Glycine attenuates impairments of stimulus-evoked gamma oscillations in the ketamine model of schizophrenia

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ABSTRACT

Although a substantial number of studies suggests some clinical benefit concerning negative symptoms in schizophrenia through the modulation of NMDA-receptor function, none of these approaches achieved clinical approval. Given the large body of evidence concerning glutamatergic dysfunction in a subgroup of patients, biomarkers to identify those with a relevant clinical benefit through glutamatergic modulation are urgently needed. A similar reduction of the early auditory evoked gamma-band response (aeGBR) as found in schizophrenia patients can be observed in healthy subjects following the application of an NMDA-receptor antagonist in the ketamine-model, which addresses the excitation / inhibition (E/I) imbalance of the disease. Moreover, this oscillatory change can be related to the emergence of negative symptoms. Accordingly, this study investigated whether glycine-related increases of the aeGBR, through NMDA-receptor co-agonism, accompany an improvement concerning negative symptoms in the ketamine-model. The impact of subanesthetic ketamine doses and the pretreatment with glycine was examined in twenty-four healthy male participants while performing a cognitively demanding aeGBR paradigm with 64-channel electroencephalography. Negative Symptoms were assessed through the PANSS. S-Ketamine alone caused a reduction of the aeGBR amplitude associated with more pronounced negative symptoms compared to placebo. Pretreatment with glycine attenuated both, the ketamine-induced alterations of the aeGBR amplitude and the increased PANSS negative scores in glycine-responders, classified based on relative aeGBR increase. Thus, we propose that the aeGBR represents a possible biomarker for negative symptoms in schizophrenia related to insufficient glutamatergic neurotransmission. This would allow to identify patients with negative symptoms, who might benefit from glutamatergic treatment.

1. Introduction

Schizophrenia places a substantial burden on people with this illness, more so as there is no satisfactory pharmacotherapy for some of its core symptoms, namely cognitive and negative symptoms (Correll and Schooler, 2020). A prominent challenge in the pursuit of an effective pharmacotherapy is that patients respond heterogeneously to treatments (Kumar et al., 2020; McCutcheon et al., 2020). Therefore, biomarkers to identify the subgroup of patients that would benefit from certain treatments are urgently needed.

A promising approach to new treatments is the modulation of aberrant neural glutamatergic activity in patients with schizophrenia. The glutamate hypothesis of schizophrenia presumes a hypofunction of the N-methyl-d-aspartate receptor (NMDAR) which is essential for glutamate neurotransmission. On this account, there were several at-

tempts to modulate its function both directly (through co-agonists such as glycine or D-serine) and indirectly (through glycine-reuptake inhibitors), which both yielded improvements in negative symptomatology (Kantrowitz, 2017; Umbricht et al., 2014). However, none of these pharmacological agents achieved clinical approval despite the repeated confirmation of the viability of this treatment approach through recent studies (Chang et al., 2020; Krogmann et al., 2019). This reinforces the need for biomarkers to detect patients with a putative clinical benefit from glutamatergic modulation.

Gamma band oscillations (GBO) play an essential role in cognition, consciousness, and perception and have been found to be impaired in schizophrenia (Dienel and Lewis, 2019; Uhlhaas and Singer, 2010). The generation of those 30 to 100 Hz frequencies substantially involves NMDARs which are frequently expressed on the inhibitory parvalbumin- (PV+) (Sohal et al., 2009) and somatostatin-expressing

(SST+) γ-aminobutyric acid (GABA) interneurons (Alherz et al., 2017). Based on the foundational hypotheses on the generation of fast cortical oscillations (cf. Buzsaki and Wang, 2012; Traub et al., 1996; Whittington and Traub, 2003), the micro-circuital interplay of the respective interneurons with glutamatergic pyramidal cells produce said frequency oscillations (Lisman et al., 2008). Further, impaired generation of gamma oscillations due to NMDAR knockout on PV+ interneurons can be linked to cognitive deficits in mouse models (Carlen et al., 2012).

The auditory evoked gamma-band response (aeGBR) is among the sensory evoked GBOs which have been of special interest, given that they do not only reflect sensory processes but appear to be affected by attention and memory (Cho et al., 2006; Herrmann et al., 2010). The aeGBR appears 25 to 100 ms after an auditory stimulus and exemplifies the top-down operations in sensory processing, as its magnitude is profoundly altered by task difficulty (Mulert et al., 2007). Several studies have proven that aeGBRs are impaired in all stages of schizophrenia (Leicht et al., 2015, 2010), high risk subjects (Leicht et al., 2016), and even first-degree relatives of people with the illness (Leicht et al., 2011). It is noteworthy, that these detriments are accompanied by a reduced activity of a network including the anterior cingulate cortex (ACC) (Leicht et al., 2015), which has repeatedly been implicated in the pathophysiology of schizophrenia and its cognitive dysfunctions (Reid et al., 2019; Takayanagi et al., 2017).

The ketamine model of schizophrenia offers the opportunity to study this illness without having to test patients. It utilizes the administration of subanesthetic doses of the NMDAR antagonist ketamine (or its eutomer S-Ketamine) to reduce NMDAR dependent glutamatergic neurotransmission, which causes the emergence of schizophrenia-like positive, negative, and cognitive symptoms in healthy volunteers (Krystal et al., 1994) or aggravating symptom severity in patients (Lahti et al., 1995). This model particularly depicts aberrant GBOs since under physiological conditions ketamine demonstrates the highest affinity for the NMDARs expressing GluN2C and GluN2D subunits, which are most frequently expressed on the aforementioned PV+ and SST+ GABAergic interneurons (Khlestova et al., 2016; Kotermanski and Johnson, 2009). The inhibition of the respective interneurons is supposed to result in a disruption of the local microcircuits (Lisman et al., 2008) and leads to an impaired generation of GBO resembling deficiencies observed in patients with schizophrenia (Jadi et al., 2016).

Glycine binds to an allosteric binding site of the NMDAR, thus enabling signal transduction following the engagement of glutamate as well as promoting and enhancing the binding of glutamate to the NMDAR (Leeson and Iversen, 1994). These characteristics allow glycine to attenuate neurophysiological impairments in patients suffering from schizophrenia (Greenwood et al., 2018; Kantrowitz et al., 2018) and neurophysiological impairments related to (induced) NMDAR hypofunction as animal studies have proven (Lee et al., 2018).

Based on the strong interplay of schizophrenia symptoms, NMDAR dysfunction and receptor co-agonists, this study aimed to delineate the impact of an exogenously induced NMDAR dysfunction on the generation of the aeGBR and the effect of glycine thereon. This was achieved by means of a cognitively demanding auditory choice reaction task after a pretreatment with glycine and during the continuous infusion of S-ketamine. We hypothesized that glycine-pretreatment mitigates the disturbances of the aeGBR and the interrelated emergence of schizophrenia-like symptoms during S-ketamine administration in healthy volunteers.

2. Methods and materials

2.1. Participants

Twenty-six healthy male participants were enrolled in this study. The general procedure was approved by the Ethics Committee of the Medical Association Hamburg and carried out in accordance with the latest version of the Declaration of Helsinki. Written informed consent was obtained from all participants after the nature of the procedures had been fully explained. One participant dropped out due to adverse effects caused by S-ketamine (dissociative effect/headache). Another participant had to be excluded due to poor EEG data quality. Accordingly, we included 24 healthy male participants in EEG data analysis. Volunteers were either students or medical staff of the University Medical Center Hamburg and received a monetary compensation for the EEG recording sessions.

The inclusion criteria included male sex, an age between 18 – 40 years (mean 24 years, 19 - 32 years, SD 3.7 years), right-handedness as assessed by means of the Edinburgh Handedness Scale (mean 77.2%, 48 - 100%, SD 16.8%), normal or corrected visual acuity and German at native speaker level. All participants had normal IQ as tested with the German WST vocabulary test (mean 111.5 points, 99 - 122 points, SD 6.3 points). and they had experienced an average of 16.5 educational years (13 - 21 years, SD 2.4 years). The sample was limited to participants of male sex based on safety concerns regarding the application of S-ketamine in case of an undetected pregnancy.

Exclusion criteria were any acute or previous psychiatric disorders, a family history of schizophrenia or bipolar disorder, the use of illegal drugs, active medication or health conditions representing a contraindication to the administration of S-ketamine. All subjects were screened with pure tone audiometry for seven frequencies ranging from 120 to 8000 Hz (Esser Home Audiometer 2.0) in order to ensure normal hearing in both ears. Participants with an auditory threshold higher than 20 dB in any of the frequencies were excluded from the study.

The required sample size was calculated using G*Power 3.1 (Faul et al., 2007). With respect to the reduction of the aeGBR amplitude through the application of S-ketamine and the potential attenuation of this effect via pretreatment with glycine, we expected a medium effect size in the range of $\eta_p^2 = 0.1$. This estimation was based on a previous study comparing the effect of the administration of S-ketamine on the aeGBR to placebo ($d = 0.6$) (Curic et al., 2019). We planned to apply regular statistical analyses and error probabilities (ANOVA, $\alpha = 0.05$; 1-$\beta = 0.95$). Thus, the sample size analyses led to a total sample size of $n = 21$ required for a repeated measure, within factors ANOVA for one group and four measurements. To completely counterbalance the order of the four experimental conditions, we decided to include 24 participants.

2.2. Study design

This study followed a double-blind (regarding pretreatment), randomized, placebo-controlled crossover design. All participants underwent four EEG recording sessions, which differed regarding the pretreatment and continuous infusions. A median nine-day and minimum five-day interval separated each of the four test sessions.

The four corresponding experimental conditions were: (i) placebo-pretreatment followed by placebo (Pla-Pla), (ii) placebo-pretreatment followed by S-ketamine (Pla-Ket), (iii) glycine-pretreatment followed by placebo (Gly-Pla), and (iv) glycine-pretreatment followed by S-ketamine (Gly-Ket). The order of sessions was randomized and overall counterbalanced.

The glycine-pretreatment was administered at a dosage of 200 mg/kg bodyweight (Greenwood et al., 2018) as an intravenous infusion in 500 ml 0.9% sodium chloride (NaCl) solution over one hour. Placebo was administered analogously as a NaCl infusion. Both pretreatments were prepared by an unblinded third person prior to the recording sessions and the ready-to-use infusions were indistinguishable. Subsequently, during the S-ketamine sessions a subanesthetic dose of S-ketamine hydrochloride (Ketanest® S, Pfizer) was administered intravenously in a 0.9% NaCl solution for a duration of 75 min. The infusion was started with an initial bolus of 10 mg over 5 min followed by a maintenance infusion of 0.006 mg/kg/min, reduced by 10% every 10 min (Curic et al., 2019). Placebo was administered analogously as NaCl in-

fusions. Heart rate, blood pressure and oxygen saturation were continuously monitored during all sessions. The clinical rater was blinded with respect to the pretreatment but not regarding the continuous infusion condition due to the clinical effects of S-ketamine.

2.3. Psychometric assessment

Immediately after each EEG-recording session, the psychiatric symptomatology was always assessed by the same experienced psychiatrist (S.C.) using the Positive and Negative Syndrome Scale (PANSS) (Kay et al., 1987). Based on our previous studies the PANSS scores were evaluated using the five-factor model (van der Gaag et al., 2006).

2.4. Stimuli

An attentionally demanding auditory reaction task used in previous studies of our group (Curic et al., 2019; Leicht et al., 2015; Mulert et al., 2007) was employed 10 min after the start of the maintenance infusion. Three tones of different pitch (800, 1000 and 1200 Hz; 40 repetitions of each) were generated and presented to participants through electrostatic headphones in pseudorandomized sequences and interstimulus intervals (ISI: 2.5–7.5 s, mean: 5 s), resulting in a task duration of 10 min. The duration of each tone was 250 ms, and the sound pressure level was set to 85 dB. Participants were instructed to respond to these stimuli as quickly and accurately as possible, by pressing a button with the left index finger when the low tone was presented, and by pressing another button whenever the high tone occurred (target tones). The middle tone was not a target for the pressing of a button. Reaction times (the amount of time from stimulus onset to the pressing of a button) and errors (pressing no button or pressing the wrong button within a timeframe of 150 and 2000 ms after onset of the stimulus) were registered. Only trials with correct responses to target tones were considered for further analyses.

2.5. EEG recording

In a sound-attenuated and electrically shielded room subjects were seated in a reclined chair with a head rest with their eyes open and asked to look at a fixation cross presented at a monitor 1 m in front of them. The EEG was recorded at a sampling rate of 1000 Hz including an analog band-pass filter (0.1 – 1000 Hz), with 64 active electrodes mounted on an elastic cap (actiCap, Brain Products, Gilching, Germany) in an extended 10/20 system (Curic et al., 2019), using the Brain Vision Recorder software Version 1.21 (Brain Products). Eye movements were recorded through four EOG channels. An electrode at the FCz position was used as reference, the electrode at position AFz served as ground. Impedances were kept below 5 kΩ.

2.6. Data analysis

Data preprocessing and analysis was carried out using the Brain Vision Analyzer (BVA) Version 2.1 (Brain Products). The preprocessing procedure was conducted in accordance with previous studies (Curic et al., 2019; Leicht et al., 2010). After band-pass filtering (1–80 Hz) and down-sampling to 500 Hz, a topographic interpolation (spherical splines) of up to five channels was performed (the mean number of interpolated channels did not differ between conditions). Channels were selected for interpolation if more than 5% of data in the respective channel was affected by non-physiological artifacts (e.g., flat lines or electrode "pop"), or muscle artifacts exceeding amplitudes of ±70 μV. An independent component analysis (ICA) was performed to identify and remove blinks, drifts, muscle artifacts and saccadic spike potentials based on their characteristic topographies, time courses, and frequency distributions (the mean number of removed ICA components did not differ between conditions: PlaPla 6.8 components, Pla-Ket 7.6 components, Gly-Pla 6.0 components, Gly-Ket 6.9 components). The continuous EEG was segmented into epochs of 3000 ms starting 1000 ms prior to the

auditory stimulus. Segments including amplitudes exceeding ±70 μV within a 600 ms window starting 200 ms pre-stimulus in any channel were automatically rejected. After re-referencing to common average reference and baseline correction (using an interval of −210 to −10 ms pre-stimulus), averaged event-related potential (ERP) waveforms were computed. Only waveforms based on at least 35 segments were accepted and included in further analyses.

2.7. aeGBR amplitude and phase-locking factor (PLF)

The aeGBR amplitude and PLF were computed using a complex Morlet wavelet transformation (Morlet parameter $c = 5$, instantaneous amplitude (Gabor) normalization) as applied in several previous studies (Curic et al., 2019; Leicht et al., 2010; Mulert et al., 2010). This wavelet transformation was performed on averaged ERPs to obtain the phase-locked evoked gamma amplitude. The analysis followed our previously published procedures and extracts the highest value within the timeframe of 30–100 ms post-stimulus at the electrode Cz of the wavelet layer with the central frequency of 40 Hz (frequency range 32–48 Hz) (Curic et al., 2019; Leicht et al., 2016, 2015, 2011, 2010; Mulert et al., 2007; Roach and Mathalon, 2008; Spencer et al., 2008;). The PLF was calculated by performing a wavelet transformation at the single trial level and extracting complex-phase information with all vector lengths normalized to the unit circle before averaging the phase information (Curic et al., 2019; Leicht et al., 2010). Gamma PLF peaks were defined as the highest value of the wavelet layer centered around 40 Hz within the timeframe of 30–100 ms post-stimulus at the electrode Cz.

2.8. Source analysis of the aeGBR

Source-space localization analyses were performed with the low-resolution brain electromagnetic tomography (LORETA) KEY software package v2017 (Pascual-Marqui, 2002). The EEG source localization of the aeGBR across 30–50 Hz was executed for every subject within a time window of 30 to 70 ms post-stimulus.

The voxel-wise comparison of cortical activities between conditions was conducted using a one-tailed t-test for paired groups (statistical significance threshold $p < 0.05$) provided in the sLORETA software, which was based on the direction of the effects in our previous results (Curic et al., 2019) and the corresponding hypothesis. A statistical non-parametric mapping randomization method was used to automatically adjust for multiple comparisons with a Fisher's random permutation test with 5000 randomizations.

2.9. Statistical analyses

All statistical analyses were performed using IBM SPSS Statistics Version 24. Comparisons of reaction times and error rates, PANSS scores, aeGBR amplitude, and PLF between all conditions were conducted using 2 (ketamine) x 2 (glycine) repeated measure analyses of variance (RM-ANOVA). All follow-up simple main effect analyses and post-hoc contrasts were subject to a Bonferroni correction.

Based on our previous study, we specifically investigated the Pearson correlations between the PANSS negative scores and the relative changes of the aeGBR (Curic et al., 2019). To calculate the percental changes, the Pla-Pla condition was defined as baseline and used as the reference for all contrasts.

3. Results

3.1. Behavioral performance

We observed a significant main effect of ketamine on reaction times ($F(1,23) = 28.4$, $p < 0.001$), with increased reaction times occurring during the application of S-ketamine (Fig. 1A). Regarding error rates, a significant main effect of ketamine occurred ($F(1,23) = 17.7$, $p < 0.001$)
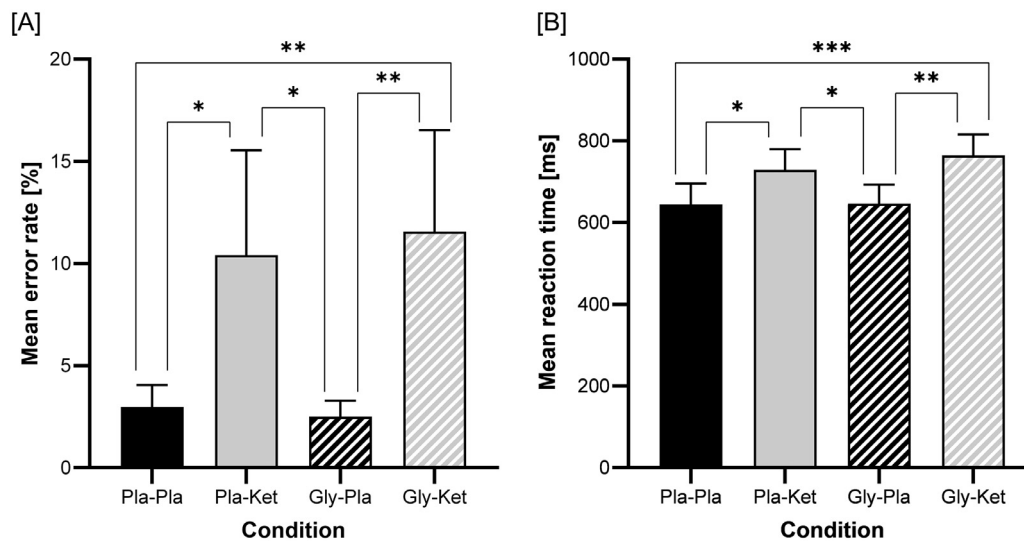
Fig. 1. Bar charts of the mean values of the error rate [A] and the reaction time [B]. ($^{***}p_{adjusted} < 0.001$, $^{**}p_{adjusted} < 0.01$, $^{*}p_{adjusted} < 0.05$).
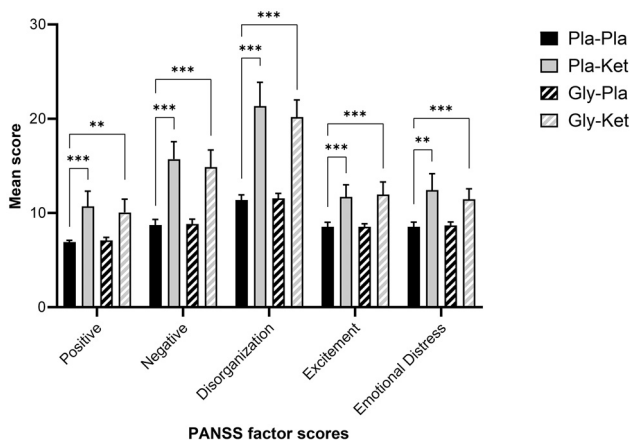

Fig. 2. Bar charts of the mean values of the five Positive and Negative Syndrome Scale (PANSS) factor scores. Only significant differences between the Pla-Pla and both ketamine conditions (Pla-Ket and Gly-Ket) are displayed. ($^{***}p_{adjusted} < 0.001$, $^{**}p_{adjusted} < 0.01$, $^{*}p_{adjusted} < 0.05$).

with S-ketamine increasing the error rate (Fig. 1B). Neither glycine pretreatment nor the interaction between glycine and ketamine affected the behavioral performances.

3.2. Ketamine-induced psychopathology

Concerning PANSS total score as well as all factor scores (Fig. 2), there was a significant main effect of ketamine (PANSS Total: $F(1,23) = 121$, $p < 0.001$; Positive: $F(1,23) = 33.7$, $p < 0.001$; PANSS Negative $F(1,23) = 100.9$, $p < 0.001$; Disorganization $F(1,23) = 130.4$, $p < 0.001$; Distress $F(1,23) = 35.5$, $p < 0.001$; Excitement $F(1,23) = 41$, $p < 0.001$). Neither glycine pretreatment nor the interaction between glycine and ketamine affected the PANSS total or factor scores.

3.3. Auditory evoked GBR amplitude and PLF

Around 50 ms after stimulus presentation in all four conditions, the evoked gamma activity increased at electrode Cz (Fig. 3B). Regarding the peaks of the evoked gamma band amplitude, a significant interaction effect between ketamine and glycine occurred ($F(1,23) = 6.2$, $p = 0.02$, $\eta_p^2 = 0.21$). Simple main effects analysis revealed a signifi-

cantly reduced aeGBR amplitude due to the application of S-ketamine following both placebo ($p_{adjusted} < 0.001$, 95% CI [−0.203, −0.086]) and glycine ($p_{adjusted} = 0.017$ 95% CI [−0.126, −0.014]) pretreatment. Glycine-pretreatment led to an increased aeGBR only when preceding the application of S-ketamine ($p_{adjusted} = 0.004$, 95% CI [0.022, 0.102]) but not the application of placebo ($p_{adjusted} = 0.718$, 95% CI [−0.058, 0.083]) (Fig. 3A).

Regarding the PLF, neither the interaction between ketamine and glycine ($F(1,23) = 3.6$, $p = 0.071$, $\eta_p^2 = 0.14$) nor the ketamine main effect ($F(1,23) = 4.2$, $p = 0.053$, $\eta_p^2 = 0.15$) nor the glycine main effect ($F(1,23) = 2.3$, $p = 0.141$, $\eta_p^2 = 0.09$) reached statistical significance (Fig. 3C).

### 3.4. LORETA whole head analysis

The reduction of the aeGBR due to administration of S-ketamine (Pla-Pla minus Pla-Ket) involved a significant reduction of gamma activity (30–50 Hz) within the ACC (4 voxels within Brodmann areas 24 and 33, $t > 3.436$, $p_{adjusted} < 0.05$, Fig. 4A). Glycine-pretreatment before S-ketamine administration increased the activity of this aeGBR source compared to placebo-pretreatment (Gly-Ket minus Pla-Ket) (Brodmann area 33, $t = 3.85$, $p_{adjusted} = 0.021$, Fig. 4B).

3.5. Association between neurophysiological and psychopathological variables

To assess the association between neurophysiological (aeGBR) and psychopathological (PANSS negative score) changes following the application of S-ketamine, we compared the Pla-Ket and the Pla-Pla condition. The correlation analysis revealed that increases of the PANSS negative score were negatively correlated with the relative reduction of the aeGBR under the influence of S-ketamine (Pearson's $r = −0.53$, $p = 0.008$) (Fig. 5A).

When contrasting the pretreatments of both ketamine sessions (Gly-Ket minus Pla-Ket) to assess the effect of glycine on this association, the reduction of the PANSS negative score correlated with the relative increase of the aeGBR after pretreatment with glycine (Pearson's $r = −0.67$, $p < 0.001$) (Fig. 5B).

In an explorative post-hoc analysis, we divided all individuals into two groups with regard to their glycine-dependent reduction of the PANSS negative score comparing both ketamine sessions, using a clinically significant reduction (of > 5.5 points corresponding to the scale intervals of the Clinical Global Impressions-Severity (CGI-S) rating) as the
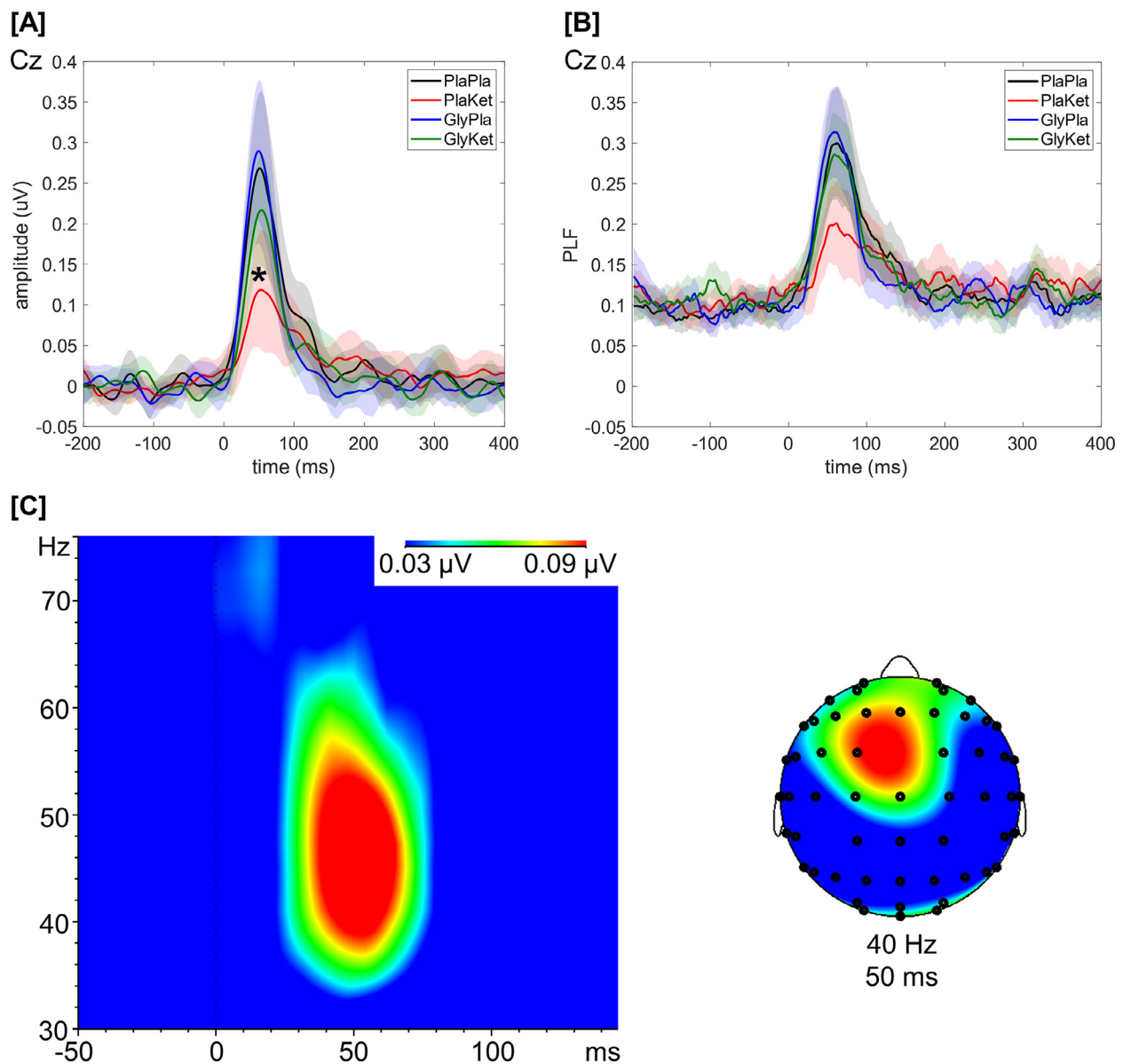
Fig. 3. The aeGBR is an increased activity about 50 ms after stimulus presentation. The aeGBR amplitude [A] and phase-locking factor (PLF) [B] are displayed as the results of the wavelet analysis focused on the activity around 40 Hz for the Pla-Pla condition (black line), Pla-Ket (red line), Gly-Pla (blue line) and the Gly-Ket condition (green line). The shaded areas depict the corresponding 95% confidence intervals of each condition. The aeGBR amplitude was significantly altered by an interaction between glycine and S-ketamine as well as S-ketamine alone [A] while the PLF was not significantly affected by glycine or S-ketamine [B]. The time–frequency analysis of the mean difference (Gly-Ket minus Pla-Ket) of the auditory evoked gamma-band response (aeGBR) amplitude and the corresponding topography at 40 Hz and 50 ms after stimulus presentation [C]. (*$p_{adjusted} < 0.05$).

cut-off (Leucht et al., 2019). Employing binary logistic regression, the relative increase of the aeGBR (ΔaeGBR) predicted a clinically relevant attenuation of the PANSS negative score comparing both ketamine conditions (Chi-Square = 13.91, df = 1, $p < 0.001$). This model determined a ΔaeGBR increase of 64,5% as the cut-off, which correctly predicted 18 out of 19 (94.7%) cases where there was no clinically significant attenuation of the PANSS negative score, and four out of five (80%) cases where there was a significant attenuation.

4. Discussion

We have presented evidence that pretreatment with glycine mitigates ketamine-induced impairments of the aeGBR. Furthermore, we have demonstrated that the relative changes of the aeGBR correlate with the severity of schizophrenia-like symptoms associated with the administration of ketamine. While glycine-pretreatment did not affect behavioral performance and PANSS factor scores at the group level, the aeGBR amplitude allowed us to identify individuals with a relevant psychopathological benefit from the pretreatment with glycine.

Cortical and subcortical GBO both at rest or task-driven (e.g. aeGBR) are critically involved in cognitive functions such as working memory (Howard et al., 2003; van Vugt et al., 2010) and are found to be altered in schizophrenia (Dienel and Lewis, 2019; Uhlhaas and Singer, 2010). The early aeGBR is known to be reduced across all stages of schizophrenia (Leicht et al., 2015, 2010) and corresponding impairments can be found in the ketamine model (Curic et al., 2019). Moreover, the activity of the aeGBR generators within the dACC is reduced in patients with schizophrenia (Leicht et al., 2015, 2010) and in healthy subjects following the acute infusion of S-ketamine (Curic et al., 2019). Accordingly, we were able to replicate these findings in the present study.
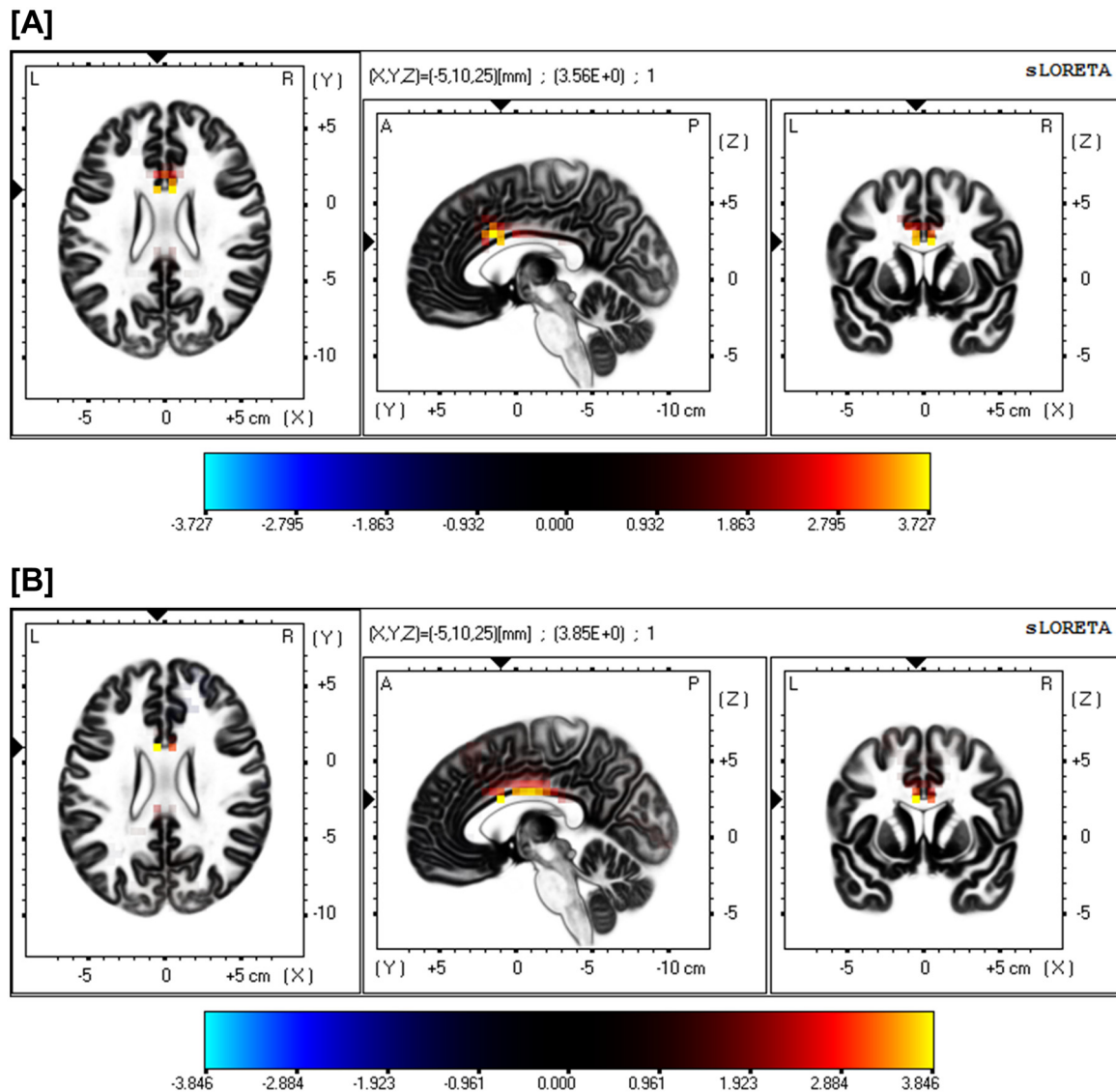
Fig. 4. Difference map of low-resolution brain electromagnetic tomography (LORETA) source activity in the gamma-frequency band (30–50 Hz) contrasting the current source density (CSD) of the aeGBR (30 – 70 ms) between the Pla-Pla and the Pla-Ket conditions [A] as well as the Gly-Ket and the Pla-Ket conditions [B]. Yellow voxels depict a significantly increased CSD in the anterior cingulate cortex (ACC) comprising the Brodmann areas 24 [A] and 33 [A and B].

Based on these observations the aeGBR has been proposed as a correlate to disrupted glutamatergic neurotransmission in schizophrenia, since the generation of oscillations in the gamma frequency range depends on a feedback-loop encompassing pyramidal cells and GABAergic interneurons (Lisman et al., 2008). This premise is further supported by our finding that the alleviation of ketamine-related aeGBR impairments follows the modulation of glutamatergic neurotransmission through the NMDAR co-agonist glycine. Yet, the effect of glycine on humans had neither been studied in the ketamine model of schizophrenia (Haaf et al., 2018) nor in light of aberrant GBO. Regarding another neurophysiological measure, the acute administering of glycine normalized the reduced duration mismatch negativity (MMN) amplitudes in patients suffering from schizophrenia (Greenwood et al., 2018). This was paralleled by findings of an improved frequency MMN after continuous treatment with D-serine (another NMDAR glycine-binding site agonist) (Kantrowitz et al., 2018). Further, MMN-deficits can be observed in rodents following the application of ketamine and can be attenuated through the application of glycine (Lee et al., 2018). These findings support the translational application of electrophysiological biomarkers, which could facilitate drug development (Javitt et al., 2020).

In the aforementioned feedback-loop, PV$^+$ and SST$^+$ interneurons significantly contribute to the model of NMDAR-dysfunction in schizophrenia, since these subtypes express high densities of NMDARs containing either GluN2C (PV$^+$) or GluN2D (SST$^+$) subunits for both of which ketamine demonstrates a higher affinity under physiological conditions compared to other subunits (Bygrave et al., 2019). Conversely, both types of interneurons are reduced in schizophrenia according to postmortem studies (Konradi et al., 2011). One might speculate that the disruption of inhibitory feedback might lead to an excessive yet uncoordinated downstream release of glutamate through pyramidal cells, causing an imbalance of glutamatergic excitation and GABAergic inhibition (E/I imbalance) (Kehrer et al., 2008). This offers a feasible explanation for the counterintuitive observation that the acute administering of racemic ketamine increases extracellular glutamate levels in several brain regions assessed by means of 1H-MRS (Stone et al., 2012), paralleled by findings in schizophrenia (Merritt et al., 2016). Further, the reports of increased resting-state gamma band oscillations in schizophrenia (Andreou et al., 2015; Baradits et al., 2019) as well as corresponding findings in the ketamine model (Bianciardi and Uhlhaas, 2021) – seemingly contradicting the notion of a hypo-glutamatergic state – might be
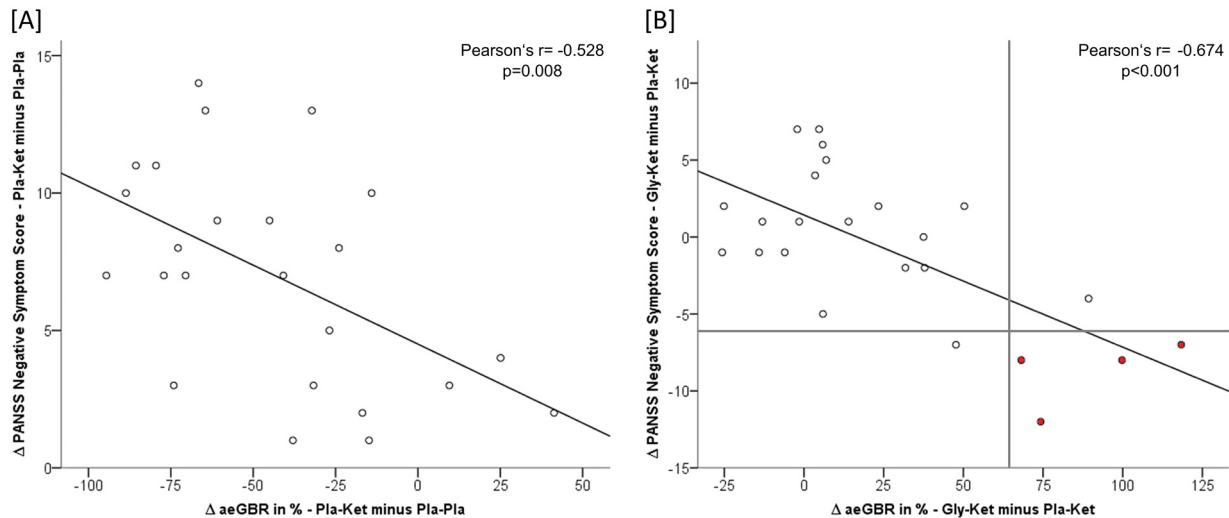
Fig. 5. Negative correlation between [A] the relative changes of the aeGBR and changes of the PANSS negative factor comparing the Pla-Ket and the Pla-Pla conditions (Pearson's $r = -0.528$ $p = 0.008$) as well as [B] the Gly-Ket and the Pla-Ket conditions (Pearson's $r = -0.674$ $p < 0.001$). A 64.5% relative increase of the aeGBR (vertical line) correctly identifies four out of five individuals with a clinically relevant glycine response (red dots) of the PANSS negative factor score (horizontal line, > 5.5 points corresponding to the scale intervals of the Clinical Global Impressions-Severity (CGI-S)).

attributed to a putative lack of synchronized neuronal activity. Hence, the concurrent inhibition of PV[+] and SST[+] GABAergic interneurons offers an explanation implementing both the incapacity to increase GBO following a cognitive-demanding stimulus and elevated GBO at rest in light of the E/I imbalance resembling a reduced signal/noise ratio.

Accordingly, animal studies found the ability to increase stimulus-evoked gamma to be diminished ensuing global disruption of NMDAR signaling or the administering of NMDAR-antagonists or selective reduction of PV[+]-cell activity by means of either optogenetic methods (Sohal et al., 2009) or gene knockout (Bygrave et al., 2019). Whereas, the results of animal studies focusing on SST[+] interneurons remain limited and inconclusive (Alherz et al., 2017). Nonetheless, a dysfunction restricted to a specific neuron subtype is likely not enough to explain the pathophysiology and complex neurophysiological changes seen in schizophrenia (Bygrave et al., 2019).

Bearing all considerations outlined above in mind, the association between an impaired early aeGBR and the emergence of schizophrenia-like negative symptoms as seen in this study leads to the presumption that glutamatergic dysfunction, aberrant neural oscillations, and negative symptoms are interdependent. Accordingly, our results contribute to a growing body of evidence that suggests an interrelation of impaired evoked GBO in a cognitive demanding task based on NMDAR dysfunction and negative symptoms as reported in both the ketamine model (Curic et al., 2019) and schizophrenia itself (Leicht et al., 2015). These assumptions are further substantiated by our discovery that the NMDAR co-agonist glycine mitigates ketamine-related aeGBR impairments which in turn presents as a recovery of PANSS negative scores.

Remarkably, four out of five individuals showing a clinically relevant attenuation of the PANSS negative score (Leucht et al., 2019) following glycine-pretreatment could be identified post-hoc by the relative aeGBR-increase with a high specificity and sensitivity (Fig. 5B). Hence, the aeGBR could serve as a putative biomarker for target-engagement of glutamatergic remedies in schizophrenia and could help to identify individuals who might benefit from the corresponding treatment. Insufficient target-engagement or competing target-engagement of co-administered medication (i.e. clozapine) might explain the varying results of glycine and D-serine treatments in schizophrenia (Kantrowitz, 2017). Nevertheless, further verification of our observations is required given the heterogeneous response to the paradigm and considering we are the first to report a mitigating effect of a glutamatergic substance on psychopatho-

logical and neurophysiological aberrations in the ketamine model of schizophrenia.

An extensive body of research supports the acute application of ketamine to healthy subjects as a pharmacological model of schizophrenia given that it produces changes in behavior, clinical symptoms, as well as neurophysiology that mimic changes seen in patients (Frohlich and Van Horn, 2014; Moghaddam and Javitt, 2012). In line with these findings, we observed schizophrenia-like symptoms, assessed by means of the PANSS, accompanied by increases in reaction time and error rates following the application of S-ketamine. Still, our results were tested in healthy subjects using a disease model and require a replication in patients with schizophrenia. Glycine-pretreatment however, paralleled by unsuccessful clinical trials of glutamatergic agents (Kantrowitz, 2017), did not reverse these effects at the group level, while clearly mitigating ketamine-associated aeGBR reductions. Nonetheless, glycine-pretreatment reduced PANSS negative scores in the subset of individuals that also demonstrated a profound increase of the aeGBR, whilst no individuals with an increase of the PANSS negative factor by more than two points responded to glycine-pretreatment regarding the aeGBR.

Finally, our study was limited by several factors that merit consideration. First, the small sample size of the experiment represents an important limitation, possibly contributing to the fact that we did not observe a mitigating effect of glycine on behavioral results (e.g., error rate or reaction time) due to medium or small effect sizes. Moreover, we acknowledge that the results of our post-hoc binary logistic regression analysis demand replication with a larger sample size. Secondly, the homogenous group of participants (male sex and highly educated) limits the generalizability of our results and warrants replication with a larger and more heterogeneous subject collective. Thirdly, the psychotomimetic effects of S-ketamine could have led to the partial unblinding of participants. While blinding for the pretreatment was thoroughly realized, further studies could implement dopaminergic agonists or non-glutamatergic psychotomimetic agents in place of saline infusions as the control for ketamine.

In conclusion, this is the first study to report a normalizing effect of glycine, an NMDAR co-agonist, on ketamine-related decreases of the aeGBR peak amplitude and on the activity of its generator located in the ACC in healthy human subjects during the performance of a cognitively demanding auditory choice reaction task. Further, we found an association between glycine-related aeGBR increases and improvements in

schizophrenia-like negative symptoms. Remarkably, an increase of the aeGBR predicted a clinically relevant psychopathological response to glycine-pretreatment. This points to the applicability of the aeGBR as a putative rapid biomarker for responders to a glutamatergic therapy for negative symptoms. To this end, our intriguing results call for the investigation of the transferability of this effect to patients suffering from schizophrenia. Prior to a clinical trial we suggest a proof-of-concept investigation of glycine-effects concerning gamma oscillations in a small to medium sample of patients with schizophrenia as being the next logical step.

Author contributions

Moritz Haaf: Data – acquisition, analysis, and interpretation, Writing – original draft, review and editing **Stjepan Curic**: Data – acquisition and analysis, Writing – review and editing **Saskia Steinmann**: Data – analysis, Writing – review and editing **Jonas Rauh:** Data – analysis, Writing – review and editing **Gregor Leicht:** Conceptualization, Supervision, Data – analysis and interpretation, Writing – review and editing **Christoph Mulert:** Conceptualization, Supervision, Data – interpretation, Writing – review and editing.

Data and software availability

Data and task paradigm-sets may be provided to interested researchers upon reasonable request to the corresponding author and approval of a data-sharing agreement.

The following software used in our analyses is publicly available: Brain Vision Analyzer 2.0 (Brain Products, www.brainproducts.com), LORETA-KEY software (http://www.uzh.ch/keyinst/loreta), G*Power 3.1 (https://www.psychologie.hhu.de/arbeitsgruppen/allgemeine-psychologie-und-arbeitsgruppen/gpower).

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Declaration of Competing Interest

None.

References

Alherz, F., Alherz, M., Almusawi, H., 2017. NMDAR hypofunction and somatostatin-expressing GABAergic interneurons and receptors: a newly identified correlation and its effects in schizophrenia. Schizophr. Res. Cogn. 8, 1–6.

Andreou, C., Nolte, G., Leicht, G., Polomac, N., Hanganu-Opatz, I.L., Lambert, M., Engel, A.K., Mulert, C., 2015. Increased resting-state gamma-band connectivity in first-episode Schizophrenia. Schizophr. Bull. 41, 930–939.

Baradits, M., Kakuszi, B., Balint, S., Fullajtar, M., Mod, L., Bitter, I., Czobor, P., 2019. Alterations in resting-state gamma activity in patients with schizophrenia: a high-density EEG study. Eur. Arch. Psychiatry Clin. Neurosci. 269, 429–437.

Bianciardi, B., Uhlhaas, P.J., 2021. Do NMDA-R antagonists re-create patterns of spontaneous gamma-band activity in schizophrenia? A systematic review and perspective. Neurosci. Biobehav. Rev. 124, 308–323.

Buzsaki, G., Wang, X.J., 2012. Mechanisms of gamma oscillations. Annu. Rev. Neurosci. 35, 203–225.

Bygrave, A.M., Kilonzo, K., Kullmann, D.M., Bannerman, D.M., Kätzel, D., 2019. Can N-methyl-D-aspartate receptor hypofunction in Schizophrenia be localized to an individual cell type? Front Psychiatry 10, 835 -835.

Carlen, M., Meletis, K., Siegle, J.H., Cardin, J.A., Futai, K., Vierling-Claassen, D., Ruhlmann, C., Jones, S.R., Deisseroth, K., Sheng, M., Moore, C.I., Tsai, L.H., 2012. A critical role for NMDA receptors in parvalbumin interneurons for gamma rhythm induction and behavior. Mol. Psychiatry 17, 537–548.

Chang, C.H., Lin, C.H., Liu, C.Y., Chen, S.J., Lane, H.Y., 2020. Efficacy and cognitive effect of sarcosine (N-methylglycine) in patients with schizophrenia: a systematic review and meta-analysis of double-blind randomised controlled trials. J. Psychopharmacol. 34, 495–505.

Cho, R.Y., Konecky, R.O., Carter, C.S., 2006. Impairments in frontal cortical gamma synchrony and cognitive control in schizophrenia. Proc. Natl. Acad. Sci. U.S.A. 103, 19878–19883.

Correll, C.U., Schooler, N.R., 2020. Negative symptoms in Schizophrenia: a review and clinical guide for recognition, assessment, and treatment. Neuropsychiatr. Dis. Treat. 16, 519–534.

Curic, S., Leicht, G., Thiebes, S., Andreou, C., Polomac, N., Eichler, I.C., Eichler, L., Zolliner, C., Gallinat, J., Steinmann, S., Mulert, C., 2019. Reduced auditory evoked gamma-band response and schizophrenia-like clinical symptoms under subanesthetic ketamine. Neuropsychopharmacology 44, 1239–1246.

Dienel, S.J., Lewis, D.A., 2019. Alterations in cortical interneurons and cognitive function in schizophrenia. Neurobiol. Dis. 131, 104208.

Faul, F., Erdfelder, E., Lang, A.G., Buchner, A., 2007. G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behav. Res. Methods 39, 175–191.

Frohlich, J., Van Horn, J.D., 2014. Reviewing the ketamine model for schizophrenia. J. Psychopharmacol. 28, 287–302.

Greenwood, L.M., Leung, S., Michie, P.T., Green, A., Nathan, P.J., Fitzgerald, P., Johnston, P., Solowij, N., Kulkarni, J., Croft, R.J., 2018. The effects of glycine on auditory mismatch negativity in schizophrenia. Schizophr. Res. 191, 61–69.

Haaf, M., Leicht, G., Curic, S., Mulert, C., 2018. Glutamatergic Schizophrenia - biomarkers and pharmacological interventions within the ketamine model. Curr. Pharm. Biotechnol. 19, 293–307.

Herrmann, C.S., Frund, I., Lenz, D., 2010. Human gamma-band activity: a review on cognitive and behavioral correlates and network models. Neurosci. Biobehav. Rev. 34, 981–992.

Howard, M.W., Rizzuto, D.S., Caplan, J.B., Madsen, J.R., Lisman, J., Aschenbrenner-Scheibe, R., Schulze-Bonhage, A., Kahana, M.J., 2003. Gamma oscillations correlate with working memory load in humans. Cerebral Cortex 13, 1369–1374.

Jadi, M.P., Behrens, M.M., Sejnowski, T.J., 2016. Abnormal gamma oscillations in N-methyl-D-aspartate receptor hypofunction models of Schizophrenia. Biol. Psychiatry 79, 716–726.

Javitt, D.C., Siegel, S.J., Spencer, K.M., Mathalon, D.H., Hong, L.E., Martinez, A., Ehlers, C.L., Abbas, A.I., Teichert, T., Lakatos, P., Womelsdorf, T., 2020. A roadmap for development of neuro-oscillations as translational biomarkers for treatment development in neuropsychopharmacology. Neuropsychopharmacology 45, 1411–1422.

Kantrowitz, J., 2017. Managing negative symptoms of Schizophrenia: how far have we come? CNS Drugs 31, 373–388.

Kantrowitz, J.T., Epstein, M.L., Lee, M., Lehrfeld, N., Nolan, K.A., Shope, C., Petkova, E., Silipo, G., Javitt, D.C., 2018. Improvement in mismatch negativity generation during d-serine treatment in schizophrenia: correlation with symptoms. Schizophr. Res. 191, 70–79.

Kay, S.R., Fiszbein, A., Opler, L.A., 1987. The positive and negative syndrome scale (PANSS) for schizophrenia. Schizophr. Bull. 13, 261–276.

Kehrer, C., Maziashvili, N., Dugladze, T., Gloveli, T., 2008. Altered excitatory-inhibitory balance in the NMDA-hypofunction model of Schizophrenia. Front. Mol. Neurosci. 1, 6.

Khlestova, E., Johnson, J.W., Krystal, J.H., Lisman, J., 2016. The role of GluN2C-containing NMDA receptors in ketamine's psychotogenic action and in schizophrenia models. J. Neurosci. 36, 11151–11157.

Konradi, C., Yang, C.K., Zimmerman, E.I., Lohmann, K.M., Gresch, P., Pantazopoulos, H., Berretta, S., Heckers, S., 2011. Hippocampal interneurons are abnormal in schizophrenia. Schizophr. Res. 131, 165–173.

Kotermanski, S.E., Johnson, J.W., 2009. Mg2+ imparts NMDA receptor subtype selectivity to the Alzheimer's drug memantine. J. Neurosci. 29, 2774–2779.

Krogmann, A., Peters, L., von Hardenberg, L., Bodeker, K., Nohles, V.B., Correll, C.U., 2019. Keeping up with the therapeutic advances in schizophrenia: a review of novel and emerging pharmacological entities. CNS Spectr. 24, 38–69.

Krystal, J.H., Karper, L.P., Seibyl, J.P., Freeman, G.K., Delaney, R., Bremner, J.D., Heninger, G.R., Bowers Jr., M.B., Charney, D.S., 1994. Subanesthetic effects of the noncompetitive NMDA antagonist, ketamine, in humans. Psychotomimetic, perceptual, cognitive, and neuroendocrine responses. Arch. Gen. Psychiatry 51, 199–214.

Kumar, J., Liddle, E.B., Fernandes, C.C., Palaniyappan, L., Hall, E.L., Robson, S.E., Simmonite, M., Fiesal, J., Katshu, M.Z., Qureshi, A., Skelton, M., Christodoulou, N.G., Brookes, M.J., Morris, P.G., Liddle, P.F., 2020. Glutathione and glutamate in schizophrenia: a 7T MRS study. Mol. Psychiatry 25, 873–882.

Lahti, A.C., Koffel, B., LaPorte, D., Tamminga, C.A., 1995. Subanesthetic doses of ketamine stimulate psychosis in schizophrenia. Neuropsychopharmacology 13, 9–19.

Lee, M., Balla, A., Sershen, H., Sehatpour, P., Lakatos, P., Javitt, D.C., 2018. Rodent mismatch negativity/theta neuro-oscillatory response as a translational neurophysiological biomarker for N-methyl-D-aspartate receptor-based new treatment development in Schizophrenia. Neuropsychopharmacology 43, 571–582.

Leeson, P.D., Iversen, L.L., 1994. The glycine site on the NMDA receptor: structure-activity relationships and therapeutic potential. J. Med. Chem. 37, 4053–4067.

Leicht, G., Andreou, C., Polomac, N., Lanig, C., Schottle, D., Lambert, M., Mulert, C., 2015. Reduced auditory evoked gamma band response and cognitive processing deficits in first episode schizophrenia. World J. Biol. Psychiatry 1–11.

Leicht, G., Karch, S., Karamatskos, E., Giegling, I., Moller, H.J., Hegerl, U., Pogarell, O., Rujescu, D., Mulert, C., 2011. Alterations of the early auditory evoked gamma-band response in first-degree relatives of patients with schizophrenia: hints to a new intermediate phenotype. J. Psychiatr. Res. 45, 699–705.

Leicht, G., Kirsch, V., Giegling, I., Karch, S., Hantschk, I., Moller, H.J., Pogarell, O., Hegerl, U., Rujescu, D., Mulert, C., 2010. Reduced early auditory evoked gamma-band response in patients with schizophrenia. Biol. Psychiatry 67, 224–231.

Leicht, G., Vauth, S., Polomac, N., Andreou, C., Rauh, J., Mussmann, M., Karow, A., Mulert, C., 2016. EEG-informed fMRI reveals a disturbed gamma-band-specific network in subjects at high risk for psychosis. Schizophr. Bull. 42, 239–249.

Leucht, S., Barabassy, A., Laszlovszky, I., Szatmari, B., Acsai, K., Szalai, E., Harsanyi, J., Earley, W., Nemeth, G., 2019. Linking PANSS negative symptom scores with the Clin-

ical Global Impressions Scale: understanding negative symptom scores in schizophrenia. Neuropsychopharmacology 44, 1589–1596.

Lisman, J.E., Coyle, J.T., Green, R.W., Javitt, D.C., Benes, F.M., Heckers, S., Grace, A.A., 2008. Circuit-based framework for understanding neurotransmitter and risk gene interactions in schizophrenia. Trends Neurosci. 31, 234–242.

McCutcheon, R.A., Krystal, J.H., Howes, O.D., 2020. Dopamine and glutamate in schizophrenia: biology, symptoms and treatment. World Psychiatry (WPA) 19, 15–33.

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

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

Mulert, C., Leicht, G., Hepp, P., Kirsch, V., Karch, S., Pogarell, O., Reiser, M., Hegerl, U., Jager, L., Moller, H.J., McCarley, R.W., 2010. Single-trial coupling of the gamma-band response and the corresponding BOLD signal. Neuroimage 49, 2238–2247.

Mulert, C., Leicht, G., Pogarell, O., Mergl, R., Karch, S., Juckel, G., Moller, H.J., Hegerl, U., 2007. Auditory cortex and anterior cingulate cortex sources of the early evoked gamma-band response: relationship to task difficulty and mental effort. Neuropsychologia 45, 2294–2306.

Pascual-Marqui, R.D., 2002. Standardized low-resolution brain electromagnetic tomography (sLORETA): technical details. Methods Find. Exp. Clin. Pharmacol. 24, 5–12.

Reid, M.A., Salibi, N., White, D.M., Gawne, T.J., Denney, T.S., Lahti, A.C., 2019. 7T Proton magnetic resonance spectroscopy of the anterior cingulate cortex in first-episode Schizophrenia. Schizophr. Bull. 45, 180–189.

Roach, B.J., Mathalon, D.H., 2008. Event-related EEG time-frequency analysis: an overview of measures and an analysis of early gamma band phase locking in schizophrenia. Schizophr. Bull. 34, 907–926.

Sohal, V.S., Zhang, F., Yizhar, O., Deisseroth, K., 2009. Parvalbumin neurons and gamma rhythms enhance cortical circuit performance. Nature 459, 698–702.

Spencer, K.M., Salisbury, D.F., Shenton, M.E., McCarley, R.W., 2008. Gamma-band auditory steady-state responses are impaired in first episode psychosis. Biol. Psychiatry 64, 369–375.

Stone, J.M., Dietrich, C., Edden, R., Mehta, M.A., De Simoni, S., Reed, L.J., Krystal, J.H., Nutt, D., Barker, G.J., 2012. Ketamine effects on brain GABA and glutamate levels with 1H-MRS: relationship to ketamine-induced psychopathology. Mol. Psychiatry 17, 664–665.

Takayanagi, Y., Kulason, S., Sasabayashi, D., Takahashi, T., Katagiri, N., Sakuma, A., Obara, C., Nakamura, M., Kido, M., Furuichi, A., Nishikawa, Y., Noguchi, K., Matsumoto, K., Mizuno, M., Ratnanather, J.T., Suzuki, M., 2017. Reduced thickness of the anterior cingulate cortex in individuals with an at-risk mental state who later develop psychosis. Schizophr. Bull. 43, 907–913.

Traub, R.D., Whittington, M.A., Stanford, I.M., Jefferys, J.G.R., 1996. A mechanism for generation of long-range synchronous fast oscillations in the cortex. Nature 383, 621–624.

Uhlhaas, P.J., Singer, W., 2010. Abnormal neural oscillations and synchrony in schizophrenia. Nat. Rev. 11, 100–113.

Umbricht, D., Alberati, D., Martin-Facklam, M., Borroni, E., Youssef, E.A., Ostland, M., Wallace, T.L., Knoflach, F., Dorflinger, E., Wettstein, J.G., Bausch, A., Garibaldi, G., Santarelli, L., 2014. Effect of bitopertin, a glycine reuptake inhibitor, on negative symptoms of schizophrenia: a randomized, double-blind, proof-of-concept study. JAMA Psychiatry 71, 637–646.

van der Gaag, M., Hoffman, T., Remijsen, M., Hijman, R., de Haan, L., van Meijel, B., van Harten, P.N., Valmaggia, L., de Hert, M., Cuijpers, A., Wiersma, D., 2006. The five-factor model of the Positive and Negative Syndrome Scale II: a ten-fold cross-validation of a revised model. Schizophr. Res. 85, 280–287.

van Vugt, M.K., Schulze-Bonhage, A., Litt, B., Brandt, A., Kahana, M.J., 2010. Hippocampal gamma oscillations increase with memory load. J. Neurosci. 30, 2694–2699.

Whittington, M.A., Traub, R.D., 2003. Interneuron diversity series: inhibitory interneurons and network oscillations in vitro. Trends Neurosci. 26, 676–682.