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Ethyl-Eicosapentaenoic Acid in First-Episode Psychosis. A 1H-MRS Study

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Ethyl-eicosapentaenoic acid (E-EPA) is an omega-3 fatty acid that has been used in a range of neuropsychiatric conditions with some benefits. However, its mechanism of action is unknown. Here, we investigate its effects on *in vivo* brain metabolism in first-episode psychosis (FEP). Proton magnetic resonance spectroscopy at 3 T was performed in the temporal lobes of 24 FEP patients before and after 12 weeks of treatment in the context of a larger double-blind, placebo-controlled E-EPA augmentation study. Treatment group effects for glutathione ($F1,12 = 6.1$, $p = 0.03$), and a hemisphere-by-group interaction for glutamine/glutamate ($F1,20 = 4.4$, $p = 0.049$) were found. Glutathione increased bilaterally and glutamate/glutamine increased in the left hemisphere following E-EPA administration. Improvement in negative symptoms correlated with metabolic brain changes, particularly glutathione ($r = -0.57$). These results suggest that E-EPA augmentation alters glutathione availability and modulates the glutamine/glutamate cycle in early psychosis, with some of the metabolic brain changes being correlated with negative symptom improvement. Larger confirmatory studies of these postulated metabolic brain effects of E-EPA are warranted.

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INTRODUCTION

Omega-3 fatty acids are essential for normal brain development, synaptic plasticity, and function (Bazan, 2005; Bazan *et al*, 1997). Cell biological and molecular studies suggest that omega-3 fatty acids modulate membrane fluidity (Hashimoto *et al*, 1999), dopaminergic (Piomelli, 1994) and serotonergic (Yao *et al*, 2004) neurotransmission, and differentially alter gene expression (Kitajka *et al*, 2002; Salvati *et al*, 2004). Furthermore, preclinical studies suggest that omega-3 fatty acids have neuroprotective properties (Lonergan *et al*, 2002; Lynch *et al*, 2007; Martin *et al*, 2002). Animal and human studies provide ample evidence that essential fatty acid deprivation during pregnancy is associated with developmental and behavioral abnormalities (Innis *et al*, 1999; Wainwright *et al*, 1994a, b) that are ameliorated by essential fatty acid supplementation (Helland *et al*, 2003; Wainwright *et al*, 1994a, b).

Decreased omega-3 fatty acid levels have been found in blood and postmortem brain cell membranes in several neuropsychiatric conditions (Schachter *et al*, 2005), in particular schizophrenia (Berger *et al*, 2006; Fenton *et al*, 2000; McNamara *et al*, 2007), bipolar affective disorders (Chiu *et al*, 2003; Hitzemann *et al*, 1984; Mahadik *et al*, 1996; Ranjekar *et al*, 2003), major depression (Frasure-Smith *et al*, 2004; McNamara *et al*, 2006; Mischoulon and Fava, 2000; Peet *et al*, 1998), and attention deficit (hyperactivity) disorder (Burgess *et al*, 2000; Stevens *et al*, 1995).

Controlled clinical trials in established schizophrenia indicate that either sole or augmentation therapy with omega-3 fatty acids may be beneficial (Emsley *et al*, 2002; Joy *et al*, 2006; Mellor *et al*, 1996; Peet *et al*, 2001), with some conflicting results (Emsley *et al*, 2006; Fenton *et al*, 2001; Peet and Horrobin, 2002a, b). Furthermore, controlled clinical trials in treatment-resistant depression (Nemets *et al*, 2002; Peet and Horrobin, 2002a, b), bipolar depression (Keck *et al*, 2006), bipolar affective disorder (Stoll *et al*, 1999), borderline personality disorder (Zanarini and

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Previous presentation: Preliminary analyses have been presented at the Congress International Neuropsychopharmacology (CINP) in Paris (2004), the International Society of Magnetic Resonance in Medicine (ISMRM) meeting in Kyoto (2004), the World Federation of Societies of Biological Psychiatry (WFSBP) in Vienna (2005), and the International Congress on Schizophrenia Research in Savannah (2005).

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Frankenburg, 2003), incarcerated young males (Gesch *et al*, 2002), and children with developmental coordination disorders (Richardson and Montgomery, 2005) also suggest that omega-3 fatty acids may modulate mood, impulsivity, and aggression, while potential neuroprotective effects were found in Huntington's disease (Puri *et al*, 2002, 2005). A recently presented, yet unpublished, study (Amminger *et al*, 2007) suggests that omega-3 fatty acids may delay the onset of frank psychosis in adolescents at ultra-high risk of psychotic disorders.

The underlying *in vivo* mechanisms of action of omega-3 fatty acids are still speculative. A recent study investigating T2 relaxation time the *in vivo* brain effects of omega-3 fatty acids using T2 relaxation time in patients with bipolar affective disorder supports preclinical findings that omega-3 fatty acids modulate membrane integrity (Hirashima *et al*, 2004), thereby potentially altering signal transduction and receptor binding (Vereb *et al*, 2003). One way to further investigate the potential *in vivo* brain effects of omega-3 fatty acids is proton magnetic resonance spectroscopy (1H-MRS), which can estimate regional concentrations of various brain metabolites. For example, alterations in *N*-acetylaspartate (NAA) most likely reflect changes in neuronal integrity, changes in glutamate/glutamine (GLX) may be linked to excitotoxicity, and changes in glutathione (GSH) related to oxidative stress (Nakamura *et al*, 1997) and/or apoptotic activity (Dringen, 2000). GSH synthesis is mainly regulated in astrocytes, and is important as a defense mechanism against excess NO, while functioning to protect glial and neuronal mitochondria (Gegg *et al*, 2003, 2005).

1H-MRS studies in established schizophrenia found altered metabolite profiles (Abbott and Bustillo, 2006; Bertolino and Weinberger, 1999; Keshavan *et al*, 2000; Lyoo and Renshaw, 2002; Stanley, 2002). However, 1H-MRS studies of first-episode psychosis (FEP) are less conclusive (Renshaw *et al*, 1995). The stage of disease at which metabolite abnormalities are established is unclear; however, studies in young, untreated FEP patients suggests that the neuronal integrity is largely preserved before onset of psychosis (Wood *et al*, 2003), while the majority of 1H-MRS studies in chronic schizophrenia show reduced levels of NAA.

Here, we present a study investigating the metabolic *in vivo* brain effects of ethyl-eicosapentaenoic acid (E-EPA) using this technique in drug-naïve or early treated FEP. We speculated that E-EPA would show neuroprotective properties *in vivo* by maintaining neuronal integrity, protecting the brain against excitotoxicity, and support antioxidative defense. We chose E-EPA because previous controlled studies in schizophrenia have shown some benefits (Emsley *et al*, 2002, 2006; Horrobin *et al*, 2002; Peet, 2003, 2001; Peet and Horrobin, 2002a, b), and because treatment response has been associated with an increase in EPA (Arvindakshan *et al*, 2003).

PATIENTS AND METHODS

Patients

All study participants were patients of the Early Psychosis Prevention & Intervention Centre (EPPIC), Melbourne, Australia that covers a service area with an approximate population of 880 000. Study inclusion criteria were (1) age between 15 to 29 years (inclusive) and (2) currently psychotic as reflected by the presence of at least one psychotic symptom daily for more than 1 week (either delusions, hallucinations, disorder of thinking, and/or speech other than simple acceleration or retardation, and disorganized bizarre, or markedly inappropriate behavior). Psychotic diagnoses were confirmed using the semi-structured DSM-IV interview for research (First *et al*, 2002). Exclusion criteria were cases of drug-induced psychosis (self-limiting drug-related psychotic experiences that resolved within less than 7 days of drug abstinence), first episode mania, organic disorders presenting with psychotic symptoms (eg, temporal lobe epilepsy, significant neurological conditions), history of intellectual disability, or history of head injury with loss of consciousness.

Participants of this study were part of a larger randomized double-blind placebo-controlled clinical trial (Berger *et al*, 2008) investigating the augmenting effects of E-EPA ((5Z,8Z,11Z,14Z,17Z)-eicosa-5,8,11,14,17-pentaenoic acid) in 80 drug-naïve or early-treated FEP patients who completed repeated MRI/MRS scans. Twenty-four patients agreed to perform 1H-MRS assessments before commencement of the study medication and after 12 weeks. Twelve in each group either received 2 g oral E-EPA or 2 g placebo oil, taken as separate 1-g doses in the morning and evening (see Table 1 for details). All participants received atypical antipsychotic medication (taken nightly) according to the guidelines of the Early Psychosis Prevention & Intervention Centre, Melbourne, Australia (McGorry and Warner, 2002). Full details of the larger clinical trial design, patient characteristics, and the clinical outcome measures can be found elsewhere (Berger *et al*, 2008). The local research and ethics committee approved this protocol and each subject (or their guardian) provided written informed consent.

Table 1 Demographic Details for the Two Treatment Groups

	E-EPA group	Placebo group
Age (years)[a]	19.6 ± 2.9	21.4 ± 4.1
Proportion male	67%	100%
Proportion schizophrenia, schizophreniform psychosis	58%	83%
Proportion smokers at baseline[b]	36%	55%
Proportion antipsychotic-naïve at baseline	42%	58%
Time between scans (days)[a]	86.2 ± 6.8	83.2 ± 3.9
Proportion receiving risperidone/quetiapine/olanzapine	6/4/2	4/3/5
Number of days on antipsychotic medication at baseline[c]	1 (0–16)	0 (0–11)
Total antipsychotic dose between scans (mg of CPZ equivalent)[a]	15634 ± 8264	19241 ± 6976
GAF at baseline[a]	43.7 ± 13.1	44.7 ± 8.9
PANSS total at baseline[a]	81.5 ± 14.3	82.2 ± 17.6
Duration of untreated psychosis (months)[c]	3 (0.25–7)	5 (0.25–36)

[a]Data presented as mean and standard deviation.
[b]Data unavailable for two participants (one in each group).
[c]Data presented as median and range.

Proton Magnetic Resonance Spectroscopy

Short-echo (TE 30 ms) acquisition proton MRS was performed on a 3 T GE LX Horizon scanner (GE Healthcare, Milwaukee) using a PRESS sequence with two chemical-shift-selective imaging pulses for water suppression. Spectra were acquired with 128 transients of 2 k data points over a frequency width of 5000 Hz with TR = 3 s. Spectra were recorded from single isotropic 2-cm voxels placed in each temporal lobe. Three-plane localizing images were acquired to allow prescription of regions of interest (ROI) for spectra. Sagittal plane, 2-cm thick scout images (T1 spin echo), followed by 2-cm thick coronal images, centered in the plane of the ponto-medullary junction, were acquired. An ROI in each temporal lobe was selected in the coronal plane, with the lateral aspect of the hippocampus in the center of the ROI. The sagittal image was viewed to ensure that the ROI did not include petrous temporal bone. This region of interest consisted largely of the anterior hippocampus (>50%). Spectra were analyzed with LCModel (Provencher, 1993), using a basis set of 15 metabolites acquired on-site, incorporating the standard macromolecule and baseline fitting routines of LCModel. Metabolite concentrations were estimated following calibration using the tissue water signal as an internal standard. Results are presented in institutional units approximating millimolar concentration, and were rejected if the Cramer–Rao lower bound was greater than 30%. Full-width-half-maxima and signal-to-noise ratios averaged 0.093 ± 0.015 and 11.3 ± 1.9, respectively, across both time points and both hemispheres. Only the following metabolites were reliably estimated in sufficient participants at both time points to allow analysis: NAA (encompassing N-acetylaspartylglutamate and N-acetyl-aspartate, NAA; $n = 24$), trimethylamines (TMA; $n = 24$), creatine/phosphocreatine (Cr/PCr; $n = 24$), *myo*-inositol (mI; $n = 24$), glutamate/glutamine (GLX; $n = 23$), and glutathione (GSH; $n = 15$). Metabolite concentrations were corrected for CSF and gray matter fraction within each voxel using SPM analysis of segmented T1 images (see also Supplementary Figure).

Analysis

Change scores were calculated as a percentage of the baseline metabolite concentration for each metabolite for each voxel. Repeated-measures ANCOVA (with hemisphere as the repeated measure) covarying for age was performed for each metabolite to compare differences in change between the two treatment groups. One-sample *t*-tests were used on the total group (collapsed across treatment) to test whether the mean change in each metabolite significantly differed from 0.

Partial correlations (controlling for age) were performed between the change scores and change in clinical variables regardless of treatment group.

RESULTS

The two treatment groups did not differ on baseline metabolite levels (all $p > 0.1$) except for Cr/PCr ($F1,19 = 8.2$, $p = 0.01$), where the placebo group had significantly higher concentrations than the EPA group.

No difference in percentage change between the two treatment groups was identified for TMA ($F1,21 = 1.7$, $p = 0.212$), Cr/PCr ($F1,21 = 2.7$, $p = 0.115$), mI ($F1,21 = 2.5$, $p = 0.128$), or NAA ($F1,21 = 0.0$, $p = 0.952$). However, a significant treatment group effect was found for GSH ($F1,12 = 6.1$, $p = 0.03$) and a significant hemisphere-by-group interaction for GLX ($F1,20 = 4.4$, $p = 0.049$). Inspection of the data (see Table 2) demonstrated that whereas the E-EPA group showed a bilateral increase in GSH, the increase in GLX was limited to the left hemisphere. When the patients with affective psychotic disorders were excluded and the analyses repeated, the effects were very similar although not quite reaching significance.

Symptom scores generally showed an improvement, ranging from a median improvement on the GAF scale of 14.5 points to a median change of 0 on the SAS. The change in the PANSS negative symptom subscale significantly correlated with three metabolites, GSH ($r = -0.57$, $p = 0.041$), TMA ($r = -0.48$, $p = 0.025$), and Cr/PCr ($r = -0.46$, $p = 0.032$), indicating that the reduction of negative symptoms correlated strongly with percentage increase in these metabolites (see Figure 1). No other significant correlations (controlling for age) between percentage metabolite change (collapsed across hemisphere) and PANSS total, positive and general subscale scores, or CGI and GAF were identified, although the largest correlations for these latter two variables were with GSH ($r = 0.28$ and $r = -0.24$ respectively). Furthermore, there were no correlations between percentage metabolite change and cumulative antipsychotic dose.

Correlations between percentage change for GSH and glutamate/glutamine were positive ($r = 0.64$, $p = 0.01$), indicating that the changes in both metabolites were closely linked. One-sample *t*-tests showed no significant change in any metabolite when the two groups were combined, although the increase in NAA approached significance ($t23 = 1.9$, $p = 0.064$; see Table 2), indicating that NAA may have increased between baseline and follow-up scan. Correlations between age and percent change were positive for all metabolites, ranging from $r = 0.46$ ($p = 0.024$) for NAA to $r = 0.33$ ($p = 0.122$) for GLX, indicating that younger age was associated with bigger metabolic changes then in older participants (see Figure 2).

Table 2 Effects of 12-Week E-EPA Treatment in FEP

	TMA	Cr/PCr	NAA	mI	GLX	GSH
E-EPA						
Left	9.8 (15.7)	16.3 (14.9)	3.3 (11.5)	14.4 (18.6)	22.0 (19.2)	45.1 (39.3)
Right	17.7 (19.1)	6.2 (13.2)	13.8 (18.2)	21.9 (24.2)	1.5 (12.7)	30.3 (24.0)
Placebo						
Left	3.0 (15.7)	−2.2 (14.9)	12.6 (11.5)	−2.4 (18.6)	−9.1 (19.2)	7.5 (39.3)
Right	−0.7 (19.1)	−3.9 (13.2)	3.5 (18.2)	0.8 (24.2)	−2.7 (12.7)	−20.7 (24.0)

Mean percentage change (95% CI) for each metabolite from each voxel.

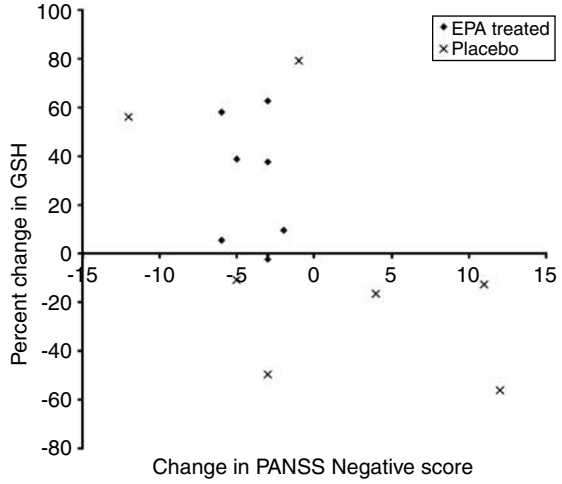

Figure 1 Scatterplot of the relationship between change in glutathione (GSH) and change in the PANSS negative syndrome score for both treatment groups.

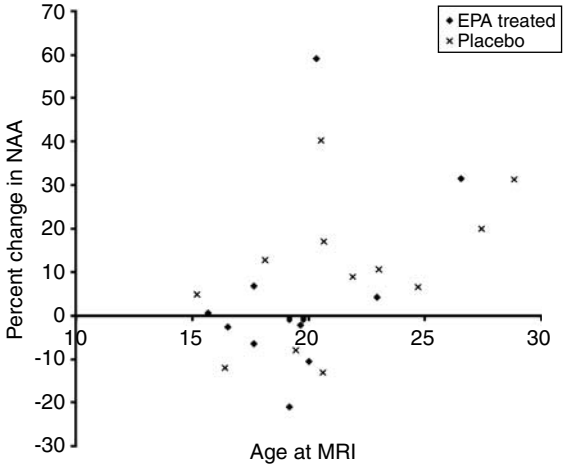

Figure 2 Scatterplot of the relationship between change in NAA and age at scan for both treatment groups.

DISCUSSION

This study demonstrates metabolic *in vivo* brain effects of 2 g E-EPA on GSH and GLX in FEP, in which there was a significant increase in these metabolites in subjects receiving E-EPA augmentation treatment compared with those on treatment as usual. Further, we demonstrated that negative symptom improvement was correlated with increases in GSH, TMA, and Cr/PCr, and were not related to cumulative dose of antipsychotic medication. The moderate to strong correlation of negative symptom improvement with metabolic brain changes suggests that the E-EPA-associated increase in GSH may partially be responsible for its clinical benefits. Our findings are of particular interest in light of previous findings that glutathione levels are reduced (by 27%) in the cerebrospinal fluid of medication-free schizophrenia patients (Do *et al*, 2000), and postmortem brain tissue of patients with schizophrenia (Yao *et al*, 2006). Glutathione protects dopaminergic neurons from oxidative and excitatory damage (Grima *et al*, 2003; Nakamura *et al*, 1997). Taken together, these findings indicate that while altered GSH metabolism might play a role in schizophrenia and related disorders, augmentation therapy with E-EPA may be able to normalize this and protect dopaminergic neurons. One can speculate that the large effects of the recently presented (not yet published) omega-3 fatty acid supplementation study in prodromal adolescents (Amminger *et al*, 2007) may partially be explained by such a neuroprotective mechanism that may be sufficient to protect dopaminergic neurons in individuals at incipient risk for psychotic disorders.

Interestingly, the changes in GSH and GLX were positively correlated ($r = 0.64$, $p = 0.01$), indicating that the metabolic changes of these two metabolites were tightly coupled. This would be expected for metabolites involved in the same metabolic pathway (Dringen and Hirrlinger, 2003) when neither is at limiting concentrations for subsequent metabolic processes. Another potential explanation for the linked change in 1H-MRS signals may be that the changes occur at the site of production of both metabolites. Glutathione is mainly produced by the reaction of glutamate with cysteine and glycine, a process occurring predominantly in astrocytes (Dringen and Hirrlinger, 2003). Astrocytes are also responsible for the conversion of glutamate into glutamine (Hertz, 2004; Hertz and Zielke, 2004). Astrocytes constitute nearly half of the cells in our brain, and play a crucial role in synapse formation and functioning (Ullian *et al*, 2004, 2001). A relatively minor change in glial cell number (eg, via antiapoptotic mechanisms) and/or glial metabolic activity could therefore explain our findings (Berger *et al*, 2003; Jarskog, 2006).

The NAA increase of the combined group between baseline and follow-up scan, although only a trend, was not expected. Schizophrenia may be associated with a progressive decrease in NAA (excessive loss compared with the decrease associated with normal aging) and it has been postulated that in particular the onset of schizophrenia is associated with excessive synaptic pruning (Feinberg, 1982; Keshavan *et al*, 1994). However, our findings suggest that the neuronal integrity was maintained or even improved in the recovery phase of our treated FEP sample. The latter is in line with a recent study suggesting that atypical antipsychotic medication may protect the brain of first-episode schizophrenia patients from gray matter loss (Lieberman *et al*, 2005). The positive correlation between age and change of *in vivo* brain metabolites may indicate an advantage for younger FEP patients with neuroprotective treatment strategies, indicating that the potential for a restoration of synaptic integrity may decrease with adulthood (Nakamura *et al*, 1999; Savvateeva *et al*, 2000). While this would have particular importance for early intervention strategies of psychotic disorders, it needs to be confirmed by additional clinical trials.

This study has several potential limitations. First, the sample size, although similar to previous studies (Steen *et al*, 2005), is still relatively small, and we were not in the position to match the groups on diagnosis because the current study was embedded in a larger double-blind, placebo-controlled study of 2 g EPA augmentation in 80 drug-naïve or early treated FEP patients, limiting any stratification procedures without breaking the blindness. The results of our own larger clinical study suggest that

E-EPA has more impact on non-affective psychotic disorders, accelerates treatment response, and reduces the amount of prescribed antipsychotic medication, as well as results in better tolerability (Berger *et al*, 2008). It is therefore noteworthy that the placebo group of the embedded Magnetic Resonance Spectroscopy study had a higher proportion of non-affective psychosis patients than the E-EPA group, meaning that we are likely to be underestimating the metabolic effect of E-EPA in the current study (as E-EPA seems to be beneficial mainly in non-affective psychosis). Second, the restriction of our ROI to the medial temporal lobes means that we cannot determine whether E-EPA has an effect throughout the brain or only in the temporal lobe. Third, the lack of a longitudinal healthy control arm means that we cannot address the question of whether the E-EPA effects were specific to early psychosis or a more general 'healthy' brain response (Yehuda *et al*, 1999). Fourth, MRS measures show normal physiological variation of between 5 and 28% (Wellard *et al*, 2005), meaning that it is potentially possible that our findings are merely due to normal fluctuations in metabolites. However, it seems unlikely that we would have found treatment group differences if this were the case. Finally, we had no measure of EPA adherence so it was not possible to establish a dose–response relationship. Furthermore, we did not assess dietary intake of essential fatty acids (although the dose given was 10 to 20 times the amount of EPA found in 100 g of tuna).

The MRS analysis used in this study utilizes a library of complex multipeak metabolite spectra that are matched and scaled to fit the observed subject spectrum (Provencher, 1993). Although peaks from other metabolites overlap with the glutathione spectrum, it is possible to detect contributions from metabolites with complex spectra, such as glutathione, even when the individual metabolite is not clearly visible in the spectrum (Pfeuffer *et al*, 1999a, b). Comparisons of a short-echo single-voxel acquisition method, as used in this study, with spectral editing techniques for glutathione measurement showed that the method used in our study gives comparable measurements (Oz *et al*, 2006).

In conclusion, the addition of E-EPA to standard treatment in early psychosis results in a large increase in glutathione in both temporal lobes, and to a lesser extent, an increase in glutamate/glutamine that reaches significance only in the left hippocampus. Negative symptom improvements correlated with increases in the concentration of a number of metabolites, in particular glutathione. We speculate that the tightly coupled increase in GSH and GLX can be explained via a protective effect of E-EPA on astrocytes, which promotes antioxidative defense mechanisms and secures a proper functioning of the glutamate/glutamine cycle in early psychosis. Our results provide encouragement to further investigate E-EPA as a potential neuroprotective agent.

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DISCLOSURE/CONFLICT OF INTEREST

Dr Berger has no conflict of interest with any manufacturer or distributor of omega-3 fatty acids. The presented data were an investigator-initiated trial. Laxdale Ltd (now owned by Amarin Cooperations) provided the study medication for free. ORYGEN Research Centre did not receive any financial support from Laxdale Ltd. Dr Berger has received grant/research support from Astra-Zeneca, Janssen-Cilag, and Eli Lilly (not related to this trial).

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Supplementary Information accompanies the paper on the Neuropsychopharmacology website (http://www.nature.com/npp)