Published in final edited form as:

Neurosci Lett. 2016 February 26; 615: 55–59. doi:10.1016/j.neulet.2016.01.013.

Novel environment influences the effect of paradoxical sleep deprivation upon brain and peripheral cytokine gene expression

Noah T. Ashley^{1,*}, David W. Sams¹, Audrey C. Brown¹, and Jennie E. Dumaine¹

¹Dept. of Biology, Western Kentucky University, Bowling Green, KY 42101

Abstract

Sleep loss increases inflammatory mediators in brain and peripheral tissues, but the mechanisms underlying this association are not fully understood. Male C57BL/6j mice were exposed to paradoxical sleep deprivation (PSD) for 24 h using the modified multiple platform (MMP) technique (platforms over water) or two different controls: home cage or a dry platform cage, which constituted a novel environment. PSD mice exhibited increased IL-1 β and TNF- α pro-inflammatory gene expression in brain (hypothalamus, hippocampus, pre-frontal cortex), as well as in peripheral tissues (liver, spleen), when compared with home-cage controls. In addition, among PSD mice, TGF β 1, an anti-inflammatory cytokine, was increased in pre-frontal cortex, liver, and spleen in conjunction with elevated serum corticosterone concentration relative to home-cage controls. However, these differences were nearly abolished when PSD mice were compared with control mice subjected to a dry MMP cage, suggesting that simply exposing mice to a novel environment can induce an acute inflammatory response.

Keywords

glucocorticoids; IL-1; inflammation; novel environment; paradoxical sleep deprivation; TNF

Introduction

Sleep loss has been associated with increased inflammation in humans and acts as a risk factor for development of chronic disease, notably cardiovascular and metabolic chronic diseases [8, 21, 25]. Although the exact mechanisms linking sleep loss to these various pathologies are incompletely understood, epidemiological and empirical studies have suggested that sleep loss promotes exaggerated inflammatory responses in brain and in the periphery [13, 14, 30, 31].

In animal models, sleep loss can be achieved using a range of methodologies that involve gentle handling, forced movement, exposure to noxious stimuli, and pharmacological and

*Author for correspondence. Dept. of Biology, Western Kentucky University, 1906 College Heights Blvd. #11080, Bowling Green, KY 42101-1080 USA; noah.ashley@wku.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

genetic interventions [5, 23]. Many of these techniques have been devised for use in rodents, and involve partial or nearly-complete deprivation [23]. Sleep in mammals is organized into two states: rapid-eye movement (REM) and non-REM sleep. Paradoxical sleep deprivation (PSD) involves suppression of the REM phase of sleep and can have negative impacts upon cognition and spatial memory [29].

Among animals that are sleep deprived, activation of the hypothalamic-pituitary-adrenal (HPA) axis is a common occurrence [7, 17, 18, 20], leading to the secretion of glucocorticoids from the adrenals. HPA activation from sleep loss is likely due to a multitude of factors that are related to the method of sleep deprivation employed (e.g., flower-pot, multiple platform, human handling, moving bar, etc.) and is not necessarily directly related to sleep loss *per se*, as well as the type of animal model used and duration of sleep loss [12]. In addition, chronically-elevated concentrations of glucocorticoids are well-known to reduce inflammatory responses [3, 6]. Thus, measuring and accounting for the effect of circulating glucocorticoids is important when assessing immune and inflammatory responses to sleep loss.

The aims of this study were to investigate the upstream effects of paradoxical sleep deprivation (PSD) upon pro- and anti-inflammatory cytokine gene expression in the brain and periphery as well as serum corticosterone concentration using a mouse model. Specifically, it was predicted that 24 h of acute PSD would increase interleukin-1 (IL-1) and tumor necrosis factor-alpha (TNF) pro-inflammatory gene expression and decrease transforming growth factor-beta-1 (TGF β 1) expression, an anti-inflammatory cytokine in peripheral tissues and brain (hippocampus, hypothalamus, pre-frontal cortex)-select areas previously shown to be sensitive to effects of sleep loss [7, 32]. In addition, it was further predicted that elevated plasma corticosterone would accompany these changes in immune responses. Lastly, this study involved controls that were either held in the sleep deprivation chamber (representing a novel environment) or remained in their home cage with other animals. It was hypothesized that exposure to novel environments may influence how mice respond to sleep loss.

Materials and Methods

Animals

Male C57BL/6j mice (>8 weeks of age) were obtained from our breeding colony and maintained on a 12 h photoperiod (lights on at 7 am) with *ad libitum* rodent food (Cincinnati labs, RM1800 diet) and tap water.

Paradoxical Sleep Deprivation

Mice were deprived of sleep for 24 h by using the modified multiple platform (MMP) method starting at 9 am. This method has been verified to abolish PSD, as well as reduce SWS in rodents [15]. Briefly, this technique entails placing mice from their home cage (27.3 $\times$ 16.8 $\times$ 12.7 cm) in a larger polycarbonate cage (43.2 $\times$ 21.6 $\times$ 21.0 cm) that contains elevated PCV circular platforms (diameter= 2.54 cm; height = 2.54 cm) that are spaced far enough apart so that mice are prevented from sleeping on them. Tap water is added until

reaching the top of the platforms. Three to four littermates were placed in the chamber to lessen effects from social isolation [22]. Two controls were used in separate experiments. The first control was mice that were briefly handled (at 9 am) and then returned to their home cage. The second control was mice exposed to the MMP cage for 24 h (at 9 am) except that no water was added to the cage. A third control, mice exposed to MMP cage involving larger platforms to permit sleep, was not used because previous studies have shown that this control still induces reductions in non-REM sleep [15].

Tissue Collection

After 24 h of PSD (or control treatments), mice were exposed to isoflurane vapors for <1 min and then rapidly decapitated. Trunk blood was collected and immediately placed on ice (<2 min relative to initial handling). Liver, spleen, epididymal fat, and brain were rapidly removed and stored in RNAlater solution (ThermoScientific) at 4°C. Hypothalamus, hippocampus, and prefrontal cortex were then dissected from the brain and stored in RNA later at -20°C. Serum was separated from blood by centrifugation at 4°C, and then stored at -80°C for future assessment of corticosterone concentration (see below).

RNA Isolation

RNA was extracted from brain tissues, liver, spleen, and fat using an RNeasy mini Kit (Qiagen). RNA concentration was assessed using a Nanodrop 2000 Spectrophotometer (ThermoFischer Scientific).

Reverse Transcription

Total RNA was reverse transcribed into cDNA using either a Moloney Murine Leukemia Virus (M-MLV) Reverse Transcription Kit (Invitrogen) or a high-capacity cDNA reverse transcription kit (ThermoFischer Scientific). Total RNA concentrations were diluted (brain: 11.2 ng/μl; liver, spleen, fat: 10.6 ng/μl). Reactions were carried out according to manufacturer's instructions using a thermocycler.

RT-PCR

Real-time PCR was conducted using an ABI 7300-RT PCR system. Relative cytokine gene expression was determined using Taqman gene expression RT-PCR master mix the and the following primer/probe sets (ThermoFischer Scientific): IL-1β (Mm00434228_m1), TNF-α (Mm00443260_g1), and TGF-β (Mm01178820_m1), or 18s endogenous primer-limited (Mm03928990_g1). The cytokine probes were labeled with 5-FAM (5' end) and the quencher MGB (3' end). These probes were selected based upon previous sleep deprivation studies [7, 31]. The 18s control involved a Vic-labeled probe with MGB quencher. Amplification conditions were as follows: 50°C for 2 min, 95°C for 10 min, 40 cycles of 95°C for 15 s, and 60°C for 1 min. The reaction proceeded according to the manufacturer's instructions, except that final reaction volumes were halved to 25 μl per well. To determine relative expression of cytokines, a standard curve was generated using serial dilutions of pooled cDNA (1:1, 1:10, 1:100, 1:1,000, 1:10,000). Mean Ct scores were calculated from duplicates of each sample. All values were normalized relative to the 18s ribosomal RNA levels of each sample.

ELISA

Serum corticosterone concentration was determined using an ELISA kit (ab108821; Abcam). Reagents and standards were prepared according to the manufacturer's instructions. Cross reactivities for the kit were <30% deoxycorticosterone, <2% aldosterone, <2% progesterone, and <1% cortexolone. Recoveries were 96% with a sensitivity of 0.3 ng/ml. After diluting samples by 200x, the reaction was carried out in duplicate according to kit instructions. The average absorbance of each plate was determined using a plate reader and subtracting the absorbance at 450 nm from the absorbance at 570 nm (from manufacturer's instructions) using a BioTek Synergy H1 Hybrid Reader. Corticosterone concentrations were extrapolated from a standard curve using a log-log transformation (better fit than four-parameter logistic curve fit in this instance), and then corrected for the dilution factor (1:200). Intra- and interassay variations were 5.5% and 8.3%, respectively.

Statistical Analyses

Unpaired t-tests (two-tailed) were used to test for differences between control versus PSD mice in IL-1, TNF, and TGF- β gene expression in peripheral (spleen, liver, fat) and brain (hippocampus, hypothalamus, pre-frontal cortex) tissue. For the first experiment, PSD mice were compared with "home-cage controls" (no exposure to the MMP cage). For the second experiment, PSD mice were compared with controls exposed to a dry MMP cage (no water added; termed "dry MMP controls"). In some cases, logarithmic transformation was necessary to satisfy requirements of normality and/or homogeneity of variances. A smaller proportion of comparisons required the use of non-parametric Mann-Whitney U tests. Two-tailed t-tests were also employed for assessing differences in serum corticosterone concentrations. Values are represented as mean $\pm$ SEM. Statistical significance was achieved when $P < 0.05$. Samples sizes in each group ranged from 6-8 animals.

Results

Home-cage controls versus PSD

Twenty-four hours of PSD produced widespread alterations in cytokine gene expression in brain and periphery compared with home-cage controls (Table 1). PSD increased IL-1 expression in liver and brain (hippocampus, hypothalamus, pre-frontal cortex) compared with home-cage controls. Similarly, TNF expression was upregulated in liver, spleen, and pre-frontal cortex among mice exposed to PSD versus home-cage controls (Table 1). Tgf β 1 mRNA expression was upregulated in liver, spleen, and pre-frontal cortex of PSD mice relative to home-cage mice (Table 1).

Dry-MMP controls versus PSD

In a separate experiment, there were few differences in cytokine gene expression between PSD and dry-MMP controls. IL-1 expression did not significantly differ between groups for all tissues measured (Table 2). However, PSD mice displayed an increase in TNF mRNA expression in the periphery (liver and spleen), but not in brain (Table 2). Hippocampal and hypothalamic Tgf β 1 expression was significantly down-regulated in PSD mice compared with dry MMP controls (Table 2).

Serum corticosterone concentration

In the first experiment (home cage vs. PSD), PSD mice had significantly elevated concentrations of baseline corticosterone compared with home-cage controls (Fig. 1A). In the second experiment (dry-MMP vs. PSD), there were no significant differences in serum baseline corticosterone levels between PSD mice and dry MMP controls (Fig 1B), and levels were similar to PSD mice in the first experiment.

Discussion

This study demonstrates that exposure to a novel environment can influence peripheral and central inflammatory responses, and that this effect is similar to mice that have been subjected to PSD using the modified multiple platform method (cage filled with water). Although sleep architecture was not evaluated in this study, changes in environment have been shown to alter sleep quality and quantity. For example, humans generally display reduced ability to sleep during the first night of a sleep study in a laboratory setting [1, 26]. In mice and rats, presentation of a novel object or placement in a new environment is typically followed by a rapid increase in the latency to NREM and REM sleep, and a corresponding decrease in NREM and REM sleep amounts [19, 27, 28]. In effect, it is probable that the novel cage induced short-term insomnia that was potent enough to promote an inflammatory response that was nearly indistinguishable from a response produced in PSD mice. Alternatively, other physiological responses, such as hormonal alterations, could influence inflammatory processes. Further study is needed to understand the factors that underlie this response.

Sleep loss is known to induce alterations in the immune system, including activation of a pro-inflammatory response [8, 12, 25]. When compared with home-cage controls, mice exposed to the MMP method for 24 hours exhibited increases in proinflammatory IL-1 and TNF mRNA in peripheral and brain tissues. These results are consistent with previous studies in rodents that have examined downstream effects of paradoxical sleep deprivation upon cytokine proteins in serum or plasma [31, 32]. In addition, there was an unanticipated elevation in anti-inflammatory cytokine (Tgf β 1) gene expression in the pre-frontal cortex. Previous work in our laboratory using a different method to induce sleep loss (horizontally-sweeping bar that moves across home cage at specified intervals to periodically awaken mice) also reported increased gene expression of pro-inflammatory (IL1, but not TNF) cytokines in the periphery and anti-inflammatory Tgf β 1 in brain [7]. Upregulation of anti-inflammatory cytokines may occur to homeostatically dampen inflammatory responses as a result of sleep deprivation and provide a protective effect, as prolonged inflammation can lead to tissue damage [2].

Serum corticosterone levels were elevated in mice exposed to 24 h of the multiple platform method compared with control mice remaining in their home cage, which indicates that this method of sleep deprivation activates the HPA axis. In contrast, control mice subjected to a dry multiple platform cage exhibited similar serum corticosterone concentrations relative to mice subjected to experimental PSD. These data indicate that exposure to a novel environment is sufficient to trigger release of glucocorticoids from the adrenals, although it is unspecified what aspects of this novel environment are involved with HPA activation.

There is good evidence that acute exposure to novel environments activates the HPA axis in rodents [4, 11, 16].

Given that chronically elevated levels of glucocorticosteroids suppress inflammation [3, 6], the finding that IL-1 and TNF gene expression was upregulated in the presence of elevated levels of serum corticosterone is surprising. Two explanations are proposed that revolve around whether elevated corticosterone acts as a cause of inflammation or an effect thereof. The first possibility involves circulating corticosterone inducing a short-term pro-inflammatory effect by increasing inflammatory mediators in brain. There is increasing evidence that glucocorticoids act permissively to prime neuroinflammation under some conditions [9, 10]. For example, glucocorticoids potential inflammasomes, which are activated by products of stress or damaged cells in brain [9]. The alternative explanation is that elevated glucocorticoids increase *in response to* inflammation, eventually providing negative feedback to inhibit the initial inflammatory response [24]. Exposure to novel environment elevated corticosterone titers and altered inflammatory profiles, which diminished the effect of PSD upon brain and peripheral inflammatory responses. More specifically, IL-1 gene expression was confounded by this effect. However, TNF upregulation was still documented in PSD mice even when comparing with controls exposed to the MMP cage. Future studies that manipulate corticosterone levels surgically or pharmacologically in sleep-deprived subjects will help resolve these two hypotheses.

Conclusions

Inflammatory responses to PSD were dampened when accounting for the role that novel environment plays in altering inflammatory profiles and stress reactivity. These results indicate that sleep researchers should consider the effect of novel environments when carrying out sleep deprivation or restriction experiments, especially when evaluating immune-neuroendocrine interactions.

Acknowledgements

This research was funded by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20GM103436. We thank Naomi Rowland for assistance with RTPCRs. In addition, we thank Dr. Bruce O'Hara for advice regarding experimental design.

Abbreviations

IL-1	interleukin-1-beta
MMP	modified multiple platform
PSD	paradoxical sleep deprivation
REM	rapid-eye movement
TGFβ1	transforming growth factor-beta-1
TNF	tumor necrosis factor-alpha

Literature Cited

- [1]. Agnew HW Jr, Webb WB, Williams RL. The first night effect: an EEG study of sleep. *Psychophysiology*. 1966; 2:263–266. [PubMed: 5903579]
- [2]. Ashley NT, Weil ZM, Nelson RJ. Inflammation: mechanisms, costs, and natural variation. *Annu. Rev. Ecol. Evol. Syst.* 2012; 42:385–406.
- [3]. Barnes PJ. Anti-inflammatory actions of glucocorticoids: molecular mechanisms. *Clin. Sci.* 1998; 94:557–572. [PubMed: 9854452]
- [4]. Brett LP, Chong GS, Coyle S, Levine S. The pituitary-adrenal response to novel stimulation and ether stress in young adult and aged rats. *Neurobiol. Aging*. 1983; 4:133–138. [PubMed: 6685230]
- [5]. Colavito V, Fabene PF, Grassi-Zucconi G, Pifferi F, Lamberty Y, Bentivoglio M, Bertini G. Experimental sleep deprivation as a tool to test memory deficits in rodents. *Front. Syst. Neurosci.* 2013; 7:106. [PubMed: 24379759]
- [6]. Coutinho AE, Chapman KE. The anti-inflammatory and immunosuppressive effects of glucocorticoids, recent developments and mechanistic insights. *Mol. Cell. Endocrinol.* 2011; 335:2–13. [PubMed: 20398732]
- [7]. Dumaine JE, Ashley NT. Acute sleep fragmentation induces tissue-specific changes in cytokine gene expression and increases serum corticosterone concentration. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 2015; 308:R1062–1069. [PubMed: 25876653]
- [8]. Faraut B, Boudjeltia KZ, Vanhamme L, Kerkhofs M. Immune, inflammatory and cardiovascular consequences of sleep deprivation and recovery. *Sleep Med. Rev.* 2012; 16:137–149. [PubMed: 21835655]
- [9]. Frank MG, Watkins LR, Maier SF. The permissive role of glucocorticoids in neuroinflammatory priming: mechanisms and insights. *Curr. Opin. Endocrinol. Diabetes Obes.* 2015; 22:300–305. [PubMed: 26087336]
- [10]. Frank MG, Watkins LR, Maier SF. Stress- and glucocorticoid-induced priming of neuroinflammatory responses: potential mechanisms of stress-induced vulnerability to drugs of abuse. *Brain Behav. Immun.* 2011; 25:S21–28. [PubMed: 21256955]
- [11]. Hennessy MB. Sensitization of the plasma corticosterone response to novel environments. *Physiol. Behav.* 1991; 50:1175–1179. [PubMed: 1798772]
- [12]. Hurtado-Alvarado G, Pavon L, Castillo-Garcia SA, Hernandez ME, Dominguez-Salazar E, Velazquez-Moctezuma J, Gomez-Gonzalez B. Sleep loss as a factor to induce cellular and molecular inflammatory variations. *Clin. Dev. Immunol.* 2013; 2013:801341. [PubMed: 24367384]
- [13]. Irwin MR, Wang M, Campomayor CO, Collado-Hidalgo A, Cole S. Sleep deprivation and activation of morning levels of cellular and genomic markers of inflammation. *Arch. Intern. Med.* 2006; 166:1756–1762. [PubMed: 16983055]
- [14]. Irwin MR, Wang M, Ribeiro D, Cho HJ, Olmstead R, Breen EC, Martinez-Maza O, Cole S. Sleep loss activates cellular inflammatory signaling. *Biol. Psych.* 2008; 64:538–540.
- [15]. Machado RB, Hipólido DC, Benedito-Silva AA, Tufik S. Sleep deprivation induced by the modified multiple platform technique: quantification of sleep loss and recovery. *Brain Res.* 2004; 1004:45–51. [PubMed: 15033418]
- [16]. Marquez C, Nadal R, Armario A. Influence of reactivity to novelty and anxiety on hypothalamic-pituitary-adrenal and prolactin responses to two different novel environments in adult male rats. *Behav. Brain Res.* 2006; 168:13–22. [PubMed: 16303185]
- [17]. McEwen BS. Sleep deprivation as a neurobiologic and physiologic stressor: Allostasis and allostatic load. *Metabolism*. 2006; 55:S20–23. [PubMed: 16979422]
- [18]. Meerlo P, Koehl M, van der Borcht K, Turek FW. Sleep restriction alters the hypothalamic-pituitary-adrenal response to stress. *J. Neuroendocrinol.* 2002; 14:397–402. [PubMed: 12000545]
- [19]. Michaud JC, Muiyard JP, Capdevielle G, Ferran E, Giordano-Orsini JP, Veyrun J, Roncucci R, Mouret J. Mild insomnia induced by environmental perturbations in the rat: a study of this new model and of its possible applications in pharmacological research. *Arch. Int. Pharmacol. Therap.* 1982; 259:93–105.

- [20]. Minkel J, Moreta M, Muto J, Htaik O, Jones C, Basner M, Dinges D. Sleep deprivation potentiates HPA axis stress reactivity in healthy adults. *Health Psych.* 2014; 33:1430–1434.
- [21]. Mullington JM, Haack M, Toth M, Serrador JM, Meier-Ewert HK. Cardiovascular, inflammatory, and metabolic consequences of sleep deprivation. *Prog. Cardiovasc. Dis.* 2009; 51:294–302. [PubMed: 19110131]
- [22]. Nunes JGP, Tufik S. Validation of the modified multiple platform method method, MPM, of paradoxical sleep deprivation in rats. *Sleep Res.* 1994; 23:419.
- [23]. Revel FG, Gottowik J, Gatti S, Wettstein JG, Moreau JL. Rodent models of insomnia: a review of experimental procedures that induce sleep disturbances. *Neurosci. Biobehav. Rev.* 2009; 33:874–899. [PubMed: 19428498]
- [24]. Silverman MN, Sternberg EM. Glucocorticoid regulation of inflammation and its functional correlates: from HPA axis to glucocorticoid receptor dysfunction. *Ann. N. Y. Acad. Sci.* 2012; 1261:55–63. [PubMed: 22823394]
- [25]. Simpson N, Dinges DF. Sleep and inflammation. *Nutr. Rev.* 2007; 65:S244–S252. [PubMed: 18240557]
- [26]. Tamaki M, Nittono H, Hayashi M, Hori T. Examination of the first-night effect during the sleep-onset period. *Sleep.* 2005; 28:195–202. [PubMed: 16171243]
- [27]. Tang X, Xiao J, Liu X, Sanford LD. Strain differences in the influence of open field exposure on sleep in mice. *Behav. Brain Res.* 2004; 154:137–147. [PubMed: 15302119]
- [28]. Tang X, Xiao J, Parris BS, Fang J, Sanford LD. Differential effects of two types of environmental novelty on activity and sleep in BALB/cJ and C57BL/6J mice. *Physiol. Behav.* 2005; 85:419–429. [PubMed: 16019041]
- [29]. Tufik S, Andersen ML, Bittencourt LR, Mello MT. Paradoxical sleep deprivation: neurochemical, hormonal and behavioral alterations. Evidence from 30 years of research. *An. Acad. Bras. Cienc.* 2009; 81:521–538. [PubMed: 19722021]
- [30]. Wisor JP, Schmidt MA, Clegern WC. Evidence for neuroinflammatory and microglial changes in the cerebral response to sleep loss. *Sleep.* 2011; 34:261–272. [PubMed: 21358843]
- [31]. Yehuda S, Sredni B, Carasso RL, Kenigsbuch-Sredni D. REM sleep deprivation in rats results in inflammation and interleukin-17 elevation. *J. Interfer. Cytok. Res.* 2009; 29:393–398.
- [32]. Zhu B, Dong Y, Xu Z, Gompf HS, Ward SA, Xue Z, Miao C, Zhang Y, Chamberlin NL, Xie Z. Sleep disturbance induces neuroinflammation and impairment of learning and memory. *Neurobiol. Dis.* 2012; 48:348–355. [PubMed: 22776332]

Highlights

- Paradoxical sleep deprivation elevated proinflammatory responses in brain/periphery
- Paradoxical sleep deprivation increased baseline serum corticosterone
- Inflammation from sleep loss was dependent upon the type of control used
- Exposure to novel environment induces an inflammatory response

Figure 1. Serum baseline corticosterone levels (ng/ml) of mice subjected to (A) home cage environment versus 24 hours of PSD or (B) mice subjected to a dry MMP cage versus 24 hours of PSD. Values are expressed as means $\pm$ sem. Asterisk indicates $P < 0.05$ relative to other group.

Table 1

Comparison of cytokine gene expression (IL-1, TNF, Tgf β 1) in peripheral (liver, spleen) and brain (hippocampus, hypothalamus, prefrontal cortex) tissue between mice experiencing PSD versus mice taken from home cages (“home-cage control”). Expression data were normalized to a 18s endogenous control (see methods). Values are expressed as mean $\pm$ SEM. Bolded *P*-values denote a significant difference ($P < 0.05$) between the two groups in gene expression using unpaired t-tests (two-tailed).

IL-1		Periphery		Brain		
		Liver	Spleen	Hippocampus	Hypothalamus	Prefrontal cortex
Home-cage control	Mean	0.255	2.682	0.068	0.093	0.070
	SEM	0.012	0.765	0.009	0.016	0.005
24h PSD	Mean	3.335	4.132	0.104	0.294	0.254
	SEM	0.943	0.789	0.011	0.040	0.048
	<i>P</i> -value	<0.001 †	0.217	0.031	<0.001 †	0.003
Up or down regulation		↑	---	↑	↑	↑
TNF						
Home-cage control	Mean	0.005	0.437	0.329	0.007	0.050
	SEM	0.001	0.006	0.143	0.002	0.004
24h PSD	Mean	0.492	0.917	0.148	0.021	0.278
	SEM	0.152	0.045	0.080	0.007	0.051
	<i>P</i> -value	<0.001 †	0.001 †	0.501 †	0.103 †	<0.001 †
Up or down regulation		↑	↑	---	---	↑
Tgfβ1						
Home-cage control	Mean	0.352	6.355	0.316	0.431	0.356
	SEM	0.020	2.588	0.028	0.019	0.031
24h PSD	Mean	0.667	22.997	0.341	0.377	0.589
	SEM	0.049	3.429	0.029	0.028	0.074
	<i>P</i> -value	<0.001	<0.001	0.556	0.139	0.015
Up or down regulation		↑	↑	---	---	↑

† indicates that logarithmic transformation was necessary to meet requirements of parametric tests (normality, homogeneity of variances).

Table 2

Comparison of cytokine gene expression (IL-1, TNF, Tgf β 1) in peripheral (liver, spleen, white adipose tissue (WAT)) and brain (hippocampus, hypothalamus, prefrontal cortex) tissue between mice experiencing PSD versus exposed to a dry MMP cage (“dry MMP control”). Expression data were normalized to a 18s endogenous control (see methods). Values are expressed as mean $\pm$ SEM. Bolded *P*-values denote a significant difference (*P* < 0.05) between the two groups in gene expression using unpaired t-tests (two-tailed).

IL-1		Periphery			Brain		
		Liver	Spleen	WAT	Hippocampus	Hypothalamus	Prefrontal cortex
Dry-MMP control	Mean	1.728	1.091	3.448	0.745	0.093	0.338
	SEM	0.362	0.196	1.321	0.602	0.032	0.267
24h PSD	Mean	1.794	1.615	4.064	0.143	0.145	0.136
	SEM	0.681	0.201	1.184	0.029	0.025	0.023
	<i>P</i> -value	0.937	0.083	0.733	0.907 [‡]	0.224	0.142 [‡]
Up or down regulation		---	---	---	---	---	---
TNF							
Dry-MMP control	Mean	0.001	0.214	0.0004	0.001	0.003	0.009
	SEM	0.0002	0.062	0.00009	0.001	0.001	0.006
24h PSD	Mean	0.017	0.892	0.001	0.013	0.003	0.009
	SEM	0.014	0.449	0.0002	0.001	0.001	0.005
	<i>P</i> -value	0.028 [‡]	0.038 [‡]	0.119	0.078 [‡]	0.851	0.951
Up or down regulation		↑	↑	---	---	---	---
Tgf β 1							
Dry-MMP control	Mean	0.406	11.337	3.627	2.137	1.218	0.610
	SEM	0.162	1.986	0.696	1.256	0.266	0.109
24h PSD	Mean	0.414	8.817	5.382	0.568	0.390	0.605
	SEM	0.103	4.088	0.896	0.052	0.039	0.083
	<i>P</i> -value	0.968	0.573	0.154	0.004 [‡]	<0.001 [‡]	0.971
Up or down regulation		---	---	---	↓	↓	---

[‡] indicates that logarithmic transformation was necessary to meet requirements of parametric tests (normality, homogeneity of variances).

[‡] indicates use of non-parametric Mann-Whitney test.