflammatory agents like omega-3-fatty acids, D-serine and cannabidiol[7–10] were obtained,[10–12] aggregation of the available evidence in meta-analyses showed benefits for various experimental interventions.[3,13,14]

In this context, *N*-acetylcysteine (NAC) provides an intriguing pathway for potential treatment in CHR-P. The neuroprotective effects of NAC are mediated by three distinct mechanisms[15]: (1) Mitigation of oxidative stress through cysteine donation; (2) decrease of neuroinflammation by attenuating cytokine levels; and (3) modulation of glutamatergic signaling by activating the cysteine-glutamate antiporter. All three pathways have been shown to be involved in the pathophysiology of schizophrenia on several occasions.[16–19] Glutamatergic signaling can also be manipulated using NMDA-receptor antagonists like ketamine.[20] Subanesthetic ketamine induces psychotomimetic states in humans and rodents similar to schizophrenia.[21] Interestingly, perinatal ketamine treatment and subsequent NAC application in mice prevented the development of cognitive and social behavioral deficits.[22] Additionally, a transgenic mouse model with a glutathione deficit showed recovery of oxidative damage by applying NAC.[23]

The compound was also shown to improve mismatch negativity,[24] processing speed,[25] and working memory[26] in patients with schizophrenia. In chronic schizophrenia, improvement of negative symptoms and neurocognitive functioning were demonstrated.[27] For individuals with CHR-P, clinical trials demonstrated that (1) NAC supplementation increases glutathione levels, (2) has a positive effect on functional connectivity within the cingulate cortex,[28] and (3) improves negative and disorganized symptoms.[29] Due to its assumed neuroprotective nature and positive effects on cognition and symptoms, NAC is thus a promising agent in the prevention of psychosis. A case report with five CHR-P patients found a potential benefit for the treatment.[30]

Psychological treatments also meet the criterion of a low side-effects profile and are generally recommended as the first-line treatment of CHR-P.[3] Psychological interventions for CHR-P that have been investigated in randomized controlled trials are cognitive behavioral therapy (CBT),[31] integrated psychological treatment,[32] and family therapy.[33] While all interventions showed generally favorable effects, no specific intervention was superior in preventing psychosis in CHR-P patients so far.

Several studies indicated that social functioning is a crucial target for preventive approaches.[34] It is predictive for transition to psychosis, impaired in CHR-P states, and persists even after remission of CHR-P symptoms.[35–37] Generally, the effects of various cognitive behavioral therapies on social functioning were shown to be rather small.[38] However, in a cohort of youth with CHR-P, a remediation intervention was recently shown to have favorable effects on mentalizing.[39] Integrated preventive psychological intervention (IPPI) is a novel psychotherapeutic intervention to provide disorder-related knowledge, improve social functioning, and stress/symptom management, and applies social-cognitive remediation.[40]

The aim of this study was to investigate individual and combined effects of the two different interventions (NAC or IPPI vs Placebo or PSM) on the transition to psychosis within CHR-P patients by focusing on amelioration of glutamatergic signaling with NAC, symptom management, and improving social cognition with IPPI. The application of both interventions in combination with a control-condition or in combination with each other, aimed to study their individual as well as their combined effects simultaneously. We hypothesized that treatment groups receiving both treatments (NAC and IPPI), would show significantly fewer transitions to psychosis, less deterioration of CHR-P symptoms (primary outcome), and improved social functioning, social cognition, and neurocognitive capabilities (secondary outcome) compared to patients in one or both placebo groups.

Methods

Participants

Between 2016 and 2021, eleven German trial sites recruited 48 subjects in this double-blind (single-blind for psychotherapeutic intervention) placebo-controlled, randomized clinical trial. Participants were recruited via the center's early detection facilities and either self-referred or referred via practitioners in stationary or ambulant settings. Inclusion criteria were (1) fulfilling criteria for CHR-P as assessed by the Structured Interview for Psychosis-Risk Syndromes (SIPS)[41] and

the Schizophrenia Proneness Instrument, Adult version (SPI-A)[42] and (2) decreased social functioning as measured with the Social and Occupational Functioning Assessment Scale[43] (SOFAS) and the Global Assessment of Functioning[44] (GAF). Exclusion criteria were, among others, a past psychotic episode spanning more than 7 days, lifetime antipsychotic medication with a cumulative dosage of over 30 times the minimum effective dose according to S3-Guidelines for schizophrenia, and any past psychotherapeutic training for prevention purposes. Further details on inclusion and exclusion criteria, as well as trial design and recruitment, can be found in Schmidt et al[40] and in Supplementary table 1. A CONSORT chart is available in the supplement.

Trial Design

The trial features a 2 × 2 factorial design with four arms to assess combined and single effects of NAC vs Placebo (PLC) and integrated preventive psychological intervention (IPPI) vs psychological stress management (PSM) (see figure 1). PSM is believed to enhance coping mechanisms and stress management among patients grappling with psychotic symptoms, potentially contributing to a reduction in the severity of these symptoms.[45] It was selected as the active control-condition for the psychological intervention, aiming to discern the specific impact of enhanced social cognition on symptoms in individuals at risk for psychosis presented only in the IPPI sessions. The intervention period spanned 26 weeks, with a follow-up period of up to 52 weeks. Randomization to one of four arms was done stratified by trial center via an internet service (ALEA; FormsVisionBV, Abcoude, NL; https://www.aleaclinical.eu/) and took place after obtaining informed consent and a baseline visit. For randomization, blocks of varied lengths were permuted to create allocation sequences. Results of the randomization were displayed on screen and communicated to approved staff members through e-mail. Follow-up assessments took place at weeks 13, 26, 52, and 78. Raters remained blinded to all conditions, as IPPI and PSM were carried out by trained therapists. To this end, generated data from psychotherapeutic sessions was kept separate from data obtained by raters in bi-weekly visits.

Interventions and Questionnaires

NAC (Hexal, Holzkirchen, Germany) or PLC were provided as two capsules containing 500 mg of the compound two times a day, amounting to 2000 mg/day. Mode of ingestion and dosage were chosen in accordance with earlier research[27] demonstrating safety, tolerability, and good bioavailability.[46] Capsules were manufactured and provided by the pharmacy of the University Hospital in Heidelberg. PLC capsules contained a filling agent (mannitol and aerosil), frequently used for medical trials.

IPPI was developed with the goal of preventing a transition to psychosis by improving stress management, symptom management as well as social cognition. This manualized therapy is comprised of 21 weekly sessions and a final booster session, and each module focuses on motivation using multi-sensory materials in social cognition domains (Theory of Mind and empathy, affect recognition, social perception, social attributions, and social problem solving) as well as symptom and stress management—further details are described in Schmidt et al.[40] A psychological stress management (PSM) intervention was introduced as an unspecific control-condition and spanned across 11 bi-weekly sessions and a final closing-session. It aims at improving coping with stressful situations in patients leaning on the vulnerability-stress-coping model of the development of psychosis.[45,47–49] Psychotherapists with at least advanced postgraduate training conducted both IPPI and PSM, ensuring their professional adherence to the highly manualized protocols. Throughout the trial period, therapists had the option to seek supervision from SJS at any time. Additionally, therapists received supervision during monthly meetings that involved participating therapists from all centers. Co-primary outcome variables assessing social functioning were operationalized by

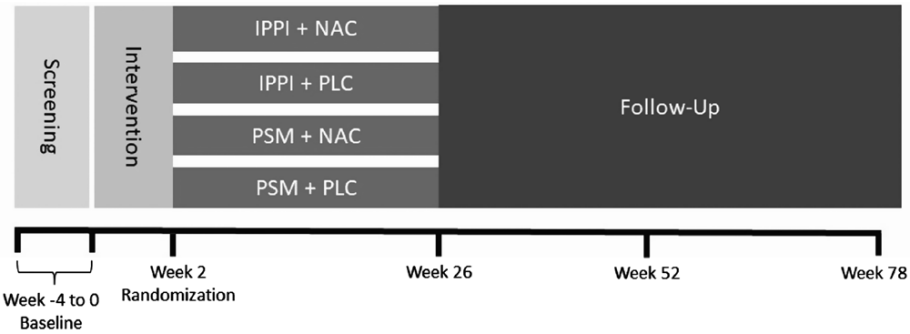

Fig. 1. Study design: the trial comprises a 2 × 2 factorial design with four study arms. The intervention spans 26 weeks with a follow-up period of up to 52 weeks. IPPI, integrated preventive psychological intervention; NAC, *N*-acetylcysteine; PSM, psychological stress management; PLC, placebo.

the Social and Occupational Functioning Assessment Scale (SOFAS) and Functional Remission of General Schizophrenia (FROGS) questionnaire. While the FROGS contains five subscales (daily life, activities, relationships, quality of adaption, and health and treatments), the SOFAS consists of a single scale ranging from low social functioning (score of 0) to perfect functioning (score of 100). A significant change from baseline in either instrument indicated improvement or worsening of social functioning. Secondary variables were quantitative changes in scores of neurocognitive assessments, ie, Digit Symbol Substitution Test[50] (DSST), Verbal Learning and Memory Test[51] (VLMT), Digit Span,[50] Trail Making Test Versions A + B[52] (TMT); improvement of negative and disorganization symptoms assessed by the Brief Negative Symptom Scale[53] (BNSS) and SIPS; remission of CHR-P-criteria, depressive symptoms in the Calgary Depression Scale for Schizophrenia[54] (CDSS), and social cognition assessed by the Movie for the Assessment of Social Cognition[55] (MASC), the Social Attribution Test Multiple Choice[56] (SAT-MC), and the Pictures of Facial Affect[57] (PFA). Further secondary outcomes were the occurrence of adverse events (AE),[58] adherence assessed with the Drug Attitude Inventory[59] (DAI) and the Patient Questionnaire on Therapy Expectations and Evaluation (PATHEV), subjective quality of life according to the WHO-Quality-of-life Questionnaire (WHO-QOL[60]), laboratory assessments and body weight from baseline over time. A comprehensive overview of all outcome variables and their operationalization is available in Supplementary table 2.

Statistical Analysis

Originally, a transition risk of 22% within 18 months had been assumed. During recruitment, new research[61] led us to assume a transition risk of about 30% within the same timeframe for patients with impaired social and role functioning, as measured with the GAF. Since the probability of transition increased when impaired social functioning was introduced as an inclusion criterion (see Supplementary table 1), less patients per group were required to measure primary and secondary outcomes. To detect a relative reduction in transition risk of 80%, at a two-sided level of 2.5%, an uncorrected chi-square test would have required 48 patients to be recruited per group (IPPI/NAC; IPPI/PLC; PSM/NAC; PSM/PLC). To compensate for the influence of about 25% drop-out, it was planned to include $n = 32$ patients per study group. This resulted in $n = 128$ patients as the adjusted aim for the trial, with 32 patients per study arm. A futility analysis was performed in January 2020. The Data Safety Monitoring Board decided to terminate the trial prematurely, as the conditional power for the primary analysis was below 80% due to a lower number of eligible patients than anticipated during the specified time frame.

Primary analysis was based on the full analysis set, as derived from the intention-to-treat (ITT) principle. All randomized patients were included. Prior to this analysis, patient data was reviewed in a blind manner to determine evaluability. Patients who withdrew or showed protocol violations were included in the ITT population. One patient was accidentally unblinded, as they received a wrong medication kit due to an error in the randomization software and were consequently dropped from the study. Data of dropouts was analyzed using all available data. The primary outcome variable is the time from randomization to transition to psychosis or deterioration of symptoms defined by SPI-A and SIPS within up to 18 months. Based on the assumed progressive temporal link of symptom complexes "cognitive disabilities" (COGDIS), "attenuated psychotic symptoms" (APS), and "brief limited intermittent psychotic symptoms" (BLIPS),[62] deterioration was defined as (1) fulfilling the diagnostic criteria for APS if COGDIS had been present before and (2) fulfilling the criteria of BLIPS if APS had been present before. The inclusion of symptom deterioration to the primary endpoint was deemed important due to the relatively truncated follow-up period of up to 12 months, which falls short of the average duration required for transition in the CHR-P demographic.[6] Transition to psychosis was defined as the presence of at least one SIPS-positive symptom with a severity score of 6 ("severe and psychotic") for >7 days. The comparisons of IPPI vs PSM and NAC vs PLC were based on stratified (by center) Cox-regression with main effects IPPI/PSM and NAC/PLC. Centers with fewer patients were pooled for this analysis. In this model, transitions and deterioration were defined as events within a survival analysis. As an estimate of effect size, hazard ratios were expressed in percentage of intervention groups showing event-free survival. Possible interactions were explored in the regression model. The proportional hazards assumption was explored by examining Kaplan–Meier plots and tested by introducing time-dependent covariates.

High censoring in data, leading to possible selection bias, was adjusted with inverse probability weighted (IPW) estimation.[63] Inverse probability weights were used to create a pseudopopulation that is random with regard to the measured determinants of loss to follow-up, applying adjusted weights to each participant not lost to follow-up. These weights were then imputed into stratified (by center) cox-regression with covariates age and sex.

Both, co-primary and secondary endpoints were analyzed using mixed models for repeated measures with corresponding contrasts (assuming sufficient approximation by normal distributions, supported by visual inspection of the data) or using generalized estimating equations to describe and evaluate differences between groups and changes over time. Cohen's d was calculated as effect size for visits at week 12, 26, and 78 and then averaged across visits. Data were analyzed with SPSS version 26 (IBM Corp., Armonk, NY, USA) and SAS.

Safety and Tolerability

Adverse events were mainly specified by (1) items on the Udvalg for Kliniske Undersogelser side effect rating scale (UKU-SERS),[58] which explores different domains of functioning within psychopharmacology, and (2) abnormal laboratory values.

The trial protocol was approved by the local ethics committees of lead centers Bonn and Cologne and subsequently approved by all ethics departments of participating trial sites. It was registered as Phase III trial with the Federal Institute for Drugs and Medical Devices and is registered with clinicaltrials.gov (NCT03149107) and European Eudra-CT (2014-003076-22). It was carried out in compliance with the Good Clinical Practice guidelines of the Declaration of Helsinki. The trial was sponsored by the Federal Ministry of Education and Research (Grant/Award Number: 01EE1407C, 01EE14071).

Results

Recruitment and Demographics

49 Participants were recruited, informed, and consent was obtained. 48 Patients were randomly assigned to one treatment group (NAC + IPPI, NAC + PSM, PLC + IPPI, PLC + PSM) after a baseline-visit. One patient terminated study participation before randomization due to the prescription of antipsychotic medication. In total, 23 patients received NAC and 24 patients participated in IPPI (for details, see Supplementary table 3). A total of 32 patients dropped out of the study. Of these, 23 dropped out during the intervention period. The most frequent reason named for drop-out was "termination by patient" ($n = 9$), followed by "loss to follow-up" ($n = 3$), and "protocol violations" ($n = 3$).

A Kruskal–Wallis test revealed no relevant differences between treatment groups in key demographic factors, even though age [range group means: 20.9 (PSM + PLC)—27.1 (NAC + PSM); $P = .016$] and urbanization [range small towns (<5.000): 0 (NAC + IPPI/NAC + PSM)—5 (PSM + PLC); range big cities (>1.000.000): 1 (PSM + PLC)—8 (IPPI + PLC/PSM + NAC); $P = .015$] showed statistical significance before multiplicity correction (ie, according to Bonferroni, see Supplementary table 3).

Primary Endpoints

Intention-to-treat Kaplan–Meier analysis of the primary outcome "transition to psychosis" revealed 16 events (transition to psychosis) ($n = 46$, 30 censored times) at the end of the maximum follow-up period of up to 78 weeks. The overall median time-to-event was 43.0 weeks ($SE = 9.6$ weeks). For the primary endpoints data is presented as the rate of event-free survival, showing percentages of patients that did not transition to psychosis.

Overall event-free survival for IPPI was 62.3 ± 11.0% after 18 months, while this probability for the control-condition (PSM) was 57.6 ± 11.8% ($P = .398$, log-rank test; hazard ratio IPPI vs PSM 0.827, 95% CI 0.295–2.314). For NAC, the total event-free survival probability was 73.0 ± 9.4%, with its control-condition presenting at 50.5 ± 11.4% ($P = .333$; hazard ratio NAC vs PLC 0.879, 95% CI 0.281–2.756). Event-free survival probability after 18 months for the combined interventions was 72.7 ± 13.4% for NAC + IPPI ($P = .674$, hazard ratio vs PLC + PSM 0.707, 95% CI 0.141–3.549), 72.7 ± 13.4% for NAC + PSM ($P = .730$, hazard ratio vs PLC + PSM 0.785, 95% CI 0.197–3.119), 56.1 ± 15.3% for PLC + IPPI ($P = .814$, hazard ratio vs PLC + PSM 0.815, 95% CI 0.149–4.457), and 39.0 ± 17.4% for PLC + PSM ($P = .504$, overall log-rank test, see figures 2 and 3). In summary, no statistically significant difference between the transition rates of the intervention groups was found.

To adjust for possible selection bias due to high censoring, inverse probability weighting was used for stratified (by center) cox-regression with covariates age and sex.[63] Inverse probability weighted time-to-event curves appeared congruent to unweighted (conventional) time-to-event (Kaplan–Meier) curves upon visual inspection, indicating that bias due to informative censoring may be negligible. In an exploratory analysis, (1) the effect of

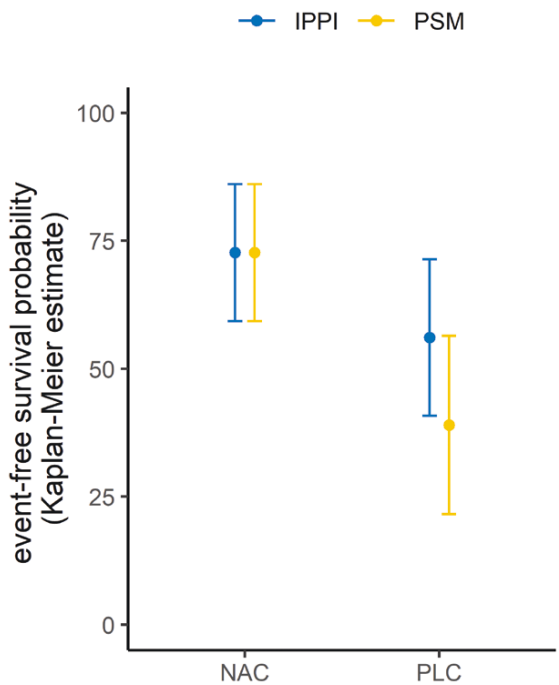

Fig. 2. Estimates of event-free survival probability as derived from Kaplan–Meier analysis indicating lower probability of transition to psychosis in patients receiving *N*-acetylcysteine as compared to patients receiving placebo. NAC, *N*-acetylcysteine; IPPI, integrated preventive psychological intervention; PLC, placebo; PSM, supportive counseling.

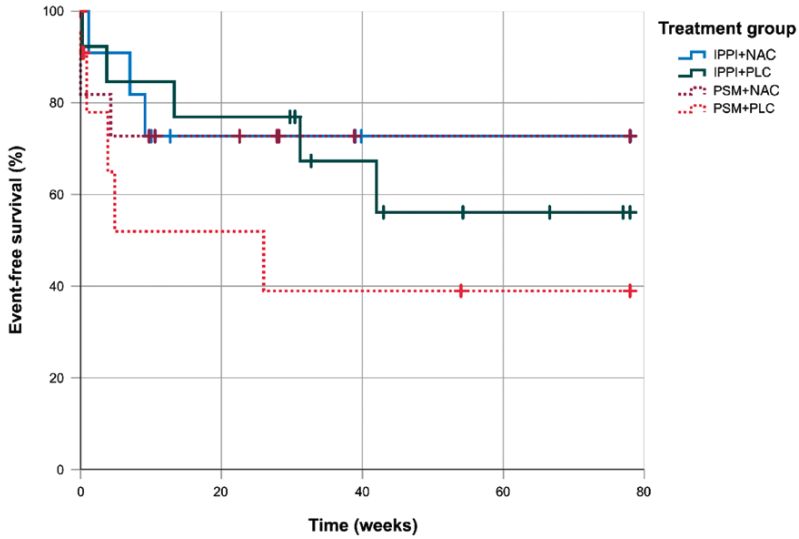

Fig. 3. Kaplan–Meier survival analysis showing a tendency for higher survival probability within NAC treatment-groups. NAC, *N*-acetylcysteine; IPPI, integrated preventive psychological intervention; PLC, placebo; PSM, supportive counseling.

sex was as expected (male vs female HR = 0.778, 95% CI 0.248–2.439, $P = .667$) albeit not statistically significant, (2) the influence of center (pooled Wald-test = 1.110 with 3°C of freedom, $P = .775$) appeared unobtrusive, and (3) the effect of compliance, defined as having attended at least 80% of all expected therapy sessions or having taken at least 80% of medication provided, was again as expected (HR 0.405, 95% CI 0.137–1.196, $P = .102$), however, not statistically significant, either.

Co-primary Endpoints

To calculate co-primary endpoints, a model with main effects for both treatments (compound and psychotherapy), using the baseline value as a covariate, was fitted. Then, an interaction between both treatments was added. No main effects or interactions yielded significant results for social functioning [FROGS: $F(1, 27.88) = 0.01$, $P = .909$; SOFAS: $F(1, 27.97) = 0.50$, $P = .485$].

Secondary Endpoints

In total, 95 AEs were recorded. A majority of the recorded AEs were items on the UKU-SERS, used to assess different possible side effects in patients. The most frequent AE were abnormal dreams ($n = 7$), disturbance in attention ($n = 6$), tension ($n = 5$), and memory impairment ($n = 4$). Any other AEs were named a maximum of three times (see Supplementary table 4). The most frequent organ system class were psychiatric disorders ($n = 28$), nervous system ($n = 13$), and gastrointestinal system ($n = 10$). Three serious AE leading to hospitalization of the patient were reported. Reasons stated for hospitalization were "acute stress disorder" ($n = 2$) and "prodromal stage" ($n = 1$). None of the stated SAE were defined as having a certain or probable causal relationship to any of the applied treatments. Seven (S)AEs were classified as of "moderate" intensity, the rest as "mild."

Pairwise comparisons between groups (NAC vs PLC and IPPI vs PSM) of different types of AE did not yield significant differences in frequency. A one-factorial Analysis of Variance (ANOVA) did not show any significant differences between the groups (NAC + IPPI vs PLC + IPPI vs NAC + PSM vs PLC + PSM: $F(3, 42) = 0.70$, $P = .560$), pointing towards good tolerability of the compound.

Psychopathological and Psychological Measures

To assess the effect of treatments on different domains, every score was fed into a mixed model ANOVA with and without interaction (see Supplementary tables 5 and 6).

While no statistically significant differences were identified, interactions (group*visit) in mixed models showed tendencies towards differences between groups (IPPI vs PSM) for the BNSS reaction scale [$F(2, 9.60) = 3.98$, $P = .055$, $d = 0.09$], leaning towards stronger remission of lacking emotional reactions to stressful events in participants receiving psychotherapeutic treatment. Similarly, the total CDSS value showed a tendency for greater reduction in participants receiving IPPI [$F(1, 95.28) = 3.43$, $P = 0.067$, $d = 0.09$], indicating a stronger decline of depressive symptoms. However, participants of the control group (PSM + PLC) showed a shift towards stronger improvement in the WHO-QOL environment scale [$F(2, 17.88) = 3.56$, $P = .050$, $d = 0.26$]. This scale measures the quality of the physical environment surrounding the patient. Lastly, group differences between NAC vs PLC showed a tendency for significant interaction in the PATHEV hopefulness scale

[$F(2, 31.46) = 0.54$, $P = .041$, $d = 0.30$], showing higher increments of hopefulness about the future in the PLC group. When Bonferroni correction for multiple testing was applied ($n = 36$), the critical P-value for all measures was reduced to P_{crit} .0014.

Lastly, we examined non-significant psychological measures whose effect sizes exceeded $d = 0.50$ (medium effect size) and did not exhibit floor effects and compared the outcomes between the contrasts IPPI vs PSM and NAC vs PLC. Our results showed that patients in the IPPI group demonstrated higher scores in SAT-MC II [$F(2, 23.92) = 0.52$, $P = .476$, $d = 0.63$] and PFA [$F(2, 11.84) = 0.25$, $P = .780$, $d = 0.82$], which are indicative of better social functioning. Interestingly, the alogy [$F(2, 13.08) = 0.95$, $P = .410$, $d = 0.55$] and avolition [$F(2, 6.07) = 0.89$, $P = .457$, $d = 0.70$] scales of the BNSS demonstrated high effect sizes, suggesting a stronger reduction of negative symptoms in patients receiving PSM. When comparing NAC vs PLC, the avolition scale [$F(2, 5.75) = 0.31$, $P = .743$, $d = 0.75$] of the BNSS was also slightly more reduced in patients receiving placebo than in the treatment group. Additionally, in the PLC group, the WHO-QOL measure indicated improvements for its quality of life [$F(2, 13.60) = 1.32$, $P = .299$, $d = 0.61$] and psychology [$F(2, 13.70) = 0.334$, $P = .722$, $d = 0.53$] scales, both of which demonstrated higher scores in PLC at the last visit than in the NAC group.

Discussion

In this randomized multicenter trial, we aimed at evaluating the individual and combined effects of pharmacotherapy with NAC and the integrated preventive psychological intervention (IPPI) for the treatment of CHR-P-patients. The primary endpoint was the transition to psychosis defined as the probability for event-free survival. No significant differences between the treatment groups (IPPI vs PSM/NAC vs PLC) were found.

However, visual inspection of the Kaplan–Meier plot and comparison of survival probabilities indicated that patients receiving NAC (IPPI + NAC: 72.7%, PSM + NAC: 72.7%) showed lower transition rates to psychosis as compared to patients receiving PLC (IPPI + PLC: 56.1%, PSM + PLC: 39.0%). Even though the beneficial effects of NAC are not statistically significant, our findings are in line with the effects of NAC on symptoms in schizophrenia in a recent meta-analysis comparing several anti-inflammatory and antioxidative agents across all stages of schizophrenia.[64] A meta-analysis by Yolland et al[65] also showed significantly improved scores on the positive, negative, and total symptom scale of the Positive and Negative Symptom Scale[66] in patients with schizophrenia receiving NAC. However, even though the overall effects for treatment with NAC might be beneficial, a recent trial comparing NAC and placebo augmentation in clozapine-resistant patients with schizophrenia targeting negative symptoms did not yield significant differences between the groups,[67] which points to higher efficacy of NAC in early stages of schizophrenia.[68,69] Nonetheless, to date only a small case series investigated the effects of NAC on CHR-P with mixed results.[28]

NAC Effects

Comparing the effect size of NAC vs PLC (OR = 0.525) in our study to previous findings in CHR-P patients indicates potentially superior effects compared to a clinical trial that investigated the impact of omega-3 fatty acids on preventing transition to psychosis.[70] Another study investigated olanzapine as a treatment for CHR-P patients and reported an OR vs control of 0.314,[71] which is comparable to the effect of NAC in the present study. Thus, considering the advantageous side-effects profile compared to olanzapine, NAC might be a promising treatment for future studies.

In general, previous studies indicate good tolerability of NAC. For example, a study modeling the effects of NAC on neurodegenerative illnesses in various clinical trials found only mild AE, such as gastroesophageal reflux and mild indigestion among patients at dosages between 1800 and 36 000 mg/day.[72] Similarly, another systematic review reported various smaller side effects of NAC pertaining to different clinical phenotypes.[27,73] Among these, schizophrenia trials were reporting none or only mild AE. Correspondingly, Miyake et al[30] did not report serious AE in their case study with CHR-P-patients. In line with these previous findings, our study indicated a similar number of AE in the treatment groups, suggesting good tolerability of NAC among CHR-P patients.

As stated earlier, NAC works as a donator for glutathione (GSH) catalyzing antioxidative and anti-inflammatory effects by modulating glutamate pathways. Low GSH levels in erythrocytes have been shown to predict lower transition rates in individuals with CHR-P.[74] A remaining question, however, pertains to how fast these NAC-modulated changes can be detected in patients with schizophrenia. In a clinical trial for patients with schizophrenia, a single application of NAC did not alter GSH levels significantly in the medial prefrontal cortex or dorsal anterior cingulate cortex when applying in vivo proton MRS.[75] Interventions showing good effect sizes for reduction of GSH-levels in patients with schizophrenia were spanning between 2 and 6 months,[24,27] which is in accord with this study.

IPPI Effects

Comparing survival probabilities indicated that in patients receiving no active pharmacological compound (PLC), IPPI (IPPI + PLC: 56.1%) was associated with slightly lower transition rates as compared to PSM (PSM +

PLC: 39.0%), whereas in patients receiving NAC, there were no differences (IPPI + NAC: 72.7%, PSM + NAC: 72.7%). It is important to note that these results must be interpreted carefully given the small sample size. Nevertheless, in existing research, CBT was often shown to have robust effects on the reduction of transition risk in multiple meta-analyses[76,77] and is generally recommended for the treatment of CHR-P.[3] Favorable outcomes of CBT towards preventing transition to psychosis were shown at 12+ months, however, not at 6 months.[78]

In a recent meta-analysis that compared CBT against cognitive remediation therapy and multi-component psychosocial interventions for CHR-P, the latter showed favorable outcomes when looking at measures of social functioning, especially when these therapies exhibited a high degree of manualization.[79] Even though the present study did not demonstrate improved social functioning as measured with SOFAS and FROGS, we found patients specifically trained in improved perception of emotions with IPPI were presenting with a small tendency towards higher sum-scores in the PFA, which is in accord with existing research.[80] Future trials with adequate power might additionally be able to demonstrate how the various manualized modules of IPPI[40] are advantageous to generalized CBT in this regard.

Patients receiving IPPI additionally showed a tendency towards more emotional reactions when faced with stressful events as measured by the BNSS distress scale, which is indicative of reduced negative symptom load.[81] However, it should be noted that distress did not increase Cronbachs α significantly in confirmatory factor analysis of the BNSS.[81] Furthermore, IPPI tended to decrease depressive symptoms as measured with CDSS. If this result can be replicated in a larger, more adequately powered trial, IPPI might prove to be beneficial to other psychotherapeutic treatments in this regard. This is due to the fact that many psychosocial interventions did not decrease depressive symptoms when compared to treatment as usual at end of trial or follow-up.[82] It is important to mention, that the results of all aforementioned secondary analyses, however, did not stay significant after correction for multiple testing. When specifically looking at negative symptoms, both interventions failed to show significant decreases of symptom load in the respective intervention groups. Effect sizes indicate that PLC and PSM groups might possibly be showing higher decreases in the BNSS avolition and alogy scale and higher decreases for the PSM group in the BNSS alogy scale than their respective treatment groups.

Combined Effects

Even though due to low power we can only take the Kaplan–Meier plot in figure 3 as an indication towards a certain trend of effects, it is interesting that the synergistic effects of NAC and IPPI are similar to those with NAC and PSM. This implies that IPPI might primarily demonstrate effectiveness when used as a supplementary therapy, whereas NAC exhibits efficacy independently. However, it's important to approach these findings cautiously, as the absence of statistical significance limits interpretation strongly in that regard.

Drop-out and Transition Rates

Finally, in this study, all groups showed high drop-out rates with two-thirds of all participants dropping out over the course of the study. This warrants attention, as these rates are higher than to be expected in trials with CHR-P patients, that usually present with one-third of participants dropping out over the course of the study.[83] One reason for high drop-out rates could be the large number of visits during the trial period, as patients sometimes had to appear twice to complete a bi-weekly visit.[84] As patients did not receive financial compensation, the cost for repeated transportation might have been an issue as well.

Another factor we would like to address is that the total transition rate of all groups after 18 months was higher (34.78%) than in most CHR-P trials, which averages around 20% transitions during the same timespan.[85] One reason for the higher number of transitions in comparison to other trials might be that the timespan between a first screening and enrollment in the study was rather long. In single cases, it spanned about 6 months when medication had to be tapered off due to strict exclusion criteria. Additionally, because only patients that were showing impairments in social functioning were included in the study,[61] it is highly likely that these constitute a group that is afflicted by CHR-P more strongly and thus more probable to transition.

Limitations

The current study has several limitations that warrant attention. Foremost, the present analysis relies on a limited sample size, necessitating a cautious interpretation of all findings within this context.

A reason for the lack of recruitment within this study might be that only a fraction of all patients that were pre-screened went on to participate in the study. Besides not fulfilling inclusion criteria, reasons named for not participating in the trial were: frequent presence of exclusion criteria (in particular due to psychopharmacological treatment), high time requirement for screenings and therapy, commute to the hospital too costly/long or not wanting to participate in either pharmacological or psychotherapeutic study arm. The inclusion criteria in this study were rather restrictive compared to other CHR-P trials. This was due to the fact that criteria were being harmonized along several clinical trials to make comparisons between trials possible. Even though the 2 × 2 design of the trial might be beneficial to investigate the interplay of intervention and compound, future trials

should reduce the number of arms and focus on the beneficial effects of NAC or IPPI in isolated studies to reduce the number of participants needed for each study arm.

Another limitation pertains to the exclusion of adolescents <18 years from the study: CHR-P is highly prevalent within this age group and including adolescents might thus have aided in (1) easier recruitment of patients for the study[6] and (2) enable more integral conclusions about effectiveness of therapies in CHR-P within the generally affected clinical population.

Generally, low recruitment is a problem, frequently encountered by studies with CHR-P patients.[71] For future clinical trials it might thus be beneficial to allow for longer periods of recruitment to enable meaningful statistical analysis. Conversely, researchers should have a clear idea on how knowledge management and transfer are implemented to preserve recruitment efforts in participating centers when staff is replaced during recruitment periods.

Conclusion and Future Directions

In conclusion, our study design offered a psychological and pharmacological intervention for CHR-P patients, revealing slightly reduced hazard ratios compared to the corresponding placebo groups. We successfully established the safety and tolerability of NAC in CHR-P patients. Although statistically significant effects of NAC were not observed, the noteworthy effect sizes suggest the potential efficacy and favorable tolerability of NAC as a treatment option for CHR-P patients. This outcome holds promise for guiding future intervention trials.

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Conflict of interests:

None.

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Author contributions

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Data availability

Data can be made available upon request.

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4. Discussion

The aim of this thesis was to investigate the link between deficits in social- and metacognition and glutamatergic signaling and test a potential treatment of these deficits.

Study 1 showed that a single dose of intravenous ketamine impaired mentalizing and reliably evoked psychotomimetic symptoms. Even though ketamine was also impaired in a control task, participants receiving the substance showed significantly more answers indicating an absence of mentalizing. In the brain, higher activity of the posterior superior temporal sulcus (pSTS) during the question phase of the task was found in the ketamine-group, a finding congruent with the notion that hyperactivity within pSTS might constitute an endophenotype of schizophrenia (Yan et al., 2020). This area was then used as a seed region to analyze task-based functional connectivity, which was increased with the anterior precuneus. Co-activity of certain brain areas with precuneus has been associated with shifts of attention between concretistic and other layers of attention, like emotion or understanding abstract concepts (Mashal et al., 2014; Ferri et al., 2016). With respect to Q1, we can thus conclude that ketamine influences mentalizing and its neural correlates and up-regulates functional connectivity between pSTS and precuneus, which may represent a dysfunctional shift of attention leading to mentalizing deficits.

The second study showed that metacognitive sensitivity for encoded items was reduced under ketamine in a task probing episodic memory. In the brain, posterior regions linked to visual and spatial attention and mental rotation like the superior parietal lobe, left calcarine gyrus, bilateral lingual gyrus and left inferior parietal lobule were found to be up-regulated in the ketamine-group during metacognition. However, these regions were active during report and follow (i.e. control) trials of metacognitive judgments. Hence it is likely that activation of these areas reflects visual presentation of the scales rather than metacognition itself. A possible explanation for these results is that ketamine might change blood oxygen level-dependent (BOLD) activity in reaction to visual stimuli, which would lead to altered interpretation of these perceived stimuli. Results would then suggest a limited influence of ketamine on important subsystems of metacognition by altering sensory reliability and neural correlates of conscious experience (Koch et al., 2016). For Q2 it can thus be inferred that there appears to be a discernable influence of ketamine on

metacognitive sensitivity, even though its influence may be smaller than initially anticipated. Interestingly, in schizophrenia, recent studies have been questioning the overall existence of online metacognition deficits (Rouy et al., 2023).

Study 3 unfortunately failed to show significantly reduced transition rates for patients with clinical high risk for psychosis (CHR-P). Premature termination of the trial left the remaining sample underpowered, possibly contributing to the lack of significant findings. However, visual inspection of Kaplan-Meier survival curves indicates a tendency for NAC and the integrated preventive psychological intervention to have promising effects on reducing transition in patients with CHR-P. Additional studies involving a sufficient sample of CHR-P individuals are necessary to determine the presence of these effects at last. It is noteworthy that meta-analyses of clinical trials with NAC in schizophrenia show mixed results (Yolland et al., 2020; Zhang et al., 2024). However, it is more likely for NAC to be an effective adjunctive therapy during early stages of the disorder than during late or chronic schizophrenia (Bradlow et al., 2022). Analysis of secondary endpoints also revealed no significant effects after correcting for multiple testing; however, effect sizes indicated improved facial affect recognition and enhanced mentalizing capabilities following the intervention period. Adverse events did ultimately not significantly differ between groups, hinting at good tolerability of NAC. At last, we cannot conclude for Q3 that transition to psychosis can be prevented with NAC and IPPI.

4.1 Limitations

Previous studies had shown a discernable increase of glutamate availability following application of ketamine in pharmacological studies employing magnetic resonance spectroscopy (MRS). As such, alteration of glutamatergic transmission through ketamine was assumed by all studies in this thesis (Ford et al., 2017; Stone et al., 2012). However, all three studies in the present thesis did not directly determine glutamate availability. In this regard, MRS provides the possibility for future research to not only to determine the accuracy of the ketamine-model, but also to compare glutamate availability more directly in key brain regions to patients with schizophrenia (Kruse and Bustillo, 2022).

Interestingly, in studies 1 and 2, ketamine was associated with hyperactivity of brain regions employed during social cognition and metacognition. However, in schizophrenia

and CHR-P the mentalizing-network has repeatedly been shown to be less activated during mentalizing (Dodell-Feder et al., 2014; Vucurovic et al., 2021). Additionally, frontoparietal regions normally show less activations during metacognition in patients with schizophrenia (Jia et al., 2020). More research directly comparing schizophrenia with the ketamine-model is needed to better understand the role of glutamatergic transmission in the pathophysiology of schizophrenia and psychosis.

Taken together, these limitations guide a way to focus future research to better understand the characteristics shared by clinical high risk for psychosis, schizophrenia and the ketamine model, but also to understand its differences.

4.2 Conclusion

The primary aim of this thesis was to examine social cognition deficits potentially influenced by altered glutamatergic signaling, aiming for a deeper understanding of their manifestation and potential treatment in schizophrenia. Studies 1 and 2 revealed that metacognition and mentalizing exhibit deficits following subanesthetic doses of ketamine. Albeit these deficits were smaller than initially anticipated, neurobiological correlates revealed hyperactivity in corresponding brain regions. The third study suggested that a combined approach of psychotherapeutic and pharmacological interventions could be advantageous in addressing these deficits early in the course of the disorder. While the results of this investigation remained inconclusive, they underscore the importance of further exploration in future research.

4.3 References

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