Brain, Behavior, and Immunity 35 (2014) 182–188

Contents lists available at ScienceDirect

Brain, Behavior, and Immunity

journal homepage: www.elsevier.com/locate/ybrbi

The inhibitory effect of sleep deprivation on cell proliferation in the hippocampus of adult mice is eliminated by corticosterone clamp combined with interleukin-1 receptor 1 knockout

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ARTICLE INFO

Article history:
Received 26 July 2013
Received in revised form 22 September 2013
Accepted 1 October 2013
Available online 8 October 2013

Keywords:
Rapid-eye-movement sleep deprivation
Interleukin-1-β
Cell proliferation
Hippocampus
Corticosterone
BrdU
Ki67

ABSTRACT

Deprivation or fragmentation of sleep for longer than 2 days significantly inhibits cell proliferation and neurogenesis in the hippocampus of adult rats and mice. Signaling pathways that mediate these effects have yet to be clarified. Although deprivation procedures can stimulate adrenal corticosterone (CORT) release, suppression of cell proliferation by sleep deprivation does not require elevated CORT. We examined a role for interleukin-1β (IL-1β), a pro-inflammatory cytokine that is increased by sleep loss and that mediates effects of stress on hippocampal neurogenesis. Wild type (WT) and IL-1 receptor 1 knockout (IL1RI-KO) mice were subjected to rapid-eye-movement sleep deprivation (RSD) for 72-h using the multiple platform-over-water method. Mice were administered BrdU (100 mg/kg) i.p. at hour 70 of RSD and were sacrificed 2-h later. New cells were identified by immunoreactivity (ir) for BrdU and Ki67 in the granular cell layer/subgranular zone (GCL/SGZ) and the hilus. In Experiment 1, WT and IL1RI-KO mice, by contrast with respective control groups, exhibited significantly fewer BrdU-ir and Ki67-ir cells. In Experiment 2, WT and IL1RI-KO mice were adrenalectomized (ADX) and maintained on constant low-dose CORT by osmotic minipumps. RSD reduced cell proliferation by 32% ($p < 0.01$) in ADX-WT animals but did not significantly reduce proliferation in ADX IL1RI-KO animals ($p > 0.1$). These results imply that RSD suppresses cell proliferation by the presence of wake-dependent factors (either elevated CORT or IL-1β signaling are sufficient), rather than the absence of a REM sleep-dependent process. The generality of these findings to other sleep deprivation methods and durations remains to be established.

1. Introduction

In adult rats and mice, disruptions of sleep for periods exceeding 2 days can inhibit cell proliferation and neurogenesis in the hippocampus by 36–70%. Effects of this magnitude are observed following total sleep deprivation (Guzman-Marin et al., 2003; Tung et al., 2005; Garcia-Garcia et al., 2011), rapid-eye movement (REM) sleep deprivation (RSD) (Mirescu et al., 2006; Mueller et al., 2008; Guzman-Marin et al., 2008) and sleep fragmentation (Guzman-Marin et al., 2007). The question arises whether reduced cell proliferation is due to the presence of wake-dependent factors, or the absence of sleep-dependent factors. Potential wake-dependent factors include correlates of non-specific stress, such as elevated adrenal glucorticoid (CORT) release. Plasma CORT is elevated during at least some sleep deprivation procedures (Mirescu et al., 2006; Mueller et al., 2008; Meerlo et al., 2002; Andersen et al., 2005). Many, although not all stress procedures as well as elevated levels of CORT have been shown to inhibit hippocampal cell proliferation (Mirescu and Gould, 2006; Hanson et al., 2011). We previously showed using rats that a 96-h RSD procedure was associated with elevated plasma CORT and reduced hippocampal cell proliferation but that the latter effect was not reduced by adrenalectomy (ADX) when continuous low dose CORT replacement was provided by a subcutaneous osmotic minipump (Mueller et al., 2008). The antineurogenic effect of sleep fragmentation has also been reported to be independent of adrenal stress hormones (Guzman-Marin et al., 2007). These results indicate that suppression of cell proliferation and neurogenesis by sleep disruption does not require elevated CORT. The signaling pathways that do mediate the effect of sleep disruptions remain to be determined.

Recent studies have established an important role for the immune system in the regulation of hippocampal neurogenesis (Ekdahl et al., 2003, 2009; Kempermann and Neumann, 2003; Battista et al., 2006; Kaneko et al., 2006; Bachstetter et al., 2011; Yirmiya and Goshen, 2011; Wu et al., 2012). Of special interest here is evidence that pro-inflammatory cytokines are activated by sleep deprivation (Hu et al., 2003; Everson, 2005; Krueger,

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http://dx.doi.org/10.1016/j.bbi.2013.10.001

2008; Imeri and Opp, 2009; Yehuda et al., 2009), and that IL-1β mediates behavioral and antineurogenic effects of different types of acute and chronic stress in rodents (Goshen et al., 2008; Koo and Duman, 2008; Ben Menachem-Zidon et al., 2008). IL-1β may suppress cell proliferation either directly via adult hippocampal progenitor cells that express the IL-1 receptor IL1R1 and subsequent NFκB signaling (Koo and Duman, 2008), or indirectly by induced CORT secretion (Goshen et al., 2008).

We tested whether the pro-inflammatory cytokine IL-1β mediates the antineurogenic effect of RSD. In Experiment 1, we subjected mice bearing an IL1RI receptor knock out (IL1-RI-KO) to 72-h RSD, using the platform-over-water method. The IL1R1 receptor appears to mediate all cellular responses to IL-1β, and IL1-R1-KO mice therefore fail to respond to IL-1β (Labow et al., 1997). The KO is reportedly associated with a very small reduction of daily NREM sleep (about 4%), but no effect on REM sleep (Fang et al., 1998). In Experiment 2, we eliminated both IL-1β signaling and elevated CORT, by adrenalectomizing IL1-R1-KO mice. Cell proliferation was quantified by immunolabeling exogenous 5-bromo-2-deoxyuridine (BrdU), which labels the *S* phase of the cell cycle, and the endogenous proliferation marker Ki67, which labels G_0 and early G_1. The results indicate that either IL-1β signaling or elevated CORT are sufficient for RSD to inhibit hippocampal cell proliferation.

2. Methods

2.1. Subjects

All experiments and procedures were approved by the Simon Fraser University Animal Care Committee. Male 7 to 8-week old homozygous IL1RI null mice (IL1RIKO), bearing a C57BL/6 background, were obtained from the Jackson Laboratory (B6.129S7-IL1R1tm1Imx/J). Age-matched C57/BL6J mice were used as wild type (WT) controls. Animals were housed in groups of 4 in standard plastic cages with bedding and were entrained to a 12:12 h light:dark (LD) cycle for 4 weeks. Room temperature was kept constant at 21.5 ± 1 °C, unless otherwise specified. During the last week the mice were handled daily and weighed during the first or last hour of the light period.

2.2. RSD procedure and perfusion

For Experiment 1, unoperated IL1RIKO and WT mice were sleep deprived in stable social groups of 4, using multiple-platform-over-water cages (24 × 45 × 15 cm) equipped with 12 platforms (3.3 cm in diameter) equally distributed across the area. This enabled the animals to move freely across platforms. The cages were filled with water (room temperature) to a depth of 3.5 cm, and the tops of the platforms were 1 cm above water level. The platform-over-water method suppresses REM sleep because the loss of muscle tone that occurs during this sleep stage is incompatible with staying on the platform (Mueller et al., 2008). Platform size and spacing were based on previous reports that provided EEG validation of RSD in mice by this method (Zager et al., 2009). Two RSD cages were equipped with video cameras and recordings were made throughout the procedure to verify that mice contacted the water.

A graphical representation of the experimental protocol is provided in Fig. 1A. Two hours after lights-on, half of the IL1RIKO and WT animals (8/group) started 72 h RSD (IL1RIKO-RSD and WT-RSD), staying with their social littermates. Home cage control (CC) animals were housed in parallel in cages of the same design but without the water (IL1RIKO-CC and WT-CC). Each day at lights-on, the mice were weighed, and water in the RSD cages was replaced. At lights-on 70-h after the start of RSD, all animals received a single i.p. injection of BrdU (100 mg/kg a 10 mg/ml, ZT0). Two hours later a blood sample was rapidly taken from the saphenous vein, animals were perfused and brains were harvested for BrdU and Ki67 immunolabeling.

For Experiment 2, the 72-h RSD procedure was performed as described above on adrenalectomized IL1RIKO and ADX WT mice with the following modifications. To prevent ADX mice from mutual grooming of sutures, all animals were single-housed in standard mouse cages (20 × 35 × 14). For RSD, the cages were equipped with 6 platforms. Previous studies have established that RSD suppresses hippocampal cell proliferation equivalently in single-housed and group housed animals (Mirescu et al., 2006; Mueller et al., 2008). RSD and control animals stayed in separate, but identical rooms. To minimize weight loss or hypothermia during RSD, the mice received breeding chow and the room temperature was raised to 25 °C. At lights-on, after 70-h of RSD, the mice received a BrdU injection. Two hours later, a blood sample was taken from the jugular vein, and the mice were perfused for BrdU and Ki67 immunolabeling.

2.3. ADX surgery

For surgical anesthesia, mice in Experiment 2 were administered the narcotic analgesic Butorphanol (1 mg/kg, SQ), followed by Ketamine (100 mg/kg, IP) and Xylazine (10 mg/kg, IP). When necessary, narcotic depth was maintained with isoflurane (0.5–2%). At the height of the kidneys, two small muscle incisions were placed on either side of the spinal cord, and the adrenal glands were exposed and removed by a lateral approach. Subcutaneous osmotic minipumps (model 1002, Alzet) that provided a constant low-dose of CORT were implanted. CORT (>92%, Sigma–Aldrich) was dissolved in polyethylene glycol 400 containing 5% ethanol to achieve a concentration that provides 35 μg/kg/h. Incisions were closed with absorbable sutures and Antisedan (1 mg/kg, SQ) was used to reverse the anesthesia. The mice received post-operative analgesic and anti-inflammatory treatment (Buprenorphine 0.1 mg/kg, Metacam 1 mg/kg, SQ) for 2 and 3 days, respectively. Additional Metacam was only given on indication. To maintain salt balance, ADX animals received 0.9% saline in the water bottles. One day after surgery, the mice were returned to the home cage condition for a week of recovery prior to RSD. WT and KO mice were assigned randomly to 72-h RSD or cage control (CC) conditions (ADX-WT-CC, ADX-WT-RSD, ADX-IL1R1KO-CC, ADX-IL1IKO-RSD).

2.4. CORT assay

Blood samples were taken within 3 min, placed on ice immediately after collection and stored overnight at 4 °C. The serum was extracted the following day and stored at −20 °C until analysis. CORT concentrations were assayed using a radio-immunoassay kit (MP Biomedicals, Orangeburg, NY, USA). The minimal detectable concentration of CORT was 0.7 μg/dl, and intra- and inter-assay coefficients of variation were less then 7.1% and 6.5%, respectively.

2.5. Immunolabeling

Brains were sectioned by cryostat in the coronal plane at 40 μm (6 series) through the entire rostro-caudal extent of the hippocampus. Brain sections were stored in 0.1 M PB (pH 7.4) at 4 °C for immediate use. A portion of the series was transferred to cryoprotectant solution (30% ethylene glycerol and 20% glycerol in 0.1 M PB buffer, pH 7.4) and maintained at −20 °C for long-term storage.

For visualization of BrdU in mice, a mouse-on-mouse kit (M.O.M immunodetection kit, Vector) was used according to the following immunostaining protocol: brain slices were incubated in 0.6% H_2O_2