Title:

Hippocampal glutamatergic/NMDA receptor functioning in bipolar disorder: A combined MMN and ^{1}H-MRS study

Kate M. Chitty,* BSc (Hons), Jim Lagopoulos , PhD, Ian B. Hickie , MD, Daniel F. Hermens PhD

Clinical Research Unit, Brain and Mind Research Institute, The University of Sydney, Sydney, Australia.

Abstract

Disturbances in the hippocampal glutamate (Glu)/N-methyl-*d*-aspartate (NMDA) system have been implicated in the pathophysiology of bipolar disorder (BD). Here we aim to provide a targeted integration of two measures of glutamatergic functioning in BD; the association between mismatch negativity (MMN) measured over temporal lobes (temporal MMN) and frontal lobes (frontocentral MMN) and *in vivo* hippocampal-Glu measured via proton magnetic resonance spectroscopy (^{1}H-MRS). Thirty-three patients with BD and 23 matched controls underwent a two-tone passive, duration deviant MMN paradigm and ^{1}H-MRS. Levels of Glu/creatine (Cr) in the hippocampus were determined. Pearson's correlations were used to determine associations between MMN and Glu/Cr. In controls MMN amplitude was positively associated with Glu/Cr at the left temporal site. We did not find any significant associations with Glu/Cr and frontocentral MMN nor did we find any significant associations in BD. The results provide further insight into the neurophysiology of MMN, with evidence supporting the role of hippocampal-Glu signalling through the NMDA receptor in temporal MMN. Our data also demonstrate that Glu/Cr regulation

* Correspondence: Kate Chitty, Clinical Research Unit, Brain and Mind Research Institute, University of Sydney. Address: 94 Mallett Street, Camperdown, NSW 2050, Australia Email: kate.chitty@sydney.edu.au Telephone: +61 2 9351 0724 Facsimile: +61 2 9351 0652

of MMN is dampened in BD which may indicate a lack of tightly regulated hippocampal NMDA functioning. These findings provide insight into the underlying basis of glutamatergic transmission disturbances implicated in the disorder.

Keywords
Hippocampus, glutamate, proton magnetic resonance spectroscopy, mismatch negativity

1. Introduction

There is evidence to suggest that disturbances in hippocampal glutamate (Glu) in bipolar disorder (BD) may be associated with its pathology (Ng et al., 2009; Scarr et al., 2003). The hippocampal-Glu disturbances recognized in BD are largely evidenced by abnormalities in the expression and signalling through N-methyl-*d*-aspartate (NMDA) receptors (Ng et al., 2009). The specifics of these findings are inconsistent however, with a report of decreased NMDA receptor mRNA expression (Law and Deakin, 2001), one showing no change in NMDA receptor density but reduced channel opening (Beneyto et al., 2007) and another showing reductions in receptor density (Scarr et al., 2003). Overall the neuropharmacology of the relationship between Glu, NMDA receptors and the hippocampus in BD is considered to be an area that requires further research, with recommendations of targeted integration between investigatory methods (Ng et al., 2009).

Mismatch negativity (MMN) is an event-related potential, consistently implicated in schizophrenia neurophysiology (Naatanen and Kahkonen, 2009; Umbricht and Krljes, 2005), and gaining increased attention in BD literature (Chitty et al., 2013; Kaur et al., 2012a; Kaur et al., 2011), with our meta-analysis supporting a decreased frontal MMN in BD compared to controls (Chitty et al., 2013) Evidence suggests that Glu signalling through the NMDA receptor channel is necessary for MMN elicitation (Javitt et al., 1995; Javitt et al., 1996), hence this brain potential is thought to provide a unique avenue to probe disturbances in receptor functioning (Javitt et al., 2011). There is extensive evidence that implicates two sources of MMN, a frontocentral source and a temporal source (Baldeweg et al., 2002; Deouell et al., 1998; Giard et al., 1990, Naatanen et al., 2007; Rinne et al., 2000). It is generally accepted that these two sources of MMN reflect distinct components of the response (Garrido et al., 2009; Naatanen et al., 2007; Rinne et al., 2000). The majority of research into the modulation of MMN by NMDA receptor agonists has looked at frontal MMN, leaving only speculations as to the role of the glutamatergic/NMDA receptor system in temporal MMN (Kenemans and Kahkonen, 2011).

Furthermore, spatial resolution of MMN is poor, so while there is limited evidence specifically implicating the hippocampus in its generation, multichannel magnetoencephalography and electroencephalography (EEG) studies have identified the temporal cortex as a source of MMN generation (Giard et al., 1995; Giard et al., 1990; Rinne et al., 2000). Recently an animal study has identified the hippocampus as

a source of MMN, which was elicited within the same latency range as auditory cortical MMN responses (Ruusuvirta et al., 2013). In addition, the hippocampus is strongly implicated in NMDA receptor-mediated long-term potentiation (Bennett, 2000), which is integral to the formation of memory and cortical plasticity, both of which are neural processes hypothesised as being indexed by MMN (Baldeweg and Hirsch, 2014). Indeed, temporally generated MMN is hypothesised to be the result of matching incoming stimuli to the sensory memory trace (Giard et al., 1990).

Glu is the brain's most abundant excitatory neurotransmitter, and the primary agonist at NMDA receptors. Measurement of *in vivo* Glu via proton magnetic resonance spectroscopy (^{1}H-MRS) has enabled advancements of our understanding of Glu abnormalities in BD, however Glu concentration measured this way has a number of limitations to its interpretation. Importantly, ^{1}H-MRS is a static measure that does not take into account dynamic changes in the metabolite (e.g. intracellular vs extracellular) and hence, does not reflect glutamatergic activity (Ongur et al., 2008). Additionally, depending on field strength of the scan, ^{1}H-MRS studies vary between reporting Glu versus Glx, the overlapping resonance of Glu and glutamine. The latter, associated with obvious difficulties in determining whether the results reflect Glu, glutamine or glial-cycling abnormalities. With those limitations in mind, our recent meta-analyses of ^{1}H-MRS studies found Glu/Glx to be increased in the frontal lobes in BD (Chitty et al., 2013). Studies looking at hippocampal-Glu have not been investigated extensively, though a recent ^{1}H-MRS study of first mania episode patients did not find any differences in this region compared to controls (Gigante et al., 2014).

Here we aim to provide a targeted integration of two measures of glutamatergic functioning in BD, by exploring the association between MMN recorded frontally and temporally and *in vivo* hippocampal-Glu (and Glx for comparison), and comparing this relationship to controls. It is entirely plausible that a brain region could have an abnormality in Glu levels, which is then related to an abnormal MMN. While the two measures cannot be directly compared, we propose that concurrently interpreting Glu concentration and NMDA receptor output (measured by MMN) will provide a more holistic approach to investigating glutamatergic activity in the disorder. We aimed to address this gap in the understanding of *in vivo* glutamatergic/NMDA receptor abnormalities in BD. We hypothesise that in controls a positive association will exist between hippocampal-Glu

and temporal MMN due to an increased availability of Glu in the hippocampus (a temporal region) to bind to the NMDA receptor, and hence elicit a larger temporal MMN. We do not expect to find an association between frontocentral MMN and hippocampal-Glu, due not only to the spatial location of the hippocampus, but also due to the hippocampal role in plasticity and sensory memory which reflect temporal MMN rather than frontal MMN. In BD we propose that a positive relationship between hippocampal-Glu and temporal MMN may be absent, given our meta-analysis suggests that BD have increased levels of Glu and decreased MMN amplitudes (albeit frontally) (Chitty et al., 2013), hence indicating there may not be a positive linear relationship between the two."

2. Materials and Methods

2.1. Sample

Patients with BD were recruited from the metropolitan and surrounding areas of Sydney as part of a larger 'Youth Mental Health' cohort study (Hermens et al., 2011; Lee et al., 2013; Scott et al., 2013). All patients were referred by a psychiatrist who made a diagnosis of a bipolar disorder using DSM-IV criteria (APA, 2000) as follows: bipolar I (n = 14), bipolar II (n = 15), bipolar not otherwise specified (n = 4), or bipolar spectrum with family history of BD (n =2), defined as an illness pattern consisting of periods of both elevated and depressed mood consistent with a bipolar spectrum disorder (Angst, 2007). To confirm diagnosis, a research psychologist subsequently conducted a structured interview including the Hamilton Depression Rating Scale (HDRS; (Hamilton, 1967)), the Brief Psychiatric Rating Scale (BPRS; Overall and Gorham, 1962)) and the Young Mania Rating Scale (YMRS; Young et al., 1978). Controls were recruited from the community via word of mouth and local newspaper advertisement. Patients and controls were then selected from the Youth Mental Health database for the current study based on whether they fitted the age range for the study (18 – 30 years) and had an MMN acquisition within 3 months of ^{1}H-MRS.

Mood state at time of testing was determined based on an algorithm using patients YMRS and HDRS scores, with a YMRS total score greater than 12 suggestive of elevated mood (Young et al., 1978) and a HDRS score greater than 16 suggestive of moderate depression (Zimmerman et al., 2013). Mood states were defined as follows: euthymic, YMRS total score less than 12 (Young et al., 1978) and HDRS less than 17; hypomanic, YMRS greater than 11 and HDRS less than 17;

depressed, YMRS less than 12 and HDRS greater than 16; and mixed mood state, YMRS was greater than 11 and HDRS greater than 16. Patients' normal psychotropic medication regimens were not interrupted in any way.

Exclusion criteria for patients and controls were medical instability (i.e. not medically or mentally well enough to complete the assessment), history of neurological disease, medical illness known to impact cognitive and brain function, intellectual disability and insufficient English for assessment. All participants were asked to abstain from drug or alcohol use for 48 hours prior to testing and informed that they may be asked to under-take an alcohol breath test and/or a saliva drug screen if the researcher had reason to believe the participant was under the influence or intoxicated.

The University of Sydney ethics committee approved the study. Participants gave written informed consent before participation.

2.2. Self-report measures

Participants completed a self-report questionnaire which contained demographic information such as age, gender and years of education as well as the Alcohol Use Disorders Identification Test (AUDIT), items from the WHO-ASSIST (Edwards et al., 2003), the depression anxiety stress scale (DASS; Lovibond and Lovibond, 1995) and the Kessler-10 (K-10), a psychological distress scale (Kessler et al., 2002).

2.3. Neurophysiological measures

Participants were fitted with a 64-channel Quik-Cap (Neuroscan) and headphones and told they will be watching a silent movie for 20 minutes and they will be asked to report back the storyline at the end of the task. Participants were then presented with 2,500 binaural pure tones (1,000 Hz, 75 dBSPL, 10 ms rise/fall) with stimulus onset asynchrony of 500ms. Two hundred of these tones were duration deviant tones (100ms) presented pseudo-randomly within 2,300 standard tones (50ms).

Continuous EEG activity was recorded from sites according to the standard 10–20 International system (including mastoids), referenced to a nose electrode. Activity was sampled and digitized at 500Hz (SynAmps2, Scan 4.3.1 software) and filtered using a bandpass filter (0.1 – 30Hz). Data were processed offline using

Neuroscan Scan 4.3.1 (Compumedics, Charlotte, North Carolina) software. Epochs were constructed at -100 to 450ms relative to stimulus onset and baseline corrected. MMN was derived from Fz (frontal site), Cz (central site), M1 (left temporal site) and M2 (right temporal site). Epochs that contained activity ± 100 μV at these sites were rejected. Additional electrodes were placed above and below the left eye and at the outer canthi of both eyes to monitor for eye-blink artifacts and contaminated data was corrected using established algorithms (Semlitsch et al., 1986). Mismatch difference waveforms were obtained by subtracting waveforms elicited by standards from those elicited by duration-deviant stimuli. Peak amplitude was chosen as the primary outcome measure to maintain consistency with our previous MMN studies, and was determined using automated peak picking within an established epoch window of 135–205ms (Hermens et al., 2010; Kaur et al., 2011; Kaur et al., 2012b).

2.4 ^{1}H-MRS data acquisition

Imaging was conducted within three months of EEG recording (mean difference in days = 10.8, SD = 21.7). Participants were scanned on a 3Tesla GE Discovery MR750 MRI (GE Medical Systems, Milwaukee, WI). Firstly, a 3D sagittal whole-brain scout was undertaken for orientation and positioning of scans (TR=50ms; TE=4ms; 256matrix; no averaging, z=5mm thickness). Next a T1-weighted Magnetization Prepared RApid Gradient-Echo (MPRAGE) sequence producing 196 sagittal slices (TR=7.2ms; TE=2.8ms; flip angle = 10°; matrix 256x256; 0.9mm isotropic voxels) was acquired for the purpose of localization of the hippocampus. A 1.5 x 3.0 x 1.0 cm voxel was placed in the left hippocampus (Figure 1 insert). Spectroscopy data was acquired using Point-RESolved Spectroscopy (PRESS) with the following parameters; TE=35ms, TR=2000ms, 128 averages, along with two chemical shift-selective imaging pulses for water suppression. All spectra were shimmed to achieve full-width half maximum (FWHM) of <13Hz and visually inspected by independent raters. Data with Cramer–Rao Lower Bound greater than 20% were excluded. Hippocampal data could not be grey matter (GM)-corrected due to the angulation of the acquisition voxel. However since hippocampal tissue composition is predominantly GM, left hippocampal volumes were calculated and used as a proxy for individual differences.

2.5 ^{1}H-MRS data processing

Data were transferred offline for post processing using the LCModel software

package (Provencher, 1993). All spectra were quantified using a GAMMA-simulated PRESS TE 35 basis set of 15 metabolites (including Glu, Glx, N-acetylaspartate [NAA] and Inositol [Ins]) and incorporated macromolecule and baseline fitting routines (for spectra see Figure 1). Metabolite concentrations were determined as a relative ratio to creatine-phosphocreatine (Cr). We also calculated Glu/H_20 to assess whether results differed when normalising to water instead of Cr.
Spectra with the following features were excluded from selection in the present study: Cramer–Rao Lower Bound greater than 20%, poor spectral morphology, poor spectral fit, large variation in residuals, poor signal-to-noise ratio and presence of artefact.

2.6 Hippocampal volumes

Left hippocampal volume was determined using FMRIB Software Library (FSL) (Smith, 2002). Subcortical volumes for the left hippocampi were extracted using a semi-automated segmentation routine based on the principles of the Active Shape and Appearance Models within a Bayesian framework as implemented by ‘‘FIRST’’ in FSL. As part of the segmentation routines, all data were aligned into the MNI standard space, using a 12- degree-of-freedom affine transformation. A mask based on shape models and voxel intensities was then applied to isolate the subcortical structures. Absolute volumes of the left hippocampi were then calculated from spatially transformed original data using FIRST as implemented in FSL. Finally, a boundary correction (z -value =3) was applied (corresponding to a 99.998% certainty) to determine the boundary voxels. Data were visually inspected for errors.

2.7. Statistical analyses

Statistical analyses were carried out using SPSS for Windows 21.0 (SPSS Inc., Chicago, Illinois, USA). Group differences in demographics, clinical measures, alcohol and tobacco use, neurophysiological measures, metabolite concentrations and left hippocampal volumes were assessed by independent t-test or χ^2 tests where relevant. Homogeneity of variance was determined using the Levene test. If the assumption of homogeneity was violated, Welch’s statistic was used to adjust degrees of freedom and p-values. Values for MMN, ^{1}H-MRS concentrations and hippocampal volumes were converted to z-scores to inspect for outliers (a z-score of +/- 3.00). Outliers were then removed from subsequent analysis to reduce the impact of influential cases.

Pearson's correlations were performed for the entire sample; co-varying for age, hippocampal volume and smoking status. A simple bootstrapping method based on 1000 samples was used to obtain bias-corrected and accelerated (BCa) 95% confidence intervals. These correlations were also performed for BD and controls separately. Alpha was set to 0.05 and BCa 95% confidence intervals were also used to assess significance.

Post-hoc analysis was then conducted to assess the influence of different psychotropic medication classes on the MMN and hippocampal-Glu correlations. These correlations were re-analysed three times as follows: 1) excluding those on an antipsychotic; 2) excluding those on an anticonvulsant; and 3) excluding those on an antidepressant.

3. Results

3.1. Demographics, symptoms and medications

Seventy-one participants (41 BD and 30 controls) were selected from the Youth Mental Health database having had MMN and ^{1}H-MRS conducted within 3 months and fitting the age criteria for the study. From those selected, valid hippocampal Glu data was available for 33 participants with BD and 23 controls. There were no significant differences in demographics and symptom scores between those who had valid (n = 56) vs. invalid data (n = 15). However BD participants excluded due to invalid data had a significantly longer duration of illness. See supplementary material for these statistical analyses as well as detailed reasons for invalid and excluded data.

The demographics and symptom scores for included participants are shown in Table 1. There were no differences in age, gender, proportion of smokers, and alcohol use between BD and controls. As expected, t-test revealed significant differences in K-10 and DASS scores and years of education, with BD showing higher symptom scores and lower years of education.

The average BPRS scores suggest that on average the patient population was moderately ill according to the Clinical Global Impression equivalent (Leucht et al., 2005), with the highest score in the markedly ill range (BPRS = 54) and the lowest score in the normal range (BPRS = 24).

At the time of testing 17 (51.5%) patients were taking antidepressants, 14 (42.4%) were on anti-convulsants, ten (30.3%) patients were on atypical

antipsychotics, nine (27.3%) were on lithium, one (3.0%) was on benzodiazepine and one was on a simulant. Seven (21.2%) patients were medication free.

3.2. Neurophysiology, structural imaging and spectroscopy results

There were no outliers in terms of M1 amplitude (z-score range: -1.77 to 2.76), M2 amplitude (-1.86 to 1.82), Glu/Cr (-1.66 to 2.55) or hippocampal volume (-2.34 to 2.11). Table 2 shows the results from the t-test for between-group differences in the neurobiological measures. There were no significant differences between BD and controls in metabolite concentrations, hippocampal volumes, average epochs accepted for MMN or temporal MMN amplitude (see Figure 2 for waveforms). BD and controls also did not differ in times between MMN and ^{1}H-MRS acquisitions ($t(54) = -0.849$, $p > 0.05$).

The correlation matrix is shown in Table 3. The combined analysis (patients and controls) showed a statistically significant correlation between Glu/Cr at M1, and trend level at M2 ($p < 0.1$). In controls, a significant correlation was found between M1 and Glu/Cr and a trend was found between M2 and Glu/Cr ($p < 0.1$). There were no significant correlations in BD. Scatterplots for left temporal MMN vs Glu/Cr are shown in Figure 3.

3.3 Post-hoc analysis

In order to further assess the aforementioned non-significant correlations in BD we re-analysed the correlations between M1 and Glu/Cr excluding patients on different classes of psychotropic medications. The correlations remained non-significant when excluding patients on an antipsychotic ($r = 0.117$, $p > 0.05$, 95% BI: -0.44, 0.58) and patients on an anticonvulsant ($r = 0.139$, $p > 0.05$, 95% BI: -0.43, 0.69). When excluding patients on an antidepressant a trend-level positive correlation was observed ($r = 0.490$, $p < 0.1$, 95% BI: -0.22, 0.87).

All results remained significant when we re-ran the correlations normalised to water instead of Cr (see Supplementary Table 2).

4. Discussion

To our knowledge, this is the first study to look at the relationship between MMN and *in vivo* concentrations of Glu/Cr in BD. We sought to explore this relationship in order to shed light on hippocampal Glu/NMDA receptor disturbances

in the disorder. The results of our study demonstrate the presence of an association between the aforementioned variables in control subjects, but not in BD, and thus provide important considerations and avenues for further research. Firstly, these findings provide new insight into the neurophysiology of MMN, with evidence supporting a relationship between Glu concentration in the hippocampus and temporal MMN amplitude. Furthermore, the lack of a correlation (in either group) between frontal MMN and hippocampal-Glu suggests that this association is localised to the temporal region. Secondly, our data demonstrate that the metabolite system regulating MMN is disturbed in BD, irrespective of differences in MMN amplitude, metabolite concentrations or hippocampal volume, none of which were significantly different between the groups. Thirdly, through integrated interpretation of these measures we provide possible neurochemical hypotheses for the basis of hippocampal Glu/NMDA abnormalities in BD. It is important to note that due to the variation in time between MMN and ^{1}H-MRS acquisitions in the present study (i.e. up to 3 months), any assumptions about the potential state related associations between these measures should be made with caution. Despite this, there evidence from similar research (Stone et al., 2010) to suggest that the relationship between these measures is better accounted for by the trait-related aspects of the underlying neurobiology.

The results demonstrate that within a "healthy" range of temporal-MMN amplitudes and *in vivo* Glu concentrations, an increased MMN is associated with an increased Glu/Cr. This association is in line with the predominant neurochemical hypothesis of MMN generation, namely, that MMN represents Glu signalling through the NMDA receptor (Garrido et al., 2009; Javitt et al., 2011; Kenemans and Kahkonen, 2011). Specifically, higher levels of Glu would result in greater NMDA receptor activation and accordingly, increased MMN amplitude. This is in agreement with research by Stone et al., (2010), who reported a positive association between *in vivo* Glx and MMN in the thalamus of prodromal patients. It is noteworthy that in the present study the ^{1}H-MRS voxel was located in the left hippocampus, and accordingly, the association between left temporal MMN amplitude was stronger than with right temporal MMN in controls and there was no relationship between frontocentral MMN and hippocampal-Glu. This underscores the idea of two dipolar sources of MMN located in the temporal lobes of each hemisphere (Rinne et al., 2000).

As hypothesised there were no significant associations between Glu/Cr and

temporal MMN in BD but surprisingly, the patients in the present sample did show a significantly higher frontal MMN than controls which is in contrast to our previous meta-analysis of this measure in BD (Chitty et al., 2013), the reason for this is unclear and warrants further investigation. However, there were no group differences in temporal MMN amplitudes, neurometabolites of interest or hippocampal volume. In light of the positive and significant temporal correlation in controls, these null findings are consistent with the BD model of neuroprogression, which proposes that as the illness progresses so do underlying neurobiological changes associated with more chronic presentations of the disorder (Berk et al, 2011). Hence in the present sample consisting of patients in early stages of BD, pathophysiological changes may not be distinct enough to differ from matched controls. This may suggest that deregulation in the Glu/NMDA system precedes impairments in Glu levels or MMN previously reported in BD samples (for a review see Chitty et al., 2013).

Alternatively, these results could suggest that MMN in BD is influenced by different neurochemicals. Indeed, there are other theories behind the pharmacological generation of MMN, not only in terms of other neurobiological systems, such as GABA, serotonin and dopamine (Garrido et al., 2009; Kenemans and Kahkonen, 2011), but also at the level of the NMDA receptor. Modulation of NMDA receptor function is complex, with multiple regulatory sites embedded within the ionophore. One such modulatory chemical that has received recent attention is glutathione (GSH), the brains primary anti-oxidant. Accordingly, a recent study has found a positive correlation between serum GSH and MMN amplitude in controls (Ballesteros et al., 2013), and another has shown that treatment with N-acetyl cysteine (NAC), a precursor for GSH synthesis, can increase MMN amplitudes in schizophrenia (Lavoie et al., 2008). It should be noted that NAC is believed to be taken up through the Glu-cysteine exchanger, resulting in a higher amount a Glu release into the extrasynaptic space (Bauzo et al., 2012; Javitt et al., 2011), and hence, the latter finding is in agreement with the present result in controls.

Use of psychotropic medications could be another potential cause for the non-significant correlations seen in patients, with all but seven of our BD participants medicated at time of testing. Psychotropic medications may impact metabolite levels and as a result, the entire MMN regulatory system. That said if psychotropic medications do affect our neurobiological measures of interest it is not evident in between-group differences in this sample of young people. However in our *post hoc*

analysis to address this issue, we did note a trend-level positive correlation in BD between M1 and Glu/Cr (similar to that seen in controls) when excluding the 17 patients on anti-depressants. This highlights a potential role for anti-depressants in the "deregulation" between Glu/Cr and M1 amplitude. Readers should bear in mind that with our relatively low sample size we were unable to thoroughly assess the influence of different subtypes of medications on the correlations and this is an avenue that requires further investigation. There are some limitations in this study. Firstly, 41% of participants did not have their EEG and 1H-MRS acquired within a 24-hour period. The degree to which Glu/Cr concentrations change over time is unclear, so there is a possibility that the concentrations of this neurometabolite may have changed between the recordings. Future studies that determine the amount of change in each of these measures over time would be of considerable importance.

Secondly, there are some limitations concerned with our ^{1}H-MRS procedure. We are not able to quantify absolute concentration of Glu, so we used ratio over Cr. This method is considered to be disadvantageous due to reported differences in frontal Cr in BD (Frey et al., 2007; Frye et al., 2007), though to our knowledge this has not been found in the hippocampus, nor were there any differences in Cr concentration between groups in the present study. It should be noted that hippocampal data was not GM-corrected, which presents a limitation to the analysis in this region. Thirdly, readers should be advised that a structured research diagnostic interview was not part of the clinical assessment, and therefore the reliability of the diagnoses made in this study may be limited. Finally, as our study into the relationship between MMN and ^{1}H-MRS was largely exploratory we have undertaken multiple correlations to explore our hypotheses and not corrected alpha, hence there is a risk of Type I error.

4.3 Conclusion

The results from this study suggest that regulation of temporal MMN by Glu is abnormal in BD. Our data does not allow us to make any firm statements as to why this occurs, or importantly, how it may be treated. Future studies should aim to collect MMN and ^{1}H-MRS data simultaneously in order to better define these associations.

The seemingly disparate and unconnected Glu/MMN system in BD noted here may indicate a lack of tightly regulated NMDA functioning in the hippocampus, and hence provides insight into the underlying basis of glutamatergic transmission disturbances implicated in the disorder

Acknowledgements

The authors would like to express their gratitude to individuals who participated in this study. This study was funded by an NHMRC Australia Fellowship awarded to IBH (No. 464914). KC was supported by an Australian Postgraduate Award and DFH by a grant from the NSW Health, Mental Health and Drug & Alcohol Office. These funding agencies had no role in the analysis or interpretation of these data; or in the writing of this report; or the decision to submit the paper for publication. IBH is a Senior Principal Research Fellow of the Australian National Health & Medical Research Council (AppID 1046899).

Contributors

DH put conception of the article forth. KC undertook the statistical analysis, KC wrote the first draft of the manuscript. All authors contributed to data interpretation and have approved the final manuscript.

Conflict of interest

IBH is the executive director of the Brain and Mind Research Institute (BMRI), at the University of Sydney, which operates two early-intervention youth services under contract to headspace. He is a commissioner of the Australian National Mental Health commission and was previously the CEO of beyondblue: the national depression initiative and a director of headspace: the national youth mental health foundation until January 2012. Previously, he has led a range of community-based and pharmaceutical industry-supported depression awareness and education and training programs. He has led depression and other mental health research service evaluation or investigator-initiated research projects that have been supported by a variety of pharmaceutical partners. Current investigator-initiated studies are supported by Servier (manufacturers of agomelatine) and Pfizer. He has received honoraria for his contributions to professional educational seminars related to depression, youth mental health and circadian-rhythms research. He has received travel support from Servier to attend scientific meetings related specifically to circadian-rhythm disorders.

References

Angst, J., 2007. The bipolar spectrum. Brit J Psychiat 190, 189-191.

APA, 2000. Diagnostic and statistical manual of mental disorders. American Psychiatric Association, Washington DC.

Baldeweg, T., Klugman, A., Gruzelier, J.H., Hirsch, S.R., 2002. Impairment in frontal but not temporal components of mismatch negativity in schizophrenia. International Journal of Psychophysiology 43, 111-122.

Baldeweg, T., Hirsch, S.R., 2014. Mismatch negativity indexes illness-specific impairments of cortical plasticity in schizophrenia: A comparison with bipolar disorder and Alzheimer's disease. International Journal of Psychophysiology 95, 145-155.

Ballesteros, A., Summerfelt, A., Du, X., Jiang, P., Chiappelli, J., Tagamets, M., O'Donnell, P., Kochunov, P., Hong, L.E., 2013. Electrophysiological intermediate biomarkers for oxidative stress in schizophrenia. Clinical Neurophysiology 124, 2209-2215.

Bauzo, R.M., Kimmel, H.L., Howell, L.L., 2012. The cystine-glutamate transporter enhancer N-acetyl-L-cysteine attenuates cocaine-induced changes in striatal dopamine but not self-administration in squirrel monkeys. Pharmacology Biochemistry Behaviour 101, 288-296.

Beneyto, M., Kristiansen, L.V., Oni-Orisan, A., McCullumsmith, R.E., Meador-Woodruff, J.H., 2007. Abnormal glutamate receptor expression in the medial temporal lobe in schizophrenia and mood disorders. Neuropsychopharmacology 32, 1888-1902.

Bennett, M.R., 2000. The concept of long term potentiation of transmission at synapses. Progress in Neurobiology 60, 109-137.

Berk, M., Kapczinski, F., Andreazza, A.C., Dean, O.M., Giorlando, F., Maes, M., Yucel, M., Gama, C.S., Dodd, S., Dean, B., Magalhaes, P.V., Amminger, P., McGorry, P., Malhi, G.S., 2011. Pathways underlying neuroprogression in bipolar disorder: focus on inflammation, oxidative stress and neurotrophic factors. Neuroscience and Biobehavioural Reviews. 35, 804-817.

Chitty, K.M., Lagopoulos, J., Lee, R.S., Hickie, I.B., Hermens, D.F., 2013. A systematic review and meta-analysis of proton magnetic resonance spectroscopy and mismatch negativity in bipolar disorder. European Neuropsychopharmacology 23, 1348 – 1363

Deouell, L.Y., Bentin, S., Giard, M.H., 1998. Mismatch negativity in dichotic listening: evidence for interhemispheric differences and multiple generators. Psychophysiology. 35, 355-365.

Edwards, S.H., Humeniuk, R., Ali, R., Poznyak, V., Monterio, M., 2003. The Alcohol, Smoking and Substance Involvement Screening Test (ASSIST): Guidelines for Use in Primary Care (Draft Version 1.1 for Field Testing). World Health Organization, Geneva, Switzerland.

Frey, B.N., Stanley, J.A., Nery, F.G., Monkul, E.S., Nicoletti, M.A., Chen, H.-H., Hatch, J.P., Caetano, S.C., Ortiz, O., Kapczinski, F., Soares, J.C., 2007. Abnormal cellular energy and phospholipid metabolism in the left dorsolateral prefrontal cortex of medication-free individuals with bipolar disorder: an in vivo 1H MRS study. Bipolar Disorder 9 Suppl 1, 119-127.

Frye, M.A., Watzl, J., Banakar, S., O'Neill, J., Mintz, J., Davanzo, P., Fischer, J., Chirichigno, J.W., Ventura, J., Elman, S., Tsuang, J., Walot, I., Thomas, M.A., 2007. Increased anterior cingulate/medial prefrontal cortical glutamate and creatine in bipolar depression. Neuropsychopharmacology 32, 2490-2499.

Garrido, M.I., Kilner, J.M., Stephan, K.E., Friston, K.J., 2009. The mismatch negativity: a review of underlying mechanisms. Clinical Neurophysiology 120, 453-463.

Giard, M.H., Lavikahen, J., Reinikainen, K., Perrin, F., Bertrand, O., Pernier, J., Näätänen, R., 1995. Separate Representation of Stimulus Frequency, Intensity, and Duration in Auditory Sensory Memory: An Event-Related Potential and Dipole-Model Analysis. Journal of Cognitive Neuroscience 7, 133-143.

Giard, M.H., Perrin, F., Pernier, J., Bouchet, P., 1990. Brain generators implicated in the processing of auditory stimulus deviance: a topographic event-related potential study. Psychophysiology 27, 627-640.

Gigante, A.D., Lafer, B., Yatham, L.N., 2014. (1)H-MRS of hippocampus in patients after first manic episode. World Journal of Biological Psychiatry 15, 145-154.

Hamilton, M., 1967. Development of a rating scale for primary depressive illness. Brit Journal of Social and Clinical Psychology 6, 278-296.

Hermens, D.F., Ward, P.B., Hodge, M.A., Kaur, M., Naismith, S.L., Hickie, I.B., 2010. Impaired MMN/P3a complex in first-episode psychosis: cognitive and psychosocial associations. Progress in Neuropsychopharmacology and Biological Psychiatry 34, 822-829.

Javitt, D.C., Schoepp, D., Kalivas, P.W., Volkow, N.D., Zarate, C., Merchant, K., Bear, M.F., Umbricht, D., Hajos, M., Potter, W.Z., Lee, C.M., 2011. Translating glutamate: from pathophysiology to treatment. Science Translational Medicine 3, 102mr102.

Javitt, D.C., Schroeder, C.E., Steinschneider, M., Arezzo, J.C., Ritter, W., Vaughan, H.G., Jr., 1995. Cognitive event-related potentials in human and non-human primates: implications for the PCP/NMDA model of schizophrenia. Electroencephalography and Clinical Neurophysiology. Supplement 44, 161-175.

Javitt, D.C., Steinschneider, M., Schroeder, C.E., Arezzo, J.C., 1996. Role of cortical N-methyl-D-aspartate receptors in auditory sensory memory and mismatch negativity generation: implications for schizophrenia. Proceedings of the National Academy of Sciences 93, 11962-11967.

Kaur, M., Battisti, R.A., Lagopoulos, J., Ward, P.B., Hickie, I.B., Hermens, D.F., 2012a. Neurophysiological biomarkers support bipolar-spectrum disorders within psychosis cluster. Journal of Psychiatry and Neuroscience 37, 313-321.

Kaur, M., Battisti, R.A., Ward, P.B., Ahmed, A., Hickie, I.B., Hermens, D.F., 2011. MMN/P3a deficits in first episode psychosis: comparing schizophrenia-spectrum and affective-spectrum subgroups. Schizophrenia Research 130, 203-209.

Kaur, M., Lagopoulos, J., Ward, P.B., Watson, T.L., Naismith, S.L., Hickie, I.B., Hermens, D.F., 2012b. Mismatch negativity/P3a complex in young people with psychiatric disorders: a cluster analysis. PloS one 7, e51871.

Kenemans, J.L., Kahkonen, S., 2011. How human electrophysiology informs psychopharmacology: from bottom-up driven processing to top-down control. Neuropsychopharmacology 36, 26-51.

Kessler, R.C., Andrews, G., Colpe, L.J., Hiripi, E., Mroczek, D.K., Normand, S.L., Walters, E.E., Zaslavsky, A.M., 2002. Short screening scales to monitor population prevalences and trends in non-specific psychological distress. Psychological Medicine 32, 959-976.

Lavoie, S., Murray, M.M., Deppen, P., Knyazeva, M.G., Berk, M., Boulat, O., Bovet, P., Bush, A.I., Conus, P., Copolov, D., Fornari, E., Meuli, R., Solida, A., Vianin, P., Cuenod, M., Buclin, T., Do, K.Q., 2008. Glutathione precursor, N-

acetyl-cysteine, improves mismatch negativity in schizophrenia patients. Neuropsychopharmacology 33, 2187-2199.

Law, A.J., Deakin, J.F., 2001. Asymmetrical reductions of hippocampal NMDAR1 glutamate receptor mRNA in the psychoses. Neuroreport 12, 2971-2974.

Leach, M.J., Marden, C.M., Miller, A.A., 1986. Pharmacological studies on lamotrigine, a novel potential antiepileptic drug: II. Neurochemical studies on the mechanism of action. Epilepsia 27, 490-497.

Leucht, S., Kane, J.M., Kissling, W., Hamann, J., Etschel, E., Engel, R., 2005. Clinical implications of Brief Psychiatric Rating Scale scores. British Journal of Psychiatry187, 366-371.

Lovibond, P.F., Lovibond, S.H., 1995. The structure of negative emotional states: comparison of the Depression Anxiety Stress Scales (DASS) with the Beck Depression and Anxiety Inventories. Behavioural Research and Therapy 33, 335-343.

Naatanen, R., Paavilainen, P., Rinne, T., Alho, K., 2007. The mismatch negativity (MMN) in basic research of central auditory processing: a review. Clinical Neurophysiology 118, 2544-2590.

Naatanen, R., Kahkonen, S., 2009. Central auditory dysfunction in schizophrenia as revealed by the mismatch negativity (MMN) and its magnetic equivalent MMNm: a review. International Journal of Neuropsychopharmcology 12, 125-135.

Ng, W.X., Lau, I.Y., Graham, S., Sim, K., 2009. Neurobiological evidence for thalamic, hippocampal and related glutamatergic abnormalities in bipolar disorder: a review and synthesis. Neuroscience and Biobehavioural Reviews 33, 336-354.

Ongur, D., Jensen, J.E., Prescot, A.P., Stork, C., Lundy, M., Cohen, B.M., Renshaw, P.F., 2008. Abnormal glutamatergic neurotransmission and neuronal-glial interactions in acute mania. Biological Psychiatry 64, 718-726.

Overall, J., Gorham, D., 1962. The Brief Psychiatric Rating Scale. Psychological Reports 10, 799 - 812.

Rinne, T., Alho, K., Ilmoniemi, R.J., Virtanen, J., Naatanen, R., 2000. Separate time behaviors of the temporal and frontal mismatch negativity sources. Neuroimage 12, 14-19.

Ruusuvirta, T., Lipponen, A., Pellinen, E., Penttonen, M., Astikainen, P., 2013. Auditory cortical and hippocampal-system mismatch responses to duration deviants in urethane-anesthetized rats. PloS one. 8, e54624.

Scarr, E., Pavey, G., Sundram, S., MacKinnon, A., Dean, B., 2003. Decreased hippocampal NMDA, but not kainate or AMPA receptors in bipolar disorder. Bipolar Disorder 5, 257-264.

Semlitsch, H.V., Anderer, P., Schuster, P., Presslich, O., 1986. A solution for reliable and valid reduction of ocular artifacts, applied to the P300 ERP. Psychophysiology 23, 695-703.

Smith, S.M., 2002. Fast robust automated brain extraction. Human Brain Mapping 17, 143-155.

Stone, J.M., Bramon, E., Pauls, A., Sumich, A., McGuire, P.K., 2010. Thalamic neurochemical abnormalities in individuals with prodromal symptoms of schizophrenia — Relationship to auditory event-related potentials. Psychiatric Research: Neuroimaging 183, 174-176.

Umbricht, D., Krljes, S., 2005. Mismatch negativity in schizophrenia: a meta-analysis. Schizophrenia Research 76, 1-23.

Vayisoglu, S., Anil Yagcioglu, A.E., Yagcioglu, S., Karahan, S., Karci, O., Gurel, S.C., Yazici, M.K., 2013. Lamotrigine augmentation in patients with schizophrenia who show partial response to clozapine treatment. Schizophrenia Reseach 143, 207-214.

Young, R.C., Biggs, J.T., Ziegler, V.E., Meyer, D.A., 1978. A rating scale for mania: reliability, validity and sensitivity. British Journal of Psychiatry 133, 429-435.

Zimmerman, M., Martinez, J.H., Young, D., Chelminski, I., Dalrymple, K., 2013. Severity classification on the Hamilton depression rating scale. Journal of Affective Disorders 150, 384-388.

Table 1: Demographic and symptoms scores for each group, tested using t-test or chi-square

	BD (n = 33)	Controls (n = 23)	t / χ^2
Sex ratio (M: F)	7:26	7:16	χ^2 (1) = 0.62
Age (SD)	23.3 (3.5)	24.3 (2.8)	t (54) = 1.13
Years of education (SD)	13.1 (2.1)	14.3 (2.2)	t (54) = 2.15*
AUDIT total score (SD)	9.42 (8.1)	9.17 (5.6)	t (54) = 0.05
Current tobacco smokers (%)	16 (48.5)	8 (24.8)	χ^2 (1) = 1.04
K-10	24.9 (8.3)	14.0 (4.2)	t (46.7) = 6.4***
DASS – Stress (SD)	17.6 (11.8)	7.05 (7.0)	t (49.4) = 4.2***
DASS – Anxiety (SD)	10.7 (7.2)	4.10 (4.9)	t (50) = 4.1***
DASS – Depression (SD)	15.4 (13.3)	2.10 (2.3)	t (32.6) = 5.6***
HDRS – Total (SD)	9.15 (7.7)		
BPRS – Total (SD)	35.9 (8.5)		
YMRS – Total (SD)	4.63 (5.3)		
Duration of illness, y (SD)	8.0 (3.8)		
Mood state, n (%)			
Euthymic	12 (36.4)		
Hypomanic	2 (6.1)		
Depressed	11 (33.3)		
Mixed	2 (6.1)		

*p< 0.05, **p < 0.01, ***p <0.001

AUDIT, alcohol use disorder identification test; BPRS, brief psychiatric rating scale; DASS, depression anxiety stress scales; HDRS, Hamilton depression rating scale; K-10, Kessler psychological distress scale; Mood state, mood state at time of testing.

Note: HAM-D, BPRS, YMRS scores were not conducted within 2 weeks of MRI for 6 patients and therefore their scores and mood states were not included. K-10 and DASS scores are missing for 2 patients and 2 controls

Table 2: MMN amplitudes, metabolite concentrations and hippocampal volume

	BD (n = 33)	Controls (n = 23)	*t*
M1 MMN amplitude (μV)	2.23 (1.3)	2.70 (1.5)	$t(54) = 1.22$
M2 MMN amplitude (μV)	2.22 (1.3)	2.54 (1.2)	$t(54) = 0.94$
Fz MMN amplitude (μV)	-4.21 (1.8)	-5.62 (2.3)	$t(54) = 1.69$*
Cz MMN amplitude (μV)	-4.01 (2.1)	-5.01 (2.2)	$t(54) = 1.69$
Average epochs accepted[&]	181.5 (24.0)	189.1 (10.3)	$t(54) = 1.43$
Hippocampal [Glu/Cr]	1.48 (0.20)	1.49 (0.18)	$t(54) = 0.20$
Hippocampal [Cr]	10.5 (1.2)	10.5 (1.1)	$t(54) = 0.15$
Hippocampal [Glx/Cr][$]	1.79 (0.34)	1.71 (0.22)	$t(47) = 0.97$
Hippocampal [NAA/Cr][$]	1.20 (0.11)	1.27 (0.15)	$t(47) = 1.76$
Hippocampal [Ins/Cr][$]	1.08 (0.16)	1.06 (0.15)	$t(47) = 0.43$
Hippocampal volume (mm^3)	3873 (416)	3862 (484)	$t(54) = 0.09$

* $p < 0.05$

[&] out of 200 duration deviant tones presented pseudo-randomly within 2300 standard tones

[$] data available for 28 patients and 21 controls

BD, bipolar disorder; Cr, creatine; Glu, glutamate; Glx, combined glutamate and glutamine signal; Ins, Inositol; M1, left temporal; M2, right temporal; MMN, mismatch negativity; NAA, N-acetylaspartate.

Table 3: Correlation matrix between temporal MMN and neurometabolite concentration in the hippocampus

		M1		**M2**		**Fz**		**Cz**	
		r	BCa 95% C.I	*r*	BCa 95% C.I	*r*	BCa 95% C.I	*r*	BCa 95% C.I
Glu/Cr	**Whole sample**	0.292*	0.017, 0.52	0.235#	-0.07, 0.53	-0.068	-0.39, 0.27	-0.006	-0.29, 0.31
	Controls	0.512*	0.15, 0.78	0.469*	-0.12, 0.83	-0.166	-0.62, 0.55	-0.141	-0.61, 0.46
	BD	0.147	-0.25, 0.48	0.115	-0.27, 0.45	-0.040	-0.42, 0.40	0.062	-0.31, 0.41
Glx/Cr[$]	**Whole sample**	0.161	-0.20, 0.47	0.070	-0.26, 0.367	-0.244	-0.53, 0.06	-0.252	-0.54, 0.03
	Controls	0.494*	0.03, 0.77	0.248	-0.38, 0.69	-0.195	-0.79, 0.48	-0.245	-0.74, 0.30
	BD	0.060	-0.48, 0.52	0.102	-0.45, 0.51	-0.278	-0.67, 0.17	-0.269	-0.65, 0.16

# $p < 0.1$; * $p < 0.05$

[$] data available for 28 patients and 21 controls

BD, bipolar disorder; Cr, creatine; Cz, central mismatch negativity amplitude; Fz, frontal mismatch negativity amplitude; Glu, glutamate; Glx, overlapping resonance of glutamate-glutamine; MMN, mismatch negativity; M1, left temporal mismatch negativity amplitude; M2, right temporal mismatch negativity.

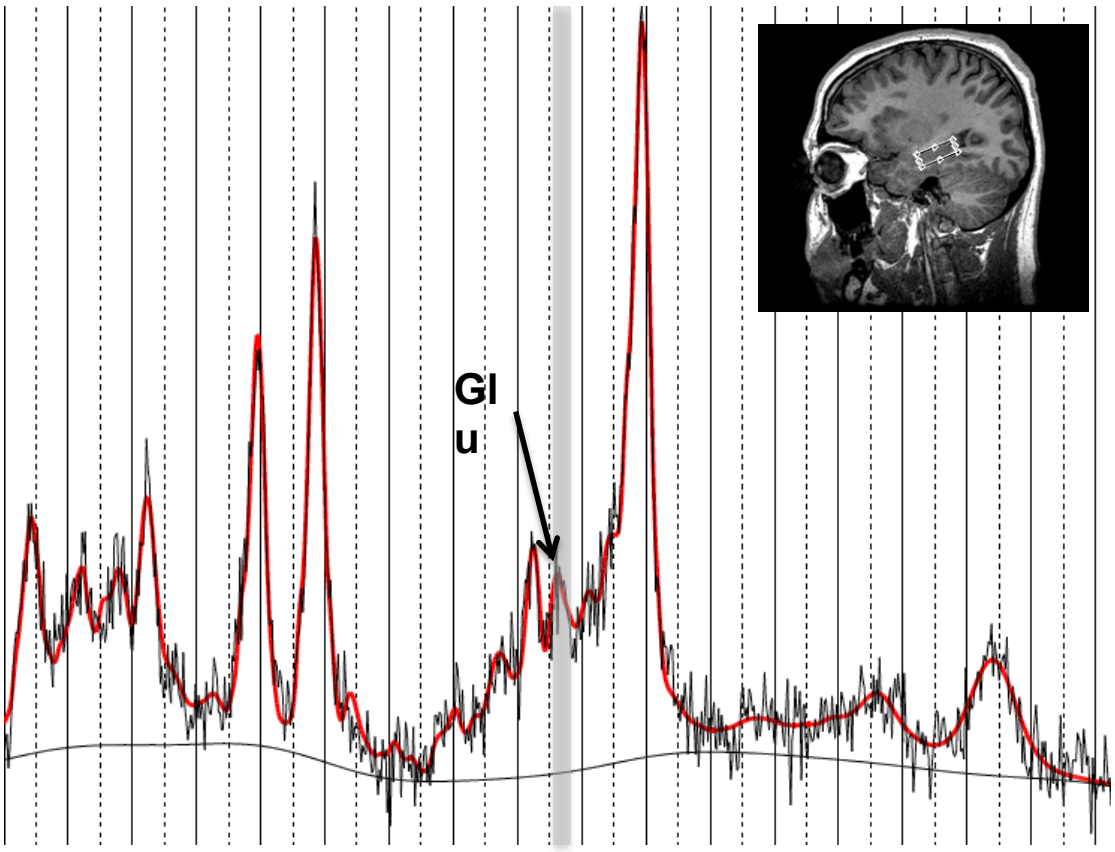

Figure 1: Water supressed glutamate spectra sampled from the left hippocampus, processed using LCModel. The glutamate metabolite peak is resolved at around 2.35ppm. The insert shows a sagittal view of the representative T1-weighted image illustrating the voxel size (1.5 x 3.0 x 1.0 cm) and placement for the hippocampus.

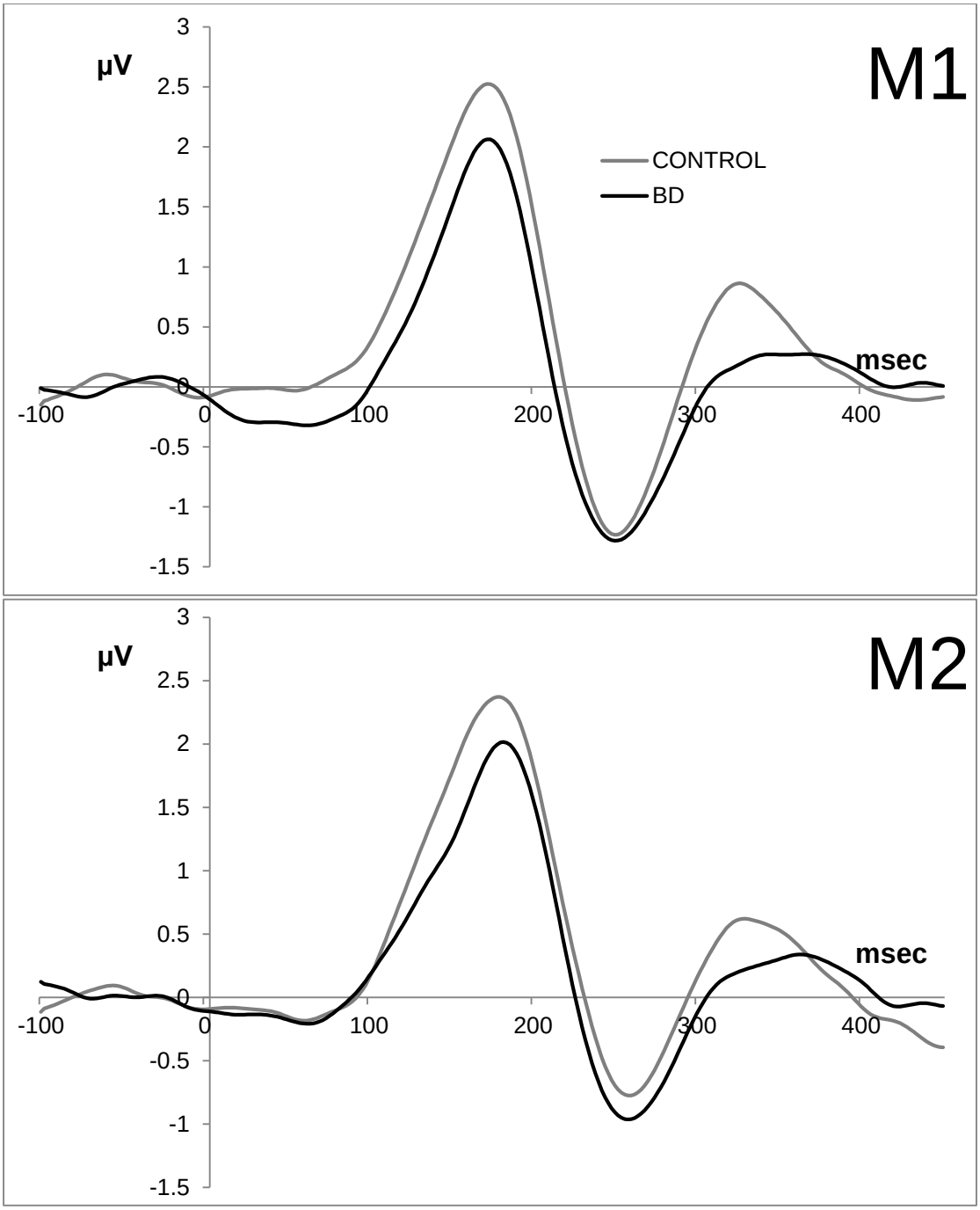

Figure 2: Average event-related potentials for controls (grey line) and BD (black line) at left temporal (M1) and right temporal (M2) electrode sites.
Note that the MMN waveforms recorded at M1 and M2 are reversed in polarity.

BD, bipolar disorder; M1, left temporal; M2, right temporal; MMN, mismatch negativity; msec, milliseconds; μV, microvolts

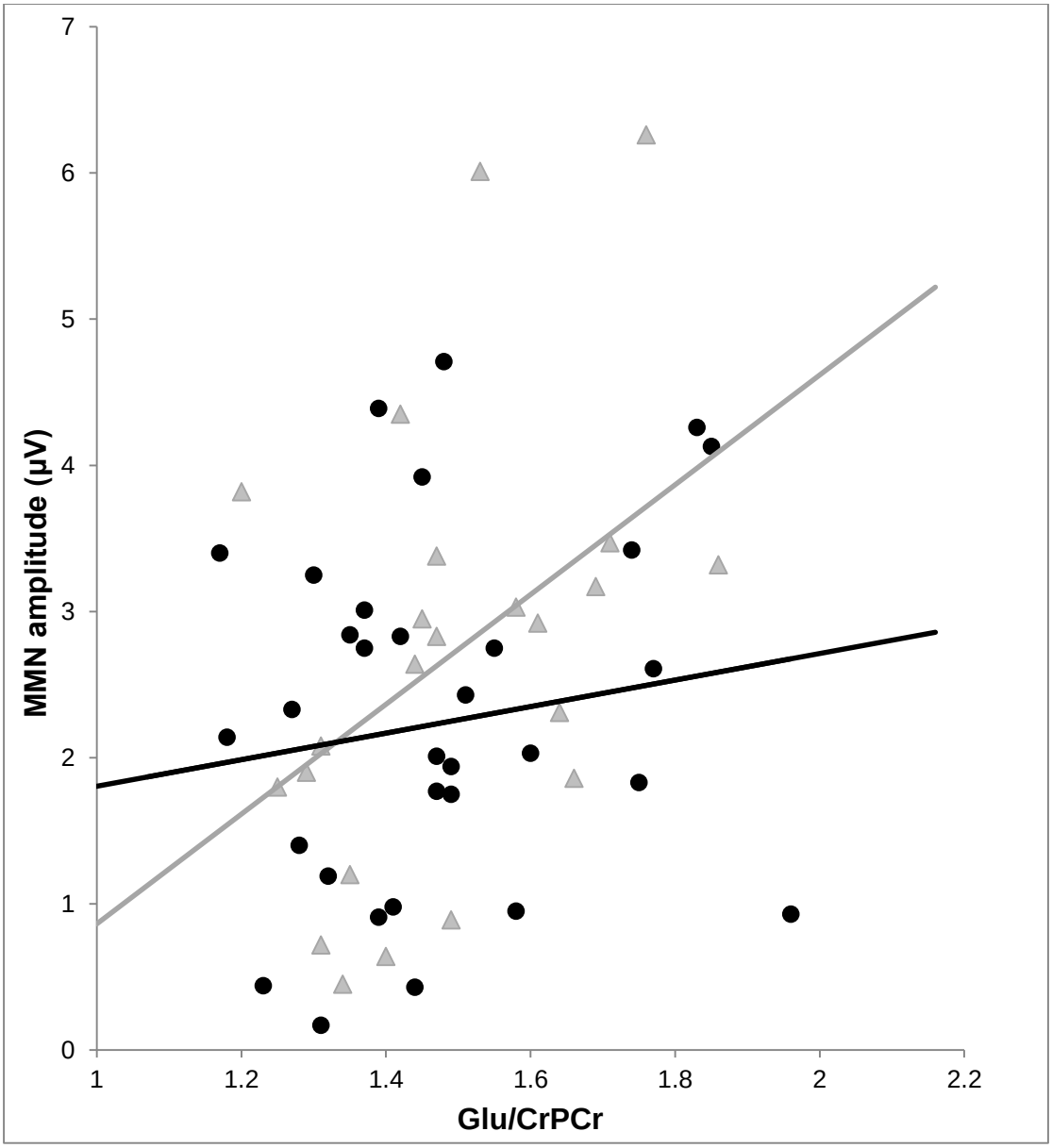

Figure 2: Scatterplot of hippocampal Glu/CrPCr vs. peak MMN amplitude at the left temporal site in controls (grey triangles and grey line) and BD (black circles and black line).
BD, bipolar disorder; Glu/CrPCr, glutamate/creatine-phosphocreatine; I.U., international units; MMN, mismatch negativity.

Supplementary Material 1: Inclusion vs exclusion

	Excluded (n =15)	Included (n=56)	t / χ^2
Reasons for exclusion (n)[&]	Cramer–Rao Lower Bound greater than 20% (7)	N/A	N/A
	Artifact (7)	N/A	N/A
	Poor spectra morphology (9)	N/A	N/A
	Poor fit to raw data (3)	N/A	N/A
	Large variation in residuals (1)	N/A	N/A
Age (SD)	24.2 (2.8)	23.7 (3.3)	t (69) = 0.58
M:F	6:9	14:42	χ^2 (1) = 1.32
BD:HC	8:7	33:23	χ^2 (1) = 0.15
Years of education	13.5 (2.5)	13.5 (2.2)	t (66) = 0.13
K-10 (SD)	23.2 (18.1)	20.5 (8.7)	t (62) = 1.05
DASS – Stress (SD)	15.5 (10.3)	13.3 (11.3)	t (62) = 0.60
DASS – Anxiety (SD)	6.83 (6.7)	8.04 (7.1)	t (62) = 0.53
DASS – Depression (SD)	13.7 (11.7)	10.0 (12.3)	t (62) = 0.93
HDRS – Total (SD)	7.56 (8.4)	6.24 (7.2)	t (57) = 0.49
BPRS – Total (SD)	36.1 (13.9)	31.9 (8.35)	t (57) = 1.24
Duration of illness, y (SD) [$]	9.63 (1.3)	7.97 (3.8)	t (38) = 1.22*

$p< 0.05$*

[&] Categories are not mutually exclusive

[$] Only relevant for proportion of sample with bipolar disorder, n = 8 excluded and n = 33 included.

BD, bipolar disorder; BPRS, brief psychiatric rating scale; DASS, depression anxiety stress scales; HC, healthy controls; HDRS, Hamilton depression rating scale;

K-10, Kessler psychological distress scale