PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Effects of adjunctive brexpiprazole on sleep-wake and circadian parameters in youth with depressive disorders: Study protocol for a clinical trial
AUTHORS	Carpenter, Joanne; Zmicerevska, Natalia; Crouse, Jacob; Nichles, Alissa; Garland, Alexandra; Song, Yun; Wilson, Chloe; Rohleder, Cathrin; McHugh, Catherine; Leweke, F. Markus; Koethe, Dagmar; Scott, Elizabeth; Hickie, Ian

VERSION 1 – REVIEW

REVIEWER	Fornaro, M Columbia University,, New York State Psychiatric Institue
REVIEW RETURNED	22-Sep-2021

GENERAL COMMENTS	The protocol looks fine, my one quibbles regard the adopted rating. The QIDS-16 should be coupled with a standard rating such as the MADRS. In addition, I expect TRD to be an inclusion criterion. Please details. Finally, one pivotal reference should appear in the updated rationale: https://pubmed.ncbi.nlm.nih.gov/31431092/

REVIEWER	Brietzke, Elisa Queen's University
REVIEW RETURNED	17-Dec-2021

GENERAL COMMENTS	This is an open label, single arm trial with brexipirazole as an adjunctive medication for the treatment of insomnia in young individuals with depression. It is sponsored by Lundbeck under an investigator-initiated grant. This study was designed aiming to fulfill an unmet need in the care of individuals with depression: circadian rhythms abnormalities. It also follows a trend in the psychiatric practice which is the prescription of second generation antipsychotics for insomnia and circadian abnormalities, more notably quetiapine. Even thinking that it is valid to investigate brexpiprazole as a circadian regulator, this trial has numerous limitations in its design and description. For example, the adoption of outcomes related both to general depressive severity and circadian rhythm makes the trial confuse. It would make much more sense simply have a trial of brexpiprazole for insomnia (a study that is really needed). There is no assessment of sleep and the circadian assessment is very simple and restrict. I added more comments below, which I believe that could be used to improve some aspects of this trial. Major comments: 1. The study is about MDD in youth and the introduction references about treatment resistance in depression are from

	studies with adults. Please try to use the most appropriate references in this section. 2. There is no mention to sleep architecture abnormalities in MDD, specially REM changes. These are classic phenotypes in MDD and should be mentioned. 3. The authors should explain in more detail what happen with the circadian rhythm during brain maturation specially during adolescence. 4. please include at least one sentence talking about the brain anatomy and physiology of circadian control. 5. Please include one sentence describing the effect of brexpiprazole in sleep. 6. In the introduction it is unclear if the data support that brexpiprazole has an effect in the circadian rhythm which is independent of the global improvement of depression. 7. It is unclear if the circadian abnormalities could be induced by the antidepressants and not part of the depressive psyhcoipathology. 8. The inclusion criteria are not explained in detail. For example, it is not clear if this population is considered as having treatment-resistant depression. More importantly, there is no definition of the circadian abnormalities. 9. As the definition of circadian abnormalities is very broad, I suspect that simple sleep hygiene measures would help most of the patients, without exposing them to the risks of the medication. 10. CAARMS is not a good instrument to assess suicidal ideation. Please consider the adoption of a more commonly use questionnaire such as Columbia Suicide Severity Rating Scale. 11. It is unclear what the authors consider as a "significant" use of alcohol and substances. Specifically the use of cannabis should be addressed, as it is very prevalent in this age range. 12. What is the definition of "recent" travel? 13. what medications are considered an exclusion criterium due to risk of drug-drug interaction? 14. What kind of recommendations about driving are being provided to the research subjects? 15. What kind of recommendations about contraception are being provided to the research subjects? 16. Please describe the actigraphy device that will be used in this study. 17. Please include detailed information about the blood analysis. 18. Please explain of the biomarkers will be collected in a standardized moment of the day and make a comment of possible influence of the menstrual cycle. 19. please provide more information about the psychoeducation section. Is this is groups? individual? in person or online? who is providing this intervention? What is the rationale behind it? What is its effect in the primary outcome? 20. The information about the dose of brexpiprazole is very scarce. 21. The description of the trial is not clear. There are several secondary outcomes and no assessment of sleep (e.g. polysomnography). 22. There are several instruments and questionnaires which are not related with the outcomes. 23. The safety and tolerability piece is remarkably under described. Minor comments:

	1. Prefer second generation antipsychotics instead of atypical antipsychotics. 2. There is no mention to biomarkers in the abstract.

REVIEWER	Stoiljkovic, Milan Yale University School of Medicine, Comparative Medicine
REVIEW RETURNED	04-Jan-2022

GENERAL COMMENTS	This is a study protocol for an open-label single-arm trial investigating the effects of the brexpiprazole as an adjuvant treatment for sleep-wake cycle disturbance in young subjects with MDD on stable antidepressant therapy with SSRI or SNRI drugs. The protocol is well designed and described in detail. Proposed multidimensional assessments and statistical analyses are appropriate for valid estimation of trial outcomes. I do not have any major concerns related to this protocol. However, I will suggest the following issues to be addressed: 1. discussion of potential limitations of the study 2. explanation of scoring scales for clinical assessment tests wherever missing

VERSION 1 – AUTHOR RESPONSE

Reviewer 1

R1.1: The QIDS-16 should be coupled with a standard rating such as the MADRS. In addition, I expect TRD to be an inclusion criterion. Please details. Finally, one pivotal reference should appear in the updated rationale:

R1.1 Response: We thank the reviewer for their suggestion. We would like to highlight that the MADRS is already included in the study protocol as one of the main depressive symptom measures under "Clinical Assessments", together with the QIDS (both clinician-rated and self-report; see Page 12 of the manuscript).

Regarding treatment-resistant depression (TRD) being an inclusion criterion, for the purposes of the current trial and to maximise recruitment, we are including young people who fail to respond to at least one adequate trial of pharmacological treatment (minimum four weeks), rather than a formal diagnosis of TRD.

Finally, we have added reference to the following paper to the rationale:

Fornaro M, Fusco A, Anastasia A, Cattaneo CI, De Berardis D. Brexpiprazole for treatment-resistant major depressive disorder. Expert Opin Pharmacother. 2019 Nov;20(16):1925-1933. doi: 10.1080/14656566.2019.1654457. PMID: 31431092.

The text (now including reference to the Fornaro et al., 2019 paper) now reads:

"While multiple psychological and pharmacological treatments are commonly provided, a large proportion of patients fail to respond to first-line psychotherapy or antidepressant treatments [3-10], and augmentation with a second generation antipsychotics is often recommended in these treatment-resistant cases [11,12]". (Page 4, Lines 90-94)

Reviewer 2

This is an open label, single arm trial with brexipirazole as an adjunctive medication for the treatment of insomnia in young individuals with depression. It is sponsored by Lundbeck under an investigator-

initiated grant. This study was designed aiming to fulfill an unmet need in the care of individuals with depression: circadian rhythms abnormalities. It also follows a trend in the psychiatric practice which is the prescription of second generation antipsychotics for insomnia and circadian abnormalities, more notably quetiapine.

Even thinking that it is valid to investigate brexpiprazole as a circadian regulator, this trial has numerous limitations in its design and description. For example, the adoption of outcomes related both to general depressive severity and circadian rhythm makes the trial confuse. It would make much more sense simply have a trial of brexpiprazole for insomnia (a study that is really needed). There is no assessment of sleep and the circadian assessment is very simple and restrict. I added more comments below, which I believe that could be used to improve some aspects of this trial.

Major comments:

R2.1. The study is about MDD in youth and the introduction references about treatment resistance in depression are from studies with adults. Please try to use the most appropriate references in this section.

R2.1 Response: We thank the reviewer for highlighting this. We now include a larger number of references to treatment non-response in youth populations. The following included studies refer to youth populations specifically:

- Kennard et al. (2000). JAACAP [PMID: 19127172]
- March et al. (2004). JAMA [PMID: 15315995]
- Bear et al. (2020). JAACAP [PMID: 31881268]
- Eckshtain et al. (2020). JAACAP [PMID: 31004739]
- Wolpert et al. (2019). Lancet Psychiatry [PMID: 30522980]
- Cuijpers et al. (2020). JAMA Psychiatry [PMID: 32186668]
- Murphy et al. (2021). Lancet Psychiatry [PMID: 34419187]

The text now reads:

"While multiple psychological and pharmacological treatments are commonly provided, a large proportion of patients fail to respond to first-line psychotherapy or antidepressant treatments [3-10], and augmentation with a second generation antipsychotics is often recommended in these treatment-resistant cases [11,12]." (Page 4, Line 92)

R2.2. There is no mention to sleep architecture abnormalities in MDD, specially REM changes. These are classic phenotypes in MDD and should be mentioned.

R2.2 Response: We have now added references in the Introduction regarding alterations in sleep architecture in MDD, including the following:

- Zhang et al. (2020). Sleep Medicine Reviews [PMID: 32739826]
- Wang et al. (2015). Current Neuropharmacology [PMID: 26412074]
- Palagini et al. (2013). Sleep Medicine Reviews [PMID: 23391633]
- Pillai et al. (2011). Biological Psychiatry [PMID: 21937023]

The text now reads:

While disturbances in electrophysiology-based measures of sleep (e.g., REM sleep) have been considered by some to represent biomarkers for depression [40-43], the current trial focuses instead on the investigation and measurement of biological and behavioural changes associated with the circadian system (including rhythms of rest/activity, melatonin, cortisol, and core body temperature), rather than changes in electrophysiological sleep architecture (which is beyond the scope of this study)." (Page 6, Lines 142-147)

R2.3. The authors should explain in more detail what happen with the circadian rhythm during brain maturation specially during adolescence.

R2.3 Response: We agree with the reviewer and have provided more detail regarding changes in the circadian rhythm during adolescence, including the following new references:

- Roenneberg et al. (2004). Current Biology [PMID: 15620633]
- Crowley et al. (2007). Sleep Medicine [PMID: 17383934]

The text now reads:

"During adolescence there is a phase shift in the circadian rhythm of the sleep-wake cycle, such that adolescents typically develop a bio-behavioural preference for going to sleep later and waking later (a manifestation of changes in the underlying biology of the circadian system) [28, 29]." (Page 4, Lines 111-113)

R2.4. please include at least one sentence talking about the brain anatomy and physiology of circadian control.

R2.4 Response: This was an important omission; we now include greater detail about the neurobiology of the circadian system in the Introduction, which now reads:

"The human circadian system is controlled by a master oscillator in the brain's hypothalamus (called the suprachiasmatic nucleus) which projects to a range of brain regions that govern many bio-behavioural processes altered in depression, including mood, vigilance, and the sleep-wake cycle. The circadian system is primarily entrained by bright light and its function and timing can be disrupted by factors including aberrant light exposure and irregular sleep-wake behaviours [23-24]." (Page 4, Lines 102-106)

R2.5. Please include one sentence describing the effect of brexpiprazole in sleep.

R2.5 Response: We have added a sentence describing the reported effects of brexpiprazole on sleep in the Introduction. The text now reads:

"As an adjunctive treatment in MDD patients with inadequate response to antidepressant treatment, brexpiprazole has been reported to lead to clinical improvement of sleep disturbances (e.g., insomnia) and depressive symptoms, as well as improvements of daytime alertness and functioning" [39]. (Page 5, Lines 127-130)

R2.6. In the introduction it is unclear if the data support that brexpiprazole has an effect in the circadian rhythm which is independent of the global improvement of depression.

R2.6 Response: At present, there is a lack of data to answer this question, with one exploratory study reporting that some measures of sleep and daytime alertness were improved in patients with sleep disturbances treated with adjunctive brexpiprazole (Krystal et al., 2016; PMID: 27835722). Accordingly, the goal of the current trial will be to provide the first mechanistic study of the potential effects of brexpiprazole on circadian rhythms and depressive symptoms. This trial will allow us to examine whether sleep/wake and/or circadian rhythm changes are associated with changes in depressive symptoms during brexpiprazole treatment.

R2.7. It is unclear if the circadian abnormalities could be induced by the antidepressants and not part of the depressive psyhcoipathology.

R2.7 Response: As this clinical trial includes an assessment of sleep-wake circadian rhythm disturbances (via actigraphy) *before* prescription of brexpiprazole, we will be able to examine the extent to which sleep-wake circadian rhythms were abnormal prior to, during, and after brexpiprazole treatment.

R2.8. The inclusion criteria are not explained in detail. For example, it is not clear if this population is considered as having treatment-resistant depression. More importantly, there is no definition of the circadian abnormalities.

R2.8 Response: We thank the reviewer for seeking clarification and note that this comment echoes a comment by reviewer 1. For the purposes of this study and to maximise recruitment, we are including young people who fail to respond to respond to at least one adequate trial of pharmacological treatment (minimum four weeks), rather than those formally diagnosed with treatment-resistant depression (TRD).

The definition of sleep-wake circadian abnormalities is provided in the inclusion criteria section of the manuscript (Page 7), which reads: "Perturbed 24-hour sleep cycle as evidenced by: delayed sleep onset; delayed sleep offset; disrupted sleep; high day-to-day variability of the sleep-wake cycle; non-restorative sleep; or daytime fatigue."

R2.9. As the definition of circadian abnormalities is very broad, I suspect that simple sleep hygiene measures would help most of the patients, without exposing them to the risks of the medication.

R2.9 Response: We agree with the Reviewer that improving sleep hygiene is one element of correcting the sleep-wake cycle; as such, participants in the current trial will be provided a personalised psychoeducation session based on the patients' actigraphy data as well as a general sleep-wake and circadian psychoeducation session comprising aspects of sleep hygiene (e.g., sleep environment, effects of light). While sleep hygiene is one important behavioural approach, poor sleep hygiene is not believed to be the primary cause of circadian rhythm sleep-wake disturbances (PMID: 16490003), and as such, a multimodal approach involving sleep hygiene education and other measures to treat sleep-wake disturbances such as hypersomnia are recommended for the successful management of sleep-wake disturbances (PMID: 17395535).

R2.10. CAARMS is not a good instrument to assess suicidal ideation. Please consider the adoption of a more commonly use questionnaire such as Columbia Suicide Severity Rating Scale.

R2.10 Response: We would like to point out that Columbia Suicide Severity Rating scale is already included in the study as one of the self-report measures. Several studies have also found that the CAARMS demonstrated adequate predictive validity and good inter-rater reliability (PMIDs: 16343296; 29413807; 22239569). Moreover, we have successfully used CAARMS to assess suicidal ideation in the youth mood disorder population in the past studies, namely the *Fish Oil Youth Depression Study* (PMID: 25130262) and several publications from the *Transitions Study* (PMIDs: 23889887; 24673851). Finally, the current study has been approved by the Sydney Local Health District Human Research Ethics Committee who deemed the CAARMS to be an appropriate measure for the suicidal ideation assessment in this trial.

R2.11. It is unclear what the authors consider as a "significant" use of alcohol and substances. Specifically the use of cannabis should be addressed, as it is very prevalent in this age range.

R2.11 Response: We thank the reviewer for highlighting this point. We have changed the wording in text from 'significant use' to 'substance dependence', which will be assessed by the *Structured Clinical Interview for DSM* (SCID). The SCID will also be used to assess the level of cannabis use and other substances, and any patients diagnosed with any substance dependency will be excluded from the study.

R2.12. What is the definition of "recent" travel?

R2.12 Response: We have added greater information to the text regarding recent travel. The text now reads:

"… or (viii) recent transmeridian travel (i.e., participants will be required to wait three days for each jet lag hour before entering the study)." (Page 8, Lines 194-195)

R2.13. What medications are considered an exclusion criterium due to risk of drug-drug interaction?

R2.13 Response: We thank the Reviewer for highlighting this important point. We have added a further statement to the exclusion criteria section of the manuscript, which now reads:

"…taking CYP2D6 or CYP3A4 inhibitors (or other contraindicated medications listed in the Rexulti investigational product information)." (Page 8, Lines 196-197)

R2.14. What kind of recommendations about driving are being provided to the research subjects?

R2.14 Response: We now make mention of this in the "*Safety and Side Effects Monitoring*" section of the paper; the text now reads:

"According to the investigational product information, participants will be advised to not drive a car, operate machinery, or do other dangerous activities until they know how the investigational product affects them, as it may induce drowsiness in some subjects." (Page 21, Lines 489-491)

R2.15. What kind of recommendations about contraception are being provided to the research subjects?

R2.15 Response: We thank the reviewer for highlighting this point. We have provided further information on this in the "*Safety and Side Effects Monitoring*" section of the manuscript.

The text now reads:

"Throughout the trial the study doctor will monitor participants for pregnancy. It is not currently included in the protocol, but the trial Standard Operating Procedures manual explains that the study doctor will be giving relevant contraception advice to the participants based on the current investigational product information." (Page 21, Lines 484-487)

R2.16. Please describe the actigraphy device that will be used in this study.

R2.16 Response: We thank the Reviewer for pointing this out and have included the description of the actigraphy device in the text, which now reads:

Participants will be asked to complete a sleep diary and to wear an actigraph (GENEActiv device; Activinsights, Kimbolton, UK) on the non-dominant wrist for at least 10 days prior to commencing the study, and continuously for the 8-week treatment phase. The device is comfortable and easy to use, battery operated wrist-worn device, similar in appearance to Fitbit, designed to record and provide data on movement, light, temperature and sleep patterns. The device will provide objective monitoring of participants' 24-hour circadian rhythm, including their sleep onset and duration, rise time, any night-time sleep interruptions, and activity patterns (for details, see Table 1). The device allows for the outputting of real time raw measurement data for up to a month without charging. Instructions will be provided to participants on how to use the device upon their recruitment into the study." (Page 17, Lines 401-405; Page 18, Lines 406-410)

R2.17. Please include detailed information about the blood analysis.

R2.17 Response: Greater detail about the blood collection analysis can now be found in the Supplementary Materials.

The text in the Supplementary Materials now reads:

"Bloods will be collected for the assessment of metabolic and inflammatory measures, and for genomic analysis. Metabolic blood markers including triglycerides, cholesterol (total, LDL, HDL), and Homeostasis Model of Insulin Resistance (HOMA2-IR) calculated from fasting glucose and insulin measures. Inflammatory blood markers that will be collected include IL-1β, IL-6, TNF-α and CRP."

Blood samples will be collected from the participants in the morning, in fasting state.

Blood samples for inflammatory markers will be analysed by the team at NSW Health Pathology. Luminex cytokine assays will be conducted to assess inflammatory markers. Any remaining samples following analysis will be disposed of in a bio-hazardous waste bin.

Blood samples for genomic analysis will be shipped to the Human Studies Unit within the Institute of Molecular Biosciences at the University of Queensland.

Genomic data will be generated using the Illumina platform for both genome wide associations scans and whole genome methylation analysis. Genomic data generated within the Human Studies Unit is then passed through a series of quality control steps before being released to analysts to progress the computational analyses. Creation and sharing of genomic data are done using the unique identifier assigned by the Human Studies Unit and is separate to the local site ID under which the samples are collected. Records linking clinical information with genomic data will be hosted on the University of Queensland server using a secure, purpose-built database. Clinical and genomic data will be held and used for analyses under previous governance approvals held by Professor Naomi Wray." (Page 2, Supplementary Materials)

R2.18. Please explain of the biomarkers will be collected in a standardized moment of the day and make a comment of possible influence of the menstrual cycle.

R2.18 Response: We agree with the reviewer on the need to provide further explanation on biomarker collection, which we now include in the Supplementary Materials.

The text in the Supplementary Materials now reads:

"Dim Melatonin Light Onset (DLMO) and Cortisol will be measured relative to the individual sleep-wake cycle.

Participants will attend the sleep laboratory approximately 6 hours prior to their habitual sleep time. Meals, lighting, and posture will be controlled. Food or beverages containing caffeine will be prohibited starting at noon on the circadian assessment day.

Saliva samples will be collected every hour over an 8-hour period (i.e. until 2-hours after habitual sleep onset) with salivette tubes (Sarstedt, Nümbrecht, Germany). Saliva samples will then be centrifuged and frozen for subsequent analysis of melatonin and cortisol by radioimmunoassay. DLMO is defined by interpolation with a threshold of 3 pg/mL (stable for the three subsequent samples).

Core body temperature will be measured at a 60-second sampling rate across the evening, night, and morning with an ingestible capsule-size sensor and the Equivital monitoring system (Equivital, Cambridge, UK). Data will be transmitted wirelessly from this sensor to a recording unit. Temperature recording using ingestible sensors has been validated previously (Darwent at al, 2011)". (Pages 2-3, Supplementary materials)

The influence of the menstrual cycle on the biomarker collection is, regrettably, beyond the scope of the present study.

R2.19. Please provide more information about the psychoeducation section. Is this is groups? individual? in person or online? who is providing this intervention? What is the rationale behind it? What is its effect in the primary outcome?

R2.19 Response: More information about the psychoeducation session can now be found in the Supplementary Materials.

The text in the Supplementary Material now reads:

"The psychoeducation session is an individual, online 1 hr session delivered via Zoom and facilitated by the trained researcher from the Brain and Mind Centre. It will involve information on sleep, circadian rhythms and their importance for healthy lifestyle, as well as advice on regulating the body clock for mental health, based on published circadian research findings specific to youth population, and customised to every participant based on their individual actigraphy and sleep-wake cycle data.

Psychoeducational interventions focusing on healthy lifestyle habits including diet, physical activity and sleep practices have been previously shown to ameliorate both the physical and mental health concerns of young people with psychiatric disorders (Bersani et al., 2017; De Rosa et al., 2017; Rönngren et al., 2018; Fiorillo et al., 2019; Taylor at al., 2018; Goracci et al, 2016).

We hope that the delivery of the psychoeducation session will lead to participants implementing the changes and advice to optimise their individual sleep-wake cycle, which will contribute to their mental and physical wellbeing and a possible reduction in depressive symptom severity.

We will compare the effects sizes from our study to those reported in simple psychoeducation interventions in the literature from similar studies." (Page 4, Supplementary materials)

R2.20. The information about the dose of brexpiprazole is very scarce.

R2.20 Response: We agree with the reviewer and have added further detail on Brexpiprazole dosage. The text now reads:

"Patients will receive 2mg/day, once daily as tablets, for oral use. The brexpiprazole dosage will be steadily increased from 1mg/day during week 1, to 2mg/day during weeks 2-8 (up-titration). This is the dosage and titration regime recommended for adjunctive use in major depression by the Federal Drug Administration (USA). Several previous clinical trials have used this titration regime from 1mg to 2mg (33, 39, 50), and a dose of 2mg has been shown to be effective in reducing depressive symptoms (32). As doses higher than 2mg have been shown to increase incidence of akathisia (36), a maximum dose of 2mg will be used in the present study to minimise side effects." (Page 9, Lines 225-231)

R2.21. The description of the trial is not clear. There are several secondary outcomes and no assessment of sleep (e.g. polysomnography).

R2.21 Response: We thank the reviewer for highlighting this point and we note that this echoes a previous comment.

While there is no electrophysiologic assessment of sleep in this trial, the primary focus of the study is on circadian rhythm sleep-wake parameters, of which there are multiple measures (e.g., ambulatory sleep-wake cycles via actigraphy, core body temperature) including gold-standard assessments (dim-light melatonin onset). Together, these measures of circadian rhythm sleep-wake cycles will allow us to robustly examine associations between changes in circadian rhythms and changes in depressive symptoms among depressed young people administered brexpiprazole.

We have added further details to make this clearer in the Introduction. The text now reads:

"Accordingly, in this clinical trial we aim to investigate whether the effect of brexpiprazole on depressive symptoms is associated with changes in 24-hour sleep-wake cycle or circadian parameters in young people with MDD. While disturbances in electrophysiological measures of sleep (e.g., REM sleep) have been considered by some to represent biomarkers for depression [40-43], this trial focuses instead on the investigation and measurement of bio-behavioural changes associated with the circadian system (including rhythms of rest/activity, melatonin, cortisol, and core body temperature), rather than changes in electrophysiological sleep architecture (which is beyond the scope of this study)." (Page 6, Lines 139-147)

While we have included several secondary outcome measures, we note that this is common in clinical trials and will allow us to investigate whether change in sleep-wake and circadian parameters are associated with change in functioning, which is an important and unanswered question that will be examined in a subsequent outcome paper.

R2.22. There are several instruments and questionnaires which are not related with the outcomes.

R2.22 Response: All instruments and questionnaires used in the study are employed to measure either primary, secondary, or tertiary outcomes, and are listed in Table 1 of the manuscript. Some of the instruments (e.g., clinical staging, illness trajectory model) are also linked to outcomes of interest in our larger body of work regarding the onset, course, and pathophysiology of youth-onset mood disorders and will be examined in secondary analyses in subsequent papers.

R2.23. The safety and tolerability piece is remarkably under described.

R2.23 Response: We agree with the review that this important information was neglected. We have added a new subheading "*Safety and Side-effects monitoring*" in the Methods. The text now reads:

"Previous studies have shown that brexpiprazole is generally well tolerated, with no unexpected or severe side effects [32; 35; 104; 105].

Safety and tolerability of the investigational product will be closely monitored throughout the trial. For the duration of the 8-week treatment period, weekly phone calls will be conducted to participants to monitor safety and elicit information regarding side effects and potential adverse events. Any changes in concomitant medications will also be investigated and recorded. In addition, participants will be provided with a medication diary and asked to complete during the study to monitor tolerability and adherence.

Further formal assessment of side effects and potential adverse events following the end of the treatment phase will be conducted at the two follow-up visits (visit 4 and 5). At the baseline visit, participants will be informed that any serious negative side effects should be reported to the study doctor immediately and will be provided with the relevant contact details to do so.

All Adverse Events will be assessed for causality and symptom severity according to the study protocol and followed up by the study doctor if required. In the occurrence of a Serious Adverse Event, appropriate diagnostic and therapeutic measures will be taken and the participant will be kept under observation for as long as is medically indicated. The Principal Investigator will then determine if the seriousness of the event warrants the removal of the participant from the study or abandonment of the study. For serious side effects or medical problems, the patient may be taken immediately to Royal Prince Alfred Hospital for further treatment. The Principal Investigator will ensure that follow-up of the participant is appropriate to the nature of any event, and that it continues until resolution." (Page 20, Lines 461-473; Page 21, Lines 475-482)

Minor comments:

R2.24. Prefer second generation antipsychotics instead of atypical antipsychotics.

R2.24 Response: We have revised all instances of "atypical antipsychotics" to "second generation antipsychotics".

R2.25. There is no mention to biomarkers in the abstract.

R2.25 Response: We have added a paragraph mentioning the biomarkers in the abstract. Further information on the biomarkers collection is also now provided in the Supplementary Materials section. The text in the manuscript now reads:

"Circadian rhythm biomarkers including salivary melatonin, cortisol, and core body temperature will be collected during an in-lab assessment. Additionally, metabolic, inflammatory, and genetic risk markers will be collected at baseline and after 8 weeks of treatment." (Page 2, Lines 56-58)

Reviewer 3

This is a study protocol for an open-label single-arm trial investigating the effects of the brexpiprazole as an adjuvant treatment for sleep-wake cycle disturbance in young subjects with MDD on stable antidepressant therapy with SSRI or SNRI drugs. The protocol is well designed and described in detail. Proposed multidimensional assessments and statistical analyses are appropriate for valid estimation of trial outcomes. I do not have any major concerns related to this protocol. However, I will suggest the following issues to be addressed:

R3.1. Discussion of potential limitations of the study.

R3.1 Response: Thank you for bringing this to our attention. We would like to highlight that this echoes one of the Editor's comments. We have now provided a further explanation of the study limitations under the 'Study Strengths and Limitations' section as follows:

- "This trial focuses on the measurement of the 24-hour sleep-wake cycle and will not examine the sleep quality or the parameters of any sleep disorder
- Circadian assessment used in the study will provide important information about the participants current sleep wake cycle patterns but will not include the assessment of the possible factors that may be contributing to circadian rhythms disruption in the long term (Page 3, Lines 81-86)

R3.2. Explanation of scoring scales for clinical assessment tests wherever missing

R3.2 Response: Thank you for highlighting this. We have now added the explanation of scoring scales for clinical assessment tests wherever missing – notably, for the clinical staging and the illness trajectory models. The text now reads:

The clinical staging framework differentiates those in the earliest phases of mental health problems with non-specific clinical presentations (stage 1a; 'help-seeking') from those at greater-risk with more specific, sub-threshold presentations (stage 1b; 'attenuated syndromes') and those who have already reached a threshold for a progressive or recurrent disorder meeting diagnostic criteria (stage 2, 3, or 4). The illness trajectory model is a novel tripartite framework based on three proposed pathophysiological pathways leading to youth-onset mental disorders: (i) "neurodevelopmental-psychosis", (ii) "circadian-bipolar spectrum", and (iii) "hyperarousal-anxious depression". [67-69] (Page 15, Lines 327-335)

VERSION 2 – REVIEW

REVIEWER	Fornaro, M Columbia University,, New York State Psychiatric Institue
REVIEW RETURNED	08-Mar-2022

GENERAL COMMENTS	!) "Depressive syndromes°: please consider focusing on MDD only. D. syndromes introduce and apples and oranges bias in terms of sub-threshold MDD, depressive symptoms during BD, or due to medical disease or substance/drugs. 3) Is 8-week a sufficient follow-up for sleep disorder restoration in MDD? A four-week interim follow-up is too early in time in my opinion. It is also a matter of steady-state issues. Mean dose of the drug, titration modality? Control for menstrual cycle for young women?

	4) What about the forbidden and the allotted concurrent medications or psychotherapy? Sleep hygiene, control for thyroid or other medical conditions, recent stressors, PTSD? 5) Shift workers excluded? 6) Please provide additional details about the eventual washout or roll-in phases. There is no previous similar research on similar compounds (namely aripiprazole) besides agomelatine (mentioned for sample size estimate).

REVIEWER	Stoiljkovic, Milan Yale University School of Medicine, Comparative Medicine
REVIEW RETURNED	15-Mar-2022

GENERAL COMMENTS	The authors successfully implemented my suggestions in the revised manuscript. I do not have other comments about this study protocol.

VERSION 2 – AUTHOR RESPONSE

Reviewer 1 (Dr. M Fornaro, Columbia University):

R1.1: “Depressive syndromes”: please consider focusing on MDD only. D. syndromes introduce and apples and oranges bias in terms of sub-threshold MDD, depressive symptoms during BD, or due to medical disease or substance/drugs.

R1.1 Response: We agree with the Reviewer’s point and have accordingly altered the text to refer to these conditions as “disorders” rather than “syndromes.” This naming convention has been amended at the following locations: Title and Page 6 (Lines 139, 143, 147, 152).

R1.2: Is 8-week a sufficient follow-up for sleep disorder restoration in MDD? A four-week interim follow-up is too early in time in my opinion. It is also a matter of steady-state issues. Mean dose of the drug, titration modality? Control for menstrual cycle for young women?

R1.2 Response: Based on previous research on brexpiprazole, we argue that an 8-week active treatment period, and an 8-week follow-up period, will be sufficient to observe changes in sleep/wake and circadian parameters. In favour of this prediction, an open label study of adjunctive brexpiprazole for MDD observed changes in self-reported and physiologically-determined sleep (polysomnography) following 8 weeks of treatment (PMID: 27835722). Moreover, we have previously had success in detecting changes in sleep (spindle density) and circadian rhythm (DLMO) parameters following an 8-week treatment phase of agomelatine in a very similar sampling frame (PMID: 30618853, 34089546).

Regarding the comment about the 4-week interim follow-up, we would like to highlight that the 4-week visit is to administer follow-up self-report and clinical measures (e.g., depressive symptoms), rather than laboratory circadian measures.

Finally, menstrual cycle will be assessed via self-report, and may be examined as a covariate (depending on patterns of association with the primary outcome variables).

R1.3: What about the forbidden and the allotted concurrent medications or psychotherapy? Sleep hygiene, control for thyroid or other medical conditions, recent stressors, PTSD?

R1.3 Response: We will discuss each of these points separately.

1. Exclusionary medications include adjunctive antipsychotic medications in the past month and medications which affect sleep, melatonin, circadian rhythms, or alertness. We have expanded on these in text, which now reads: “Exclusion criteria are … (ii) use of medications which affect sleep, melatonin, circadian rhythms, or alertness (e.g., agomelatine, modafinil) …” (Page 7, Lines 173-175). Since these are exclusionary, we will not control for these in analyses.

2. We now expand upon the allotted concurrent medications in text, which now reads: “… (v) current antidepressant treatment with an SSRI or SNRI (including citalopram, fluoxetine, paroxetine, sertraline, escitalopram, venlafaxine, desvenlafaxine, or duloxetine) …” (Page 7, Lines 167-168). Exposure to these medications will be reported, but not controlled for. Psychotherapy and sleep hygiene will not be controlled for.

3. Participants with thyroid or other medical conditions that could affect sleep-wake function will be excluded from the study: “Exclusion criteria are … … (v) evidence of a medical condition (primary, respiratory, neurological) that could contribute to sleep-wake dysfunction …” (Page 7, Lines 176-177).

4. As tertiary outcome measures, we will examine in subsequent analyses associations between changes in sleep-wake and circadian parameters and symptoms of PTSD. Recent stressors will not be measured by a dedicated instrument. Exposure to PTSD and recent stressors will not be controlled for in primary analyses.

R1.4: Shift workers excluded?

R1.4 Response: Yes, shift workers will be excluded: “Exclusion criteria are: … (vii) shift work …” (Page 7, Line 179).

R1.5: Please provide additional details about the eventual washout or roll-in phases. There is no previous similar research on similar compounds (namely aripiprazole) besides agomelatine (mentioned for sample size estimate).

R1.5 Response: As brexpiprazole will be given as an adjunct to a stable psychotropic medication, there will be no wash-out of the previous antidepressant medication (TAU). We have added the following additional details regarding the eventual wash-out of the medication (as clinically indicated). The text now reads:

“Participants will continue to be provided brexpiprazole for up to 12 months following completion of the treatment phase as clinically indicated, at the discretion of their treating clinician. Analyses will account for whether participants have continued or discontinued the medication during the follow-up period.” (Page 9, Lines 230-233)

Reviewer 3 (Dr. Milan Stoiljkovic, Yale University School of Medicine):

The authors successfully implemented my suggestions in the revised manuscript. I do not have other comments about this study protocol.

R3 Response: We thank the Reviewer for their time and constructive comments.