

Research Article:

Evaluation of Pain Tolerance Threshold Following Administration of Anti-TNF- α in REM Sleep-deprived Male Wistar Rats



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ABSTRACT

Background and Aim: The level of Tumor Necrosis Factor-alpha (TNF- α) changes by Rapid Eye Movement (REM) sleep deprivation. TNF- α is a known biomarker of REM Sleep Deprivation (RSD). Prior studies have shown that any alteration in REM sleep can increase the amount of TNF- α . Accordingly, the Pain Tolerance Threshold (PTT) is believed to be increased in patients with insomnia after using anti-TNF- α or Infliximab (IFX). The present study aims to demonstrate the effect of IFX and its importance in the pain management of hospital inpatients.

Methods and Materials/Patients: Seventy-two male Wistar rats in 9 groups were studied after obtaining the approval of the ethics committee of Tehran University of Medical Sciences (CNS. Protocol-ICSS-940816). Remicade was used for inducing the anti-TNF- α . Multiple platform water-tank was used for REM sleep deprivation induction. Pain tolerance was measured on a hot plate apparatus.

Results: There was a significant increase in the duration of the rats' tolerance on the hot plate between the saline group and the group that received IFX (0.2 mg/kg) ($F_{2,8.363}$) ($P < 0.001$).

Conclusion: Chronic SD can cause neuronal damage due to neuroinflammatory insults. REM sleep deprivation, in the long run, sensitizes the brain to neurodegenerative insults via the inflammatory mechanism, to some extent through the TNF α -associated pathways.

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Highlights

- The hippocampus has an essential role in cognition and emotion regulation and is one of the numerous areas of the brain displaying neurogenesis from adolescence into adulthood.
- The reduction of hippocampal neurogenesis due to REM sleep deprivation causes a reduction in cognition and emotion regulation, leading to depressive states and emotional disorders.
- Concerning our pharmacological intervention, rats that received IFX 15 minutes before being submitted to the SD procedure showed less memory deficit.
- Sleep deprivation causes inflammation in many parts of the body including the nervous system. These studies emphasize sleep disorders as one presenting sign of the altered function of the immune system and hypothesize the possible mirror effects of sleep disorders and the immune system on each other.

Plain Language Summary

Tumor Necrosis Factor-alpha (TNF- α) changes by REM sleep deprivation. TNF- α is a known biomarker of REM sleep deprivation (RSD). Prior studies have shown that any alteration in REM sleep can increase the amount of TNF- α . Accordingly, the Pain Tolerance Threshold (PTT) is believed to be increased in patients with insomnia after using anti-TNF- α or Infliximab (IFX). Therefore, 72 male Wistar rats in 9 groups were studied. Remicade was used for inducing anti-TNF- α . A multiple platform water-tank was used for REM sleep deprivation induction. Pain tolerance was measured on a hot plate apparatus. There was a significant increase in the duration of the rats' tolerance on the hot plate between the saline group and the group that received IFX. Chronic SD can cause neuronal damage due to neuroinflammatory insult. REM sleep deprivation, in the long run, sensitizes the brain to neurodegenerative insults via the inflammatory mechanism, to some extent through the TNF α -associated pathways. According to the findings of this study and the observations on PTT, analgesics should be used more carefully in the treatment process.

1. Introduction

Researchers have extensively surveyed human behaviors with respect to a multitude of neuro-psycho-physiological aspects [1]. Most of the neuroinflammatory markers such as elevated Tumor Necrosis Factor-alpha (TNF- α) can remarkably affect neural factors. TNF- α is a known biomarker in the deprivation of Rapid Eye Movement (REM) sleep [2]. The relation between sleep, nutrition, and practice has been discussed considering many aspects such as neuroinflammation, neuroprotectors, and neurotransmitters. These components affect human sensation, perception, cognition, emotions, and behaviors [3] which can also be attributed to a close relationship with the immune system [4, 5].

Today, the relationship between sleep and the immune system has also been addressed by researchers in the fields of neural physiology and pathophysiology [6]. Thus, in order to improve the conditions of sleep for hospital inpatients, intensive care standards must be considered [7]. In addition, sleep disorders change the level of cytokines such as Interleukin-1 (IL1), IL6, and specifically TNF- α [4]. Remarkably, some researchers

have demonstrated that the reduction in the level of TNF- α could increase the quality of sleep.

Insomnia especially in the REM stage is considered as one of the important complaints that can affect the treatment progress of inpatients [8]. Among many reasons that cause sleep disorder, pain and stress are the major causes of REM sleep disorder, especially in patients hospitalized after surgery [9]. Managing this condition is one of the common aims of therapists [10]. Previous studies focused on the effects of REM Sleep Deprivation (RSD) and the Pain Tolerance Threshold (PTT) affected by Infliximab (IFX) [11]. Anti-TNF- α or IFX is one of the effective suppressants for this condition [12]. Therefore, this study aimed at indicating the necessity of pain management for therapists before prescribing IFX.

2. Methods and Materials/Patients

Subjects

In this study, 72 male Wistar rats, weighing between 200 and 250 g, were divided into 9 groups each including 8 rats which were sacrificed according to the ethics code in the ethics committee (CNS.Protocol-ICSS-940816).

The ethical protocol for animal interventional experiments was approved by the Ethics Committee of the School of Advanced Technologies in Tehran University of Medical Sciences, Iran. The rats were placed in Plexiglas cages (45 × 30 × 30 cm) within the study period with free access to food and water. They were maintained under a light-dark cycle of 12 hours (lights at 07:00 A.M.), with a temperature of 22±3°C and humidity of 55-60%.

Drug preparation and administration

Infliximab (IFX) is a monoclonal antibody that all of the interventional groups of rats received 15 minutes before starting the REM sleep deprivation phase [13-15]. The studied groups underwent intraperitoneal injection of IFX with different dosages. The rats subjected to TSD (Total Sleep Deprivation) and CPSR (Chronic Partial Sleep Restriction) which did not receive IFX exhibited a more noticeable memory impairment, increased serum corticosterone, and declined BDNF levels [15].

Apparatus for REM sleep deprivation by multiple platform water tank

REM sleep deprivation in rodents has been performed by multiple platform water tanks. In this method, awake animals are placed on small platforms in a water tank, in order to allow the animal's body to be at a higher level than the water level. This way prevents the awake animal to be unobserved in the water. The multiple-platform apparatus was used to induce REM SD for 24 hours [16].

After initial training with the apparatus, the animal remains undisturbed. The large platforms allow the animal to sleep during the non-REM sleep, but in the REM period,

the loss of muscle tone causes the neck to relax and the snout to touch the water, thus stimulating and arousing the animal [17]. The animal increasingly loses balance and falls in the water which exactly shows REM sleep deprivation. To decrease stress, the protocol was modified by placing multiple platforms in a large tank (150 cm in diameter, 45 cm in height of the water, and 50 cm in height of the surface of the platform). The apparatus has 10 platforms so each time a group of animals including 8 rats is inserted into the apparatus [18]. All of the cases were included in the apparatus for 24 hours. The apparatus provided two conditions. For the sham group (non-intervention), platforms with 15 cm diameter were used, and for the intervention group platform with 9 cm diameter.

Hot-plate test

Nociceptive sensitivity was assessed by the hot plate test in which the animal was placed over a heated plate and the latency to lick the paw was used as a criterion for pain threshold and sensitivity. Before analgesia tests, to preserve any stressor agents, great care was taken to control the lab environment for additional and parasite voices, lights, and smells. To assess the pain tolerance threshold, rats were placed on a hot plate at 50±1°C for less than 30 seconds. The latency for the rats to lick their paws was recorded at which point they were immediately removed from the hot plate [19]. To analyze the data, ANOVA test and Mean±SD were used by SPSS software, v. 21. The significance level was considered P<0.05.

3. Results

The three study groups included sham (Intraperitoneal Saline injection), rats which received 0.1 mg/kg of intra-

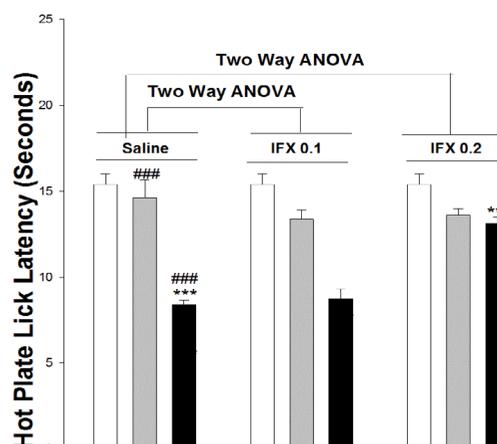


Figure 1. Comparison between normal states (white bar), sleep state (gray bar), and RSD (black bar) in three conditions

Including saline (First 3 groups), with IFX 0.1 mg/kg (Second 3 groups), and with IFX 0.2 mg/kg (Third 3 groups) on the hot-plate lick latency according to Mean±SD per each group. The comparison was done between the rate of the first 3 groups (saline), sham group, and RSD group by the hash mark (###) and in the first and third groups of RSD by asterisk mark (***). The marks over bars mean as ***P<0.001, and ###P<0.001.

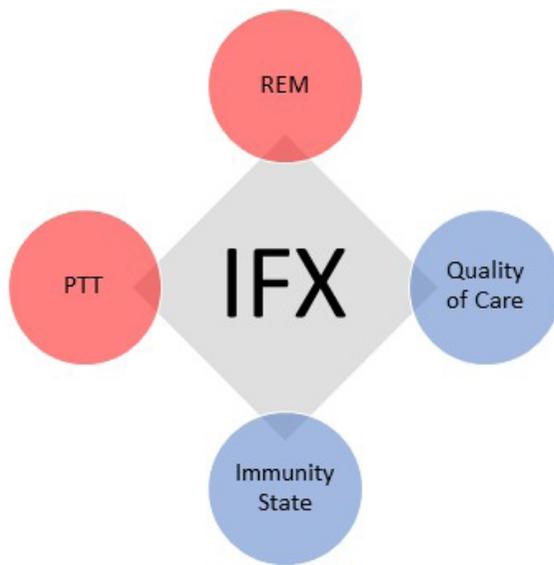


Figure 2. The association between the anti-TNF- α and other main four components. This figure shows the main four components of the study including the quality of care which is closely related to the pain tolerance threshold (PTT). Sleep deprivation affects PTT on the one hand and the immune system on the other hand. There is a close relationship between REM sleep deprivation and the immune system by anti-TNF- α (IFX).

peritoneal infliximab, and rats which received 0.2 mg/kg of intraperitoneal infliximab. No significant difference was found between the sham group and the group with 0.1 mg/kg of infliximab with respect to lick latency on hot-plate, whereas there was a significant difference between the sham group and the group with 0.2 mg/kg of Infliximab injection ($F_2=8.363$), ($P<0.001$) (Figure 1).

4. Discussion

REM sleep affects pain tolerance. From all functional regions of the brain, the hippocampus appears to be particularly sensitive and vulnerable to sleep loss. It seems that the hippocampus has an essential role in cognition and emotion regulation and is one of the numerous areas of the brain displaying neurogenesis from adolescence into adulthood. The reduction of hippocampal neurogenesis causes a reduction in this regulation, leading to depressive states and emotional disorders.

There was an important dilemma here in that whether sleep loss in the long term could at least to some extent lead to neurodegenerative disorders including Alzheimer's Disease (AD) or accelerate the process of disease development?

TNF α receptor system is believed to be one of the important pathways which can be involved in neurodegeneration in sleep-restricted rats. It remains unclear how TNF α turnover and receptor expression are affected following sleep deprivation over a short or long period. According to the findings of several researchers, TNF α elevates oxidative stress after SD [15]. Similarly, this study found that IFX at least partially prevents the behavioral consequences of SD.

The negative impacts of SD in rodents have been revealed using diverse methods of sleep deprivation. A few related studies have shown the viability of new cells under such conditions. Results from one study indicated that sleep loss reduces the survival of newly generated cells [17]. Nevertheless, contrary to the results of the present investigation, no unfavorable impact of sleep deprivation on cell survival in this area have been mentioned in several studies [20].

Several researchers have pinpointed hippocampal cell death in other disorders [21] such as Amnesia [22, 23]. Reductions have also been observed in the hippocampal volume in psychopathologies like major depression. Eventually, these studies report that these results can be related to specific symptoms in these disorders [15].

Evidence which show a connection between sleep deprivation and neurodegeneration stem from the reduced volume of the hippocampus and some other brain areas in both sleep-deprived humans and experimental animals [15].

Concerning our pharmacological intervention, rats that received IFX 15 minutes before being submitted to the SD procedure exhibited less memory impairment than that of the controls. This observation is supported by studies reporting on cell death in the CA1 (Cornu ammonis) and DG (Dentate Gyrus) of the hippocampus [15].

These findings can emphasize the key role of TNF α in the likely neurodegenerative processes which can be triggered by sleep loss [15, 21, 24]. TNF α is essential for cognitive development according to the current research focus on cognitive improvement based on sleep [15].

Based on the findings of several research studies, sleep deprivation causes inflammation in many parts of the body including the nervous system [25]. These studies focus on sleep disorders as one presenting sign of the altered function of the immune system and hypothesize the possible mirror effects of sleep disorders and the immune system on each other [25, 26].

The current evidence confirms that sleep disturbances occur over time; however, more studies are needed to understand and optimize sleep patterns in patients recovering from critical illnesses such as COVID-19 [27].

It has been proposed that patients with inflammation may benefit from monoclonal antibodies such as infliximab, which can be attributed to changes in the threshold of pain tolerance. Therefore, great attention needs to be paid to the ever-changing pain tolerance threshold [28]. The existing literature is sparse on high-quality studies on the interaction between sleep and pain with cognitive features using objective sleep measurements and pain assessment, Naranjo and many others have recognized this importance [29].

A systematic review of the available evidence suggests that sleep deprivation increases TNF- α (Tumor Necrosis Factor-alpha) in patients who need TNF- α treatment. According to the findings of this study and the observations on PTT, analgesics should be used more carefully in the treatment process. Figure 2 depicts the association between the quality of care affected by PTT (Pain Tolerance Threshold) which is a complex state on the one hand and the close relation between REM sleep deprivation and immunity system by anti-TNF- α (IFX) on the other hand.

5. Conclusion

The findings of this study demonstrated that chronic SD might result in neuronal damages as a result of neuroinflammatory insult. In addition to the possible role of TNF α , further mechanisms of this effect are yet to be determined. The results of the present research confirm the hypothesis that sleep loss, in a long period, sensitizes the brain to neurodegenerative insults. This is possible via activation of the inflammatory mechanisms to some extent through TNF α -associated pathways.

Ethical Considerations

Compliance with ethical guidelines

Compliance guidelines are approved by the Research Ethics Committee at the Institute for Cognitive Science Studies (ICSS) and registered by the code of CNS. Protocol-ICSS-940816 to refer the local guidelines for animal care and use at the Ethics Committee of the Institute for Cognitive Science Studies.

The specific ethical concern for the extent of sleep loss imposed by the multiple platform devices like DOW (Disk on the Water) is to use a practical standard instruc-

tion according to Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research. To refine the DOW method and make it the least cruel and invasive possible, guideline measures were accurately observed. All efforts were made to minimize animal suffering and to reduce the number of animals used.

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Authors' contributions

Study conception and design: Mohammadjavad Hoseinpoufard; Data analysis and interpretation: Mohammadjavad Hoseinpoufard; Drafting the article: Mohammadjavad Hoseinpoufard; Critically revising the article: All authors; Reviewing submitted version of manuscript: All authors; Approving the final version of the manuscript: All authors.

Conflict of interest

The authors declared that they have no conflict of interest.

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