Author's response to reviews

Title: Delayed circadian phase is linked to glutamatergic functions in young people with affective disorders: A proton magnetic resonance spectroscopy study

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Version: 3
Date: 6 November 2014

Author's response to reviews: see over
5th November 2014

Dear Professor Murray,

Thank you for providing reviewers’ comments for the manuscript entitled ‘Delayed circadian phase is linked to glutamatergic functions in young people with affective disorders: A proton magnetic resonance spectroscopy study.’

We appreciate the feedback from reviewer one and two and have revised the manuscript in line with their suggestions wherever possible.

Please refer to the following pages with our response to reviewers’ comments for further detail. We believe we have addressed the reviewers’ concerns and hope you now find this manuscript suitable for publication in *BMC Psychiatry*.

Sincerely

Professor Sharon Naismith
RESPONSE TO REVIEWERS

Reviewer 1:

Comment:
The introduction is too brief, and the 2nd paragraph is too general. Individuals with limited knowledge of NAA, GSH, Glx will be left behind. I would suggest a some redrafting of the 2nd paragraph to give
(a) a better insight into what is related to eg development (would young adults show relationships between sleep midpoint and neurobiological change even in the absence of mental disorders? Are abnormalities being investigated disorder specific?)
(b) a set of hypotheses that give some insights into how eg circadian dysregulation or delayed melatonin onset may relate to the selected neurobiological markers....or a clear statement that this is a proof or principle study and you are running a range of statistical tests (given this is the first study combining these measures I do not think the latter affects a decision on publication)

Response:
(a) Unfortunately, there is a dearth of research examining the relationships between sleep midpoint and neurobiological change in healthy adult or adolescent samples. However, we can certainly acknowledge this lack of research, and have added the following to page 3:

“Indeed, even in healthy adults or adolescents there is a dearth of research specifically examining the inter-relationships between such factors”.

(b) We acknowledge the need for hypothesis in the final paragraph of the introduction and have updated the manuscript to include the following hypothesis statement (page 4).

“We hypothesised that delayed sleep would be associated with decreased concentrations of NAA (indicative of neuronal compromise), GSH (indicative of oxidative stress), and increased Glx (indicative of excitatory neurotransmission)”

Comment: Sample- the sample is described as a tertiary referral population, the reference provided (#23) is about primary care. Given that most of those interested in this paper are not likely to be health service research experts, it may makes sense to rewrite this bit... the main issue is whether this is a convenience/consecutive sample, how did you arrive at the sample size and were cases selected in any way on the basis of their history of affective disorder and etc- additional information to enable readers to understand the sample might include duration of illness or mean number of prior depressive or affective episodes.
Response: A more appropriate and descriptive reference is provided. Additional detail regarding sample selection has been included in the manuscript (page 4). Please see excerpt below:

“Fifty-three young adults (age range 15-33 years, mean = 21.8, sd = 4.3) were recruited from specialised early intervention services for youth mental health (Youth Mental Health Clinic and headspace), Sydney, Australia [23]. Participants were a subset of a broader sample participating in detailed neurobiological assessments. They were specifically selected for this study if their psychiatrist considered them to have an emerging affective disorder [23] and completed actigraphy monitoring and a magnetic resonance imaging scan within a one-month period”.

Since this sample has emerging affective disorders (with largely sub-threshold symptoms) (see Hickie et al, 2013 Early Intervention in Psychiatry), data pertaining to the duration of illness and number of depressive or manic episodes is not available/applicable.

Comment: L103-113: if the sample comprises both UP and BD, were any measure of manic symptoms used. Also, overall how many individuals would currently be regarded as in a depressive episode or in any other mood episode? Were alcohol or substance use comorbidities excluded?

Response: Thank you for making this suggestion. Yes, we did record manic symptoms using the Young Mania Rating Scale. This data is available for 39 individuals. Individuals were not routinely evaluated using DSM-IV criteria for major depression or mania since this sample were specifically targeted to have emerging (rather than full-threshold) affective disorders. Alcohol and substance dependence disorders were excluded.

We have updated the file to include the YMRS data for the 39 individuals. Interestingly, when these scores were controlled for in partial correlation, the relationship between Glx and sleep midpoint was no longer significant. This suggests that manic symptoms may at least partially account for the relationship between Glx and circadian rhythm.

We have added the following to the results and discussion (page 7, 8 and 9):

Page 7:
“As expected, YMRS total score was significantly higher in bipolar participants (t = -2.2, p = 0.034). Importantly, there were also no significant differences between patients taking mood stabilising or antidepressant medication compared to those not taking medication at the time of assessment in terms of age, gender, depressive symptoms, mania symptoms, neurometabolite concentrations (Glx, GSH, NAA) or sleep midpoint (all p > 0.05)”.

Page 8:
“However, when examining the relationship between ACC Glx and sleep midpoint controlling for YMRS scores (n = 39), the resulting partial correlation was not significant (partial r = 0.064, p =
0.73). This is despite the fact that YMRS scores alone were not associated with ACC Glx ($r = -0.29$, $p = 0.862$). Thus, these latter findings may be reflective of the smaller sample with YMRS scores or alternatively may suggest that manic symptoms mediate the relationship between elevated ACC Glx and later sleep midpoint”.

Page 9: Discussion

“In this study of youth with emerging affective disorders, we did not find any group differences in neurometabolites or sleep variables between those participants considered by the psychiatrist to have either a unipolar or bipolar illness. While the reasons for this are unclear, it is possible that they reflect the fact that the symptom severity of this sample was rather mild and encompassed those with emerging rather than full-threshold illnesses. That is, even those that were considered to have a ‘unipolar’ illness may eventually progress to bipolar disorder with longitudinal follow-up. Certainly, our data in the sub-sample of 39 participants with detailed measurement of symptoms of mania suggests that manic symptoms may contribute to the association between delayed circadian phase and Glx. Thus, it is possible that those on a trajectory to bipolar disorder are accounting for the major findings of this study, and future studies focusing on the specificity of these findings within the affective disorder sub-types is warranted”.

Comment: Did the authors examine any other correlations between sleep parameters and neurobiological parameters? I think that the novelty of the study would allow some additional exploration of other variables...so it would be quite acceptable to have a single line in the results to state that other tests were not significant.

Response: As requested, we have added detail regarding Glx and other sleep parameters which demonstrates that higher Glx was associated with later sleep offset, greater sleep disturbance (WASO) and poorer sleep efficiency (page 8), as follows:

“For descriptive purposes, we secondarily examined the association between Glx and key sleep parameters. While the association with TST, TiB and sleep onset were not significant ($r = -0.03$, $p = 0.812$; $r = 0.10$, $p = 0.457$ and $r = 0.24$, $p = 0.083$ respectively), Glx was significantly associated with sleep offset ($r = 0.30$, $p = 0.030$), WASO ($r = 0.325$, $p = 0.017$), and SE ($r = -0.31$, $p = 0.023$)”.

Comment: In the results section, the significant correlation between ACC Glx and sleep midpoints was tested in separate partial correlations using severity and age- why not run one partial correlation with both variables... were these confounders selected because they have previously been shown to have any association? Given that some sleep abnormalities may be more common in males than females or in those with longer duration of illness or in BD rather than UP (??mood stabilizers or not etc)...is there a case for examining additional potential confounders? I appreciate the authors may wish to avoid multiple testing, but there needs to be clarity about why these two variables were seen as the most relevant.
Response: We do not believe we have adequate statistical power to test the effects of various medications. We chose the potential confounds since age is important when considering sleep and neurobiological changes in adolescent samples. We chose to examine depressive severity to investigate whether such relationships were mediated by state phenomena. This rationale has now been entered into the results section. We have also added in details about the YMRS, as requested above.

Comment: The authors are clearly aware of limitations to the study—although I think two things may need to be made more of—
a) The sample size means that there is probably a lack of statistical power eg several separate rather than combined analyses were used.
b) The lack of a control group is commented on—BUT it would help if a further sentence were added about what we know about the associations of sleep midpoint and ACC Glx with age or gender in controls ie without a control group how certain are the researchers that they are looking at associations that are linked to affective disorder pathway rather than associations that would be seen as normal at this stage of individual development, (or within normal range).

Response: We agree that the limitation regarding controls and sample size requires elaboration and have expanded the discussion to be more specific about interpreting these results (page 11).

“While this study represents the first to examine the association between delayed circadian rhythms and underlying neurobiology in youth depression, several methodological factors should be considered when interpreting our findings. Firstly, the lack of a control group is a limitation of this study. This would have been particularly helpful for interpretation of these findings since it is currently unclear how sleep midpoint relates to ACC Glx in healthy adolescent or even adult samples. Secondly, adolescence itself is known to be associated with circadian delay [41]. Thus, from these results, we cannot ascertain whether our findings are reflective of adolescence generally or whether they are specific to affective disorders. Third, although the sample size of 53 is not particularly small for these types of detailed neurobiological studies, this sample is rather heterogeneous since ultimate illness trajectory is unknown in these early clinical stages [42]. Also, a larger sample would have been optimal in terms of conducting multivariable analyses whereby the influence of potential confounds could be determined concurrently.

Reviewer 2

Comment: Medication effect. The sample size is quite a good number, 53 subjects participated in this study. However, the treatment history is complicated by multiple medications. Therefore, the authors should have more discussion about medication effects and do more data processing to check whether some kind of relationship exists between medication effects and MRS measurements.
Response: We have examined those taking either an antidepressant or mood stabilising medication and found no significant difference in clinical or spectroscopic measures compared to those not taking a medication. The manuscript has been updated to reflect this change, as follows:

"Importantly, there were also no significant differences between patients taking mood stabilising or antidepressant medication compared to those not taking medication at the time of assessment in terms of age, gender, depressive symptoms, mania symptoms, neurometabolite concentrations (Glx, GSH, NAA) or sleep midpoint (all p > 0.05)."

Comment: Bipolar vs. unipolar. 53 subjects were recruited for this study, 32 of which were classified as unipolar and 21 as bipolar. The authors did group-group difference analysis and found no significant difference among demography, MRS measurements and depressive symptoms. The authors should provide more interpretation about why differences were not observed and give some possible explanations.

Response: We agree these findings should be discussed and we have added the following to page 11:

"In this study of youth with emerging affective disorders, we did not find any group differences in neurometabolites or sleep variables between those participants considered by the psychiatrist to have either a unipolar or bipolar illness. While the reasons for this are unclear, it is possible that they reflect the fact that the symptom severity of this sample was rather mild and encompassed those with emerging rather than full-threshold illnesses. That is, even those that were considered to have a ‘unipolar’ illness may eventually progress to bipolar disorder with longitudinal follow-up. Certainly, our data in the sub-sample of 39 participants with detailed measurement of symptoms of mania suggests that manic symptoms may contribute to the association between delayed circadian phase and Glx. Thus, it is possible that those on a trajectory to bipolar disorder are accounting for the major findings of this study, and future studies focusing on the specificity of these findings within the affective disorder sub-types is warranted."

Comment: It is not clear how to do “Sleep-wake ambultatory assessment”, especially in Line 116-118’s description. How was sleeping monitored? Is each day of the month? And when (in the one month window) was 1H-MR performed? Did every subject follow the same procedures? This is particularly important for the female subjects considering females’ menstrual cycle.

Response: Study participants were asked to wear an activwatch-64/L/2 (Philips Respironics, USA) and complete a sleep diary for a total of 14 days within one month of completing clinical assessments and an MRI scan. Sleep was monitored using the activwatch, which is an accelerometer that also collects light intensity data. Detail regarding the duration with which participants were asked to wear the activwatch has now been added to the text (detailed below). As described, trained technicians scored various sleep measures using Actiware 5.0 software including ‘sleep onset’, ‘sleep offset’, ‘time
in bed’, ‘total sleep time’, ‘wake after sleep onset’ and ‘sleep efficiency’ (each measure defined in text). The mean duration of actiwatch data collection was 12.2 days (sd = 3.3 days; median duration = 13.0 days; described in text). Detail regarding the female participants menstrual cycle was not collected. As such, we are unable to comment on any effect this may have had on results. This has however now been added to the limitations section of the manuscript.

The following excerpts have been modified to reflect the aforementioned response to Reviewer 2’s comments:

“Participants completed sleep diary and actigraphy monitoring (Actiwatch-64/L/2, Philips Respironics, USA) for 14 days (mean duration = 12.2 days, sd = 3.3 days; median duration = 13.0 days) within one month of undergoing 1H-MRS.” Page 5.

“It is also important to note that details regarding female study participants’ menstrual cycle phase were not collected. Increasing evidence suggests that menstrual phase affects sleep in females [36] however we were unable to account for the possible influence of this factor.” Page 12

Comment: The most interesting findings are the association between circadian and Glx in the ACC. The authors gave some interpretation of how sleep is involved in brain circuitry (including ACC) in Line 194-198, but they should give a clearer discussion of how Glx and ACC regulate sleep.

Response: In regard to the ACC and sleep regulation, the manuscript has updated to include a paragraph describing the role of the ACC in sleep and affective disorders.

The authors accept this criticism re Glx, but note that as mentioned in the text, there is little evidence currently available regarding the role of Glx (or Glu) in sleep-wake regulation. We describe the current evidence in the excerpt below, and highlight that the specific role of Glx in the sleep-wake cycle is currently unknown.

“The specific role that Glx substituents assume in sleep-wake cycles is currently unknown. However, it is clear that glutamate signalling is critically involved in information transmission, plasticity and neurotoxicity. Animal studies show that it increases during both waking and rapid eye movement sleep states and when the sleep period is preceded by wake states [34, 35]. Additionally, the rate of glutamate decrease is greatest with sleepiness and when slow wave activity is high [34]. Thus, it is feasible that sleep-wake history mediates glutamatergic signalling in affective disorders, though such causative relationships have yet to be demonstrated in humans.” Page 11.

Comment: Limitations. More should be mentioned about how MRS measurements were acquired at different stages of brain sleep-wake conditions and menstrual cycles for female subjects; 2) The method of measuring GSH by PRESS 35ms echo time is questionable and cannot be fully separated from Glu/Gln’s contribution.
Response: The MRS data was collected during wake only. The mean time of MRS data collection was 3:42pm (sd = 1.8 hrs; described in text). As such, there was little variance in the sleep-wake conditions between subjects. We have acknowledged the limitation regarding the time of day individuals were scanned in the following excerpt:

“In this study, MRS measures were derived across various times of the day, and if in-vivo measures of brain neurometabolites also vary across the day, this is a potential source of noise that could be eradicated if scanning were to occur at a fixed time period.” Page 12

In response to a previous comment by Reviewer 2, the limitations have already been updated to include not collecting data regarding females’ menstrual cycle phase.

Regarding the measurement of GSH using a PRESS sequence, we have previously validated our findings and have published several papers examining GSH in different populations. Nevertheless, we have included reference to our papers validating the GSH signal detected by our scanner in the limitations of this manuscript.

“Furthermore, this study used a PRESS sequence to examine GSH concentration. Although we have previously validated the GSH signal measured using this sequence, replication of null findings using MEGA-PRESS may be warranted.” Page 12

We acknowledge the limitation of using Glx rather than Glu or Gln and had already made mention of this in the limitations section. This has been expanded to include the need for further research examining these markers, please see excerpt below:

“Additionally, since we examined Glx as a surrogate marker of glutamate, the current findings may in part be also reflective glutamine. Further research examining the roles of Glu and Gln are now required to further delineate their role in sleep-wake function.”