

## Original Research Article

## Minocycline Modulates Anxiety-Like Manifestations in Sleep Deprived Mice

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**Abstract:** Primarily, sleep allows the brain to recover and regenerate. In view of this, central nervous system disorders are the most common effects that result from sleep loss or prolonged periods of wakefulness since the system most benefits from sleep. Therefore, this increases the risk of schizoaffective disorders such as mania and bipolar disorder. This research was therefore designed to investigate the possible anti-manic and anxiolytic effect of minocycline in mice exposed to sleep deprivation. Twenty-four mice were grouped into four of six animals each (n=6) and orally treated with 10 mL/kg distilled water, 25 or 50 mg/kg minocycline. Treatment was scheduled for 7 days and three out of the four groups were subjected to the sleep deprivation protocol which lasted for 3 days. Afterwards, all groups were subjected to behavioural tests to evaluate anxiety-like behaviour. Subsequently, histology of selected brain regions was carried out. Experimental data were analysed using one-way ANOVA with statistical significance of  $p < 0.05$ . The results obtained suggested that minocycline ameliorated the manic-like behaviour in mice caused by sleep deprivation. This study therefore concluded that minocycline can be classified as an anxiolytic and anti-manic agent in mice exposed to sleep-deprivation.

**Keywords:** Anxiety, Mania, Sleep, Minocycline, Sleep deprivation.

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## INTRODUCTION

Sleep is one of the many body processes that are internally regulated by the circadian process. The circadian process is a sleep-wake independent 24-hour oscillatory rhythm that modulates sleep propensity (Sagaspe *et al.*, 2012). Studies have revealed that sleep is most beneficial to the brain and therefore is a physical and mental necessity for basic human functioning (Eduviere *et al.*, 2021a). Quality sleep reverses concentration deficits without which, several central processes are weakened. It also allows the brain to alleviate stress built up during wakefulness (Lockley *et al.*, 2012; Pollak *et al.*, 2010).

Stress has been shown to be one of the topmost causes or contributors to the pathogenesis of neuropsychiatric disorders (Akinpelu *et al.*, 2019). Sleep deprivation-induced stress is a widely studied form of stress that causes ripple defects in almost all systems of the body, particularly the brain (Dudek *et al.*, 2020). Whether it is intentionally induced (due to lifestyle/job demands) or not, sleep deprivation is detrimental to the body in many ways; it puts the body at risk of many neurobehaviours/neuropsychiatric disorders (Eduviere *et al.*, 2021a). Changes in mood and attentiveness can begin after just 36 hours of insufficient/no sleep. Lack of sleep contributes to

reduced concentration, short-term memory, decline in learning ability, and loss of behavioural self-control. Also, since it is well known that circadian rhythm disruptions and its consequent sleep disruptions are comorbid to various neuropsychiatric disorders (Palagini *et al.*, 2021), researchers have previously used various sleep deprivation paradigms in a bid to elucidate its potential adverse effects in humans (Nollet *et al.*, 2020; Casse-Perrot *et al.*, 2016).

However, since mania is a medical condition, only the symptoms of mania (most commonly, anxiety) can be modelled in experimental animals. Therefore, this study was designed to evaluate the potential benefit of minocycline on increased anxiety levels induced by sleep deprivation in mice.

## EXPERIMENTAL SECTION

## Experimental Animals

Twenty four male albino mice of ~22.0 g body weight were procured from the affiliated institution and set aside in rectangular plastic cages at room temperature with 12:12 h light-dark cycle. They were allowed to acclimatize for a few days before the outset of the experiment. The experimental procedures were performed in accordance with the NIH and institutional guidelines.

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**Drug**

Minocycline (100 mg tablets) were dissolved in 20 mL of distilled water in order to obtain the stock solution. From pilot studies, the stock solution was subjected to serial dilution in distilled water to acquire the concentrations of minocycline used in this experiment.

**Treatment groups**

The mice were randomly allotted into four (4) treatment groups of six each (i.e., n = 6).

Group I received 10 mL/kg distilled water only (i.e., control group).

Group II received 10 mL/kg distilled water and was sleep deprived,

Group III received 25 mg/kg minocycline and was sleep deprived, and

Group IV received 50 mg/kg minocycline and was sleep deprived.

All treatments were orally administered. The animals were treated for 7 days, while mice in groups II – IV were subjected to the 3-day sleep deprivation paradigm beginning from the fourth day of treatment.

**Sleep deprivation paradigm**

The physical method of sleep deprivation formerly described by Uchida (2016) was used in this study with slight modifications. On the eighth day, mice were assessed for behavioural changes using the elevated plus maze, light/dark transition box and open field test.

**Behavioural Tests****Open field test**

This test is usually used to assess the spontaneous locomotor activity of experimental animals. For 10 min, each mouse was placed in the centre of the open field chamber and allowed to explore freely. The number of square lines crossed with all paws and duration of ambulation of each mouse was recorded (Eduviere *et al.*, 2021b). Stereotype behaviour, specifically frequency of rearing and grooming, were also recorded for each mouse (Eduviere *et al.*, 2021b). In other studies, lines crossed and ambulation are considered indices of hyperactivity while stereotypy is considered an index of anxiety.

**Assessment of anxiety-like behaviour****Light/dark transition test**

Firstly, each mouse was placed in the light/dark transition box and the duration spent in the dark compartment, and frequency of dark compartment entries was measured for 5 min.

**Elevated plus maze test**

Secondly, each mouse was placed in the elevated plus maze and the duration spent in the closed arm, frequency spent in closed arm, and index of open arm avoidance was measured for 5 min.

**Histology and estimation of neuronal density**

Afterwards, mice in the respective groups were euthanized and their brains harvested. Thereafter, paraffin wax-embedded brain tissue blocks required for histology were obtained. Specific brain tissue sections (hippocampal CA3 and caudate putamen) of each treatment group were stained with Hematoxylin and Eosin to evaluate their general histology (Eltony 2016). Micrographs of stained tissues were acquired using a digital camera. Neuronal density of both brain regions for each group was also calculated as a ratio of viable neurons by simple extrapolation from the photomicrographs using the Image J software.

**Statistical Analysis**

Values are presented as mean  $\pm$  standard error of mean (S.E.M). Analysis was done using 1-way ANOVA followed by Students Newman-Keuls post test. Determination of statistical significance was carried out using the Graph Pad InStat<sup>®</sup> Biostatistics software version 7 and set at  $p < 0.05$ .

**RESULTS****Effect of minocycline on motor activity and stereotype behaviours in sleep- deprived mice**

Behavioural alterations in sleep deprived mice measured in the open field test are presented in Table 1 below. Mice in group II exhibited a significantly ( $p < 0.05$ ) higher motor activity and stereotype behaviour than mice in the control group. On the other hand, administration of minocycline to groups III and IV resulted in a significant ( $p < 0.05$ ) reduction in motor activity and stereotype behaviours in comparison to sleep deprived mice of group II.

**Table 1: Effect of minocycline on motor activity and stereotype behaviours in sleep- deprived mice**

Treatment groups	Frequency of grooming (s)	Frequency of rearing (s)	Number of lines crossed	Duration of ambulation (s)
Group I (control)	10.67 $\pm$ 0.95	19.83 $\pm$ 2.09	143.70 $\pm$ 8.19	3.52 $\pm$ 0.36
Group II	18.50 $\pm$ 0.84 <sup>#</sup>	37.67 $\pm$ 2.08 <sup>#</sup>	213.50 $\pm$ 10.22 <sup>#</sup>	6.88 $\pm$ 0.39 <sup>#</sup>
Group III	14.33 $\pm$ 1.26 <sup>*</sup>	29.83 $\pm$ 1.92 <sup>*</sup>	179.2 $\pm$ 9.13 <sup>*</sup>	5.45 $\pm$ 0.39 <sup>*</sup>
Group IV	12.33 $\pm$ 0.99 <sup>*</sup>	24.83 $\pm$ 2.77 <sup>*</sup>	168.2 $\pm$ 12.99 <sup>*</sup>	5.15 $\pm$ 0.34 <sup>*</sup>

<sup>#</sup> means significance ( $p < 0.05$ ) compared to the control group.

<sup>\*</sup> means significance ( $p < 0.05$ ) compared to group II.

**Effect of minocycline on anxiety-like behavioural parameters in sleep-deprived mice**

Behavioural alterations in sleep deprived mice measured in the elevated plus maze test and light/dark transition test are presented in Table 2 and 3 below.

From Table 2, mice in group II exhibited a significantly ( $p < 0.05$ ) higher duration of exploration of the closed arm of the maze and a higher percentage of avoiding the open arm than mice in the control group. On the other hand, administration of minocycline to groups III and IV resulted in a significant ( $p < 0.05$ )

reduction in closed arm exploration and open arm avoidance in comparison to sleep deprived mice of group II.

From Table 3, mice in group II exhibited a significantly ( $p < 0.05$ ) higher duration and frequency of exploration of the dark compartment of the box than mice in the control group. On the other hand, administration of minocycline to groups III and IV resulted in a significant ( $p < 0.05$ ) reduction in dark compartment exploration in comparison to sleep deprived mice of group II.

**Table 2: Effect of minocycline on anxiety-like behavioural parameters of the elevated plus maze in sleep-deprived mice**

Treatment groups	Duration of closed arm exploration (s)	Frequency of closed arm entries (s)	Index of open arm avoidance (IOAA, %)
Group I (control)	192.80±7.25	4.17±0.31	56.56±2.31
Group II	253.00±3.82 <sup>#</sup>	8.67±0.42 <sup>#</sup>	83.18±3.76 <sup>#</sup>
Group III	215.20±6.49*	6.33±0.50*	67.76±3.79*
Group IV	208.30±7.16*	5.50±0.43*	63.50±2.65*

<sup>#</sup> means significance ( $p < 0.05$ ) compared to the control group.  
 \* means significance ( $p < 0.05$ ) compared to group II.

**Table 3: Effect of minocycline on anxiety-like behavioural parameters of the light/dark transition box in sleep-deprived mice**

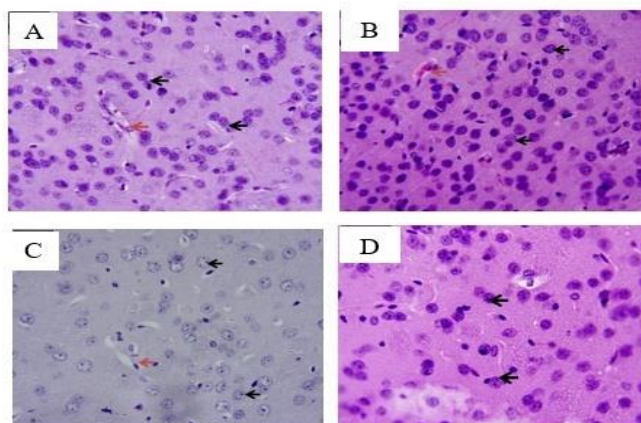
Treatment groups	Duration of exploration of the dark compartment (s)
Group I (control)	143.80±6.32
Group II	197.50±4.57 <sup>#</sup>
Group III	156.70±6.21*
Group IV	147.80±5.68*

<sup>#</sup> means significance ( $p < 0.05$ ) compared to the control group.  
 \* means significance ( $p < 0.05$ ) compared to group II.

**Effect of minocycline on brain neuronal processes in sleep-deprived mice**

Brain neuronal alterations in sleep deprived mice evaluated by histology are presented below. Figure 1 shows the histology of the caudate putamen of mice in each group. Mice in group II (slide B) showed a significantly higher number of neurons undergoing degeneration than mice in the control group (slide A). However, administration of minocycline to groups

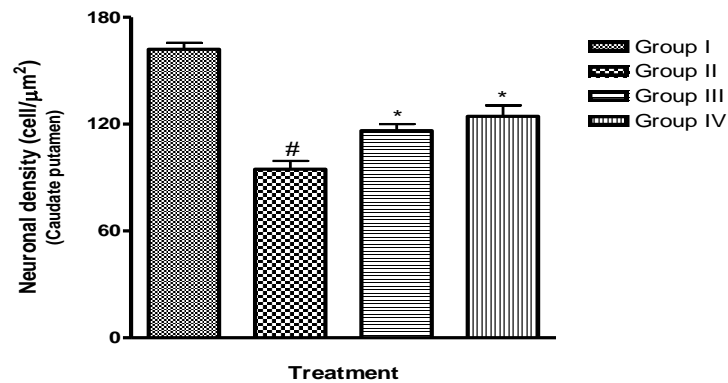
III and IV (slides C and D respectively) resulted in a significant reduction in neuronal degeneration in comparison to sleep deprived mice of group II. This result was further depicted in Figure 2 showing the estimated neuronal density of the caudate putamen of mice following exposure to sleep deprivation. These effects were identical to what was observed in the hippocampal CA3 region of mice following exposure to sleep deprivation (as seen in Figure 3 and 4).



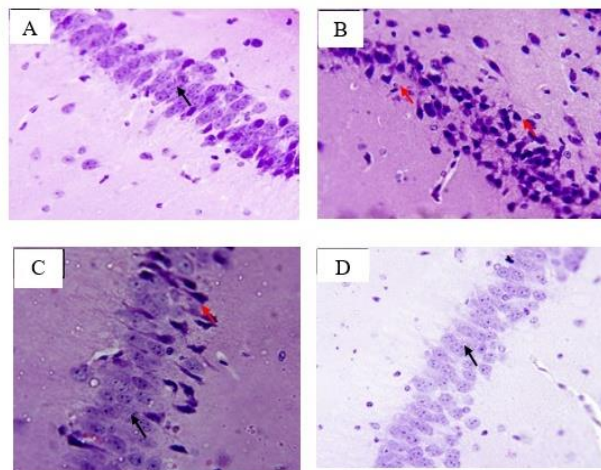
**Figure 1: Photomicrograph of the caudate putamen of sleep deprived mice**  
 Slide A= Group I (control); Slide B= Group II; Slide C= Group III; Slide D= Group IV.

Black arrows show normal neurons.  
 Red arrows show neurons experiencing degeneration.

Magnification: X400.



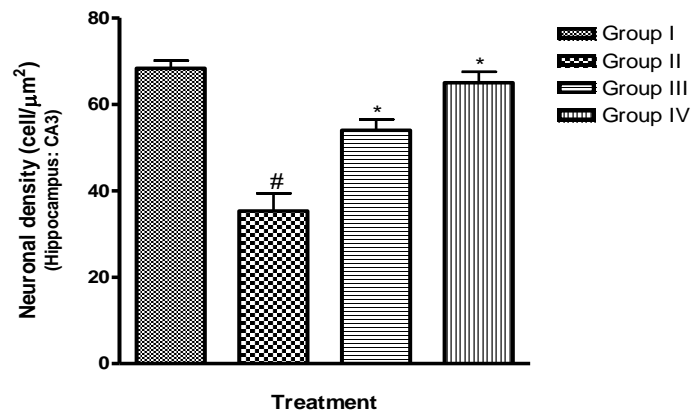
**Figure 2: Estimated neuronal density of caudate putamen of sleep deprived mice**  
 # means significance ( $p < 0.05$ ) compared to the control group.  
 \* means significance ( $p < 0.05$ ) compared to group II.



**Figure 3: Photomicrograph of the hippocampal CA3 region of sleep deprived mice**  
 Slide A= Group I (control); Slide B= Group II; Slide C= Group III; Slide D= Group IV.

Black arrows show normal neurons.  
 Red arrows show neurons experiencing degeneration.

Magnification: X400.



**Figure 4: Estimated neuronal density of hippocampal CA3 region of sleep deprived mice**  
 # means significance ( $p < 0.05$ ) compared to the control group.  
 \* means significance ( $p < 0.05$ ) compared to group II.

## DISCUSSION

The results from the present study revealed that minocycline exhibited anxiolytic property in mice subjected to sleep deprivation for 3 days. The sleep deprived mice displayed behavioural changes that have been accrued to mania (Valvassori *et al.*, 2017) as depicted by hyperactivity and stereotypy in the open field test. These observations agree with existing literature which showed that sleep deprivation can induce manic-like symptoms in animals (Gessa *et al.*, 1995; Eduviere *et al.*, 2021b; Andrabi *et al.*, 2020; Kanazawa *et al.*, 2016). This result therefore supports the valuable existing evidence that a bidirectional relationship exists between sleep deprivation and the outset of mania. Therefore, sleep deprivation represents a singular reliable precursor of anxiety.

Behavioural tests – elevated plus maze and light/dark transition test – which are notable experimental tests for anxiety in animals showed that the sleep deprived mice spent longer time in the closed arm and dark compartment of the apparatus respectively. This also agrees with literary evidence that anxious mice have a higher need to be in the dark arms of the apparatus (Anseloni and Brandao 1997; Shimada *et al.*, 1995).

Also in the present study, Hematoxylin and Eosin histological staining of selected brain regions of the mice (caudate putamen and hippocampal CA3) revealed that sleep deprivation increased the extent of neuronal damage with a consequent decline in the population of viable brain neurons in mice. However, pre-treatment of mice with both concentrations of minocycline significantly attenuated this neuronal damage in the sleep deprived mice. This agrees with existing literatures which have outlined the benefit of minocycline in neurodegenerative diseases and brain defences (Yrjanheikki *et al.*, 1999; Kim and Suh 2009; Stirling *et al.*, 2005).

## CONCLUSION

In conclusion, this research provides experimental evidence that minocycline possesses anxiolytic property and also reverses brain neuronal damage in mice exposed to sleep deprivation for three days. However, further studies investigating safety, efficacy, dosage and toxicity of minocycline in central nervous system disorders are necessary.

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**Conflict of Interests:** The authors declare the absence of any conflict of interests

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