Neurobiology of Sleep and Circadian Rhythms xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Neurobiology of Sleep and Circadian Rhythms

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

Research paper

# Acute sleep disruption- and high-fat diet-induced hypothalamic inflammation are not related to glucose tolerance in mice

Jacqueline M. Ho[a], Nicole H. Ducich[a], Nhat-Quynh K. Nguyen[a], Mark R. Opp[a,b,*]

[a] Department of Anesthesiology & Pain Medicine, University of Washington, Seattle, WA, USA
[b] Program in Neurobiology and Behavior, University of Washington, Seattle, Washington, USA

ARTICLE INFO

*Keywords:*
Astrocytes
Microglia
Cytokines
Glucose metabolism
Hypothalamus

ABSTRACT

Chronic insufficient sleep is a major societal problem and is associated with increased risk of metabolic disease. Hypothalamic inflammation contributes to hyperphagia and weight gain in diet-induced obesity, but insufficient sleep-induced neuroinflammation has yet to be examined in relation to metabolic function. We therefore fragmented sleep of adult male C57BL/6 J mice for 18 h daily for 9 days to determine whether sleep disruption elicits inflammatory responses in brain regions that regulate energy balance and whether this relates to glycemic control. To additionally test the hypothesis that exposure to multiple inflammatory factors exacerbates metabolic outcomes, responses were compared in mice exposed to sleep fragmentation (SF), high-fat diet (HFD), both SF and HFD, or control conditions. Three or 9 days of high-fat feeding reduced glucose tolerance but SF alone did not. Transient loss of body mass in SF mice may have affected outcomes. Comparisons of pro-inflammatory cytokine concentrations among central and peripheral metabolic tissues indicate that patterns of liver interleukin-1β concentrations best reflects observed changes in glucose tolerance. However, we demonstrate that SF rapidly and potently increases Iba1 immunoreactivity (-ir), a marker of microglia. After 9 days of manipulations, Iba1-ir remains elevated only in mice exposed to both SF and HFD, indicating a novel interaction between sleep and diet on microglial activation that warrants further investigation.

## 1. Introduction

The number of adults with diabetes worldwide has more than doubled in recent decades (Danaei et al., 2011) while the age of diagnosis has decreased (Carstensen et al., 2008; Holden et al., 2013; Koopman et al., 2005; Kitagawa et al., 1994; Dabelea et al., 2014). Concurrent declines in societal sleep health likely contribute to these trends (Liu et al., 2016; Basch et al., 2014; Matricciani et al., 2012; but see Youngstedt et al., 2016). Sleep disturbances such as fragmented, nonrestorative sleep are more frequently observed in individuals with type 2 diabetes than in those without (Sokwalla et al., 2017; Knutson et al., 2006; Trento et al., 2008), and glycemic control in these patients relates directly to their sleep quality (Knutson et al., 2006; Knutson et al., 2011; Tsai et al., 2012; Tang et al., 2014). Additionally, poor sleep may exacerbate metabolic outcomes when combined with other risk factors. For example, sleep fragmentation and obesity are common characteristics of obstructive sleep apnea. Patients with obstructive sleep apnea are at increased risk of type 2 diabetes (Tasali et al., 2008), whereas those that achieve weight loss through diet and exercise demonstrate improved apnea-hypopnea index and diabetes control (Foster et al., 2009).

Although associations between chronic insufficient sleep and type 2 diabetes are well established (Leng et al., 2016; Kowall et al., 2016; Anothaisintawee et al., 2015), the mechanisms underlying these relationships are less clear. Chronic, systemic inflammation is a well-recognized mechanism in diabetes and obesity pathophysiology (Donath and Shoelson, 2011; Gregor and Hotamisligil, 2011), and many of the same inflammatory pathways activated during metabolic stress are stimulated by sleep loss. For example, macrophage infiltration and release of the pro-inflammatory cytokines interleukin (IL)-1β, IL-6, and tumor necrosis factor-α (TNF-α) contribute to impaired islet beta-cell function, insulin action, and glucose homeostasis in pathogenic conditions such as hyperglycemia and obesity (Maedler et al., 2002; Larsen et al., 2007; Stanley et al., 2011; Weisberg et al., 2003; Ehses et al., 2007). Sleep fragmentation-induced insulin resistance is likewise associated with increased numbers of M1 macrophages, circulating concentrations of IL-6 (Poroyko et al., 2016), and IL-6 and TNF-α mRNA expression in visceral adipose tissue of mice (Zhang et al., 2014). A recent study that found systemic inflammation partially mediates the significant relationship between insufficient sleep and insulin resistance in women (Kim et al., 2016).

* Corresponding author. Present addrss: Department of Integrative Physiology, University of Colorado, UCB 354, 2860 Wilderness Place, 201K, Boulder, CO 80301, USA.
*E-mail address:* Mark.Opp@Colorado.edu (M.R. Opp).

http://dx.doi.org/10.1016/j.nbscr.2017.09.003
Received 27 June 2017; Received in revised form 18 September 2017; Accepted 19 September 2017

Metabolic deficits in diet-induced obesity are also associated with increased inflammatory markers in the hypothalamus (De Souza et al., 2005; Thaler et al., 2012; Milanski et al., 2009; Kleinridders et al., 2009; Zhang et al., 2008; Berkseth et al., 2014; Posey et al., 2009; Andre et al., 2017), a brain region critical to energy homeostasis (Morton et al., 2006). Chronic consumption of saturated fatty acids leads to hypothalamic insulin and leptin resistance, impaired anorectic signaling, and subsequent weight gain in rodents and are associated with increased local IL-1β, IL-6, and TNF-α expression and endoplasmic reticulum stress (De Souza et al., 2005; Milanski et al., 2009). Acute (i.e., 1 day) sleep disruption in rodents also increases cytokine gene expression in the hypothalamus (Dumaine and Ashley, 2015) and basal forebrain in general (Zielinski et al., 2014), but how these findings translate to protein levels and metabolic function remains to be determined.

To address these questions, we used a mouse model of sleep fragmentation to determine whether insufficient sleep elicits neuroinflammation in metabolically relevant brain regions and how this relates to metabolic function. We compared inflammatory responses between central and peripheral tissues, and additionally tested the hypothesis that exposure to multiple inflammatory factors exacerbates metabolic outcomes. Sleep and diet of mice were therefore manipulated for 3 or 9 days to determine individual and combined effects of sleep fragmentation and high-fat feeding on inflammation, energy balance, and glucose metabolism.

## 2. Materials and methods

### 2.1. Animals and housing

Male C57BL/6 J mice (age 6–8 weeks upon arrival) were obtained from The Jackson Laboratory (Bar Harbor, ME). Mice were acclimated to handling and individual housing in standard shoebox cages in controlled conditions of a 12:12 h light:dark cycle at an ambient temperature of 28 ± 1 ˚C. Mice were then habituated to housing in sleep disruption devices for 3 days under the same light and temperature conditions. Ad libitum rodent chow (PicoLab Rodent Diet 20; 13.1% kcal from fat; Labdiet, St. Louis, MO) and water were provided throughout habituation. During the experimental period, mice were maintained on chow or a high-fat diet (HFD; 60% kcal from fat, D12492, Research Diets, New Brunswick, NJ) with ad libitum access to food and water except where noted. All procedures involving the use of animals were approved by the University of Washington Institutional Animal Care and Use Committee.

### 2.2. Sleep fragmentation

Sleep of mice was manipulated using a novel sleep disruption device as previously described (Ho et al., 2016; Ringgold et al., 2013; Sutton and Opp, 2014). Sleep disruption devices consisted of a Plexiglas® cylindrical chamber divided into two separate compartments with each compartment housing one mouse. The floor of the chamber is a disc that is programmed to rotate for 8 s during each 30-s interval. Parameters of disc rotation are such that 1) the direction of rotation is randomized and 2) placement of the 8-s rotation within each 30-s period is varied. Mice were subjected to sleep fragmentation (SF) for 18 h per every 24-h period. Under this protocol, mice were allowed 6 h of uninterrupted sleep opportunity at the start of the light cycle in which they could freely behave, including sleep, when the disc was not rotating.

### 2.3. Experimental design

Prior to habituation in sleep disruption devices, mice were assigned to one of four experimental groups: 1) undisturbed sleep with ad libitum chow diet (Rested Chow); 2) undisturbed sleep with ad libitum HFD (Rested HFD); 3) 18-h SF with ad libitum chow diet (SF Chow); 4) 18-h SF with ad libitum HFD (SF HFD). To control for non-specific effects of housing conditions, all mice were housed in sleep disruption devices throughout the study regardless of group assignment. Mice of the same experimental group were paired together in the same sleep disruption device. After 3 days of habituation in sleep disruption devices, mice were subjected to 3 or 9 days of sleep and diet manipulation. Daily measures of body mass, food intake, and water consumption were recorded throughout habituation and manipulation periods of the study. Food spillage was taken into account by subtracting the weight of food spilled from the difference in food hopper weight between days. Due to disc rotation and shared occupancy of sleep disruption devices, food spillage could not be accurately ascribed to individual mice during periods of SF. Food intakes of SF mice were therefore calculated as a mean value among pairs of mice housed within the same device during the period of manipulation. Although this method limits accuracy of individual food intakes, mice paired together in a given device were subjected to the same experimental manipulations. Our measures thus reflect overall response patterns to sleep and diet manipulations.

### 2.4. Glucose tolerance tests

Glucose tolerance tests (GTTs) were performed after 3 (n = 12/group) and 9 (n = 6/group) days of manipulation. To habituate mice to testing procedures, mice were handled daily prior to the start of manipulations and were given an intraperitoneal (i.p.) injection of 0.9% saline on 3 separate days prior to testing. Mice were fasted at the start of the light cycle for 6 h on test days. Fasting blood glucose was measured with a glucometer (AlphaTRAK2, Abbott Laboratories, North Chicago, IL) via tail vein blood sampling. Mice were then given a bolus i.p. injection of glucose (2 g/kg, Sigma-Aldrich, St. Louis, MO) and blood glucose was sampled at 15, 30, 60, 90, and 120 min post-injection. Food was returned at the end of testing.

### 2.5. Determination of cytokine and hormone concentrations

#### 2.5.1. Blood and tissue collection

Plasma and fresh tissues were collected 2 h into the light cycle following days 3 (n = 8/group) or 9 (n = 7–8/group) of manipulation (i.e., 2 h after the last bout of SF). Mice were deeply anesthetized with isoflurane (Henry Schein, Dublin, OH) and a terminal blood sample was taken via cardiac puncture. Blood samples were collected in EDTA-coated tubes and kept on ice until centrifugation at 17910 g for 20 min at 4 ˚C to collect plasma. Immediately following blood sampling, brains were dissected on ice and brain and peripheral tissues samples were flash-frozen in liquid nitrogen. All samples were stored at -80˚C until further processing.

#### 2.5.2. Cytokine assays

Multiplex bead sets were produced in-house using Luminex (Austin, TX) MagPlex microspheres (regions 34, 38, and 65 for IL-1β, IL-6, and TNF-α, respectively). Beads were conjugated to capture antibodies from R & D Systems (Minneapolis, MN) DuoSets (DY401, DY406, and DY410 for IL-1β, IL-6, and TNF-α, respectively) as per manufacturer's instructions with the exception of overnight incubations. These were performed using the kit's wash buffer, followed by removal in wash buffer for long-term storage at 4 ˚C in Stabiliguard (product code SG01, SurModics, Eden Prairie, MN).

All reagents and samples were allowed to warm to room temperature prior to use. To generate a standard series, lyophilized recombinant protein standards from R & D Systems (401-ML, 406-ML, and 410-ML for IL-1β, IL-6, and TNF-α, respectively) were reconstituted as per manufacturer's instructions. Diluents were generated with cell lysis buffer (BioRad, Hercules, CA, catalog #171–304012). Diluents and reconstituted standards were used to generate a series of 7 standards (for IL-1β: 34, 103, 309, 926, 2,778, 8,333, and 25,000 pg/ml; for IL-6: 3, 10, 31, 93, 278, 833, and 2500 pg/ml; for TNF-α: 7, 21, 62, 185, 556,