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Electrophysiological Intermediate Biomarkers for Oxidative Stress in Schizophrenia

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Abstract

Objective—Diverse electrophysiological abnormalities have been associated with schizophrenia, but the underlying causes remain elusive. We tested whether the altered oxidative stress in schizophrenia contributes to the electrophysiological abnormalities.

Methods—We used an auditory oddball task to measure mismatch negativity (MMN) and gamma band response on 29 schizophrenia patients and 25 normal controls. Oxidative stress was assessed by monomeric glutathione (GSH, reduced form) and glutathione disulfide (GSSG, oxidized form).

Results—Patients had reduced MMN ($p=0.015$) and reduced power of gamma band responses at 21–40 Hz and 41–85 Hz (all $p<0.001$). GSH was significantly lower ($p<0.001$) while %GSSG was higher ($p=0.023$) in patients compared with controls. MMN was correlated with GSH in controls; while 21–40 Hz responses were correlated with GSH in patients. Lower GSH and higher GSSG levels were associated with low community functioning ($p=0.018$). Multivariate mediation modeling showed that gamma band at 21–40 Hz was a significant mediator for GSH effect on community functions.

Conclusions—High beta/low gamma range (21–40 Hz) responses may be an intermediate biomarker indexing oxidative stress and its effect on clinical functions.

Significance—Electrophysiological abnormalities and associated clinical functional changes may in part be associated with heightened oxidative stress in schizophrenia.

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Keywords

schizophrenia; oxidation; oscillations; MMN; GSH; GSSG

INTRODUCTION

Electrophysiological abnormalities in specific oscillatory frequencies (Spencer et al., 2004; Light et al., 2006; Cho et al., 2006; Ühlhaas and Singer, 2010) and in event-related potentials (ERPs) such as mismatch negativity (MMN) (Shelley et al., 1991; Umbricht and Krijes 2005; Javitt et al., 1995; Umbricht et al., 2000; Bramon et al., 2004; Hall et al., 2006;) are consistently reported in schizophrenia. Our understanding on the causes of these diverse electrical anomalies is limited. Schizophrenia animal models suggest that behavioral and cognitive deficits may be driven by altered excitatory/inhibitory (E/I) balance in cortical circuits (O'Donnell, 2011), and such balance can be associated with gamma frequency oscillations (Gandal et al., 2012). N-methyl-D-aspartate (NMDA) receptor blockade can yield altered excitation-inhibition balance, primarily by impairing NMDA function in inhibitory interneurons (Homayoun and Moghaddam, 2007). Parvalbumin interneurons stand out as a critical neural population responsible for E/I balance, which is directly involved in the generation of gamma oscillations (Carlen et al., 2012). NMDA receptor function is sensitively indexed by MMN in animals and humans (Javitt et al., 1996 and 2011). MMN is consistently found to be impaired in schizophrenia (Javitt et al., 1993, 1996 and 1998), and this phenomenon requires synchronized gamma activity (Nicol et al., 2012). Parvalbumin interneurons and NMDA receptors also share the common property that they are sensitive to damage from oxidative stress (Gonzalez-Burgos and Lewis, 2012; Choi and Lipton, 2000; Kohr et al., 1994; Nakazawa et al., 2012; Do et al., 2009). In this study, we tested the hypothesis that abnormal electrophysiological markers thought to be indexing NMDA receptors and interneuron functions, are at least in part driven by heightened oxidative stress in schizophrenia. If so, then some abnormal electrophysiological signals may index the intermediate biological processes connecting oxidative stress to schizophrenia clinical presentations.

Heightened oxidative stress in schizophrenia may be due to genetic factors leading to decreased capacity to synthesize antioxidant compounds (Gysin et al., 2007) and/or early developmental insults that can increase generation of reactive oxygen species in the brain (Do et al., 2009). Genetic mouse models with decreased glutathione, a major antioxidant redox substrate in the brain, showed impaired NMDA receptor function, which can be restored by glutathione (Choi and Lipton, 2000; Kohr et al., 1994). Animals with reduced glutathione (GSH) levels also showed pronounced beta and gamma oscillation deficits, secondary to parvalbumin fast spiking interneurons impairment (Steullet et al., 2010). Oxidative stress has been implicated in the schizophrenia pathophysiology. It is possible that altered excitation-inhibition balance can be initiated or exacerbated by increased oxidative stress in the patients. As such, gamma oscillations and MMN could be sensitive tools to evaluate the impact of oxidative stress in the brains of schizophrenia patients. Overall, evidence of increased oxidative stress in schizophrenia is extensive (Yao et al., 2006; Gawryluk et al., 2011; Do et al., 2000; Wood et al., 2009); however it remains to be

determined whether oxidative stress causes or is secondary to other aspects of schizophrenia pathophysiology.

Defense against oxidative stress in the brain relies on the balance between the reduced form of glutathione (GSH) and its oxidized form (GSSG). Genetic and environmental causes for the schizophrenia pathophysiology could heighten oxidative stress in cortical regions during neural development (Do et al., 2009). Post-mortem studies (Yao et al., 2006; Gawryluk et al., 2011) and in-vivo magnetic resonance spectroscopy (MRS) studies revealed reduced GSH levels in schizophrenia patients (Do et al., 2000; Wood et al., 2009). Administration of the glutathione precursor n-acetylcysteine (NAC) improved clinical symptoms and MMN deficits in schizophrenia patients (Lavoie et al., 2008; Berk et al., 2008), as well as improved EEG synchronization (Carmeli et al., 2012). Here, we tested whether gamma band anomalies and MMN deficits, commonly observed in schizophrenia, are correlated with blood levels of glutathione as peripheral measures of oxidative stress. These peripheral measures of oxidative stress have been shown to reflect the extent of brain oxidative stress (Mahadik et al., 2006). We further tested this hypothesis by modeling how GSH and GSSG abnormalities may be linked to clinical functions through neuronal mechanisms as indexed by MMN and gamma band oscillations.

MATERIALS AND METHODS

Participants

We recruited 29 schizophrenia patients and 25 normal controls, who were not significantly different in age, gender, or smoking status (Table 1). Patients were recruited from the outpatient clinics of the Maryland Psychiatric Research Center and neighboring community clinics. Normal controls were recruited using media advertisements. Major medical and neurological illnesses, history of head injury with cognitive sequelae, mental retardation, substance dependence within the past 6 months, or current substance abuse (except nicotine) were exclusionary. The Structured Clinical Interview for DSM-IV (SCID) was administered to all subjects to obtain DSM-IV diagnoses. Controls had no DSM-IV Axis I diagnosis and no family history of psychosis in 3 generations. Patients were individuals with DSM-IV schizophrenia who were medicated and clinically stable. Patients were on the following medications: 3 patients were on first generation antipsychotic medications (CPZ equivalent of daily dose = 117.1 ± 23.4 mg), the rest were on second generation antipsychotic medications, including 12 on clozapine (368.8 ± 137.5 mg), 3 on aripiprazole (18.3 ± 10.4 mg); 3 on risperidone (6.7 ± 4.6 mg); 2 on olanzapine (10.0 ± 7.1 mg); 2 on quetiapine (800.0 ± 282.8 mg); and the rest on 2 or more second generation antipsychotic medications. All subjects gave written informed consent in accordance with local Institutional Review Board guidelines.

Measurement of GSH and GSSG

Participants were instructed to avoid strenuous physical exercise 24 hours before testing, to fast overnight and not to smoke on the morning of the blood draw. The number of hours of reported fasting before blood draw and ERP was not significantly different between patients and controls (Table 1). A venous whole blood sample was drawn between 8:30AM and

10:30AM. The key step of sample preparation was to prevent GSH, the predominant form of glutathione, to be oxidized into GSSG, so that GSSG can be accurately measured. GSSG samples were mixed with a pyridine derivative thiol-scavenging reagent before freezing. This redox scavenger overcomes shortfalls of other methods associated with undesirable enzyme reactivity or slow rate reaction (Griffith, 1980; Guntherberg, 1966). Scavenger-processed (for GSSG) and unprocessed (for GSH) whole blood samples were immediately stored at -80°C to avoid artifactual oxidation by room temperature and cellular lysis (the blood draw occurred in the freezer room). At the end of the study, all samples were shipped using a secured dry ice container to Oxford Biomedicals, Rochester Hills, MI, USA, who performed GSH and GSSG assays in one batch. The individuals who performed the assays were blind to subject information. The reaction of GSH with Ellman's reagent (5,5'-dithiobis-2-nitrobenzoic acid) gave rise to a product that can be quantified spectrophotometrically at 412 nm. This reaction was used to measure the reduction of GSSG to GSH, and its rate is proportional to GSH and GSSG concentrations (Iwasaki et al., 2009). Given that the predominant form is GSH (Umbricht et al., 2006), GSSG is more meaningful when expressed as %GSSG $[(\text{GSSG}/\text{total glutathione}) \times 100]$, which was the primary measure for GSSG.

ERP recording and analysis

ERP was recorded after blood sample collection, using Neuroscan (Charlotte, NC) SynAmp² 64 channel system at 1 KHz sampling rate with bandpass at 0.1-200 Hz. Subjects sat in a semi-reclining chair inside a sound-attenuated chamber. The paradigm was a duration-deviant oddball task (Magno et al., 2008). Subjects were presented with 1000 auditory stimuli, of which 800 (80%) were standard tones presented at 75 dB, 60-ms, 1000 Hz; 200 (20%) were duration-deviant tones at 75 dB, 150-ms, 1000 Hz. All tones had a 5 ms rise/fall time, with a stimulus onset asynchrony of 300 ms. A nose electrode served as reference. Electrode impedance was kept below 5 k Ω . FZ was used for MMN measurement because this location typically shows the largest MMN (Javitt et al., 2000; Umbricht et al., 2006). Eyeblink artifacts were minimized using a VEOG-based eyeblink spatial filter routine implemented in Neuroscan software. An ICA based algorithm for correction of saccade-related transient potentials was used on each subject to identify and remove microsaccade-related electromyogenic activities (Hassler et al., 2011). Records were then filtered at 0.1-30 Hz (Umbricht et al., 2006) in 24db/octave, epoched, baseline-corrected, threshold-filtered at $\pm 75 \mu\text{V}$ for artifact rejection, followed by visual inspection to exclude any overt artifacts from muscle contraction. Over 90% of standard and deviant trials were retained after these procedures in each subject and there were no statistical significant differences in the number of trials retained between schizophrenia patients and normal controls for either standard or deviant trials (all $p < 0.05$). Standard and deviant trials were averaged separately, followed by a subtraction of the two averaged waveforms. MMN was scored by peak detection within a 100-225 ms post-stimulus window by an automatic algorithm followed by visual inspection to verify correct placement of each marker for peak detection. Scoring was blinded to clinical information.

Single-trial neural oscillatory responses were obtained from the original 0.1-200 Hz recording from FZ, epoched using a 250 ms window from 25-275 ms following standard and

deviant stimuli. This window was wider than the 100-225 ms window used for MMN peak measurement so that we could capture the energy in the waveform. An 8-level Daubechies biorthogonal discrete wavelet transform (DWT) was then applied to each single-trial to decompose the oscillatory activities. Six “details” from this DWT decomposition were used for the following frequency bands: high gamma frequency activities >85 Hz; gamma at 41-85 Hz; low gamma/high beta at 21-40 Hz; beta at 13-20 Hz; theta-alpha at 5-12 Hz; and delta at <5 Hz. Further details of this method have been fully described (Hong et al., 2008 and 2010). Energy within each DWT decomposition was measured by power spectrum density (PSD in dB/Hz) using a nonparametric Welch method (Welch, 1967). The single trial averaging represents the combined stationary and nonstationary energy post-stimulus rather than the time-locked activity as in MMN. Oscillatory measures were scored by algorithms written in Matlab environment without manual scoring.

Clinical and functional assessments

To determine whether GSH and GSSG are clinically related, we used the 20-item Brief Psychiatric Rating Scale (BPRS) to estimate overall symptom severity for schizophrenia patients. To assess functional capacity in all subjects, we used the total score of the University of California San Diego Performance-based Skills Assessment, 2nd edition (UPSA-2), which is a proxy measure of community functional capacity by assessing simulated life and community skills in household chores, communication, finance, transportation, and recreational activities (Patterson et al., 2001; Twamley et al., 2002; Harvey et al., 2007).

Statistical Analysis

ANOVA was used to assess patient-control differences on single dependent measures. A Mann-Whitney U test was used when data did not follow normal distribution. For multiple oscillatory response frequencies, repeated measures ANOVA was used for group comparisons where 6 frequency bands and 2 stimulus types (standard and deviant) were repeated measures. Greenhouse-Geisser corrections were applied. Post-hoc tests examined if patients and controls were significantly different on each frequency band / stimulus type. Bonferroni correction was applied to adjust for multiple comparisons. Correlations were performed using Spearman's Rank Order tests. To reduce multiple comparisons, correlation analyses were performed only if measures were found significantly associated with schizophrenia. All tests were two-tailed.

Contributions of oscillatory responses to MMN were tested using stepwise regression. GSH and GSSG contributions to clinical symptoms and functions were assessed by linear regressions where a clinical assessment was the dependent variable and GSH and GSSG were the predictors. GSSG instead of %GSSG was used in this analysis for maximizing the independence of the two predictors.

To formally test whether electrophysiological measures could be considered “intermediate” markers between oxidative stress and clinical outcome, we performed multivariate mediation analysis (Preacher and Hayes, 2004; Preacher and Kelley, 2011; Hayes, 2012), where a clinical measure was the outcome variable, GSH and GSSG concentration were two

independent predictors, and MMN was the mediator. For neural oscillations, all 6 frequency bands were entered and tested as potential mediators. This model estimates the total, direct, and indirect relation of multiple causal variables (GSH and GSSG) on outcome clinical variable through a proposed mediator (MMN or oscillatory responses at different frequency bands). The mediation analysis estimated whether the direct effect of oxidative stress markers on the clinical outcome was significantly mediated through the indirect effects of the electrophysiological biomarkers. Statistical significance for the mediators was established by bootstrapped 95% confidence intervals (95% CI) with 5000 iterations. To reduce type I error, this analysis is restricted to only those clinical measures that were already directly and significantly related to oxidative stress.

RESULTS

1. Blood oxidative stress markers GSH and %GSSG

Fasting GSH levels were significantly lower in schizophrenia patients compared with controls ($p < 0.001$, Table 1). Patients showed over 5-fold higher %GSSG compared with controls ($p = 0.023$, Table 1). GSH and %GSSG were not significantly correlated to each other in either the controls ($r = 0.03$, $p = 0.90$) or patients ($r = -0.09$, $p = 0.66$). Separating patients into those on clozapine and those on other antipsychotics and comparing them with normal controls, a significant group main effect was found for GSH ($F(2, 53) = 11.29$, $p < 0.001$) but not for %GSSG ($p = 0.12$). Post-hoc tests on GSH showed that lower GSH was present independently in clozapine (559.2 ± 238.3) ($p = 0.005$) and other antipsychotics subgroup (486.4 ± 241.0) ($p < 0.001$) compared with normal controls (800.5 ± 201.7). Increased %GSSG was also present in both clozapine ($10.6 \pm 23.6\%$) and other antipsychotics subgroups ($13.7 \pm 25.3\%$) compared with controls ($2.4 \pm 3.0\%$) although they were not statistically significant ($p = 0.051$ – 0.20). Correlations between antipsychotic CPZ and GSH and %GSSG were not significant ($r = -0.26$ and -0.25 , respectively, $n = 29$, all $p > 0.40$). Clozapine dose was not significantly correlated with GSH level ($r = 0.01$, $p = 0.97$), but significantly and inversely correlated with %GSSG level ($r = -0.65$, $n = 12$, $p = 0.02$) such that higher clozapine doses were associated with lower GSSG. Age was not significantly associated with blood GSH level in schizophrenia patients or controls ($r = -0.21$ – -0.26 , all $p > 0.2$).

2. MMN and correlations with GSH and %GSSG

MMN amplitude was significantly reduced in schizophrenia patients compared to controls ($F = 6.33$, $p = 0.015$, Figure 1). MMN latency was not significantly different between groups (183.2 ± 25.8 ms vs. 175.0 ± 31.5 ms, respectively, $F = 1.07$, $p = 0.31$). MMN amplitude (absolute value) and GSH were significantly correlated in controls (Spearman's $\rho = 0.45$, $n = 25$, $p = 0.024$; Figure 2). This correlation was not significant in patients ($\rho = 0.05$, $n = 29$, $p = 0.79$). %GSSG was not significantly correlated with MMN in either group (all $p > 0.31$).

3. Neural oscillations and correlations with GSH and % GSSG

Single-trial response analysis demonstrated significant stimulus $\times$ group ($F_{(1.0, 5.7)} = 7.50$, $p = 0.008$) and frequency $\times$ group ($F_{(1.9, 22.6)} = 3.14$, $p = 0.050$) interactions but no significant main effect of group ($p = 0.089$) or 3-way interaction ($p = 0.48$). Post-hoc tests showed that

patients had 1) reduced power in 41-85 Hz gamma band during standard ($p<0.001$) and deviant ($p<0.001$) stimuli compared with controls; 2) reduced power in 21-40 Hz gamma band during standard ($p<0.001$) and deviant ($p<0.001$) stimuli compared with controls (Figure 3). These were significant after Bonferroni correction using $p=0.004$ for $n=12$ comparisons (6 bands $\times$ 2 stimulus types). No other frequencies showed between-group differences at this threshold.

Of these significant responses, GSH level was correlated with 41-85 Hz during standard ($\rho=0.42$, $n=29$, $p=0.025$) and deviant ($\rho=0.40$, $p=0.032$) stimuli and also 21-40 Hz during standard ($\rho=0.59$, $p=0.001$) and deviant ($\rho=0.50$, $p=0.005$) stimuli in schizophrenia patients. In normal controls, GSH was significantly correlated only with 41-85 Hz in response to deviant stimulus ($\rho=0.41$, $n=25$, $p=0.042$). The correlations between GSH and 21-40 Hz responses to the standard ($p=0.001$) and deviant ($p=0.005$) stimuli in the patients remained statistically significant after Bonferroni corrections (4 responses $\times$ 2 groups, $p=0.05/8=0.006$). The correlation between GSH and the 21-40 Hz responses during deviant stimuli is plotted in Figure 4. Partial correlation with antipsychotics in CPZ equivalent showed that the correlation between GSH and 21-40 Hz remained about the same ($r=0.56$ for standard and $r=0.47$ for deviant stimuli). %GSSG was not significantly correlated with gamma band measures.

4. Relationship of MMN and neural oscillations

Stepwise regressions were performed where MMN amplitude was the dependent measure and the 12 responses (6 frequency bands $\times$ 2 stimulus types) were the predictors. In controls, gamma band power at 41-85 Hz during standard tone was significantly correlated with MMN amplitude ($F_{(1, 23)}=6.04$, $R^2=20.8\%$, $p=0.022$). Removing this 41-85 Hz response to standard tones, we found that 41-85 Hz responses to deviant tones were similarly and significantly correlated with MMN ($F_{(1, 23)}=6.03$, $R^2=20.8\%$, $p=0.022$). None of the other frequencies was statistically significant. In patients, none of the frequency bands showed significant correlation to MMN.

5. Clinical and functional outcome

GSH and GSSG contributions to BPRS total score in the patients was not significant ($F_{(2, 26)}=0.30$, $p=0.74$). In the regression analysis of GSH and GSSG contributions to UPSA-2 using all subjects, the model was significant ($F_{(2, 49)}=4.36$, $R^2=15.1\%$, $p=0.018$) with tolerable colinearity statistics ($VIF=1.02$). GSH level significantly contributed to higher function ($t=2.08$, $R^2=8.0\%$, $p=0.045$) while GSSG level significantly contributed to lower function ($t=-2.22$, $R^2=7.1\%$, $p=0.031$). Adding group as a dummy variable, GSH no longer significantly contributed to higher function ($p=0.065$) but GSSG still significantly contributed to lower function ($p=0.048$). The results suggest that part of the variance was due to group differences on UPSA-2 and/or oxidative stress measures, although the general relationship between GSH/GSSG and UPSA-2 remained even after considering the group effect. However, this model was not statistically significant when analyzed in controls and patients separately, likely due to smaller sample sizes.

6. Intermediate biomarkers linking oxidative stress to clinical functions

The indirect effect of oscillatory responses as mediators for the significant association between GSH/GSSG and UPSA-2 was tested by a multivariate mediation model. Oscillatory responses to standard and deviant tones were modeled separately to reduce collinearity because they were highly correlated in the respective frequencies. This analysis was only done in the combined sample due to small sample size. For the standard tone (full model depicted in Figure 5), the 21-40 Hz response significantly mediated the effect between GSH and UPSA-2 score (95% CI=0.0015 to 0.040) (red dotted lines). None of the other response frequencies was statistically significant (dark dotted lines). Testing the same model in responses to deviant tone showed a similar effect (not graphed): only the 21-40 Hz response significantly mediated GSH and UPSA-2 (95% CI=0.002 to 0.053).

The indirect effect of MMN as a mediator was also tested: neither GSH (95% CI=-0.0031 to 0.0048) or GSSG (-0.015 to 0.016) was significantly mediated via MMN for their effects on UPSA-2.

DISCUSSION

In this study, we aimed to examine the hypothesis that electrophysiological abnormalities that are frequently observed in schizophrenia patients may be associated with oxidative stress. We found that schizophrenia patients indeed had a severe imbalance in the redox substrates GSH and %GSSG, and significantly reduced 20-85 Hz gamma band responses during the passive auditory oddball task. The oscillatory response in this range was significantly correlated with GSH levels in both controls and schizophrenia patients, suggesting that decreased antioxidative capacity was associated with reduced gamma response.

These findings are largely consistent with the growing evidence of a prominent oxidative stress state in schizophrenia patients, as indicated by reduced GSH and changes in other redox enzymes (Yao et al., 2006; Gawryluk et al., 2011; Do et al., 2000; Wood et al., 2009). Our study provided new evidence that %GSSG was also substantially increased in schizophrenia patients, which was not typically measured in most previous studies. This may indicate an overall reduction in total available glutathione due to impaired synthesis, or over-stretched demand for antioxidative defense. Low GSH has been seen in treatment-naïve or unmedicated schizophrenia patients (Do et al., 2000; Hayes, 2012; Raffa et al., 2011). We also found no significant correlations between antipsychotic in CPZ equivalent and GSH or %GSSG, except a negative correlation between clozapine dose and %GSSG. The significant correlation with %GSSG may be related to a previous finding of a reduced glutathione peroxidase activity in clozapine-treated patients (Miljevic et al., 2010). Dose-dependent inhibition of glutathione peroxidase activity by clozapine might have influenced %GSSG (Yao et al., 2006; Raffa et al., 2011; Dakhale et al., 2004; Kuloglu et al., 2002; Ben Othmen et al., 2008; Ranjekar et al., 2003).

Several cellular and synaptic mechanisms are sensitive to oxidative stress, particularly parvalbumin (PV) interneurons and NMDA receptors (Choi and Lipton, 2000; Kohr et al., 2004; Nakazawa et al., 2012; Do et al., 2009). According to our observations, low GSH

level was correlated with reduced power in 21-40 Hz activities. PV interneurons are characterized by fast-spiking rates that maintain cortical microcircuits through synchronized gamma band oscillations (Sohal et al., 2009). Our analysis supports that oxidative stress may represent one of the mechanisms behind the gamma band dysfunction frequently noted in schizophrenia.

NMDA receptor is also a redox sensitive protein preserved by GSH (Choi and Lipton, 2000; Köhr et al., 1994). We found that MMN and GSH level was significantly and positively correlated, although only in normal controls. This could be viewed as consistent with previous findings that supplement of glutathione by NAC increased MMN (Lavoie et al., 2008). MMN is an established marker for NMDA integrity (Javitt et al., 1996). Mice with NMDA receptor knocked out in parvalbumin interneurons showed altered behaviors and deficits in gamma oscillations (Belforte et al., 2010) and these abnormal interneurons are recently hypothesized to be secondary to oxidative stress (Cabungcal et al., 2012; O'Donnell, 2012; O'Donnell and Sullivan, 2012). Therefore, the severe oxidative stress as indexed by reduced GSH and increased %GSSG, their correlation with gamma oscillations and MMN, support the proposal that these commonly observed ERP abnormalities in schizophrenia may be in part indexing an oxidative stress mechanism.

Our study also demonstrated that GSH and GSSG were associated with the composite functional measure UPSA. Higher GSH predicted higher scores while higher GSSG predicted lower scores, suggesting that an imbalance may be particularly detrimental. UPSA score is lower in schizophrenia patients (Twamley et al., 2002; Harvey et al., 2007) and serves as a strong predictor of community functioning outcome (Green et al., 2011). Mediation modeling effort supported that the 21-40 Hz response acted as an intermediate modulator for the relationship between GSH level and functioning. This finding is in line with results in animal models showing that oxidative stress could cause abnormalities in neurophysiological measures (Do et al., 2009) and subsequent altered social behaviors (Kulak et al., 2012). One important limitation of this analysis is that the mediation analysis was exploratory and under-powered given the small sample size, as the analysis was only possible in the combined sample and may have missed other important oscillatory components due to false negatives. Regardless, this path modeling contributes to translational research development by suggesting that 21-40 Hz gamma frequency response during the simple auditory oddball task is an intermediate biomarker that links oxidative stress to clinical functions.

In summary, we observed an association between oxidative stress in schizophrenia and changes in gamma band responses in the auditory oddball paradigm and clinical functioning. However, our study cannot confirm causality from oxidative stress to electrophysiological abnormalities and functional impairments. Experimental animal models are likely required for that purpose. Our effort only provides a clinically guided, suggestive path to establish such an animal model(s). Finally, the findings support that increased oxidative stress in schizophrenia patients should be considered as one of the important causal factors in studies of clinical electrophysiological anomalies and their treatments in schizophrenia.

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Highlights

1. Our understanding of the causes of the diverse electrophysiological abnormalities found in schizophrenia is limited.
2. Mismatch negativity and gamma band oscillations are found to be associated with oxidative stress in schizophrenia
3. Gamma oscillations may index the glutathione related oxidative stress and its impact on clinical functions.

Figure 1.

A: Grand averages of MMN waveforms in schizophrenia patients and normal controls. B: Reduced MMN amplitude in schizophrenia patients compared with healthy controls (* $p=0.015$).

Figure 2.

A significant correlation between MMN and the reduced form of glutathione (GSH) was observed in normal controls (circle and grey dotted line) (Spearman's $\rho = -0.45$, $p = 0.024$). No such relationship was observed in schizophrenia patients (square and red dotted line) ($r = 0.04$, $p = 0.85$). Black solid line is the regression line for the combined sample.

Figure 3.

Single trial neural oscillatory response to standard (A) and deviant (B) auditory tones. Schizophrenia patients showed reduced gamma band power spectrum density in 21-40 Hz and 41-85 Hz in response to both stimulus types. Y-axis is in log scale. * Significant after Bonferroni correction. PSD: power spectrum density, a measure of the power of oscillatory responses.

Figure 4.

A significant correlation between GSH and 21-40 Hz response to the deviant tone was observed in schizophrenia patients (square and red dotted line) ($\rho=0.50$, $p=0.005$). No such relationship was observed in normal control (circle and grey dotted line) ($\rho=0.18$, $p=0.38$). Black solid line is the regression line for the combined sample. PSD: power spectrum density.

Figure 5.

Multivariate mediation model where neural oscillatory responses to the standard tone were tested as multiple potential mediators between oxidative stress markers GSH and GSSG and everyday function measure UPSA-2. Red solid lines indicate direct and significant effects of GSH and GSSG on UPSA-2. Dotted lines represent the mediation model where the indirect effects were tested. Dark dotted lines indicate no significant indirect (mediating) effect. Red dotted lines indicate significant indirect effects that a biomarker directionally mediated the effect between an oxidative stress marker and the UPSA-2 score. This model showed that the 21-40 Hz oscillatory response during auditory oddball task was a significant intermediate biomarker linking GSH to community function outcome as measured by UPSA-2.

Table 1

Demographic, clinical characteristics, and glutathione levels of the participants.

	Controls (N = 25)	Patients (N = 29)	F or χ^2 values	P values
Age (years)	38.76±13.72	41.10±13.81	0.009	0.55
Sex (male:female)	11:14	20:9	2.48	0.12
Smoker:Nonsmoker	7:18	8:21	0.00	1.00
BPRS score	n/a	43.28±9.93	n/a	n/a
UPSA-2	102.9±10.3	90.7±10.5	17.62	<0.001
Fasting duration (hours)	12.18±2.81	12.48±3.64	0.11	0.74
GSH (μmol)	800.50±201.73	516.50±238.40	21.06	<0.001
GSSG (μmol)	19.96±24.82	27.94±23.19	8.56	0.005
%GSSG	2.40±3.02	12.43±24.18	2.28*	0.023

BPRS: Brief Psychiatric Rating Scale total score. UPSA-2: the University of California San Diego Performance-based Skills Assessment, 2nd edition total score (the data for controls were from 23 subjects because 2 controls did not complete UPSA-2). Values are mean ± sd.

* Based on Mann-Whitney U test.