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Treatment in early psychosis with N-Acetyl-Cysteine for 6 months improves low-level auditory processing: pilot study

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Running title: NAC improves audition in early psychosis

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Abstract

Sensory impairments constitute core dysfunctions in schizophrenia. In the auditory modality, impaired mismatch negativity (MMN) has been observed in chronic schizophrenia and may reflect N-methyl-D-aspartate (NMDA) hypo-function, consistent with models of schizophrenia based on oxidative stress. Moreover, a recent study demonstrated deficits in the N100 component of the auditory evoked potential (AEP) in early psychosis patients. Previous work has shown that add-on administration of the glutathione precursor N-acetyl-cysteine (NAC) improves the MMN and clinical symptoms in chronic schizophrenia. To date, it remains unknown whether NAC also improves general low-level auditory processing and if its efficacy would extend to early-phase psychosis. We addressed these issues with a randomized, double-blind study of a small sample (N=15) of early psychosis (EP) patients and 18 healthy controls from whom AEPs were recorded during an active, auditory oddball task. Patients were recorded twice: once prior to NAC/placebo administration and once after six months of treatment. The N100 component was significantly smaller in patients before NAC administration versus controls. Critically, NAC administration improved this AEP deficit. Source estimations revealed increased activity in the left temporo-parietal lobe in patients after NAC administration. Overall, the data from this pilot study, which call for replication in a larger sample, indicate that NAC improves low-level auditory processing in early psychosis.

1. Introduction

Low-level sensory impairments, both in auditory and in visual processing, seem to constitute part of the core dysfunctions in schizophrenia (Ethridge et al., 2015, Javitt and Freedman, 2015; Javitt, 2009). Increasing evidence indicates that oxidative stress related to glutathione (GSH) synthesis deficits in conjunction with N-methyl-D-aspartate (NMDA) hypofunction are major contributors to the pathophysiology of schizophrenia (Hardingham and Do, 2016). Previous work has shown that add-on administration of the glutathione precursor NAC in chronic schizophrenia patients improves auditory MMN generation to tone deviants (Lavoie et al., 2008) and clinical symptoms (Berk et al., 2008). However, it remains unknown whether NAC can also improve general low-level auditory processing impairments and whether its efficacy extends to the early stages of the disease. The contribution of an impaired antioxidant system in schizophrenia is supported by a variety of findings. Polymorphisms in key genes for GSH synthesis have been associated with schizophrenia (Rodriguez-Santiago et al., 2010; Do et al, 2009; Gysin et al., 2007; Tosic et al., 2006) and are related with decreased GSH levels in the cerebrospinal fluid, prefrontal cortex and post-mortem caudate of patients (Do et al., 2000; Yao et al., 2006; Flatow et al., 2013; Gawryluk et al., 2011; Xin e al., 2016; for a review see Koga et al., 2016; Yao and Keshavan, 2011). GSH-deficient animal models reproduce schizophrenia phenotypes including hypofunction of NMDA receptors (NMDAR) (Steullet et al., 2006). Several studies have demonstrated that the administration of NMDAR agonists induces in healthy controls clinical symptoms as well as sensory processing impairments similar to those observed in schizophrenia and exacerbates these symptoms in patients. Specifically, impairments in the P50, N100, and P300 components of the AEP as well as in oscillatory activity have been observed after acute administration of NMDAR agonists (Chen et al., 2015; Javitt, 2009; Krystal et al., 1994).

MMN, an AEP component generated by deviant stimuli within an oddball paradigm, has been extensively used to measure NMDAR dysfunction in schizophrenia and it is often found decreased in amplitude in both chronic schizophrenia and earlier stages of the disease (Coyle, 2012; Moghaddam and Javitt; 2012; Turetsky et al., 2007). Previous studies have shown that administering NAC to chronic schizophrenia patients led to improved MMN responses (Lavoie et al., 2008), and increased EEG synchronization (Carmeli et al., 2012), in addition to improvement of negative symptoms (Berk et al., 2008). NAC has been used in various studies as a cysteine donor and given orally is quickly absorbed; peak plasma concentration of cysteine is reached within 120

mins (Borgstrom and Kagedal, 1990). NAC crosses the blood-brain barrier, and cysteine can be used in the brain as a GSH precursor (Farr et al., 2003; Conus et al., in revision). Animal studies have previously shown that administration of NAC protects the brain against GSH depletion (Atkuri et al., 2007; Fu et al., 2006; Kamboj et al., 2006) and its neurochemical and morphological consequences (das Neves Duarte et al., 2012; Cabungcal et al. 2013).

Deficits in the P50 and N100 components of the AEPs are well established in patients with schizophrenia (Bodatsch et al., 2015; Brockhaus-Dumke et al., 2008; Rosburg et al., 2008). Schizophrenia patients show a significant diminution in the amplitude of N100, especially for long ISIs (>1 s) (Rosburg et al., 2008; Shelley et al., 1999). Two recent studies using an auditory oddball paradigm demonstrated that psychotic disorder patients show impairments in response to standard/frequent stimuli in both P50 and N100 (Geiser et al., 2017), as well as the alpha-frequency range, the latter of which is thought to reflect deficient thalamo-cortical connectivity (Lee et al., 2017). The low-level AEP deficit observed by Geiser et al. resulted from weaker responses within the left temporo-parietal lobe and it was correlated with peripheral measures of GSH levels, specifically the ratio of glutathione peroxidase and glutathione reductase activities (GPx/GR) which correlates negatively with brain GSH levels in early psychosis patients (Xin et al., 2016). This AEP deficit in response to standard stimuli within the oddball sequence points to impaired input to auditory cortex (Lee et al., 2017). Previous studies have shown that the responses to standard sounds within oddball ERP tasks are impaired not only in chronic schizophrenia, but also in first–episode schizophrenia patients and clinical high-risk individuals (Salisbury et al., 2010; del Re et al., 2015). In addition, Foxe et al. (2011) demonstrated a significant amplitude reduction of the N100 in clinically unaffected first-degree relatives and highlighted the importance of the use of high numbers of trials for the reliable quantification of the evoked responses of interest.

In the present study, we investigated if the effect of NAC on MMN responses also applies to low-level auditory deficits in psychosis, specifically to the reduced N100 response to standard sounds. Additionally, as the group of patients that participated in this study was in the early stages of the disease, we assessed whether the efficacy of NAC can extend to early psychosis (EP). We show for the first time, that NAC administration results in improved low-level auditory processing.

2. Methods and materials

2.1. Clinical Trial Protocol

NAC (2700 mg / day) and placebo were administered to EP patients for 6 months following a double-blinded, randomized design. Electroencephalographic (EEG) recordings and blood sampling were performed at the onset of the protocol (baseline measurements), and at the end of the study (after the 6 months of the NAC administration) (Swiss Medic (2008DR2308), ClinicalTrial.gov. (NCT01354132)).

2.2. Participants

Fifteen patients (13 men, 13 right-handed; aged 26±1.4 years; mean±SEM) meeting criteria for psychosis, as defined by the "Psychosis threshold" subscale of the Comprehensive Assessment of at Risk Mental States scale (CAARMS; Yung et al., 2005) at the baseline participated in this study. The patients were recruited from the Treatment and Early Intervention in Psychosis Program, (TIPP, University Hospital, Lausanne; Baumann et al., 2013), which is a 3 year program specialized in the treatment of early phase of psychosis that included patients that had not received more than 6 months of previous treatment. The diagnosis was confirmed 3 years after the data acquisition (**Table 2**). The participants we report here are thus part of a larger clinical trial (Conus et al., in revision). Only the data from patients that completed the EEG sessions both before and after treatment are reported here. Data from these patients at the onset of the protocol were compared with those from 18 gender and age-matched healthy controls (15 men, 16 right-handed; aged 27.3±2 years) (**Table 1**). Healthy controls were assessed by the Diagnostic Interview for Genetic Studies (Preisig et al., 1999). Major mood, psychotic or substance-use disorder and having a first-degree relative with a psychotic disorder were exclusive criteria for controls. All participants reported normal hearing. All participants provided their written, informed consent, and the procedures were approved by the local Ethics Committee. Some of the data collected in the baseline measurement were reported as part of a study focusing on low-level auditory impairments in EP patients (Geiser et al., 2017).

Among the 15 patients, 8 were among the group that received NAC, and the remaining 7 received placebo. Following their recruitment, patients were given an ID number, and both patients and investigators were blinded until the time of analysis. Patients that received NAC and patients that received placebo did not differ in their clinical and demographic characteristics (**Table 1**).

2.3. Stimuli and task

Participants performed an active oddball detection paradigm. An active task ensured attention to the auditory modality and to the stimulus features. Such attention has been shown to enhance early ERP components (Woldorff et al., 1993). Their task was to press a button on a response pad as fast as possible when they heard infrequent stimuli. The frequent stimulus (70% of trials) was a 1000Hz centrally-presented tone of 100ms duration. The infrequent stimuli (each type constituting 10% of trials) varied in pitch (1200Hz), perceived lateralization (700µs inter-aural timing difference), or duration (150ms). All tones were presented at an intensity of 75 dB SPL at the ear. A central visual fixation cross was present throughout the experiment. All tones were presented with an average inter-stimulus-onset-interval of 916ms jittered with a maximum of ±67ms. Stimuli were presented over insert earphones (Etymotic model ER-4P; www.etymotic.com). Stimulus presentation and response recordings were controlled by E-Prime (www.pstnet.com/eprime).

2.4. EEG acquisition and pre-processing

Continuous EEG was acquired at 1024 Hz through a 64-channel Biosemi ActiveTwo system using the internal CMS/DRL loop as reference (www.biosemi.com). EEG data pre-processing and analyses were performed with Cartool (Brunet et al., 2011) and STEN (http://www.unil.ch/line/home/menuinst/about-the-line/software--analysis-tools.html). Prior to epoching, the EEG was filtered (low-pass 60Hz; high-pass 0.1Hz; 50Hz notch; using a second-order Butterworth filter with -12 db/octave roll-off that was computed linearly in both forward and backward directions to eliminate phase shifts). As the purpose of the current study was to establish whether NAC administration can improve low-level auditory sensory processing, we focused the analysis on AEPs to frequent stimuli. Analyses of MMN will appear in a separate forthcoming article. Peri-stimulus epochs for the frequent stimuli, spanning 100ms pre-stimulus to 500ms post-stimulus were averaged from each participant to calculate the AEPs. Epochs were rejected based on an automated artifact rejection criterion of ±80µV as well as visual inspection for eye blinks, eye movements or other sources of transient noise. Prior to group averaging, data at artefact electrodes from each subject were interpolated (Perrin et al., 1987). In addition, data were baseline corrected using the 100 ms pre-stimulus period and recalculated against the average reference.

For baseline recordings, the average number (± SEM) of accepted epochs was 1594±65 for controls and 1410±85 for patients. These values did not significantly differ. Furthermore, in the baseline the average number of accepted epochs was 1560±102 for patients that later on received NAC treatment and 1230±110 for patients that later on received placebo. Following NAC treatment,

1302±110 trials were included. Following placebo treatment, 1359±168 trials were included. These values did not significantly differ.

2.5. AEP analyses

The AEP data were analysed within an electrical neuroimaging framework. Modulations in AEP strength (using Global Field Power, GFP) and topography (using global dissimilarity) were analysed separately as a function of time (Lehmann and Skrandies, 1980; Murray et al, 2008). Presentation of some voltage waveform data at specific electrodes are also included here as a function of time in order to provide readers with a sense of the general waveform shape and data quality. Analyses of voltage waveforms were not performed quantitatively given that they are reference-dependent (Murray et al., 2008). First, at the baseline, independent samples t-tests were performed between patients and healthy controls in order to verify that this group of patients exhibited impaired N100, as it has been demonstrated in a recent study from our group (Geiser et al., 2017). Second, mixed-model ANOVAs with the patient group as a between-subject factor (NAC vs. placebo) and the time of measurement (Pre vs. Post treatment administration) as a within-subject factor were performed in order to assess whether 6 months of NAC administration improved low-level auditory processing. A temporal criterion for the detection of statistically significant effects was applied (>15 ms continuously at 1024 Hz sampling) in order to correct for temporal auto-correlation (Guthrie and Buchwald, 1991).

2.6. Source estimation and analyses

We estimated the underlying intracranial sources of the AEPs in response to the patient group and the time of measurement using a distributed linear inverse solution (minimum norm) combined with the LAURA (local autoregressive average) regularisation approach (Grave de Peralta Menendez et al., 2001, 2004; see also Michel et al., 2004 for a review). The solution space was calculated on a realistic head model that included 5013 nodes, selected from a grid equally distributed within the gray matter of the Montreal Neurological Institute's average brain (courtesy of Grave de Peralta Menendez and Gonzalez Andino; http://www.electrical-neuroimaging.ch/). The head model and lead field matrix were generated within the Spherical Model with Anatomical Constraints (SMAC; Spinelli et al., 2000) as implemented in Cartool. As an output, LAURA provides current density measures; their scalar values were evaluated at each node. The time periods used for source estimation were determined from the topographic analyses. Statistical analysis of source

estimations was performed by first taking the average of the AEP across the N100 component (which was identified over the 52–140ms interval based on spatial correlation as a function of time (Michel and Murray, 2012). Then, the estimation of the source activity for this averaged time period was performed in two a priori defined regions known to participate in N100 generation, one in the left and one in the right posterior superior temporal cortex. Each of these regions included 49 solution points (see Figure 3). After that an ANOVA for the between subject factor of patient group and the within subject factor of time of measurement was conducted on the source estimations of each region.

3. Results

3.1. Baseline measurements: Controls vs. Patients

Patients compared to controls showed a weaker auditory response to frequent stimuli at P50/N100 and at later time windows (52-140, 163-463ms) as measured qualitatively at individual frontal electrodes (F3 and F4) as well as quantitatively through statistical analysis of GFP (**Figure 1**). This is consistent with the results of Geiser et al., 2017 from which a subgroup of patients was included here. No significant topographic differences were found between groups quantified by global dissimilarity.

3.2.1. Treatment efficacy: NAC vs. Placebo

Group-averaged AEPs for the two treatment groups, before and after treatment are displayed in **Figure 2** from two exemplar frontal electrodes (F3, F4). A mixed-model ANOVA on the GFP as a function of time showed a significant interaction between time of measurement and patient group over the time periods 70-86ms, 100-120ms and 270-371ms. Post-hoc paired t-tests were conducted for each treatment group separately. A significant effect of treatment was observed for the NAC group over the time periods: 75-95, 106-126, 178-198, 283-303 and 312-342ms. No reliable differences were observed for the placebo group. Analysis of dissimilarity revealed no significant topographic differences between the different conditions or groups.

Statistical contrasts of the mean source activity calculated over the 52-140ms time period for the left posterior superior temporal cortex showed a significant interaction between treatment group and time of measurement ($F= 5.11$, $p< .05$, $\eta^2=0.28$) (**Figure3**). Post-hoc t-tests showed that source activity was significantly stronger for the Post-NAC than Pre-NAC treatment within the left temporal

cortex (t=-2.5, p<. 05). In contrast, no differences were found in the placebo group before and after treatment. In the right posterior superior temporal cortex, there was no significant interaction between treatment group and time of measurement (F=0.06, p= 0.8).

4. Discussion

This study demonstrated that administration of the GSH precursor NAC improves early auditory ERP deficits in patients during the early psychosis. It has been previously shown that apart from MMN deficits, there is a general early auditory impairment in psychotic disorder patients, manifested as a significantly weaker response to standard sounds during an auditory oddball paradigm (Geiser et al., 2017; Lee et al., 2017; del Re et al., 2015; Salisbury et al., 2010). This was again the case for our patients at protocol onset; patients' AEPs were significantly weaker than those from healthy controls 52-140ms post-stimulus onset encompassing the P50/N100 complex. This result contrasts with some previous studies that have used a similar auditory oddball paradigm in chronic schizophrenia patients and have demonstrated impaired MMN generation, but intact AEP amplitude in general around N100 (Lavoie et al., 2008). This discrepancy between our findings and the previous results could be due to that this general auditory impairment within the oddball paradigm is specific to earlier stages of the disease and later on it recovers or is otherwise compensated for. However, there are a few studies showing that N100 is reduced in chronic schizophrenia especially when long ISIs are used, which makes this account less plausible (Bodatsch et al., 2015; Javitt & Sweet, 2015; Rosburg et al., 2008). Alternatively, the discrepancy between our results and the Lavoie et al., 2008 findings could be due to differences in the number of trials that were used, and therefore in signal-to-noise. In the current study we used a very large number of trials per subject (>1500 trials) which led to a high signal-to-noise for the N100 component and arguably minimized false negative findings. This highlights the importance of acquiring large numbers of trials in individual patients. As most of the previous studies using auditory oddball paradigms have focused on the MMN component, further work is needed in order to clarify the pattern of deficits across stages of the disease.

The critical finding of our study is that add-on administration of NAC for an extended period of time (6 months), improved the generation of early AEPs. This is the first study showing an improvement of low-level auditory processing by NAC. Previous clinical trials administering NAC to chronic schizophrenia patients had shown improved MMN generation, but no changes in AEP

amplitude in general (though it must be emphasized that such was not impaired to start with at least in their small group of chronic patients) (Lavoie et al., 2008). Add-on administration of NAC similar to that used here has been shown to result in increased GSH levels in the blood, though without concomitant increases in cysteine (at least at the doses we have used). That said, there is evidence to support NAC leading to increased brain levels of GSH in humans (Conus et al., in revision) as well as in mice during periods of oxidative stress (Farr et al., 2003; Lante et al., 2008; das Neves Duarte, 2012). Given the evidence that GSH can potentiate NMDAR functioning (Hardingham and Do, 2016) alongside evidence for the role of NMDAR in generation of auditory AEPs (Javitt, 2015 for review), we speculate that NAC administration here is exerting its positive effect via NMDAR activity.

It is well established that the N100 is generated by neural populations in the primary and association auditory cortices (Godey et al., 2001; Zouridakis et al., 1998) as well as frontal sources (Deouell et al., 1998; Giard et al., 1990; De Santis et al., 2007). The AEP improvements following NAC treatment resulted from changes in ERP strength but not topography, suggestive of general amplitude or gain modulations rather than compensatory network activity. Consistently, the source-localization here revealed that the improvement following NAC administration in the P50/N100 complex was the result of stronger activity in the left temporal cortex; regions well established as underlying the N100 response. In contrast, the placebo group did not demonstrate changes in the amplitude of brain activity 6 months after the first testing session within either left or right temporal cortices. This finding highlights the potential value of NAC in the treatment of psychosis, in remediating impaired responses in auditory cortices. It is worth noting that electrophysiological improvements following NAC administration seem to coincide with biochemical and neurocognitive improvements observed in an investigation of a larger sample than the limited set of patients studied here (Conus et al., in revision). Specifically, in this larger cohort of patients, it was shown that administration of NAC led to increased glutathione levels in the brain (levels of GSH in medial prefrontal cortex were assessed by H-MRS on a 3T MR scanner) and improved speed of processing (assessed with the MATRICS Consensus Cognitive Battery). AEP effects seem to be particularly robust, even in small samples and in analyses of individual patients and therefore are particularly promising as trait/state markers. For example, in Lavoie et al. (2008) AEP effects after add-on NAC administration in chronic patients preceded improvements in clinical scores by at least 2 months (Lavoie et al., 2008; Berk et al., 2008). The present study extends such a pattern to demonstrate NAC efficacy even in the early stages of the disease.

In summary, we show results indicating that long-duration treatment with NAC improves low-level auditory processing deficits over the N100 time period in this sample of EP patients. However, the small size of the sample of patients limits the generalisability of our findings. In order to establish the effects of NAC on low-level auditory processing in early psychosis a replication of these findings in a larger sample of patients is needed. Nonetheless, the present data contribute to a growing body of evidence on the efficacy of such add-on treatments, particularly for low-level sensory impairments.

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Figure Captions

Figure 1. Group-averaged voltage waveforms from frontal sites F3 and F4 as well as global field power (GFP) waveforms from control subjects and early psychosis patients (black and blue traces, respectively). The grey areas in the GFP waveforms indicate significant differences as a function of time. Responses were weaker in patients.

Figure 2. Group-averaged voltage waveforms from frontal sites F3 and F4 as well as GFP waveforms from patients who received NAC and placebo treatment (panels A and B, respectively). Gray traces display responses pre-treatment and red traces responses post-treatment. Insets display the difference topography averaged over the 52-140ms period. Blue arrows indicate the significant differences before and after NAC treatment over the N100 period.

Figure 3. Results of the mixed ANOVA on the mean source activity on the predefined left temporal cortex region. Asterisks indicate significant differences between pre and post-treatment for the patients that received NAC. Source activity was estimated on data averaged over the 52-140ms period. Inset indicates the selected points for the left hemisphere (pink nodes). A symmetric set of points was tested in the right hemisphere.

Table(s)

Table 1: Demographic and clinical characteristics of psychosis disorder patients that received NAC (n=8) and patients that received placebo treatment (n=7) at baseline. Mean and standard error are indicated.

	NAC treatment patients	**Placebo** treatment patients	p values
Daily Chlorpromazine (CPZ)-equivalent [mg/day]	385.5 ±100	397.13±111	p=.94
Education of patients in years	12.42±0.89	11.42±1.04	p=.58
PANSS: Positive Symptoms	16.6±1.6	15.3±1.3	p=.56
PANSS: Negative Symptoms	15.5±1.8	17.4±2.8	p=.57
PANSS: Total	68.62±5.4	69.14±5.5	p=.95
Time lapse between psychosis threshold and EEG recordings in days	783 ±255	771 ±280	p=.97

Table 2: Specific diagnosis of patients after 3 years and antipsychotic medication at the time of the experiment (separately for the groups following NAC and placebo treatment)

Antipsychotic medication	**Number of NAC patients**	**Number of Placebo patients**
Aripiprazole	1	1
Olanzapine		2
Quetiapine	3	1
Risperidone	1	1
None	3	2
Diagnoses		
Schizophrenia undifferentiated	2	2
Schizophrenia, paranoid type	4	2
Schizotypical personality disorder	1	1
Recurrent depressive disorder with psychotic features		1
Schizo-affective disorder, bipolar type		1
Schizo-affective disorder, depressive type	1	

Figure 1

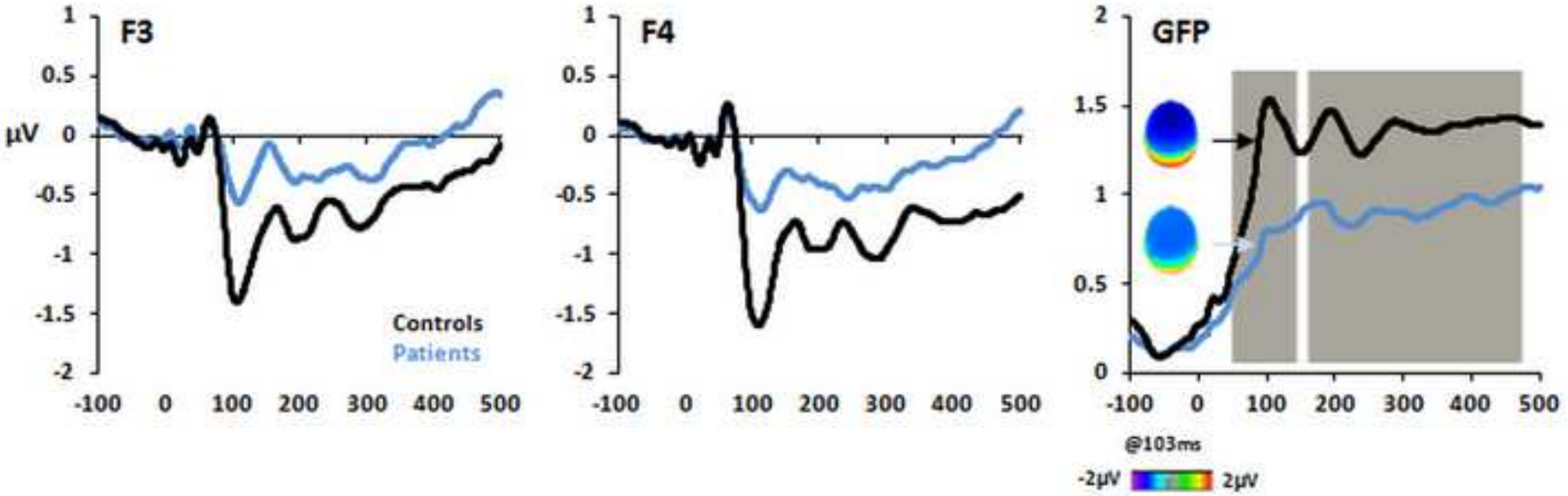

Figure 2

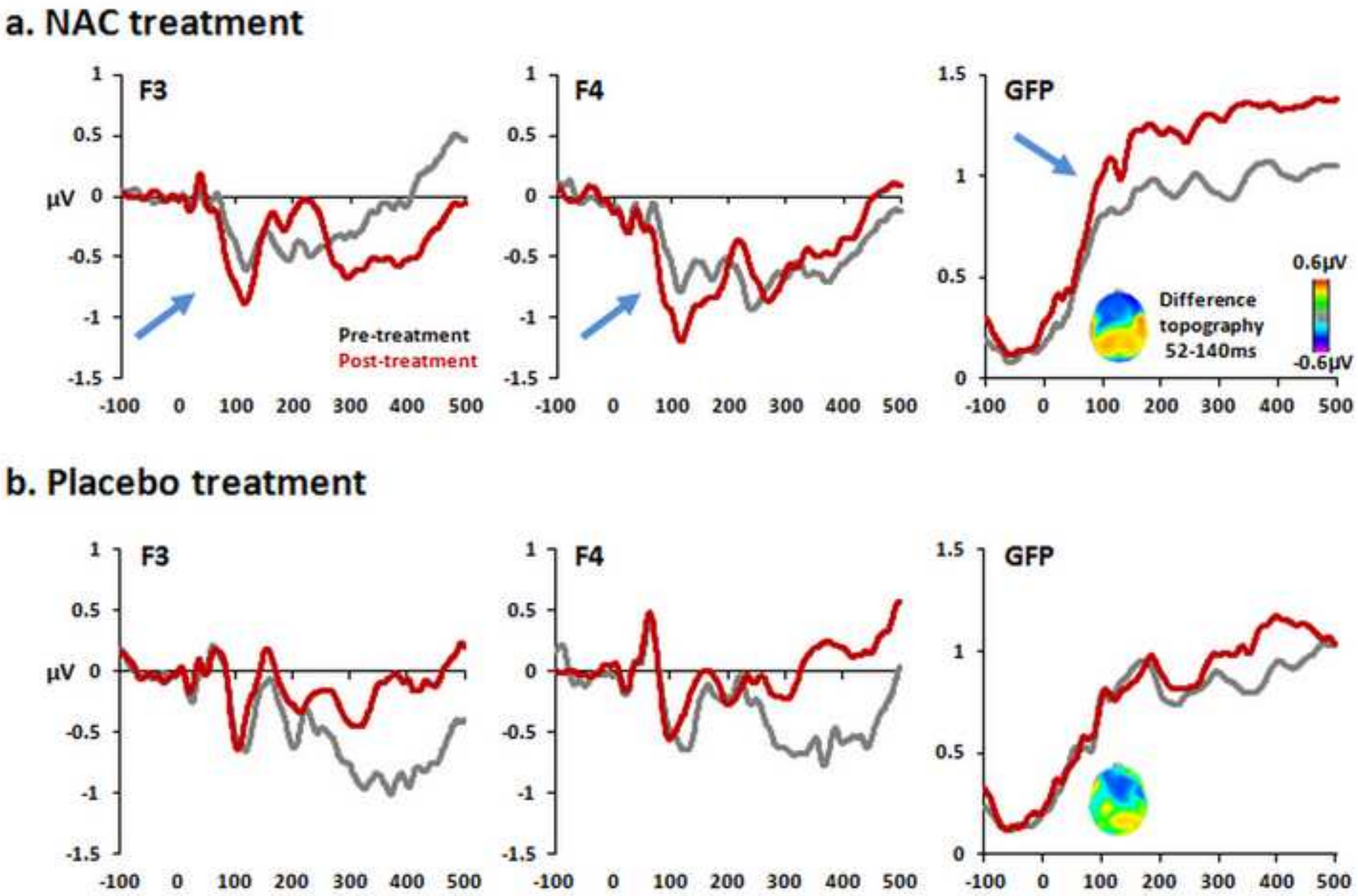
a. NAC treatment
F3
F4
GFP
μV
Pre-treatment
Post-treatment
Difference topography 52-140ms
0.6μV
-0.6μV
b. Placebo treatment
F3
F4
GFP
μV

Figure 3

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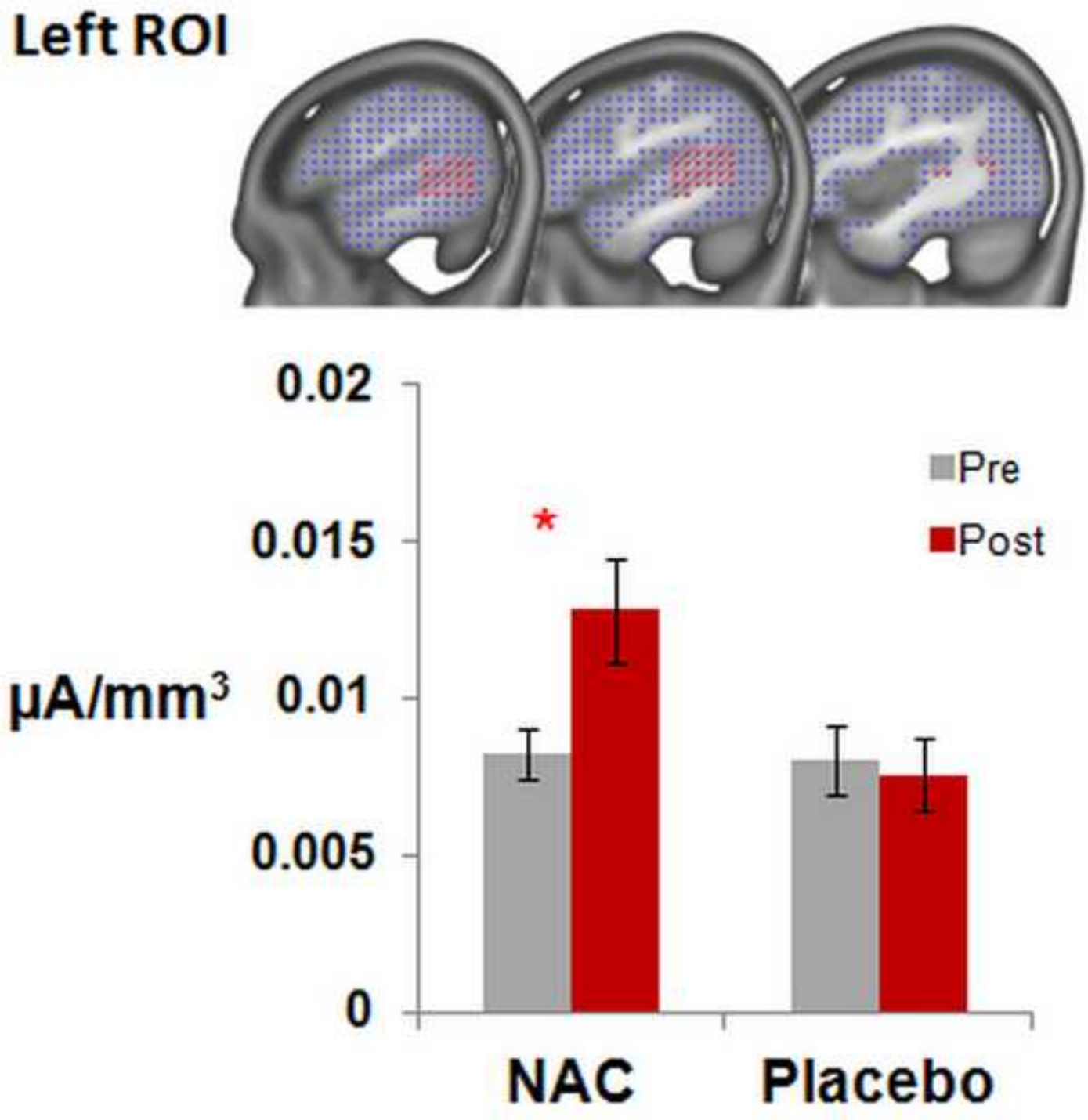

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Conflict of Interest

Authors report no conflict of interest.

*Contributors

Author Contributions

Conceived and designed the study: MMM, SC, PC, KQD

Acquired the data: CR, JFK, CF, RJ, MF, LA, PSB

Analyzed and interpreted the data: CR, JFK, MMM

Drafted the manuscript: CR, MMM

Critically revised the manuscript: all authors

Supervised the study: MMM, PC, KQD

All authors contributed to and have approved the final manuscript.

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