Dear editor,

We, the authors, appreciate the constructive comments and suggestions by you and the referee and, therefore, addressed all of them. So, in addition to Figure 1 in the original manuscript, we added Table 1 and Table 2 in the revised manuscript. As per the journal's guidelines, Figure 1, its legend & title are attached as a PowerPoint file, and Tables 1 & 2 along with their titles, are attached as a word file. Locations for the figure and tables are indicated in the revised manuscript as well as this document.

I hope the manuscript is now ready for your approval.

Kind regards,

B. R. Sastry.

Review Report:

Referee's comments are numbered and shown in green font.

Updated answers and changes for referee's suggestions are shown in red font.

Text in black font indicates continuation with the text in the main manuscript.

In the revised manuscript, places where table 1, Table 2 and Figure 1 need to be inserted, are indicated in red font.

1. Since sleep is so fundamental to this review, I feel the last paragraph on page 5 of section I. a. Sleep and Its significance could be expanded. You describe what normally occurs in the brain when one sleeps however with regards to sleep deprivation you don't explain what changes or differs with regards to the REM and SWS. What are some typical and atypical scenarios where people would

suffer from sleep deprivation? Do people who work nights or shift work suffer from more memory impairment than those who work during the day?

SECTION I a: SLEEP AND ITS SIGNIFICANCE

Disturbances in regular sleep cycles were assessed through the monitoring of EEG patterns. Waves of low amplitude and high frequency, associated with wakefulness, also occur during REM sleep. Deprivation of sleep is usually followed by a compensatory increase in REM sleep (REM rebound). This increase is interpreted to indicate an attempt in re-establishment of homeostasis in learning and memory as well as emotional balancing [5]. Non-REM sleep is associated with high amplitude and low frequency delta waves (stages 3 &4) along with stage 2 spindle activity [2,3]. Sleep deprivation increases the amplitude of the waves associated with spindle activity but reduces spindle density [6-9]. Upon chronic sleep restriction, the power density of theta wave frequency increases [10,11]. Therefore, lack of sleep seems to result in changes in REM as well as non-REM sleep.

The functional significance of sleep guides us towards the negative implications of sleep deprivation. A balanced and sufficient sleep cycle acts as one of the major factors that determine the quality of human life. There are a wide range of environmental, psychological and physiological factors that lead to sleep deprivation. Environmental changes have influenced many aspects of our day-to-day life and sleep disturbances can arise due to an increase in surrounding noise as well as fluctuations in light and temperature [12]. These factors add on to the list of causes that negatively burden the state of mind. Mental health also plays a crucial role in maintaining a regular sleep pattern since the prevalence of stress, anxiety, depression, etc., affects regular sleep cycle which can translate into insomniac conditions. Psychosis disorders like schizophrenia or neurodegenerative diseases like Alzheimer's have often been associated with issues related to sleep deprivation like a reduced REM sleep cycle as well as a lowered sleep spindle activity [13,14]. Apart from mental disorders, pathophysiological illnesses (e.g., cancer, diabetes, respiratory disorders etc.) also often result in sleep disturbances due to manifestations like pain or difficulty in breathing. Interestingly, lack of sleep can also

increase the prevalence of such physiological disorders since an important function of sleep is the regulation of the immune system [15]. Modern lifestyle changes like uneven working hours, over consumption of caffeine along with an increase in screen-time exposure have potentially interluded the quality and quantity of sleep. Homeostasis of the normal biological circadian rhythm is required for better cognition and task performance. For example, studies have implicated that night shift-workers, especially in chronic situations, experience varying levels of cognitive impairment and task performance [16,17]. Drug abuse and alcohol consumption which can lead to substance use disorder have also been recognized as a growing cause of sleep disruption. For instance, alcohol consumption is indicated to supress REM sleep which in turn causes impairment in performing procedural tasks [18]. Conversely, lack of sleep affects the activity balance of important neurotransmitters like dopamine in the brain and this has been indicated to increase vulnerability to the use of drugs [19]. The above mentioned negative influences of sleep deprivation are just few of the many but it helps in bringing the importance of sleep deprivation into perspective.

Without sufficient amount of sleep, the brain cannot adequately perform the processes that take place during the state of sleep. Sleep deprivation affects the ability to concentrate, intake information and mediate that information through neuronal signalling, learn as well as process memories for consolidation. Furthermore, it is affected by the signalling and expression profiles of many biological molecules including the GPCRs [20,21]. These functions are related to the different stages of sleep and hence there are stage specific implications of sleep deprivation. For example, lack of REM sleep has been directed towards to an impairment in the development and expression of emotional and spatial memories [22,23]. Additionally, disruption of SWS is suggested to reduce attention span, affect motor activity and task performance [24]. Since learning and task performance are dependent on the memory processing of various responsible stimuli, the concept of memory consolidation has been highlighted to understand the significance of sleep and its associated cellular machinery.

2. In the last sentence on Page 6/first sentence of Page 7 you define LTP (high frequency stimulation = high Ca2+ influx) but you don't define LTD (low frequency stimulation = low Ca2+ influx). As I continue to read the article you frequently talk about "LTP and LTD" and so describing the processes for each of these synaptic mechanisms would help the reader understand both the difference and importance of each of these processes and what happens (i.e., Ca2+ increases, AMPA receptors, etc). I strongly suggest a table or figure be created to represent this.

SECTION II: THE ROLE OF SYNAPTIC PLASTICITY IN SLEEP & LEARNING (THIS AND TABLE 1)

The post-synaptic depolarization is thought to remove a Mg^{2+} block of the N-methyl-D-aspartate (NMDA) receptor coupled ionic channel allowing a Ca^{2+} influx which activates protein kinases that sets up the expression of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors on the dendritic spines at synapses leading to an increase in response to the released glutamate, the transmitter [51]. The post-synaptic depolarization is also suggested to facilitate the release of a retrograde messenger that causes an increase in glutamate transmitter release and, thus, an increase in synaptic transmission [49]. Upon stimulation of synapses at lower frequencies than those that induce LTP, the induction of LTD also involves an influx of Ca^{2+} ions, although the surge of Ca^{2+} ions is comparatively lower than that in case of LTP [52]. The difference in the level of Ca^{2+} influx results in activation of phosphatases that are responsive to lower concentration of Ca^{2+} ions. This subsequently leads to a dephosphorylation of AMPA receptors and a reduction in their activity which decreases the response to the released glutamate and causes an overall reduction in synaptic efficacy [53]. Therefore, the phosphorylation of AMPA receptor through kinases in LTP is counteracted by the phosphatases during LTD which leads to an interference and reversal of LTP. This interplay promotes modulation of synaptic plasticity which as explained in the section below, appears to be crucial for memory processing and learning. The comparative details of LTP/LTD

associated molecular events are summarized in Table 1 and are also discussed further in subsequent sections of this review.

INSERT TABLE 1 HERE

3. In the last paragraph on page 10 you mention a hypothesis by Lynch. I felt you did not summarize the findings of the article adequately. Something is missing. What caused the increase in the number of glutamate receptors and what did it have to do with calcium proteinases?

SECTION II a: LTP AND LTD AS CELLULAR CORRELATES FOR LEARNING & MEMORY FORMATION, 2nd PARAGRAPH.

What exactly is harnessing this LTP and LTD in these regions of the brain and how these activity dependent synaptic changes relate to memory consolidation and learning? In this review, an attempt is made to find some answers. An early hypothesis put forward by Lynch et al. on the biochemistry of memory involved an examination of calcium proteinase-receptor interaction [74]. They demonstrated that in the forebrain sub-synaptic membranes, an influx of Ca^{2+} causes a long lasting increase in the number of glutamate receptors through its activation of the enzyme calpain which is a proteinase. The activated form of this enzyme degrades the membrane-anchored cytoskeleton protein fodrin and as a result, exposes the obstructed glutamate receptors. The effects of this Ca^{2+} proteinase-receptor interaction could functionally modify neuronal circuits as well as showed similar biochemical effects that are seen post learning [74]. This and other related studies examining molecular mechanisms were, therefore, examined to find correlations between synaptic strength and memory consolidation.

4. Midway through page 11 you talk about now ionotropic and metabotropic receptors are included in synaptic plasticity and have different mechanisms of action. It would be great to give the reader an example of each type of

receptor (in brackets) and the different mechanism of action it has with regards to synaptic plasticity like the ones you talk about later in the article.

SECTION II a: LTP AND LTD AS CELLULAR CORRELATES FOR LEARNING & MEMORY FORMATION, LAST PARAGRAPH.

Both ionotropic (e.g., NMDA, AMPA receptors) and metabotropic (e.g., mGluR) receptors are known to be involved in synaptic plasticity but project different mechanisms of action which are employed for mediating excitatory and/or inhibitory signals [81]. The ionotropic receptors are heteromeric compounds that consist of a ligand binding site and pore forming channel that combines the function of the receptor and ion channel into a single unit for the mediation of post-synaptic potentials. On the other hand, metabotropic receptors do not comprise of an ion channel but mediate their effects on other ion channels via intermediate effector molecules and secondary messengers. Ionotropic receptors like NMDA and AMPA receptors get activated upon binding of the neurotransmitter glutamate on the ligand-binding site and this leads to an opening of the ion channel subunit of the receptor which is permeable to Na^+ and K^+ ions to induce an inward current causing depolarization through the excitatory post-synaptic potential (EPSP) that lasts for a few milliseconds [51]. NMDA channel is permeable to the above cations and also to Ca^{2+} which affects the downstream signalling cascade that regulates synaptic plasticity [82]. Glutamate can also bind to the mGluRs which leads to an activation of signal transduction molecules like the receptor associated G-protein that gets detached from the receptor and directly activates the nearby ion channels like NMDA receptors or promotes the action of various effector molecules that indirectly activate other ion channels. mGluRs can also regulate the intracellular Ca^{2+} levels through membrane proteins and secondary messengers. The influx of ions through these receptors therefore results in post-synaptic potentials that have a slower induction and longer response time ranging from a few milliseconds to even much longer times [83]. LTP is known to be predominantly mediated by the ionotropic

receptor NMDA when high frequency stimulations result in a build-up of EPSPs due to the activation of AMPA receptors causing the removal of a Mg^{2+} block of the NMDA channel [51,58]. However, metabotropic receptor mediated LTP has also been observed with mGlu1 and mGlu5 receptors wherein mGluR activation can lead to LTP induction through NMDA receptors as well as via the modulation of intracellular Ca^{2+} levels [84,85]. As mentioned before, LTD arises upon a lower frequency stimulation of the excitatory synapses than needed to induce LTP and also involves NMDA receptors but with a resulting deactivation of the AMPA receptors. LTD mediated by metabotropic receptors like mGluRs usually involves secondary messengers that modulate intracellular Ca^{2+} levels [62]. Other metabotropic GPCRs like GABA-B receptors can also induce LTD due to their inherent nature to transmit inhibitory signals [63]. The details of these molecular and cellular features of synaptic plasticity will be discussed more in the coming sections. Since activity dependent synaptic plasticity and memory consolidation occur sequentially, cellular receptors and other associated molecules that mediate LTP and LTD consequently find themselves to be associated with learning and memory processing [61,86].

5. Within the last few lines on page 14, you say "Considering that NO has a role in so many other important physiological functions, "I feel you should give an example of an important physiological function.

SECTION III a: MOLECULAR EVENTS IN ASSOCIATION WITH SLEEP AND SYNAPTIC PLASTICITY, 3rd PARAGRAPH

More insight on such accessory molecules needs to be put forward through detailed experimental work and NO has so many other important physiological functions like decreasing vascular resistance and increasing cerebral blood flow and oxygenation rate [111] or playing a key role in mediation of an immune response against infectious diseases [112]. Thus, its definitive role in sleep deprivation through synaptic plasticity can be a major contribution to this field of research.

6. On page 15, section 4 and specifically In line number 8 you write "high in the hippocampus (300) and about 20…" what does the (300) refer to? Is it a reference? Or are you referring to how many transcripts? Please clarify

300 GPCRs: Correction made in the main manuscript.

7. I see several instances where you write "post synaptic", "post-synaptic" and "postsynaptic". Please make it consistent throughout the manuscript. The same for "pre-synaptic".

Post-synaptic: Corrections made in the text of the manuscript.

8. Regarding the last 2 sentences in section "a." on page 21, you summarize the results from reference 131. However, something is missing. Please review the 2 sentences and possibly add more detail about the study. How were the rats sleep deprived? What part of the brain did the authors study? Which method was used to measure the expression levels of the receptors?

SECTION V a: ROLE OF GABA-B AND mGLURS,

Progressing from these initial findings, recent work done on rats that were sleep deprived using gentle prodding and tapping has demonstrated that induced LTD of population excitatory post-synaptic potentials (pEPSPs) in the hippocampus requires activation of mGluRs and GABA-B Rs along with an increase in Ca^{2+} released from intracellular stores [150]. In sleep deprived conditions, western blot analysis and co-immunoprecipitation studies revealed that there were elevated expression levels of mGlu1αR and GABA-B1 receptor subunit as well as enhanced co-expression and heterodimerization between mGlu1αR & GABA-B R1 subunit and mGlu1αR & GABA-B R2 subunit [150].

9. In both Figure 1 and in the concluding paragraph on page 24-25 you talk about psychiatric therapeutics, however you don't list any examples. I strongly suggest you highlight (and give examples) of a few drugs that are used to treat the disorders you talk about in your manuscript particularly those that are or act upon GPCRs. Also what is the mechanism of action of these drugs? Do they increase the availability of neurotransmitters which in turn will cause an increase the transcription of their respective receptors? Are they GPCRs themselves?

10. I believe a much stronger link needs to be made with regards to sleep deprivation and psychiatric disorders. I can see where you are trying to go with regards to the conclusion of this review, that being that changes are occurring to GPCRs as a result of sleep deprivation and it is important to study this because it can affect the formation of memories as well as the ability of drugs that target these receptors to perform their action when it comes to treating neuropsychiatric disorders. One angle that you could take to make the link stronger is maybe highlight how one of the side effects of taking drugs that treat neuropsychiatric disorders is (likely) problems with sleeping (which you kind of touch on in the future direction paragraph). I think it would also be a good idea to briefly describe drugs that treat sleeping problems as well unless wordcount/space is an issue.

(Suggestions 9 and 10 addressed together)

SECTION VI: DISCUSSION

Activity mediated synaptic plasticity has been widely correlated to sleep associated functions of cognitive learning, memory processing and over all brain development. GPCRs like GABA-B Rs and mGluRs are types of receptors that have shown a potential to be involved in these processes through their actions on LTD and LTP. What is even more intriguing is the fluctuation in their distribution, heterodimerization and co-localization following sleep deprivation, suggesting that these receptors can exist in one condition during normal sleep and change with sleep

deprivation. GPCRs are one of the major cellular targets for drug interaction and therefore changes in the receptor expression profile can affect drug action.

For instance, the antipsychotic drug clozapine is one of the common and effective drugs used in treating disorders like schizophrenia. The cellular targets for this drug are GPCRs like 5-HT2A and DA D2 receptors with a higher affinity for the former receptor than the latter. It acts as an antagonist of DA D2 receptors in the mesolimbic pathway and although initially classified as an antagonist, clozapine is also known to be an inverse agonist of the 5-HT2A receptor present in the prefrontal cortex of the brain [166-168]. Other antipsychotic drugs like olanzapine, risperidone, aripiprazole, etc., have also been indicated to elicit their effects through these receptors [166]. Interestingly, 5-HT2A and mGluR2 receptors are suggested to form heterodimers which raise the possibility of testing if clozapine and other such antipsychotic drugs modulate similar complex formation [156]. Moreover, drugs like clozapine are known to have sleep-inducing effects [169] and since disorders like schizophrenia and other psychiatric diseases are associated with sleep disturbances [170], potential interplay between the molecular events of sleep deprivation and actions of antipsychotic drugs needs to be investigated. Other examples of drugs that act on GPCRs include ropinirole for Parkinson's disease which acts as an agonist on DA D2 receptors [171] and baclofen, a GABA-B receptor agonist suggested for treatment of depression and anxiety [172]. Whether their actions change with sleep deprivation and *vice versa* also needs to be tested. With regards to synaptic plasticity, antipsychotics and antidepressants are suggested to affect LTP upon both acute and chronic use and interestingly, these effects are different for each of these situations which may suggest differences in network behaviour with acute vs chronic exposure to the drugs (see Table 2). Moreover, their effect on LTD has not been sufficiently explored and hence similar studies on the differential effects of chronic vs acute use of such drugs on network behaviour are required with regards to LTD. Table 2 provides a brief summary on antipsychotic, antidepressant and anxiolytic drugs with their mechanisms of action and association with synaptic plasticity, learning and sleep.

INSERT TABLE 2 HERE

The relation between a drug's mechanism of action and sleep can be directly observed in drugs that are used for treating sleeping disorders like insomnia. Characterized by a lack of both quality and quantity of sleep, insomniac conditions are treated with many drugs that target GPCRs. For example, melatonin is a hormone that has been known to play a key role in the sleep-wake cycle and promoting sleep. Agonist drugs like ramelteon act on the melatonin GPCRs type 1 and 2 (MT1 and MT2) which results in reduced sleep latency in chronic insomniac patients [212]. Intriguingly, the MTI and MT2 receptors have been known to form dimers among themselves as well as heterodimers with other GPCRs like the serotonin 5-HT2C receptor [213, 214]. Thus, differences in ligand selectivity for these homomeric and heteromeric forms of the target receptors can have important consequences if the expression profile of such GPCRs change upon sleep deprivation. Other drug targets for insomnia include neuropeptides like orexins which promote wakefulness and are synthesized by neurons in the hypothalamus region of the brain. Drugs like suvorexant act as antagonists of the orexin GPCRs type 1 and 2 (OX1R and OX2R) thereby blocking their wakefulness promoting effects [215]. These GPCRs known to be associated with other GPCRs like the cannabinoid receptor type 1 (CB1) and GABA-B receptors. They form heterodimers with the CB1 receptors [216] which have been implicated in affecting memory formation and maintenance of mood. Moreover, orexin and GABA-B receptor activity are indicated to have a balancing interplay wherein inhibitory GABA-B modulates the wakefulness promoting properties in the orexin producing neurons [217]. If expression profiles of GABA-B receptors change with sleep deprivation, can there be consequences for actions of orexins? In summary, changes in receptor profiles associated with sleep deprivation can have consequences for drug action and need a thorough investigation to understand the mechanisms involved so that CNS disorders can be treated with more rationally based therapeutics.

Insert Figure 1 between Discussion & Future Scope sections.

SECTION VII b: FUTURE SCOPE FOR PSYCHONEUROLOGICAL DISORDERS & THERAPEUTICS

For example, the antidepressant drug mirtazapine exhibits its action through GPCRs by blocking 5-HT2 and adrenergic α_2 receptors, leading to an increase in the activation of 5-HT1A receptor mediated activity as well as noradrenaline release, respectively [193]. This drug is known to have sleep-promoting properties and has been associated with problems like day-time somnolence [225] potentially disrupting the normal sleep cycle. Interestingly, there are off-label therapies that employ antidepressants like mirtazapine in treating sleeping disorders due to its sedative effect [226]. Since antidepressants and antipsychotic drugs appear to directly or indirectly change activity at GPCRs, drug therapy for these disorders can affect sleep while sleep disturbances can also necessitate changes in that therapy. Therefore, sleep disturbance-induced plasticity and cross-talk between GPCRs can have consequences for drug therapy, the mechanisms for which need to be thoroughly examined. Lastly, allosteric modulation, which is another upcoming molecular interaction that is important for drug designing, can be applied to the homo and heterodimerization of GPCRs for various conditions. Hence, studies on structural, functional, receptor co-localization & remodelling, etc., will yield new insights into mechanisms involved and help improve therapeutics for a variety of CNS disorders, including in psychiatry.