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SLEEP LOSS AND THE BRAIN VULNERABILITY TO NEURODEGENERATION: BEHAVIORAL, BIOCHEMICAL AND NEURO-HISTOPATHOLOGICAL OBSERVATIONS IN A RAT MODEL

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ABSTRACT

Background: Experimentally-induced total sleep deprivation (TSD) and chronic partial sleep restriction (CPSR) leads to the emergence of cognitive impairments. This is hypothesized to result from a consequent neuroinflammation which may also hasten the neurodegenerative processes. Neuroinflammatory markers such as tumor necrosis factor-alpha (TNFα) are thought to be potential culprits in SD-induced neurodegeneration. ***Methods:*** The effect of TSD and CPSR on memory and anxiety-related behaviors (using the Elevated Plus-Maze test-retest protocol) and serum level of brain derived neurotrophic factor (BDNF) and corticosterone were assessed in male Wistar rats subjected to the modified disk-over-water (DOW) apparatus. In addition, an immunohistochemical (IHC) study was done to possibly detect the amyloid-beta (Aβ) and hyper-phosphorylated tau protein (HPτ) deposition in the dentate gyrus (DG) of the examined rats' hippocampi. Histomorphology and neuronal numerical density assessments were done at the same level across control and experimental animals. We also studied the above parameters in rats after intraperitoneal injection of the TNFα neutralizing antibody, infliximab (IFX). ***Results:*** Rats subjected to TSD and CPSR which did not receive IFX, showed a more pronounced impairment of memory, elevated serum corticosterone and decreased BDNF levels. CPSR rats which underwent delayed brain excision following behavioral testing, showed deposition of the HPτ and revealed the least numerical density in the hippocampal DG neurons. Meanwhile, IHC study revealed no Aβ deposition in the hippocampal DG of all examined rats. Interestingly, treatment with IFX, abrogated sleep restriction-induced cognitive decline, biochemical changes and the immunohistopathology in the hippocampal DG. ***Conclusions:*** Taken together, our findings indicated that CPSR (the SD model mimicking shift work) induces not only cognitive and biochemical changes, but also

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pathology in the hippocampal DG. This is possibly via activation of the inflammatory mechanisms in part through TNFα-dependent pathways.

Keywords: Sleep deprivation, elevated plus-maze, corticosterone, BDNF, immunohistochemistry, infliximab, dentate gyrus, rat(s)

INTRODUCTION

One of the crucial functions of sleep is to promote synaptic plasticity and neuronal recovery which leads to a proper brain function (Benington, 2003; Tononi and Cirelli, 2006; Meerlo et al., 2009). While behavioral studies have endorsed the above notion, the precise cellular and molecular processes governing this fundamental function of sleep are yet to be fully understood.

Based on the evidence from earlier experiments, sleep deprivation (SD) attenuates different markers of adult neurogenesis (Meerlo et al., 2009). Given this, and taking other changes into account, sleep loss is not only thought to leave detrimental effects on brain functionality but to speed up the progress of neuropathology.

When sleep restriction becomes a chronic problem, it seems to even more deleteriously affect the brain. This is clinically relevant since today's competitive life with its shift work, around-the-clock style and the overall pressures, predisposes many people to have either restricted or disrupted sleep (Bonnet and Arand, 1995; Pilcher et al., 2000; Rajaratnam and Arendt, 2001).

It has been noted that restricted sleep affects various physiological parameters including but not limited to the metabolic hormones and neuroinflammatory markers (Banks and Dinges, 2007). Therefore, SD is hypothesized to hasten neuroinflammation and subsequently the neurodegenerative processes (Livingston et al., 1993; Chang et al., 1997; Malow, 2004; Buysse et al., 2008; Germain et al., 2008; Roane and Taylor, 2008).

However, despite several studies devoted to understanding the possible relation between sleep loss and neurodegeneration, no clear picture has yet emerged. It seems difficult to draw a conclusive image from the available SD-neurodegeneration evidence, firstly because the earlier studies have employed various methods to induce SD, such as disk-over-water (DOW), tread mills, and multiple platforms each dealing with specific confounding variables. Secondly, each of the earlier studies has investigated a different sleep stage loss i.e. total- (TSD) or REM sleep deprivation (RSD) and few have modeled animals as shift-work, and thirdly, a plethora of cellular and molecular methods have been used to look for neurodegeneration. Some methods only declare the neuronal damage during DNA degeneration (within a period of hours), while others such as amino cupric silver staining need two days to show something, if any. Therefore, many of these methods are not suitable to provide evidence for the slowly cumulating neurodegeneration over time. This slowly progressive neurodegeneration is hypothesized to be one of the consequences of the chronic partial sleep restriction (CPSR), which is an issue in today's life.

SD-related neurodegeneration is thought to occur through at least two possible mechanisms: 1- altered level/effect of the neuroprotective markers and the resultant dominance in neuroinflammatory processes and 2- the heightened sensitivity to neuronal excitatory pathways. Some earlier studies have indicated an enhanced expression of hippocampal glutamate receptors and Ca^{2+}-induced excitotoxicity following SD (Ankarcrona et al., 1995; Sattler and Tymianski, 2000; Vyazovskiy et al., 2008).

Moreover, SD has led to a various levels of expression for neuroprotective factors such as nerve growth factor (NGF), brain derived neurotrophic factor (BDNF) and the

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cAMP response element-binding protein (CREB) (Kume et al., 2000; Sei et al., 2000; Guzman-Marin et al., 2006; Valera et al., 2008; Kim et al., 2009; Alhaider et al., 2011).

In the current study, we used an established rat model of TSD and CPSR which partly mimics sleep loss in humans (Meerlo et al., 2002; Roman et al., 2005a, b).

Knowing from the available data that sleep loss may influence neurogenesis, neuronal plasticity and typical neurodegenerative pathways, we hypothesized that SD in rats can increase the brain (especially hippocampal) vulnerability to neurodegeneration. Our hypotheses were: 1- SD influences memory function and anxiety level while antiinflammatory medications [here, the intraperitoneal (i.p.) injection of the anti-TNFα monoclonal antibody, Infliximab (IFX)] may rescind these effects; 2- SD leads to increased deposition of beta-amyloid peptide (Aβ) and hyperphosphorylated tau protein (HPτ) in the granular cell layer (GCL) of the hippocampal dentate gyrus (DG) and IFX can affect this possible deposition (an immunohistochemical assessment) and 3- SD leads to the altered serum level of BDNF and corticosterone and the i.p. IFX can possibly restore these changes.

To test the above hypotheses, we employed a setup consisting of the DOW apparatus to model the rats as TSD and CPSR, Enzyme-Linked Immuno-Sorbent Assay (ELISA) to assess the serum level of BDNF and corticosterone in the study arms and an immunohistochemical (IHC) method to examine the possible deposition of Aβ and HPτ in the GCL of the hippocampal DG.

MATERIALS AND METHODS

Animals

Male albino Wistar rats weighing 220-270 g, bred in the animal house of the Institute for Cognitive Science Studies (ICSS), Tehran, Iran, were used as subjects. Rats were housed in groups of four in Plexiglas home cages (45×30×15 cm) during which they had free access to food and water. Animals were kept under a light/dark cycle of 12 h (lights on at 07:00 AM), with the temperature ranging from 23±2 °C and the humidity of 55-60 %. Animal handling was limited to the time of cage cleaning (every other day), weighing and drug administration. Each study arm consisted of eight animals. Rats were handled about 3 min each day prior to the behavioral testing.

Apparatus

We used a carousel like device as a rotating disk-over-water, known as the DOW apparatus (Everson et al., 1989; Rechtschaffen et al., 1989; Rechtschaffen and Bergmann, 1995). This apparatus was used to induce TSD and CPSR in experimental rats. The apparatus housed two rats in separate Plexiglas cages which shared a round disk as a partially elevated floor. Instead of the yolk controls, we used DOW controls (i.e. animals were placed in off apparatus for the given period of time). Beneath the disk floor were 6 cm deep trays filled with shallow water. The water temperature maintained at 29±2 ºC throughout the experiments. This relatively higher than normal temperature was to let animals adjust with the well-defined thermoregulatory and metabolic syndrome possibly imposed by sleep deprivation procedure (Rechtschaffen and Bergmann, 1995). In the DOW method, when the rotating plate spins at a slow rate (4 rounds per minute), rats should keep pace when moving on the plate to avoid being sunk in the shallow water. Based on the earlier reports, the validated DOW setup (Everson et al., 1989; Rechtschaffen et al., 1989; Rechtschaffen and Bergmann, 1995) causes the experimental rats to be effectively (at least 85 % to 90 %) deprived from their sleep.

Using the DOW apparatus housing two experimental rats for TSD or CPSR models, close observation (randomly 10 to 20 blocks, each for 10 min, every day) as well as camera surveillance was done to ensure they actively and safely move around on the spinning plate or in water. The DOW control rats were housed in the off apparatus.

When in the apparatus, food (the standard lab oratory chow) and water were provided *ad libitum* and rats were closely monitored for their physical wellbeing and tolerance. Lighting schedule of the environment where the apparatus located was compliant with the standard L/D cycle, similar to the control condition.

Behavioral testing using the Elevated Plus-Maze (EPM) apparatus

Our EPM was made of black Plexiglas (floor and walls) and set elevated to the floor at a 50 cm height. It composed of two 50×10 cm open arms and two 50×10×40 cm enclosed arms, each with an open roof. The junction area of the four arms (the central platform) measured 10×10 cm. The maze was placed at the center of a quiet and dimly lit room (Zarrindast et al., 2010, 2011). All EPM experiments on rats were carried out between 14:00 h and 17:00 h.

We used the test-retest EPM protocol through which anxiety level (on test day) and the aversive memory retrieval (on retest day, 24 h later) could be evaluated. Rats who were assigned to any control or experimental arms were placed in the experimental room at least 1 h before testing. They were then individually placed at the center of the plus-maze, facing one of the open arms and allowed for 5 min free exploration in the EPM (the test session) after which were taken back to their home cages. In 24 hours, rats were returned to the test room and placed again in the EPM for a new exploration period of 5 min (the retest session). Animals' behaviors were tracked and recorded by an observer who quietly sat 1 m behind one of the closed arms of the maze, using a chronometer. The observer measured:

1- the time spent in open arms,
2- the time spent in closed arms,
3- the number of entries into open arms and
4- the number of entries into closed arms during the five minute period both upon test and retest. An entry was defined as "all four paws in the arm". In between the EPM tests and after each rat, the maze was cleaned with distilled water. The obtained data were used to calculate:

a- % OAT (the ratio of time spent in open arms to the time spent in all arms ×100);
b- %OAE (the ratio of entries into open arms to total entries×100) and
c- the total closed and open arm entries (a relatively pure index for the locomotor activity) (Zarrindast et al., 2010; Eslimi et al., 2011). The number of rearings (rats maintaining an erect posture), groomings (rats rubbing their face including ears, mouth, vibrissae, eyes with rapid circular movements of their forepaws) and defecations (the number of passed fecal boli) were manually recorded as the conventional indices for anxiety-related behaviors (Zarrindast et al., 2010, 2011; Eslimi et al., 2011).

Study design

Rats were assigned to different study arms based on: 1- whether they had no SD [cage control (CC) vs. DOW apparatus control (DOW-C)] or sleep-deprived (TSD for 48 hours vs. CPSR i.e. intermittently sleep-deprived for 7 consecutive days) and 2- whether they had received i.p. IFX (the TNFα neutralizing antibody) vs. PBO (Placebo/saline) 15 minutes prior to the interventions. Following the procedure and behavioral testing in different arms, blood sampling was done and the rats were euthanized with their brain removed for neurohistopathological studies. The sub-analysis was based on "when" the blood was drawn and brains were excised [i.e. immediately after the completion of behavioral testing (early brain excision/EBE) vs. two weeks later (late brain excision/LBE)].

For the study arms, we had two sets of stratifications leading to 12 arms. The first set of stratifications comprised:

arm 1- PBO/CC/48 h;
arm 2- PBO/DOW-C/48 h;
arm 3- PBO/CC/7 days;
arm 4- PBO/DOW-C/7 days;
arm 5- PBO/TSD/48 h and
arm 6- PBO/CPSR/7 days.

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The second set of stratifications consisted of:

arm 1- IFX/CC/48 h;
arm 2- IFX/DOW-C/48 h;
arm 3- IFX/CC/7 days;
arm 4- IFX/DOW-C/7 days;
arm 5- IFX/TSD/48 h and
arm 6- IFX/CPSR/7 days.

For TSD, the DOW apparatus was continuously rotating for 48 h and for CPSR, an automatically set timer allowed the apparatus to be 12 h on (08:00 h to 20:00 h) and 12 h off (20:00 h to 08:00 h), intermittently for 7 consecutive days. The study design has been illustrated in Figure 1.

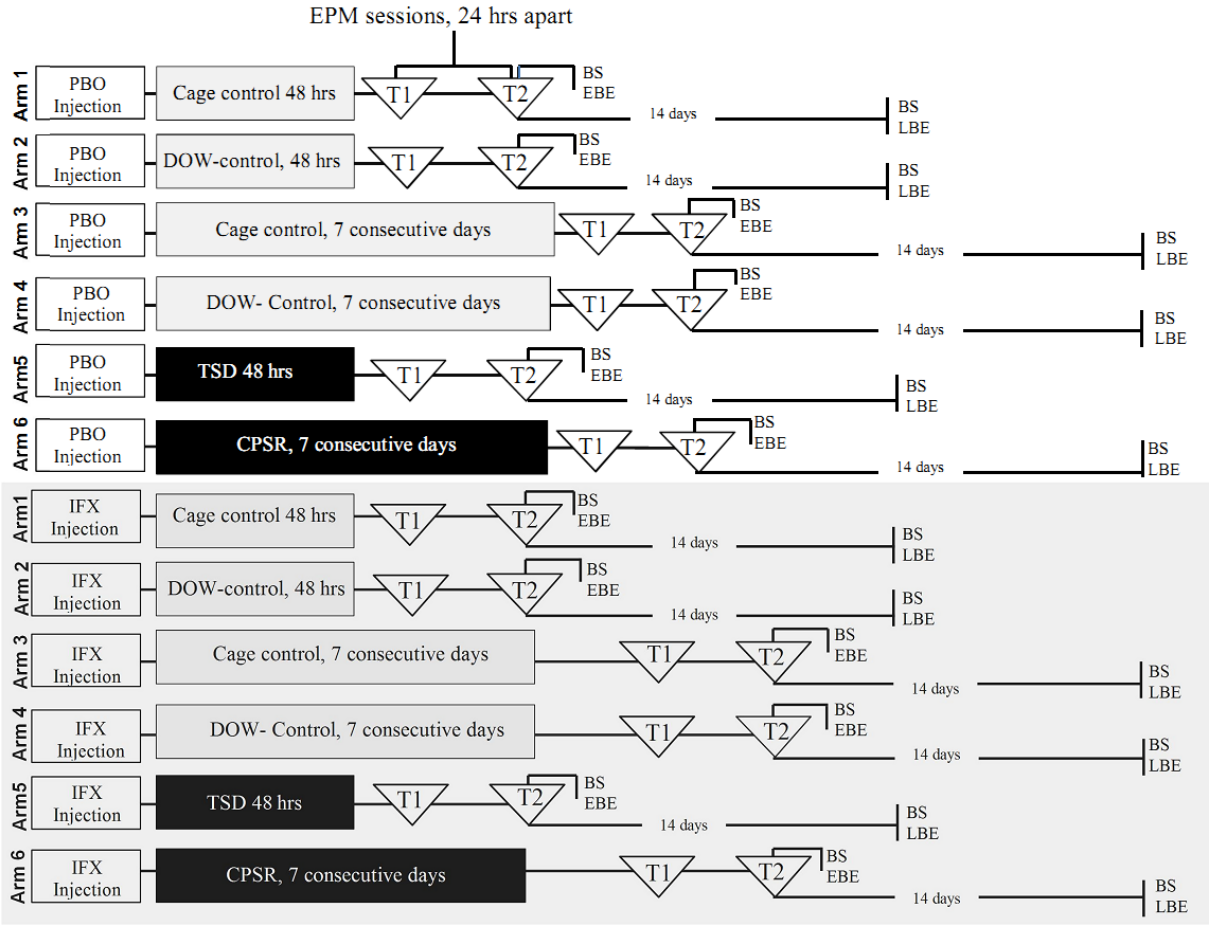

Figure 1: Study design diagram
The variables through which stratification into these arms was made were:
1- sleep deprived (either TSD or CPSR) vs. control (either CC or DOW-C);
2- the length of intervention or control condition (i.e. 48 h vs. 7 days);
3- PBO vs. IFX treatment and
4- early (immediately after EPM retest) vs. late (14 days after EPM retest) blood sampling for serum neurochemical assessment or early vs. late brain excision for IHC/histomorphological study of the hippocampus. By this, two sets of stratification (each comprising six arms) were defined. The 12 study arms with their assignment criteria are depicted in this diagram. TSD: total sleep deprivation, CPSR: chronic partial sleep restriction, CC: cage control, DOW-C: disk-over-water apparatus control, PBO: placebo, BS: blood sampling, IFX: infliximab, EPM: elevated plus-maze, IHC: immunohistochemistry

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Drugs

Infliximab (Schering Plough, NJ, USA), the chimeric human-murine anti-tumor necrosis factor-alpha (TNFα) monoclonal antibody or PBO (saline) were injected i.p. to all control (CC and DOW-C) and experimental (TSD and CPSR) rats 15 minute before submitting them to the SD procedures (TSD or CPSR) or control conditions (cage or off apparatus) (Figure 1). The TNFα-neutralizing Ab, infliximab (IFX), binds with high affinity and avidity to the soluble, transmembrane and receptor-bound forms of TNFα but not to lymphotoxin α (TNFβ). Infliximab neutralizes the TNFα heterotrimers and inhibits their inflammatory effect on target cells. Since TNFα is considered a death ligand and contributes to apoptotic pathways following the oxidative stress (Figure 2), IFX was expected to lessen the adverse effects of SD both behaviorally and histopathologically. That was the reason IFX was applied in the current investigation. IFX is a commercially available medication used for distinct immune-mediated inflammatory disorders (IMIDs) in clinical medicine at 3-5 mg/kg based on the indications such as rheumatoid arthritis, ankylosing spondylitis, psoriasis, ulcerative colitis and Crohn's disease (Infliximab summary of product characteristics, Schering Plough, 2010). As described in an earlier investigation (Karson et al., 2013), and based on our observations, since 4 mg/kg of i.p. IFX rendered no effect on anxiety-related behaviors or memory functions in rats by itself, this dose was used as the subthreshold applied dose in our experiments.

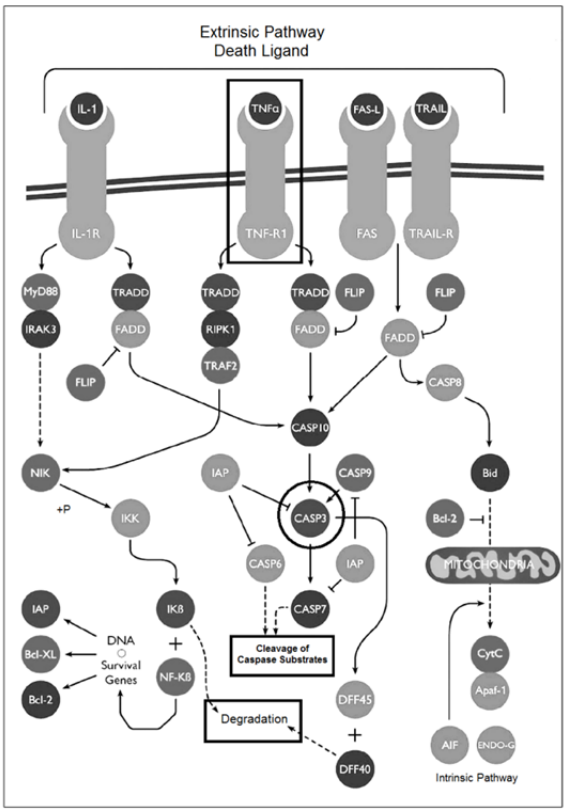

Figure 2: Extrinsic pathway of the apoptotic caspase cascade
This pathway is initiated through binding of death ligands such as TNFα to their receptors. This in turn would activate membrane-proximal caspases (caspase-9 and 10) and eventually induces the activation of caspase-3 and thereby promotes cell death. The oxidative stress is thought to contribute to this mechanism, in which TNFα seems to play an active part. The previously described neuronal cell death in the hippocampal dentate gyrus following sleep deprivation in rodents is thought to be at least partly resulted from TNFα. Therefore, anti TNFα monoclonal Ab, infliximab, was applied to investigate whether it prevents the neuroinflammatory consequences of sleep loss. Adapted from (http://ptglab.com), Jan. 2013, with permission.

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The serum neuro-biochemical, tissue immunohistochemical and stereological assessments

Neuro-biochemical measurements

According to the rats' stratification into the study arms, either immediately after EPM retest session (early blood sampling) or 14 days later (late blood sampling), each animal was euthanized using inhaled chloroform, after which 3 ml blood was obtained transcardially and centrifuged (the above process was done between 17:00 h and 18:00 h). Serum was isolated and stored at -20 °C in EDTA tubes. Serum BDNF and corticosterone levels were measured using the sandwich ELISA (Enzyme-Linked Immuno-Sorbent Assay) method. The monoclonal Anti-BDNF antibody 1B10 (Sigma-Aldrich Inc.) and Rat Corticosterone ELISA Kit, 96T (BIOTANG Inc.) were used in our biochemical assessments.

Brain collection

Either immediately after the EPM retest session (early brain excision/EBE) or 14 days later (late brain excision/LBE), animals were euthanized and their brains were removed from the skull, postfixated in 4 % paraformaldehyde for 24 h and immersed in 30 % sucrose for an additional 48 h for cryoprotection.

The brains were collected for immunohistochemical and further histological (Mouton et al., 2002) and stereological study (Namavar et al., 2012).

The immunohistochemical study for the detection of Aβ peptide and HPτ protein in the hippocampal DG

The formalin-fixed paraffin embedded brain blocks of all rats were sectioned and tissue slices at DG level of the hippocampi were mounted on slides (sections were mainly taken from -2.5 mm to -5.0 mm from bregma according to the atlas of rats' brain) (Paxinos and Watson, 2007). Sections were pretreated for 30 min with 0.3 % H_2O_2 to prevent non-specific staining before incubation with 1:200 mouse anti-beta amyloid antibody (DE2B4-ab11132, Abcam, Cambridge, UK) or with anti-tau antibody (E178-ab32057, Abcam, Cambridge, UK) for 24 h at 4 °C. When preparing negative control slides, this step was skipped. Tissues were then exposed to 1:500 mouse and rabbit specific horseradish peroxidase/diaminobenzidine (HRP/DAB) secondary antibody (ab64264, Abcam, Cambridge, UK) for 2 h. Sections were subsequently incubated for 1 h with 1:500 Streptavidine-DAB chromogen complex (ab64264, Abcam, Cambridge, UK) and 0.003 % H_2O_2. The whole immunohistochemical (IHC) staining for Aβ peptide and HPτ protein were carried out according to previously published methods and the manufacturer's manuals (Papasozomenos and Su, 1991; Li et al., 1995). Prepared slides were studied both by an IHC expert and an independent pathologist blinded to the study protocol to interpret the IHC study results of all preparations (control vs. sleep-deprived rats who had either been PBO- or IFX-treated).

Histomorphological assessments

To assess the numerical density of the granular cell layer (GCL) neurons of the DG and further histomorphological evaluations, processed brain tissues were coronally and serially sectioned, using a cryostat (SLEE, Frankfurt, Germany) at a thickness of 30 µm throughout the hippocampal DG, and were immersed in cryoprotectant solution and retained at -20 °C, till needed. Every 6th section provided a mounted slide which was stained using cresyl violet.

The numerical density assessment of the hippocampal DG neurons in sleep-deprived and control rats were done using the optical disector technique (Abusaad et al., 1999). This setup comprised an Eclipse microscope (E200, Nikon, Tokyo, Japan) possessing a high numerical-aperture (NA=1.25)×60 oil-immersion objective. The setup was connected to a video camera, transmitting the image to the monitor. For motion correction, the device was equipped with an electronic microcenter digital

readout (MT12, Traunreut, Germany). The neuronal density was defined as follows: $NV=\sum Q/[\sum P \times a(f) \times h]$. In this calculation, $\sum Q$ is the number of neurons counted, $\sum P$ is number of dissector, $a(f)=0.001369$ mm^2, is the area of the sampling frame, and h=0.015 mm, is the height of the dissector (Gundersen et al., 1988, 1999; Abusaad et al., 1999; Namavar et al., 2012). To facilitate cell counting, we employed an automated image analysis system (Leica Qwin, Rijswijk, the Netherlands). Neuronal nuclei were counted in 3±1 sections using our set-up at a 400x final magnification. Through the optical dissector technique (Mouton et al., 2002), result values were reported as the number of DG neurons per mm^3.

Ethical issues

The specific ethical concern about the extent of sleep loss imposed by a device like DOW was dealt with according to the Guidelines for the care and use of mammals in neuroscience and behavioral research (2003). To refine DOW method and make it least cruel and invasive possible, guideline measures were meticulously observed. All efforts were made to minimize animal suffering and to reduce the number of animals used.

Based on this and referring to the local guidelines for animal care and use, the study was approved by the ethics committee of the Institute for Cognitive Science Studies.

Experimental design

Experiment 1: Open-arms exploratory behaviors of sleep-deprived and control animals submitted to EPM test-retest protocol, in the presence or absence of infliximab injection

To examine the extent of TSD or CPSR involvement in the presence or absence of IFX injection in anxiety and memory function, IFX or PBO was injected to animals 15 min before being submitted to the SD procedure. The control rats similarly received either IFX or PBO. EPM testing was done following the interventions (see Figure 1). The animals' open arms exploratory behaviors on test day corresponded to anxiety while same indices during retest attributed to the EPM-associated memory retrieval.

Experiment 2: Assessing the possible effect of sleep loss on serum BDNF and corticosterone levels in the presence or absence of infliximab injection

Following the EPM retest, based on the study arm to which rats were assigned, blood sampling was done in early or late setting as described in the method section. BDNF and corticosterone serum levels were measured using the ELISA method. The effect of sleep loss in presence or absence of IFX injection on these chemicals' serum level was compared across groups.

The immunohistochemical detection of Aβ and HPτ in the hippocampal DG of sleep-deprived vs. control rats in the presence or absence of infliximab injection

Whether animals' brain were removed early or late (as described in methods), tissues were submitted to the IHC study for possible detection of Aβ or HPτ protein in the DG of the hippocampi. In this experiment, prepared tissue slides from the hippocampi of 12 groups of rats were examined. These experimental or control rats had already received either IFX or PBO. Qualitative analysis was done to interpret the IHC study results.

Histomorphological evaluations in the hippocampal DG of sleep-deprived vs. control rats in the presence or absence of infliximab injection

Brain tissues of the 12 study arm animals were sectioned and stained with cresyl violet. The numerical density of the DG neurons was measured to see whether TSD or CPSR in presence or absence of IFX injection affect this variable.

Statistical analyses

Given the normality of distribution and homogeneity of variance, to analyze the obtained behavioral tests data (i.e. EPM measures such as %OAT, %OAE, locomotion, rearing, grooming and defecation) during test and retest sessions in the presence or absence of IFX injection, we employed the repeated measure analysis of variance (ANOVA). Moreover, two ANOVA was used to analyze the serum BDNF and corticosterone data. When ANOVA revealed any significance, post hoc Tukey's test was used to assess differences between specific treatment arms.

To establish if TSD and CPSR rats vs. their corresponding controls in the presence or absence of IFX injection, show evidence of Aβ and HPτ deposition in DG, the observations across groups were qualitatively compared using the Pearson's chi-squared test. Additionally, to compare the neuronal numerical density/mm^3 amongst groups, we used two-way ANOVA. Upon significance, post hoc Tukey's test was applied to determine differences between specific groups. The level of significance for all tests was $P<0.05$. All data were expressed as mean ± S.E.M.

RESULTS

Experiment 1: Open-arms exploratory behaviors of sleep-deprived and control animals submitted to EPM test-retest protocol in the presence or absence of infliximab injection

Repeated measure analysis of variance declared that TSD and CPSR increase %OAT (Figure 3A), %OAE (Figure 3, 2B) and the number of groomings (Figure 4B) while do not alter the locomotor activity (Figure 3C).

SD (in the presence or absence of IFX injection) led to a notable decrease in the number of rearings and defecations on test and retest days as compared to controls (Figure 4A and C). Increased %OAT during the T1 EPM session indicates the anxiolytic-like response induced by TSD and CPSR (Figure 3A). However, this effect was more pronounced in CPSR as compared to TSD rats ($p<0.01$) (Figure 3, panel 1A). As compared to PBO-treated rats, TSD animals which received IFX showed even more increase in %OAT on test day which indicated a more pronounced anxiolytic-like effect of TSD in this group (Figure 3, panel 3A). Results indicated an increase in %OAT and %OAE upon retest (T2 session) in TSD and CPSR rats (Figure 3A and B). While the analysis for PBO-treated TSD and CPSR animals showed an increased %OAT in T2 (which corresponds to EPM-associated memory impairment), IFX could partially revert this SD-induced memory impairment (Figure 3, panels 4A and 4B). Taken together, the increased %OAT and %OAE (during T2) could be interpreted as impairment of memory consolidation and retrieval following TSD and CPSR which was partially abrogated by IFX injection. The results of this experiment are illustrated in Figure 3 and 4. In addition, the quantitative statistical analysis outcome for this experiment is summarized in Table 1.

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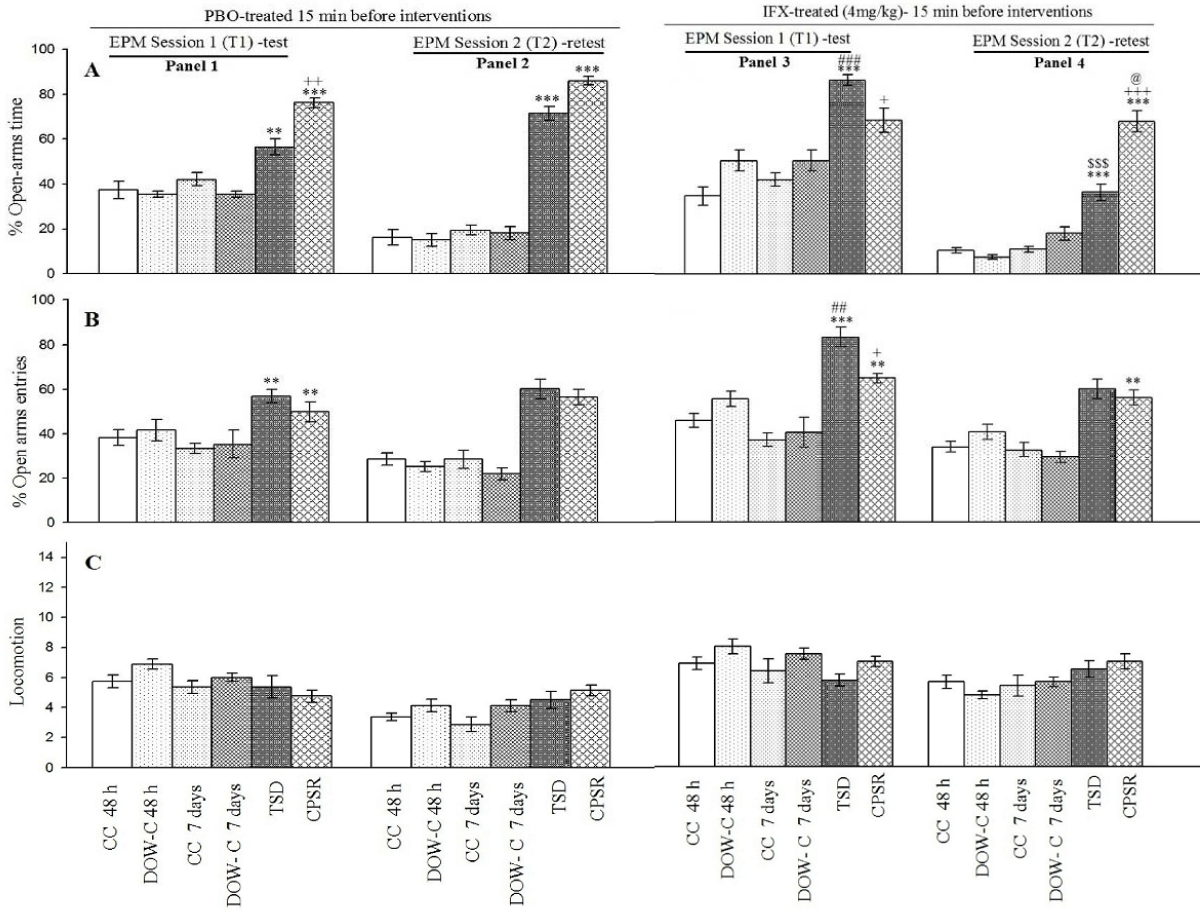

Figure 3: EPM test-retest paradigm
Open-arms exploratory behaviors following control condition or sleep deprivation (TSD or CPSR) upon PBO (panels 1 and 2) and IFX (panels 3 and 4) i.p. injection, 15 min before the intervention. %Open-Arms Time (A); %Open-Arms Entries (B) and locomotion (C). Values are expressed as mean±S.E.M (n=8 in each group). *P<0.05, **P<0.01 and ***P<0.001 different from their corresponding control arms (i.e. CC or DOW-C) in each panel. For the TSD groups, the corresponding control arms are CC 48 h and DOW-C 48 h while for CPSR, the corresponding controls are CC-7 days and DOW-C 7 days. Panel 1. ++P<0.01 different from TSD in the same panel. Panel 3, ###P<0.001 different from the corresponding arm (TSD) in panel 1. Panel 4, +++P<0.001 different from TSD in the same panel. Panel 4, $$$P<0.001 different from the corresponding arm (TSD) in panel 2. Panel 4, @P<0.05 different from the corresponding arm (CPSR) in panel 2. TSD: total sleep deprivation, CPSR: chronic partial sleep restriction, PBO: placebo, IFX: infliximab, CC: cage control, DOW-C: disk-over-water apparatus control

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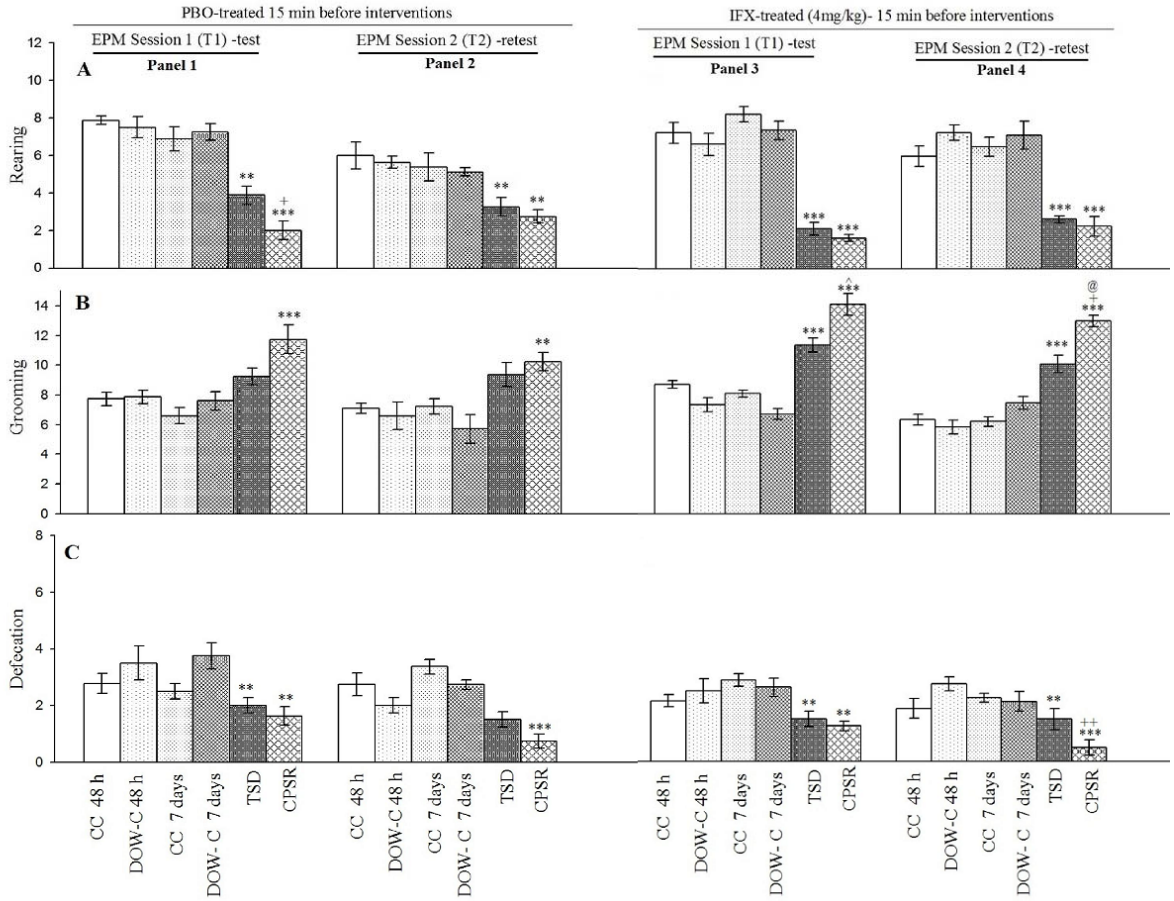

Figure 4: EPM test-retest paradigm
Auxiliary open-arms exploratory behaviors [Rearing (A); grooming (B) and defecation (C)] following control condition or sleep deprivation (TSD or CPSR) upon PBO (panels 1 and 2) and IFX (panels 3 and 4) i.p. injection, 15 min before the intervention. Values are expressed as mean±S.E.M (n=8 in each group). *P<0.05, **P<0.01 and ***P<0.001 different from their corresponding control arms (i.e. CC or DOW-C) in each panel. For the TSD groups, the corresponding control arms are CC 48 h and DOW-C 48 h while for CPSR, the corresponding controls are CC-7 days and DOW-C 7 days. Panel 1, +P<0.05 different from TSD in the same panel. Panel 3, ^P<0.05 different from the corresponding arm (CPSR) in panel 1. Panel 4, +P<0.001 different from TSD in the same panel and @P<0.05 different from the corresponding arm (CPSR) in panel 2. TSD: total sleep deprivation, CPSR: chronic partial sleep restriction, PBO: placebo, IFX: infliximab, CC: cage control, DOW-C: disk-over-water apparatus control

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Table 1: Repeated measure analysis and two-way ANOVA results with P values for the effect of sleep deprivation vs. control condition

Type of analysis	EPM indices	within-subjects effects		between-subjects effects		tests × groups	
		F (1, 42)	P	F (5, 42)	P	F (5, 42)	P
Repeated measure between panels 1 and 2	%OAT	34.88	0.0005	155.00	0.0005	19.05	0.0005
	%OAE	8.27	0.01	18.00	0.0005	3.34	0.05
	Locomotion	43.41	0.0005	2.21	0.072	3.75	0.007
	Grooming	20.10	0.0005	22.31	0.0005	3.46	0.01
	Rearing	4.48	0.040	10.76	0.0005	1.262	0.30
	Defecations	5.61	0.023	12.94	0.0005	2.57	0.041
Repeated measure between panels 3 and 4		F (1, 42)	P	F (5, 42)	P	F (5, 42)	P
	%OAT	219.10	0.0005	60.80	0.0005	11.82	0.0005
	%OAE	45.64	0.0005	25.25	0.0005	1.97	0.10
	Locomotion	18.17	0.0005	1.06	0.40	4.91	0.001
	Grooming	3.90	0.005	14.11	0.0005	1.11	0.30
	Rearing	21.61	0.0005	89.48	0.0005	2.66	0.035
	Defecations	3.10	0.09	12.77	0.0005	0.76	0.58
Two-Way ANOVA between panels 1 and 3		F (1, 84)	P	F (5, 84)	P	F (5, 84)	P
	%OAT	16.86	0.0005	42.18	0.0005	8.06	0.0005
	%OAE	24.37	0.0005	19.77	0.0005	2.00	0.088
	Locomotion	19.79	0.0005	4.19	0.002	0.838	0.53
	Grooming	1.19	0.277	38.82	0.0005	1.09	0.28
	Rearing	8.97	0.004	34.80	0.0005	3.11	0.013
	Defecations	7.47	0.008	8.20	0.0005	1.21	0.311
Two-Way ANOVA between panels 2 and 4		F (1, 84)	P	F (5, 84)	P	F (5, 84)	P
	%OAT	59.95	0.0005	195.5	0.0005	10.12	0.0005
	%OAE	7.89	0.006	37.56	0.0005	1.51	0.19
	Locomotion	46.06	0.0005	5.137	0.0005	1.058	0.39
	Grooming	2.63	0.11	12.66	0.0005	1.33	0.258
	Rearing	1.78	0.186	27.38	0.0005	3.4	0.008
	Defecations	4.38	0.04	15.18	0.0005	2.7	0.026

These interventions were in the presence (panels 3 and 4 in Figure 3 and 4) or absence (panels 1 and 2 in Figure 3 and 4) of anti TNFα (IFX) injection. The effect of such interventions on EPM indices (exploratory-like behaviors) including %OAT, %OAE, locomotion, rearing, grooming and defecation, have been demonstrated. IFX: infliximab, EPM: elevated plus-maze, %OAT: open arm time percentage, %OAE: open arm entries percentage

Experiment 2: Assessing the possible effect of sleep loss on the serum BDNF and corticosterone levels in the presence or absence of infliximab injection

In this experiment, we assessed the serum BDNF and corticosterone levels following sleep deprivation or control condition in PBO- vs. IFX-treated animals. Rats were submitted to either early (immediately after EPM retest) or late (after 14 days) blood sampling to have their serum BDNF and corticosterone levels measured (ELISA). While PBO-treated TSD and more significantly, CPSR rats, showed decreased level of BDNF and peaked corticosterone, treatment with IFX partially rescinded these changes when blood was drawn in early setting. On the other hand, in late blood sampling, IFX almost completely blocked the SD-induced changes in BDNF and corticosterone. This possibly indicates the central role of TNFα in the inflammatory processes leading to such neuro-biochemical changes following SD. The results of this experiment are demonstrated in Figure 5 and 6. In addition, the quantitative statistical analysis outcome for this experiment is summarized in Table 2.

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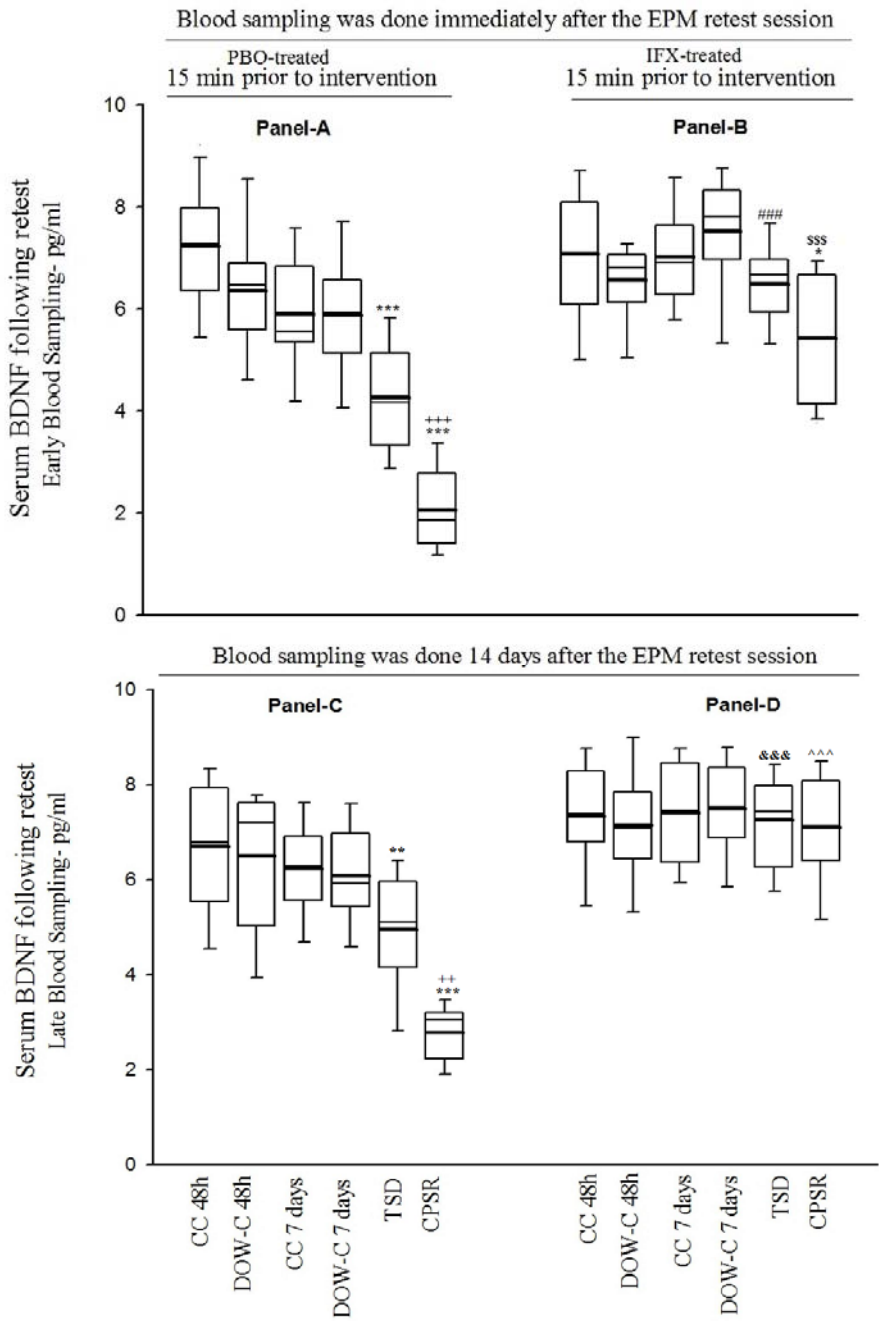

Figure 5: Serum BDNF levels following sleep deprivation or control condition in PBO- vs. IFX-treated animals

Rats were submitted to either early (immediately after the EPM retest) or late (after 14 days) blood sampling to have their serum BDNF level assessed (ELISA). Values are expressed as mean±S.E.M (n=8 in each group), pg/ml. **P<0.01 and ***P<0.001 different from corresponding control arms in the same panel. For the TSD groups, the corresponding control arms are CC 48 h and DOW-C 48 h while for CPSR, the corresponding controls are CC-7 days and DOW-C 7 days. Panel A and C, ++P<0.01 and +++P<0.001 different from TSD arm in the same panel. Panel B, ###P<0.001 different from the respective arm (TSD) in panel A. Panel B, $$$P<0.001 different from the respective arm (CPSR) in panel A. Panel D, &&&P<0.001 different from the respective arm (TSD) in panel C. Panel D, ^^^P<0.001 different from respective arm (CPSR) in panel C. BDNF: brain derived neurotrophic factor, EPM: elevated plus-maze, ELISA: enzyme-linked immunosorbent assay, pg/ml: picogram per milliliter, TSD: total sleep deprivation, CPSR: chronic partial sleep restriction, PBO: placebo, IFX: infliximab, CC: cage control, DOW-C: disk-over-water apparatus control

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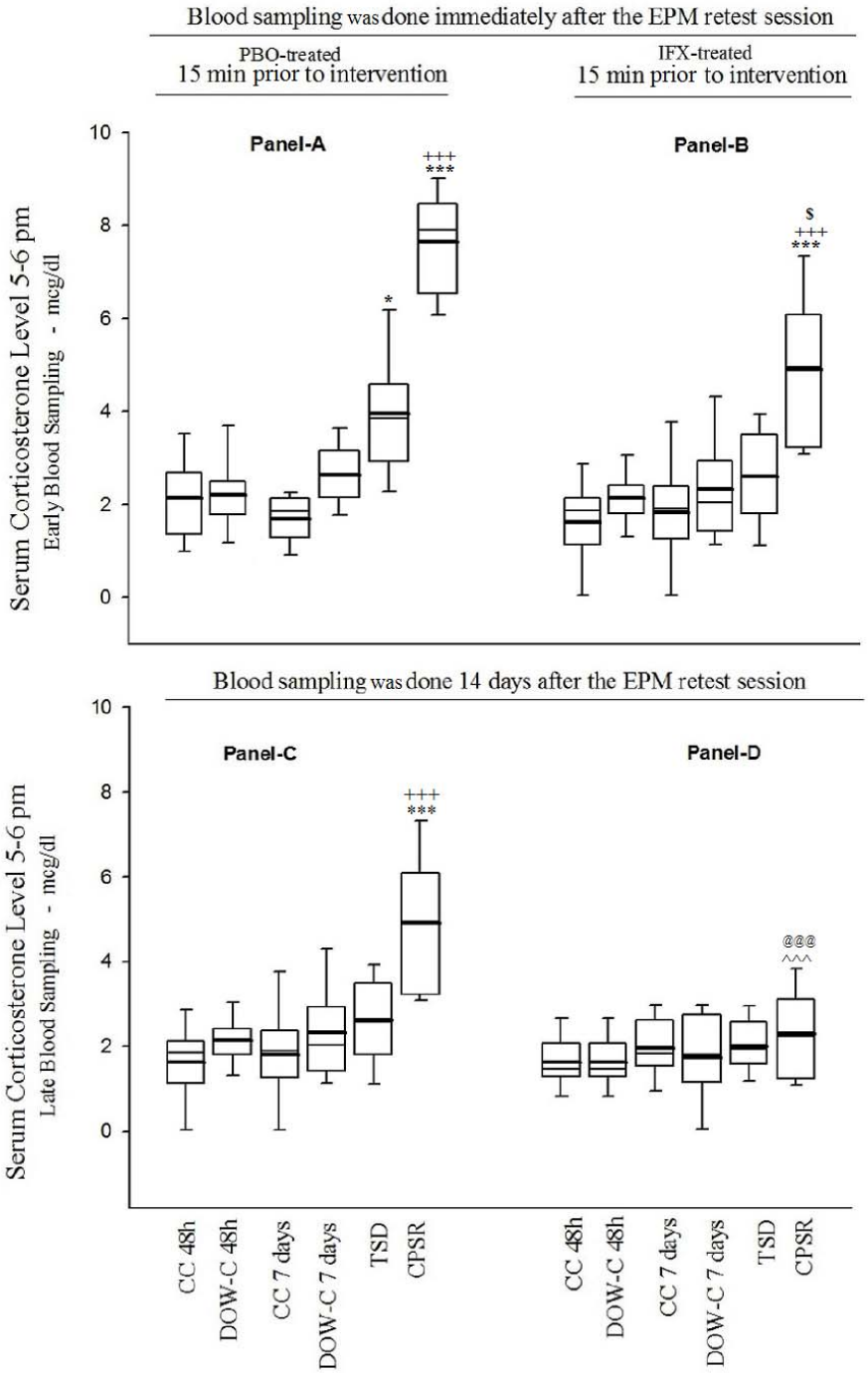

Figure 6: Serum corticosterone levels following sleep deprivation or control condition in PBO- vs. IFX-treated animals

Rats were submitted to either early (immediately after the EPM retest) or late (after 14 days) blood sampling to have their serum corticosterone level assessed (ELISA). Values are expressed as mean±S.E.M (n=8 in each group), mcg/dl. *P<0.05 and ***P<0.001 different from corresponding control arms in the same panel. For the TSD groups, the corresponding control arms are CC 48 h and DOW-C 48 h while for CPSR, the corresponding controls are CC-7 days and DOW-C 7 days. Panel A, B and C, +++P<0.001 different from TSD arm at the same panel. Panel B, $P<0.05 different from the respective arm (CPSR) in panel A. Panel D, ^^^P<0.001 different from the respective arm (CPSR) in panel C. Panel D, @@@P<0.001 different from the respective arm (CPSR) in panel B. EPM: elevated plus-maze, ELISA: enzyme-linked immunosorbent assay, mcg/dl: microgram per deciliter, TSD: total sleep deprivation, CPSR: chronic partial sleep restriction, PBO: placebo, IFX: infliximab, CC: cage control, DOW-C: disk-over-water apparatus control

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Table 2: Two-way ANOVA results with P values for the effect of sleep deprivation interventions vs. control condition

Type of analysis	Specimen collection	within-subjects effects		between-subjects effects		tests × groups	
		F (1, 84)	P	F (5, 84)	P	F (5, 84)	P
Two-Way ANOVA for BDNF	for early brain excision	47.5	0.0005	27.37	0.0005	8.18	0.0005
	for late brain excision	61.88	0.0005	12.26	0.0005	10.77	0.0005
Two-Way ANOVA for Corticosterone	for early brain excision	14.3	0.0005	46.94	0.0005	2.76	0.02
	for late brain excision	5.32	0.024	13.43	0.0005	3.84	0.003
Two-Way ANOVA for neuronal numerical density in dentate gyrus	for early brain excision	6.96	0.01	24.99	0.0005	1.024	0.41
	for late brain excision	3.72	0.07	31.10	0.0005	1.77	0.43

These interventions were in the presence or absence of anti TNFα (IFX) injection (panels A-D in Figure 5, 6 and 7), upon early (immediately after EPM retest) vs. late (14 days after EPM retest) blood sampling or early vs. late brain excision. The effect of such interventions on serum levels of BDNF/corticosterone and the numerical density of the hippocampal dentate gyrus neurons, have been demonstrated. IFX: infliximab, EPM: elevated plus-maze, BDNF: brain derived neurotrophic factor

The immunohistochemical detection of Aβ and HPτ in the hippocampal DG of sleep-deprived vs. control rats in the presence or absence of infliximab injection

The IHC study of the hippocampal DG of all examined rats (sleep deprived vs. control, PBO-treated vs. IFX-treated, early brain excision vs. late brain excision) revealed no evidence of Aβ deposition. However, in the absence of any tissue reaction in negative controls for HPτ, CPSR rats which received PBO and underwent late brain excision showed positive staining reaction for HPτ. Interestingly, IFX (4 mg/kg i.p. injection) prevented this reaction suggesting the possible role of TNFα as a pivotal proinflammatory mediator in the above process. In summary, while Aβ deposition did not occur across groups, unlike all other arms, a significant positive staining reaction with regard to HPτ, in PBO-CPSR-LBE group was noted (r^2=0.8). The IHC findings in the hippocampal DG are demonstrated in Figure 7.

Histomorphological evaluations in the hippocampal DG region of sleep-deprived vs. control rats in the presence or absence of infliximab injection

Nissl staining of the rats' hippocampal sections at the level of DG showed the presence of dark neurons, comparatively more in PBO-CPSR-LBE group animals. Measuring the numerical density (i.e. the number of neurons/mm^3) indicated that the number of DG neurons declined following SD. This decline was more pronounced in CPSR rats with late brain excision (with the least number of neurons/mm^3), however the difference was not statistically significant as compared to other CPSR groups. Interestingly, both in TSD and CPSR arms, IFX injection (4 mg/kg, i.p.) resulted in prevention of the numerical density decline in DG. This suggests the possible role of IFX to at least partly hamper the apoptotic pathways leading to the neuronal cell death in DG following an oxidative stress such as SD. The results of this experiment are summarized in Figure 8. In addition, the quantitative outcome of the statistical analysis on this experiment is outlined in Table 2.

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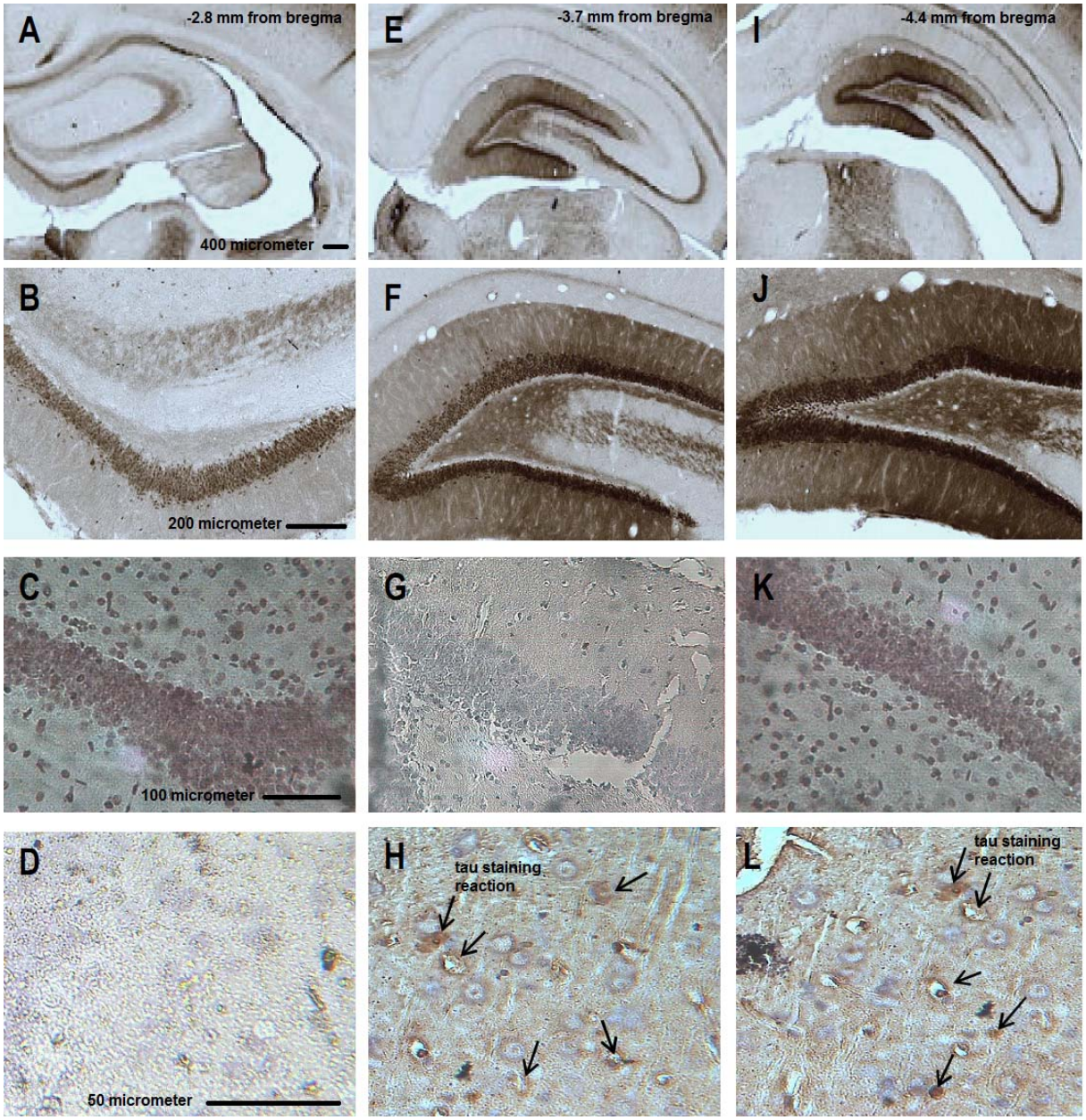

Figure 7: IHC study of the prepared tissue slides for the detection of Aβ and HPτ protein in the hippocampal DG in all examined groups (n=8/arm and n=4/group, see Figure 2, the study design)
Presence of antigen produces brown discoloration when encounters specific monoclonal antibodies (DE2B4- ab11132 for Aβ and E178-ab32057 for HPτ) in this method. Comparable to negative control slides (in which the application of primary antibody was skipped during the IHC protocol), no tissue staining reaction for Aβ was found in all slides examined (controls vs. sleep-deprived groups, on PBO vs. IFX). Meanwhile in the absence of any tissue reaction in negative controls for HPτ, CPSR rats which received PBO and underwent LBE showed positive staining reaction for HPτ (H, L: arrows). This may be a good sign of τ deposition following sleep loss in long run. Interestingly, IFX (4 mg/kg i.p. injection) prevented this reaction suggesting the possible role of TNFα in the above process. While Aβ deposition did not occur across groups, unlike all other arms, a significant positive staining reaction with regard to HPτ, in PBO-CPSR-LBE group was noted. IHC: immunohistochemical, DG: dentate gyrus, Aβ: amyloid beta peptide, HPτ: hyperphosphorylated tau protein, IFX: infliximab, PBO: placebo, CPSR: chronic partial sleep restriction, i.p.: intraperitoneally

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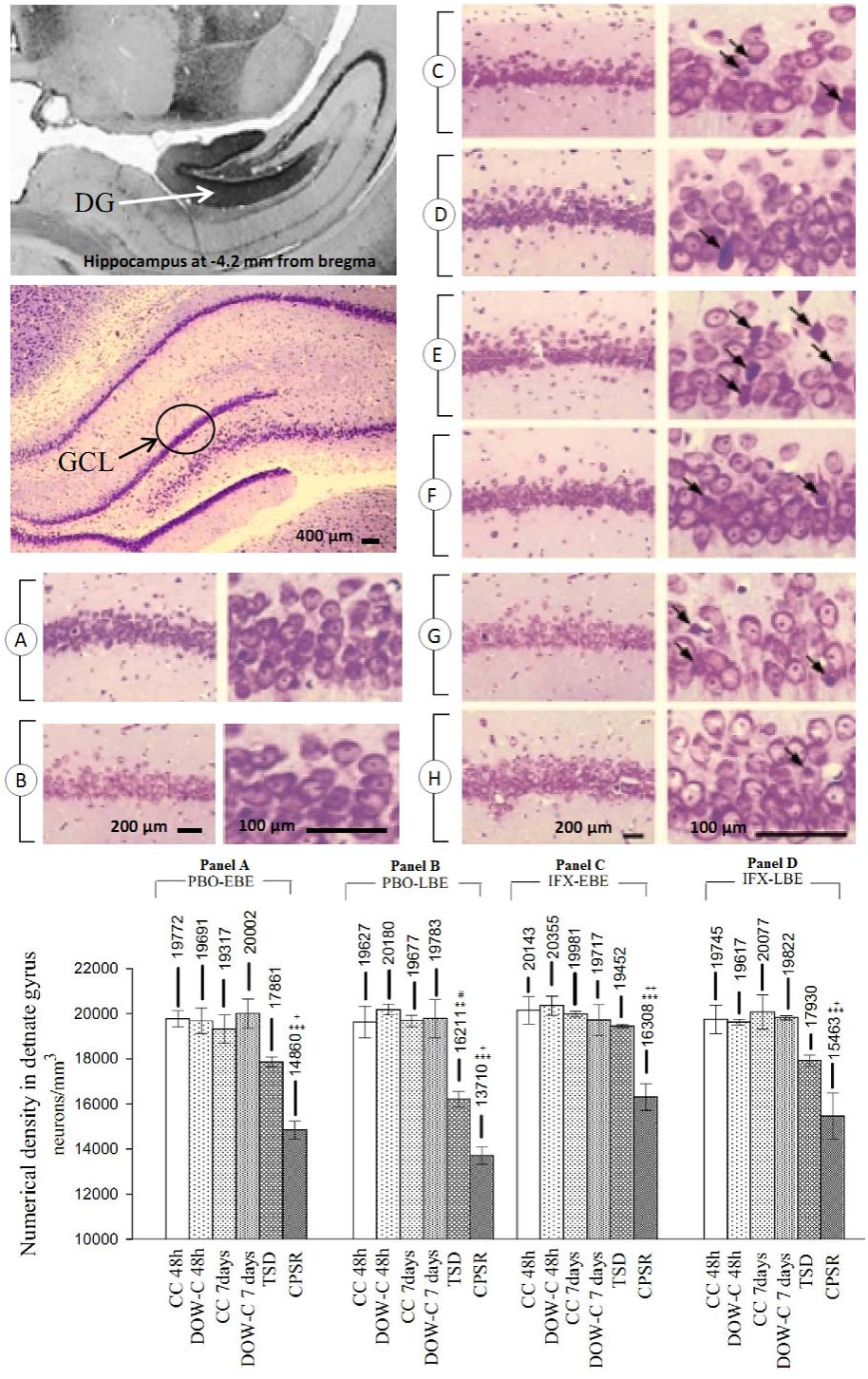

Figure 8: Nissl staining of rats' hippocampal sections at the level of DG. A-H images represent the hippocampal DG sections of the rats assigned to each arm (control vs. sleep-deprived) which had been either PBO- or IFX-treated with early (immediately after EPM retest) or late (after 14 days) brain excision. (A) cage control for 48 h or 7 days on either BPO or IFX; (B) DOW-control for 48 h or 7 days on either PBO or IFX; (C) TSD on PBO with late brain excision (LBE); (D) TSD on IFX with LBE; (E) CPSR on PBO with LBE; (F) CPSR on IFX with LBE; (G) CPSR on PBO with early brain excision (EBE); (H) CPSR on IFX with EBE. The graph beneath, represents the mean ± S.E.M for the numerical density (i.e. the number of neurons/mm^3) calculated as described in methods section. **P<0.01 and ***P<0.00, different from the corresponding control arms in the same panel. For the TSD groups, the corresponding control arms are CC 48 h and DOW-C 48 h while for CPSR, the corresponding controls are CC-7 days and DOW-C 7 days. Panels A-D, +P<0.05 and ++P<0.01, different from TSD arm in each panel. Panel B, #P<0.05 different from the corresponding arm (TSD) in panel C. As shown in the upper figure, PBO-CPSR-LBE group (E) was the most vulnerable group with 6±2 dark neurons per a high power field (HPF) (arrows point at presumably apoptotic neurons) and wider inter-cellular spaces. Likewise, as per numerical density data for DG neuron/mm^3, PBO-CPSR-LBE rats demonstrated the least value (13710 ± 391 neurons/mm^3), however not significantly different from the CPSR arms (i.e. panels A, C and D). Interestingly, both in TSD and CPSR arms, IFX injection resulted in prevention of numerical density decline in DG. DG: dentate gyrus, GCL: granular cell layer, EBE: early brain excision, LBE: late brain excision, TSD: total sleep deprivation, CPSR: chronic partial sleep restriction, PBO: placebo, IFX: infliximab, CC: cage control, DOW-C: disk-over-water apparatus control. PBO: placebo, IFX: infliximab

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DISCUSSION

This study which evaluated the consequences of sleep loss (TSD and CPSR) on EPM-associated memory and anxiety-related behaviors in a rat model, also assessed the serum neurochemical changes and hippocampal DG histopathological outcomes, in the presence or absence of i.p. IFX. We showed that PBO-treated CPSR rats had a more drastically impaired aversive memory function as well as more pronounced anxiolytic-like behaviors. Moreover, in the absence of Aβ deposition in the hippocampal DG of all examined rats, we observed a positive IHC reaction for HPτ in the DG of PBO-treated CPSR rats with late brain excision. Our results also showed that IFX abrogates this reaction. Likewise, the above group in which brains were removed 14 days after the EPM retest session, showed the least number of DG neurons/mm^3 as well as the most number of dark neurons. In addition, sleep deprived animals especially in CPSR groups, showed to have a significant decline in their serum BDNF and a surge in their corticosterone levels.

The use of elevated plus-maze (EPM) test-retest paradigm here, was an attempt to concomitantly assess the effects of such interventions on anxiety, learning and memory (Asth et al., 2012). In terms of behavioral outcome of SD, in agreement with our findings, the majority of experimental studies have shown memory impairment and they report no effect or even indicate decreased anxiety in sleep-deprived rodents (Hicks and Moore, 1979; Moore et al., 1979; Suchecki et al., 2002; Martinez-Gonzalez et al., 2004; Tartar et al., 2009). However, there seem to be some inconsistencies in the literature, which may be the result of a complex interaction between the duration and the method of sleep deprivation employed, the anxiety test involved, the differences in species and strains, and perhaps other variables and contributing factors. Apparently, explaining the relationship between restricted or disrupted sleep and different types or stages of memory function and anxiety is not straight forward thus needs further investigations to become clearer.

Many reports have suggested that sleep loss can decrease neurogenesis level by means of the changes occurred in stress systems. According to Mirescu et al. (2006), SD in rats hampers neurogenesis through a surge in the main stress hormone, corticosterone (Mirescu and Gould, 2006; Mirescu et al., 2006). Conversely, other studies have demonstrated that the neural damage due to SD is independent of corticosterone levels (Guzman-Marin et al., 2007; Mueller et al., 2008).

The adverse events seen following SD involve not only with changes in neurotransmitter systems and neuroinflammatory processes but also the cell signals regulating the neurogenesis and neurotrophic factors such as BDNF (Meerlo et al., 2009; Lucassen et al., 2010; Pathania et al., 2010).

Earlier findings have reported that, the activity of the antioxidant enzyme superoxide dismutase (SOD) in the brainstem and hippocampus can be decreased by TSD and CPSR (Alzoubi et al., 2012). The multifunctional proinflammatory cytokines such as TNFα and interleukin-1 beta (IL-1β), have not been only described as crucial inflammatory mediators, but also as factors crucial to the regulation of sleep mechanisms (Krueger, 2008). Based on this, it is hypothesized that these conditions which activate the reactive oxygen species, can trigger a cascade of events which facilitate the release of proinflammatory factors such as TNFα and interleukins (IL1 and 6) in different brain areas including the hippocampal region. These are shown to attenuate the secretion of BDNF which is a neuroprotective factor (Nagahara et al., 2009; Esumi et al., 2011; Alzoubi et al., 2012). The hippocampal BDNF is shown to critically contribute to synaptic plasticity, long-term potentiation (LTP) and hence the memory function (Laske et al., 2007; Nagahara et al., 2009; Alhaider et al., 2011; Esumi et al., 2011; Havekes et al., 2012). According to the established positive corre-

lation between the circulating BDNF level and its secretion in various brain regions (Angelucci et al., 2011), we measured the serum BDNF. Even if the cause for this positive correlation is not clearly known, our data may partly elucidate the contribution of the peripheral and central BDNF changes in possible brain insults following sleep deprivation. There are reports indicating that, after sleep deprivation, BDNF secretion and therefore its concentration is diminished in areas of the brain including brain stem, hippocampus and cerebellum (Sei et al., 2000; Alhaider et al., 2011). This suggests that sleep plays a role in secretion of BDNF. Due to the BDNF contribution to learning and memory processes, sleep deprivation is perceived to negatively affect the memory function. This is in line with what we found in our behavioral and neurobiochemical approach, as our results showed decreased BDNF post TSD and CPSR as compared to control conditions. Moreover, the SD-induced diminished level of BDNF was not seen in IFX-treated animals, suggesting the possible role of TNFα in the neuroinflammatory insults following SD. Nevertheless, the relationship between BDNF and SD-induced memory impairment needs to be more deeply addressed in further studies.

On the other hand, our results indicated an increased level of corticosterone in sleep deprived animals. Similar to our findings, some reports have indicated that the HPA axis activity occurs in sleep deprived animals although the level of such activation can in part result from the sleep deprivation method employed (Suchecki et al., 1998; Meerlo et al., 2002; Hipolide et al., 2005).

The mechanism(s) mediating these effects of sleep restriction on HPA axis regulation are yet to be fully explained and perhaps involve the HPA axis itself, as well as its regulatory systems such as serotonergic inputs (Prevot et al., 1996; Gardner et al., 1997; Lopez-Rodriguez et al., 2003; Hipolide et al., 2005; Roman et al., 2005a, b; Meerlo et al., 2008).

One of the brain regions which appears to be particularly sensitive and vulnerable to sleep loss is the hippocampus (Graves et al., 2003; McDermott et al., 2003; Ruskin et al., 2004). The hippocampus plays an important role in cognition and emotion regulation (Bannerman et al., 2004; Bast, 2007) and is one of the few brain regions that displays consistency in neurogenesis from adolescence into adulthood (Ming and Song, 2005). The diminished hippocampal neurogenesis and its reduced volume have been implicated in the etiology and symptomatology of depressive and emotional disorders (Sapolsky, 2000; Czeh and Lucassen, 2007; Perera et al., 2008; Boldrini et al., 2009; Lucassen et al., 2010).

The question raised was whether sleep loss in long run may at least partly contribute to the neurodegenerative disorders such as Alzheimer's Disease (AD) or hasten the process? To test this, we applied the IHC staining method for Aβ and HPτ in the hippocampal DG. In presence of an apparent staining reaction for HPτ in PBO-treated CPSR rats with late brain excision compared to other PBO- or IFX-treated animals, no Aβ deposition was noted across groups.

One of the crucial pathways which might involve in neurodegeneration in sleep-restricted rats is the TNFα receptor system. Again, it remains to be established how TNFα turnover and receptor expression are affected following SD over a short or extended periods of time (D'Almeida et al., 1997; Gopalakrishnan et al., 2004; Lucassen et al., 2010). Some studies have shown that TNFα increases the oxidative stress measures following SD (D'Almeida et al., 1998; Ramanathan et al., 2002). In support of the latter notion, we showed that IFX (anti-TNFα MAb) at least partially prevents the behavioral, neurochemical and even histopathological consequences of SD.

The negative effects of SD in rodents have been demonstrated with different methods of sleep deprivation (Guzman-Marin et al., 2003, 2005, 2008; Mueller et al., 2008; Junek et al., 2010; Garcia-Garcia et al., 2011). Sleep disturbance for periods

shorter than 24 hours do not appear to affect cell proliferation rates in GCL of the DG in rats' hippocampus. Only few sleep deprivation studies showed the viability of all new cells. Results from one study indicated that sleep loss reduces the survival of newly generated cells (Hairston et al., 2005). Our findings were in line with the latter one, showing that chronic partial sleep restriction leads to the neuronal cell death in GCL of the DG. However, in contrast to our results, some investigations have reported no adverse effect of SD on cell survival in this region (Roman et al., 2005a, b; Garcia-Garcia et al., 2011).

Hippocampal cell death has similarly been reported in human sleep disorders such as primary insomnia (Riemann et al., 2007) and sleep apnea (Morrell et al., 2003). Among these lines, some researchers have observed reductions in the hippocampal volume in psychopathologies such as major depression and subsequently reported these findings to be implicated in specific symptoms of such disorders (Sapolsky, 2000; Czeh and Lucassen, 2007; Perera et al., 2008; Boldrini et al., 2009; Lucassen et al., 2010).

Evidence suggesting a link between sleep loss and neurodegeneration comes from the diminished volume of the hippocampus and some other brain regions both in sleep-restricted humans and experimental animals (Sapolsky, 2000; Perera et al., 2008; Boldrini et al., 2009; Altena et al., 2010; Lucassen et al., 2010; Neylan et al., 2010; Torelli et al., 2011; Bora et al., 2012). Studies which have demonstrated a reduced hippocampal volume following SD have brought about a variety of explanations such as neuronal cell death, shrinkage, diminished arborization in dendrites, slowed-down/reduced neurogenesis, or decreases in the number of glial cells (Czeh and Lucassen, 2007).

The present findings which indicate cell loss in GCL of the hippocampal DG of rats, suggest that the hippocampal volume reduction observed in human sleep disorders and mood disorders may possibly be a consequence of disrupted sleep rather than a non-specific side-effect. This questionable notion however needs to be investigated to be proven correct.

With regard to our pharmacological intervention, rats which received i.p. IFX, 15 min before being submitted to the SD procedure, not only showed less memory impairment compared to controls (PBO-treated), but also comparatively preserved cell count in the GCL of their DG. This may further suggest the determinant role of TNFα in the possible neurodegenerative processes triggered by sleep loss.

The effects of SD on BDNF in the present study, supports the hypothesis that the reduction in neurogenesis and the corresponding cell loss may have resulted from a reduction in such trophic factors or a possible dysregulation in their signaling.

One way or another, sleep deprivation might constitute a threat to neuronal integrity which however may not be sufficient to cause neurodegeneration but to activate neuroinflammatory mechanisms (Hsu et al., 2003; Moldovan et al., 2010).

In conclusion, the present study suggests that the chronic SD results in a damage caused by a neuroinflammatory insult. Other than the possible role of TNFα, further mechanism(s) of this effect remain(s) to be established. Our findings support the hypothesis that insufficient sleep in long run sensitizes the brain to neurodegenerative insults. This is possibly via activation of the inflammatory mechanisms in part through TNFα-associated pathways.

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REFERENCES

Abusaad I, MacKay D, Zhao J, Stanford P, Collier DA, Everall IP. Stereological estimation of the total number of neurons in the murine hippocampus using the optical disector. J Comp Neurol 1999;408:560-6.

Alhaider IA, Aleisa AM, Tran TT, Alkadhi KA. Sleep deprivation prevents stimulation-induced increases of levels of P-CREB and BDNF: protection by caffeine. Mol Cell Neurosci 2011;46:742-51.

Altena E, Vrenken H, Van Der Werf YD, van den Heuvel OA, Van Someren EJ. Reduced orbitofrontal and parietal gray matter in chronic insomnia: a voxel-based morphometric study. Biol Psychiatry 2010; 67:182-5.

Alzoubi KH, Khabour OF, Rashid BA, Damaj IM, Salah HA. The neuroprotective effect of vitamin E on chronic sleep deprivation-induced memory impairment: the role of oxidative stress. Behav Brain Res 2012;226:205-10.

Angelucci F, Gelfo F, De Bartolo P, Caltagirone C, Petrosini L. BDNF concentrations are decreased in serum and parietal cortex in immunotoxin 192 IgG-Saporin rat model of cholinergic degeneration. Neurochem Int 2011;59:1-4.

Ankarcrona M, Dypbukt JM, Bonfoco E, Zhivotovsky B, Orrenius S, Lipton SA et al. Glutamate-induced neuronal death: a succession of necrosis or apoptosis depending on mitochondrial function. Neuron 1995;15:961-73.

Asth L, Lobao-Soares B, Andre E, Soares Vde P, Gavioli EC. The elevated T-maze task as an animal model to simultaneously investigate the effects of drugs on long-term memory and anxiety in mice. Brain Res Bull 2012;87:526-33.

Banks S, Dinges DF. Behavioral and physiological consequences of sleep restriction. J Clin Sleep Med 2007;3:519-28.

Bannerman DM, Rawlins JN, McHugh SB, Deacon RM, Yee BK, Bast T et al. Regional dissociations within the hippocampus - memory and anxiety. Neurosci Biobehav Rev 2004;28:273-83.

Bast T. Toward an integrative perspective on hippocampal function: from the rapid encoding of experience to adaptive behavior. Rev Neurosci 2007;18:253-81.

Benington I. Valedictory address from Ian Benington OBE of the British Dental Association. Br Dent J 2003;194:527-8.

Boldrini M, Underwood MD, Hen R, Rosoklija GB, Dwork AJ, John Mann J et al. Antidepressants increase neural progenitor cells in the human hippocampus. Neuropsychopharmacology 2009;34:2376-89.

Bonnet MH, Arand DL. We are chronically sleep deprived. Sleep 1995;18:908-11.

Bora E, Fornito A, Pantelis C, Yucel M. Gray matter abnormalities in Major Depressive Disorder: a meta-analysis of voxel based morphometry studies. J Affect Disord 2012;138:9-18.

Buysse DJ, Angst J, Gamma A, Ajdacic V, Eich D, Rossler W. Prevalence, course, and comorbidity of insomnia and depression in young adults. Sleep 2008;31:473-80.

Chang PP, Ford DE, Mead LA, Cooper-Patrick L, Klag MJ. Insomnia in young men and subsequent depression. The Johns Hopkins Precursors Study. Am J Epidemiol 1997;146:105-14.

Czeh B, Lucassen PJ. What causes the hippocampal volume decrease in depression? Are neurogenesis, glial changes and apoptosis implicated? Eur Arch Psychiatry Clin Neurosci 2007;257:250-60.

D'Almeida V, Hipolide DC, Azzalis LA, Lobo LL, Junqueira VB, Tufik S. Absence of oxidative stress following paradoxical sleep deprivation in rats. Neurosci Lett 1997;235:25-8.

D'Almeida V, Lobo LL, Hipolide DC, de Oliveira AC, Nobrega JN, Tufik S. Sleep deprivation induces brain region-specific decreases in glutathione levels. Neuroreport 1998;9:2853-6.

Eslimi D, Oryan S, Nasehi M, Zarrindast MR. Effects of opioidergic systems upon anxiolytic-like behaviors induced in cholestatic rats. Eur J Pharmacol 2011;670: 180-5.

Esumi LA, Palma BD, Gomes VL, Tufik S, Hipolide DC. Inflammatory markers are associated with inhibitory avoidance memory deficit induced by sleep deprivation in rats. Behav Brain Res 2011;221:7-12.

Everson CA, Bergmann BM, Rechtschaffen A. Sleep deprivation in the rat: III. Total sleep deprivation. Sleep 1989;12:13-21.

Garcia-Garcia F, De la Herran-Arita AK, Juarez-Aguilar E, Regalado-Santiago C, Millan-Aldaco D, Blanco-Centurion C et al. Growth hormone improves hippocampal adult cell survival and counteracts the inhibitory effect of prolonged sleep deprivation on cell proliferation. Brain Res Bull 2011;84:252-7.

Gardner JP, Fornal CA, Jacobs BL. Effects of sleep deprivation on serotonergic neuronal activity in the dorsal raphe nucleus of the freely moving cat. Neuropsychopharmacology 1997;17:72-81.

Germain A, Buysse DJ, Nofzinger E. Sleep-specific mechanisms underlying posttraumatic stress disorder: integrative review and neurobiological hypotheses. Sleep Med Rev 2008;12:185-95.

Gopalakrishnan A, Ji LL, Cirelli C. Sleep deprivation and cellular responses to oxidative stress. Sleep 2004;27:27-35.

Graves LA, Heller EA, Pack AI, Abel T. Sleep deprivation selectively impairs memory consolidation for contextual fear conditioning. Learn Mem 2003;10:168-76.

Gundersen HJ, Bendtsen TF, Korbo L, Marcussen N, Moller A, Nielsen K et al. Some new, simple and efficient stereological methods and their use in pathological research and diagnosis. APMIS 1988;96:379-94.

Gundersen HJ, Jensen EB, Kieu K, Nielsen J. The efficiency of systematic sampling in stereology - reconsidered. J Microsc 1999; 193:199-211.

Guzman-Marin R, Suntsova N, Stewart DR, Gong H, Szymusiak R, McGinty D. Sleep deprivation reduces proliferation of cells in the dentate gyrus of the hippocampus in rats. J Physiol 2003;549:563-71.

Guzman-Marin R, Suntsova N, Methippara M, Greiffenstein R, Szymusiak R, McGinty D. Sleep deprivation suppresses neurogenesis in the adult hippocampus of rats. Eur J Neurosci 2005;22:2111-6.

Guzman-Marin R, Ying Z, Suntsova N, Methippara M, Bashir T, Szymusiak R et al. Suppression of hippocampal plasticity-related gene expression by sleep deprivation in rats. J Physiol 2006;575:807-19.

Guzman-Marin R, Bashir T, Suntsova N, Szymusiak R, McGinty D. Hippocampal neurogenesis is reduced by sleep fragmentation in the adult rat. Neuroscience 2007;148:325-33.

Guzman-Marin R, Suntsova N, Bashir T, Nienhuis R, Szymusiak R, McGinty D. Rapid eye movement sleep deprivation contributes to reduction of neurogenesis in the hippocampal dentate gyrus of the adult rat. Sleep 2008;31:167-75.

Hairston IS, Little MT, Scanlon MD, Barakat MT, Palmer TD, Sapolsky RM, Heller HC. Sleep restriction suppresses neurogenesis induced by hippocampus-dependent learning. J Neurophysiol 2005; 94:4224-33.

Havekes R, Vecsey CG, Abel T. The impact of sleep deprivation on neuronal and glial signaling pathways important for memory and synaptic plasticity. Cell Signal 2012;24:1251-60.

Hicks RA, Moore JD. REM sleep deprivation diminishes fear in rats. Physiol Behav 1979;22:689-92.

Hipolide DC, Moreira KM, Barlow KB, Wilson AA, Nobrega JN, Tufik S. Distinct effects of sleep deprivation on binding to norepinephrine and serotonin transporters in rat brain. Prog Neuropsychopharmacol Biol Psychiatry 2005;29:297-303.

Hsu JC, Lee YS, Chang CN, Ling EA, Lan CT. Sleep deprivation prior to transient global cerebral ischemia attenuates glial reaction in the rat hippocampal formation. Brain Res 2003;984:170-81.

Junek A, Rusak B, Semba K. Short-term sleep deprivation may alter the dynamics of hippocampal cell proliferation in adult rats. Neuroscience 2010;170:1140-52.

Karson A, Demirtas T, Bayramgurler D, Balci F, Utkan T. Chronic administration of infliximab (TNF-alpha Inhibitor) decreases depression and anxiety-like behaviour in rat model of chronic mild stress. Basic Clin Pharmacol Toxicol 2013;112:335-40.

Kim HW, Chang YC, Chen M, Rapoport SI, Rao JS. Chronic NMDA administration to rats increases brain pro-apoptotic factors while decreasing anti-Apoptotic factors and causes cell death. BMC Neurosci 2009;10: 123.

Krueger JM. The role of cytokines in sleep regulation. Curr Pharm Des 2008;14:3408-16.

Kume T, Nishikawa H, Tomioka H, Katsuki H, Akaike A, Kaneko S et al. p75-mediated neuroprotection by NGF against glutamate cytotoxicity in cortical cultures. Brain Res 2000;852:279-89.

Laske C, Stransky E, Leyhe T, Eschweiler GW, Maetzler W, Wittorf A et al. BDNF serum and CSF concentrations in Alzheimer's disease, normal pressure hydrocephalus and healthy controls. J Psychiatr Res 2007;41:387-94.

Li GL, Farooque M, Holtz A, Olsson Y. Changes of beta-amyloid precursor protein after compression trauma to the spinal cord: an experimental study in the rat using immunohistochemistry. J Neurotrauma 1995;12:269-77.

Livingston G, Blizard B, Mann A. Does sleep disturbance predict depression in elderly people? A study in inner London. Br J Gen Pract 1993;43:445-8.

Lopez-Rodriguez F, Wilson CL, Maidment NT, Poland RE, Engel J. Total sleep deprivation increases extracellular serotonin in the rat hippocampus. Neuroscience 2003;121:523-30.

Lucassen PJ, Meerlo P, Naylor AS, van Dam AM, Dayer AG, Fuchs E et al. Regulation of adult neurogenesis by stress, sleep disruption, exercise and inflammation: Implications for depression and antidepressant action. Eur Neuropsychopharmacol 2010;20:1-17.

Malow BA. Sleep deprivation and epilepsy. Epilepsy Curr 2004;4:193-5.

Martinez-Gonzalez D, Obermeyer W, Fahy JL, Riboh M, Kalin NH, Benca RM. REM sleep deprivation induces changes in coping responses that are not reversed by amphetamine. Sleep 2004;27:609-17.

McDermott CM, LaHoste GJ, Chen C, Musto A, Bazan NG, Magee JC. Sleep deprivation causes behavioral, synaptic, and membrane excitability alterations in hippocampal neurons. J Neurosci 2003;23: 9687-95.

Meerlo P, Koehl M, van der Borght K, Turek FW. Sleep restriction alters the hypothalamic-pituitary-adrenal response to stress. J Neuroendocrinol 2002;14:397-402.

Meerlo P, Sgoifo A, Suchecki D. Restricted and disrupted sleep: effects on autonomic function, neuroendocrine stress systems and stress responsivity. Sleep Med Rev 2008; 12:197-210.

Meerlo P, Mistlberger RE, Jacobs BL, Heller HC, McGinty D. New neurons in the adult brain: the role of sleep and consequences of sleep loss. Sleep Med Rev 2009; 13:187-94.

Ming GL, Song H. Adult neurogenesis in the mammalian central nervous system. Annu Rev Neurosci 2005;28:223-50.

Mirescu C, Gould E. Stress and adult neurogenesis. Hippocampus 2006;16:233-8.

Mirescu C, Peters JD, Noiman L, Gould E. Sleep deprivation inhibits adult neurogenesis in the hippocampus by elevating glucocorticoids. Proc Natl Acad Sci U S A 2006;103:19170-5.

Moldovan M, Constantinescu AO, Balseanu A, Oprescu N, Zagrean L, Popa-Wagner A. Sleep deprivation attenuates experimental stroke severity in rats. Exp Neurol 2010;222:135-43.

Moore JD, Hayes C, Hicks RA. REM sleep deprivation increases preference for novelty in rats. Physiol Behav 1979;23:975-6.

Morrell MJ, McRobbie DW, Quest RA, Cummin AR, Ghiassi R, Corfield DR. Changes in brain morphology associated with obstructive sleep apnea. Sleep Med 2003;4:451-4.

Mouton PR, Long JM, Lei DL, Howard V, Jucker M, Calhoun ME et al. Age and gender effects on microglia and astrocyte numbers in brains of mice. Brain Res 2002;956:30-5.

Mueller AD, Pollock MS, Lieblich SE, Epp JR, Galea LA, Mistlberger RE. Sleep deprivation can inhibit adult hippocampal neurogenesis independent of adrenal stress hormones. Am J Physiol Regul Integr Comp Physiol 2008;294:R1693-703.

Nagahara AH, Merrill DA, Coppola G, Tsukada S, Schroeder BE, Shaked GM et al. Neuroprotective effects of brain-derived neurotrophic factor in rodent and primate models of Alzheimer's disease. Nat Med 2009;15:331-7.

Namavar MR, Raminfard S, Jahromi ZV, Azari H. Effects of high-fat diet on the numerical density and number of neuronal cells and the volume of the mouse hypothalamus: a stereological study. Anat Cell Biol 2012;45:178-84.

Neylan TC, Mueller SG, Wang Z, Metzler TJ, Lenoci M, Truran D et al. Insomnia severity is associated with a decreased volume of the CA3/dentate gyrus hippocampal subfield. Biol Psychiatry 2010;68: 494-6.

Papasozomenos SC, Su Y. Altered phosphorylation of tau protein in heat-shocked rats and patients with Alzheimer disease. Proc Natl Acad Sci U S A 1991;88:4543-7.

Pathania M, Yan LD, Bordey A. A symphony of signals conducts early and late stages of adult neurogenesis. Neuropharmacology 2010;58:865-76.

Paxinos G, Watson C. The rat brain in stereotaxic coordinates (6 ed). London: Academic Press, 2007.

Perera TD, Park S, Nemirovskaya Y. Cognitive role of neurogenesis in depression and antidepressant treatment. Neuroscientist 2008;14:326-38.

Pilcher JJ, Lambert BJ, Huffcutt AI. Differential effects of permanent and rotating shifts on self-report sleep length: a meta-analytic review. Sleep 2000;23:155-63.

Prevot E, Maudhuit C, Le Poul E, Hamon M, Adrien J. Sleep deprivation reduces the citalopram-induced inhibition of serotoninergic neuronal firing in the nucleus raphe dorsalis of the rat. J Sleep Res 1996;5:238-45.

Rajaratnam SM, Arendt J. Health in a 24-h society. Lancet 2001;358:999-1005.

Ramanathan L, Gulyani S, Nienhuis R, Siegel JM. Sleep deprivation decreases superoxide dismutase activity in rat hippocampus and brainstem. Neuroreport 2002;13:1387-90.

Rechtschaffen A, Bergmann BM. Sleep deprivation in the rat by the disk-over-water method. Behav Brain Res 1995;69:55-63.

Rechtschaffen A, Bergmann BM, Everson CA, Kushida CA, Gilliland MA. Sleep deprivation in the rat: X. Integration and discussion of the findings. Sleep 1989;12: 68-87.

Riemann D, Voderholzer U, Spiegelhalder K, Hornyak M, Buysse DJ, Nissen C et al. Chronic insomnia and MRI-measured hippocampal volumes: a pilot study. Sleep 2007;30:955-8.

Roane BM, Taylor DJ. Adolescent insomnia as a risk factor for early adult depression and substance abuse. Sleep 2008;31: 1351-6.

Roman V, Van der Borght K, Leemburg SA, Van der Zee EA, Meerlo P. Sleep restriction by forced activity reduces hippocampal cell proliferation. Brain Res 2005a;1065:53-9.

Roman V, Walstra I, Luiten PG, Meerlo P. Too little sleep gradually desensitizes the serotonin 1A receptor system. Sleep 2005b;28:1505-10.

Ruskin DN, Liu C, Dunn KE, Bazan NG, LaHoste GJ. Sleep deprivation impairs hippocampus-mediated contextual learning but not amygdala-mediated cued learning in rats. Eur J Neurosci 2004;19:3121-4.

Sapolsky RM. Glucocorticoids and hippocampal atrophy in neuropsychiatric disorders. Arch Gen Psychiatry 2000;57: 925-35.

Sattler R, Tymianski M. Molecular mechanisms of calcium-dependent excitotoxicity. J Mol Med (Berl) 2000;78:3-13.

Sei H, Saitoh D, Yamamoto K, Morita K, Morita Y. Differential effect of short-term REM sleep deprivation on NGF and BDNF protein levels in the rat brain. Brain Res 2000;877:387-90.

Suchecki D, Lobo LL, Hipolide DC, Tufik S. Increased ACTH and corticosterone secretion induced by different methods of paradoxical sleep deprivation. J Sleep Res 1998;7:276-81.

Suchecki D, Tiba PA, Tufik S. Hormonal and behavioural responses of paradoxical sleep-deprived rats to the elevated plus maze. J Neuroendocrinol 2002;14:549-54.

Tartar JL, Ward CP, Cordeira JW, Legare SL, Blanchette AJ, McCarley RW et al. Experimental sleep fragmentation and sleep deprivation in rats increases exploration in an open field test of anxiety while increasing plasma corticosterone levels. Behav Brain Res 2009;197:450-3.

Tononi G, Cirelli C. Sleep function and synaptic homeostasis. Sleep Med Rev 2006; 10:49-62.

Torelli F, Moscufo N, Garreffa G, Placidi F, Romigi A, Zannino S, Bozzali M, Fasano F, Giulietti G, Djonlagic I, Malhotra A, Marciani MG, Guttmann CR. Cognitive profile and brain morphological changes in obstructive sleep apnea. Neuroimage 2011;54:787-93.

Valera E, Sanchez-Martin FJ, Ferrer-Montiel AV, Messeguer A, Merino JM. NMDA-induced neuroprotection in hippo-campal neurons is mediated through the protein kinase A and CREB (cAMP-response element-binding protein) pathway. Neurochem Int 2008;53:148-54.

Vyazovskiy VV, Cirelli C, Pfister-Genskow M, Faraguna U, Tononi G. Molecular and electrophysiological evidence for net synaptic potentiation in wake and depression in sleep. Nat Neurosci 2008; 11:200-8.

Zarrindast MR, Naghdi-Sedeh N, Nasehi M, Sahraei H, Bahrami F, Asadi F. The effects of dopaminergic drugs in the ventral hippocampus of rats in the nicotine-induced anxiogenic-like response. Neurosci Lett 2010;475:156-60.

Zarrindast MR, Nasehi M, Khansari M, Bananej M. Influence of nitric oxide agents in the rat amygdala on anxiogenic-like effect induced by histamine. Neurosci Lett 2011;489:38-42.