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Sleep deprivation induces spatial memory impairment by altered hippocampus neuroinflammatory responses and glial cells activation in rats

Meetu Wadhwa, Punita Kumari, Garima Chauhan, Koustav Roy, Shahnawaz Alam, Krishna Kishore, Koushik Ray, Usha Panjwani*

Defence Institute of Physiology and Allied Sciences (DIPAS), Defence Research and Development Organization (DRDO), Lucknow Road, Timarpur, Delhi, India.

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ABSTRACT

We aimed to investigate the glial cells activation as a potential mechanism involved in the sleep deprivation (SD) induced cognitive impairment through changes in inflammatory cytokines. We analyzed the spatial memory, inflammatory cytokine levels, and gliosis during SD. SD induced spatial memory impairment, imbalance of inflammatory (increased pro- and decreased anti-) cytokines in both hippocampus and plasma in association with glial cells activation in the hippocampus of sleep-deprived rats were observed. Further analysis of the data presented a correlation between spatial memory impairment and activated microglia induced increased pro-inflammatory cytokines after 48 h of SD.

1. Introduction

Hippocampus is a critical brain region involved in the cognition (Furuya et al., 2013). Glial cells (microglia and astrocytes), located densely in the hippocampus (Belarbi and Rosi, 2013), are the major components of the neuroimmune system (O'Callaghan et al., 2015). Microglial cells in association with astrocytes, are the major source of cytokines in brain, acting as a stimulatory factor for the cytokines release (Hanisch, 2002; Vezzani et al., 2008). Other than glial cells, passage of cytokines across blood brain barrier, is an indirect source of cytokines in brain (Yarlagadda et al., 2009). Glial cells present in the resting stage normally (Belarbi and Rosi, 2013) which participate in defence against harmful stimuli, brain development (Belarbi et al., 2012; Topper et al., 2015) and sleep regulation. However, in the pathological conditions such as cardiovascular disorder, metabolic abnormalities and severe neurodegenerative diseases such as Alzheimer's disease, brain injury, cerebral ischemia, seizures etc., glial cells transform from resting to active stage, elevating the levels of pro-inflammatory cytokine (Bardou et al., 2013; Wisor et al., 2011), induce a neuroinflammatory response in the brain (Belarbi and Rosi, 2013, Wisor et al., 2011) and alter the sleep profile. Additionally, cytokine receptors are also densely located in the hippocampus (Czerniawski et al., 2015). Therefore, the hippocampus is more vulnerable to microglia activation and neuroinflammation (Bardou et al., 2013, Lam et al., 2015). Glial cells activation induces an increased level of pro-inflammatory cytokines in the brain resulting in a decline in cognitive function (Adzovic et al., 2015, Belarbi et al., 2012, Czerniawski et al., 2015, Furuya et al., 2013, Zhu et al., 2012).

Normal sleep is important for neurocognitive (Chennaoui et al., 2015, Dumaine and Ashley, 2015, Ramesh et al., 2012, Zhu et al., 2012) and immune functioning (Dumaine and Ashley, 2015). Whereas, sleep deprivation (SD) is detrimental to cognitive function (Clark and Vissel, 2014; Dumaine and Ashley, 2015; Weil et al., 2009). It also alters the immune functions (Chennaoui et al., 2015, Dumaine and Ashley, 2015) and affects the inflammatory homeostasis (Mullington et al., 2010). The pleiotropic and multifunctional nature of cytokines (Clark and Vissel, 2014), mediate crucial inflammatory processes and are critically involved in the regulation of sleep (Mullington et al., 2010, Ramesh et al., 2012). At the basal level, cytokines mediate the immune response and regulate sleep, contrarily at the pathophysiological level, the altered cytokines profile induces sickness behavior and impairs cognition (Austin et al., 2015, Czerniawski et al., 2015, Dumaine and Ashley, 2015, Moore et al., 2009).

During neuroinflammatory disease conditions, over activity of the

Abbreviations: AC, apparatus control; BSA, bovine serum albumin; CC, cage control; DAB, diaminobenzidine; ELISA, enzyme linked immunosorbent assay; EDTA, ethylenediaminetetraacetic acid; GFAP, glial fibrillary acidic protein; IHC, immunohistochemical; Iba-1, ionized calcium binding adapter molecule I; LPS, lipopolysaccharide; MWM, Morris water maze; NGS, normal goat serum; PFA, paraformaldehyde; PBS, phosphate buffered saline; SD, sleep deprivation; SD, 24 h:sleep deprived for 24 h; SD, 48 h: sleep deprived for 48 h; SD, 72 h:sleep deprived for 72 h

* Corresponding author at: Neurophysiology Division, Defence Institute of Physiology and Allied Sciences (DIPAS), Defence Research and Development Organization (DRDO), Lucknow Road, Timarpur, Delhi 110 054, India.

E-mail address: usha_panjwani@dipas.deldom (U. Panjwani).

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microglia and astrocytes (Bardou et al., 2013, Belarbi and Rosi, 2013, Moore et al., 2009, Xuan et al., 2012) induces increased production of pro-inflammatory cytokines (TNF-α, IL-1β and IL-6) (Belarbi and Rosi, 2013, Xuan et al., 2012), that hamper cognitive function (Ronnback and Hansson, 2004; Saggu et al., 2016) with signs of insufficient sleep (Wisor et al., 2011). However, the role of gliosis mediated changes in inflammatory cascades in SD-induced cognitive impairment remains poorly understood.

The present study investigated the role of glial cells activation mediated elevated pro-inflammatory cytokine levels in SD-induced cognitive impairment in rats. We assessed the spatial memory after SD at three-time points (24 h, 48 h, and 72 h) and measured inflammatory cytokine levels exhibiting significant glial cells followed by an assessment of post-SD alterations in glial morphology, immunoreactivity, and count. Finally, the correlation between microglial activation, pro-inflammatory cytokine levels, and spatial memory, was studied.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats (6–8 weeks old), weighing 220–250 g, were used. The rats were sourced from the in-house animal facility of the institute and kept in pairs in polypropylene cages in controlled conditions [temperature (23 ± 1 °C) and humidity (55 ± 5% RH)] on a 12–12 h light-dark cycle. Food and water were given to rats ad libitum. The experimental procedures were conducted in accordance with the approved institutional animal care procedures and guidelines and National Institute of Health Guide for the Care and Use of Animals and Animal Ethics Committee. All possible efforts were done to minimize animal pain and to reduce the number of experimental animals used. Prior to behavioral testing, rats were handled daily for at least a week by the experimenter to exclude the effect of stress during the experiment.

2.2. Experimental design

Rats were randomly divided into four experimental groups: cage control/apparatus control (CC/AC); sleep deprived for 24 h (SD 24 h); sleep deprived for 48 h (SD 48 h); sleep deprived for 72 h (SD 72 h). Each group contained five rats. Food intake and body weight were noted down daily. Behavioral experiments took place between 8:00 A.M. to 11:00 A.M. All rats were euthanized during the light phase of the diurnal cycle.

Throughout the study, rats were sleep deprived in a time-dependent manner for 24 h, 48 h, and 72 h and evaluated for behavioral, biochemical and histological analyses. In the first part of the study, rats were subjected to a spatial memory test. Where as in the second, rats were euthanized, and brains were quickly removed for cytokine levels determination. Further, in a subsequent study, immunohistochemical (IHC) staining was done to study glial markers such as glial fibrillary acidic protein (GFAP) and ionized calcium binding adapter molecule I (Iba1).

2.3. SD procedure

The SD apparatus used to induce SD in rodents has been previously described (Chauhan et al., 2016; Wadhwa et al., 2015). In brief, rats in the SD group were individually placed in the SD cages and employed SD stimulus such as sound, light, and vibration to deprive the sleep in rats. Rats had free access to food and water during the SD protocol. The control group rats had the same environment and condition as the SD group except for sound, light, and vibration. Food and water were freely accessible to rats.

2.4. Physiological testing

Body weight and food intake of both control and SD group rats were measured regularly at each time point as physiological test markers. A constant amount of food was maintained per animal, and the weight of remaining food pellets was measured every morning. Similarly, the body weight of each animal was taken every morning.

2.5. Behavioral testing

The rats were tested for spatial memory using MWM test apparatus in behavior test lab according to the protocol of Chauhan et al., 2016. The maze consisted of a round tank, (210 cm in diameter, 53 cm in height), that was filled with water maintained at room temperature (23 ± 3 °C). The pool was divided into four quadrants (I, II, III, IV) and the hidden platform was placed in quadrant IV. A moveable hidden circular platform was placed at a fixed location and submerged about 2 cm below the water surface. The test consisted of three phases: habituation, hidden platform training, and a probe test. Rats underwent 5 min of habituation in the water maze without the hidden platform before the starting of the training session. Briefly, a standard place training was conducted for seven days before exposure to SD. Each rat was placed in the water in all the four quadrants in a fixed order. All rats performed four consecutive training trials per day. The maximum trial duration was 60 s. Animals that failed to locate the hidden platform, were manually guided and once reached, they were allowed to remain on the platform for 15 s. Animals were allowed a 60 s inter-trial interval within a given training block. The position of the platform was kept identical throughout the training session. Each rat was then properly dried with a towel and returned to the home cage (25 °C).

Learning was assessed over seven consecutive days that had been found to produce optimal learning in rats. A probe trial was conducted on day 8 (final assessment) with and without the hidden platform. An overhead camera coupled to a computer-assisted tracking system, ANY-Maze (Stoelting, USA) monitored all performances in the following parameters: latency, path length, path efficiency to reach the platform [hidden platform test parameter] and the number of entries, time spent in the target quadrant [no hidden platform test parameter]. After the confirmation of the optimum learning, rats were divided into different groups and underwent the experimental test exposure (both control and SD) for the respective period. After completion of SD, spatial memory was finally assessed in water maze with and without hidden platform by using the same protocol and test parameters as the probe trial. For all days, the experiment was performed at the same time of the day under the same environmental conditions.

2.6. Sample collection and preparation

Rats were euthanized immediately after the behavioral testing and samples were collected for the ELISA and histochemistry. The rats used for the histochemistry were anesthetized with 1.2 g/kg of urethane, after which they were transcardially perfused with 0.01 M phosphate buffered saline (PBS) followed by 4% paraformaldehyde (PFA). The brain was then removed and post-fixed in 4% PFA, cryoprotected in PBS containing graded sucrose solution (10%, 20%, and 30%) and stored at 4 °C until processed. Tissues were placed in 30% sucrose solution immersion until the tissues sank to the bottom. The tissue would then embedded in OCT medium (Jung, Leica Biosystems) for cryosectioning. Coronal sections of 30 μm thickness were obtained using the cryostat (Leica Microsystems, Wetzlar, Germany) and collected in PBS containing 0.02% sodium azide.

For the ELISA, the brain was isolated at 4 °C immediately. The hippocampi were isolated and stored at − 80 °C till the time of analysis to prevent degradation of unstable inflammatory markers. The hippocampal brain tissues were homogenized on ice with the help of Polytron homogeniser with 1 × PBS, pH 7.4, containing protease inhibitor