Published in final edited form as:

J Mol Neurosci. 2013 November ; 51(3): 1038–1045. doi:10.1007/s12031-013-0093-6.

Hypersomnolence and reduced activity in pan-leptin receptor knockout mice

Yuping Wang, Junyun He, Abba J. Kastin, Hung Hsuehou, and Weihong Pan*

Blood-Brain Barrier Group, Pennington Biomedical Research Center, Baton Rouge, LA 70808, USA

Abstract

Excessive obesity correlates with hypersomnolence and impaired cognitive function, presumably induced by metabolic factors and cytokines. Production of the adipokine leptin correlates with the amount of adiposity, and leptin has been shown to promote sleep. To determine whether leptin plays a major role in the hypersomnolence of obesity, we measured sleep architecture in pan-leptin receptor knockout (POKO) mice that do not respond to leptin because of the production of a mutant, non-signaling receptor. The obese POKO mice had more non-rapid eye movement (NREM) sleep and less waking time than their littermate controls. This was mainly seen during the light span, although increased bouts of rapid eye movement (REM) sleep were also seen in the dark span. The increase of NREM sleep correlated with the extent of obesity. The POKO mice also had decreased locomotor activity and more immobility in the open field test, but there was no increase of forced immobility nor reduction of sucrose intake as would be seen in depression. The increased NREM sleep and reduced locomotor activity in the POKO mice suggest that it was obesity, rather than leptin signaling, that played a predominant role in altering sleep architecture and activity.

Keywords

Leptin; Activity; Sleep; Obesity

Introduction

Obesity is associated with hypersomnolence in human beings and other animals (Panossian and Veasey, 2012). Among the metabolic factors greatly elevated in obesity, leptin is a 16 kD protein mainly produced by fat cells and released into the circulation. Leptin levels are regulated by circadian rhythms, feeding, inflammation, as well as obesity (Pan and Kastin, 2013). Leptin crosses the blood-brain barrier (BBB) through a saturable transport mechanism (Banks et al., 1996) to reach the central nervous system (CNS), where it exerts diverse effects including regulation of feeding, metabolic activity, and neurobehavior. A circadian rhythm of leptin transport is present at the BBB and blood-spinal cord barrier (Pan and Kastin, 2001). Leptin is involved in the regulation of sleep architecture and the diurnal rhythmicity of feeding and locomotor activities, whereas obesity is usually associated with hyperleptinemia and leptin resistance (Banks et al., 2000). To differentiate the direct effects of leptin on sleep architecture from those of obesity, we studied mice with pan-leptin receptor knockout (POKO), a strain recently characterized in our laboratory that has early

*Corresponding author (to whom reprint request should be addressed): Weihong Pan, MD, PhD Blood-Brain Barrier Group Pennington Biomedical Research Center 6400 Perkins Road, Baton Rouge, LA 70808 Tel. 225-763-2715; Fax 225-763-0265 panw@pbrc.edu.

onset obesity resulting from the production of a mutant leptin receptor with resulting abrogation of leptin signaling (Hsueh et al., 2013).

Evidence from the literature is confusing in that both leptin treatment and obese mice with deficient leptin signaling may promote sleep. Leptin (1.3 mg/kg intraperitoneally, without time of the day specified) has been reported to increase slow wave sleep (part of non-rapid eye movement sleep, NREM) by 13 % and decrease REM by 30 % in the ensuing 9 h in fed rats but not in fasted rats (Sinton et al., 1999). However, obese ob/ob mice lacking leptin production also show an increased total amount of NREM, although there are more arousals and stage shifts as well as shorter sleep bouts. This increase of sleep is associated with a reduction of locomotor activity and body temperature (Laposky et al., 2006). The hypersomnolence in the ob/ob mice is obviously not mediated by leptin.

In parallel, db/db mice missing the ObRb receptor subtype have more sleep time, more sleep fragmentation, and less diurnal rhythmicity of REM and NREM delta power. There is attenuated recovery of sleep after 6 h of acute sleep deprivation, lower locomotor activity, and a less distinct diurnal rhythm (Laposky et al., 2008). Studies from obese Zucker rats with defective ObR also show consistent findings with those in the mutant mice (Danguir, 1989; Megirian et al., 1998). In C57 mice with diet-induced obesity, there is increased NREM correlating with weight gain but not energy intake. At 2, 4, and 6 weeks of 59 % high-fat diet starting at 6 months of age, the mice show large increases (about 80-100 min/day) of NREM, more episodes of NREM and REM, and a corresponding reduction in the duration of waking episodes during the dark span (Jenkins et al., 2006). Overall, the literature suggests that obesity may play a greater role than leptin in promoting sleep. To isolate the effect of leptin from that of obesity-induced metabolic disturbance, we determined sleep architecture and activity levels in the obese POKO mice lacking leptin signaling in all cells (Hsueh et al., 2013).

Materials and Methods

1. Mice and surgery for headmount placement

The animal studies were approved by the Institutional Animal Care and Use Committee. Mice were housed with a 12:12 h light – dark cycle, lights on from 6 am to 6 pm. The POKO mice (n = 7) were generated and characterized as described previously (Hsueh et al., 2013), and studied along with their littermate control wildtype (WT) mice on the C57 strain background (n = 8).

Mice were subjected to headmount surgery at age 3 months. After isofluorane anesthesia, a prefabricated headmount (Pinnacle Technology, Lawrence, KS) was secured to the skull with 4 stainless steel screws (0.1 inches in length). The headmount contains a plastic 6-pin connector glued to a printed circuit board (PCB), where 3 electroencephalographic (EEG) electrodes and 2 electromyographic (EMG) electrodes are affixed (Wisor et al., 2011). The screws serve as EEG electrodes, and their conductivity with the PCB is secured by application of silver epoxy. The PCB is attached to a 6-pin connector at the side of the headmount facing away from the skull. The 2 frontal screws were placed 1.5 mm lateral to the midline and 1 mm anterior to the bregma. The left frontal screw served as an internal ground. The 2 parietal screws were placed 1.5 mm lateral to the midline and approximately 2 mm anterior to lambda. Two channels of EEG data were collected, one conveying the potential between the 2 parietal leads (channel 1), and the other the potential between the left parietal and right frontal leads (channel 2). Channel 2 was used for state classification. The 2 EMG electrodes consisted of stainless steel wires, 1.5 cm in length, attached to the circuit board on one end and terminating in a bolus of epoxy (approximately 1 mm in diameter) at the other end. Insulating material was absent from approximately 2 mm of

stainless steel immediately adjacent to the bolus of epoxy; this exposed stainless steel served as the lead and was embedded in the neck musculature. The headmount was glued to the skull and sealed with dental cement. The neck incision was closed with 5-0 silk suture and a topical analgesic was applied. The mice received buprenorphine (0.05 mg/kg ip) daily for 3 days after the surgery, and were allowed to recover for 2 weeks before adaptation to cylindrical acrylic plastic sleep recording cages.

2. Sleep recording and data analysis

The mice with headmounts were individually housed in cylindrical sleep recording cages. The 8400-SL data collection system (Pinnacle Technology) was used. The headmount was connected to a preamplifier via a 6-pin connector. The EEG and EMG waveforms were amplified 100-fold and filtered. Signals were then passed through a low-torque commutator/swivel to the 8401 Data Conditioning and Acquisition system (DCAS) for final stage conditioning and filtering. After 3 days acclimation to the recording environment, data was then acquired with Serenia Acquisition software during 48 h or longer continuous recording, with or without synchronized video monitoring. For data analysis Digitized EEG (bandpass, 0.5-35 Hz) and EMG (10 – 100 Hz) were processed in 10 second (sec) epochs. Manual scoring was performed by an experienced sleep researcher, with each of the 10 sec epochs assigned as wake, NREM, or REM, following standard scoring criteria (Veasey et al., 2000; Louis et al., 2004). The distribution of NREM, REM, and wake parameters was quantified for the periods of 24-h, 12-h dark span, 12-h light span, and 2-h intervals across the dark - light phase. The Serenia Sleep Pro software (Pinnacle Technology) was used to perform sleep bout analyses.

3. Behavioral testing

3.1. Open field test for locomotor activity and anxiety-like behavior—The open field test was performed on 5 month old POKO (n = 29) and WT mice (n = 32) as described previously (Wu et al., 2010a, 2010b; Nesher et al., 2012). The open field was a square arena (50 × 50 cm) with level surface surrounded by walls 30 cm tall, evenly illuminated, and divided into 16 equal squares. The 4 squares in the center were defined as the central region, and the remaining 12 squares adjacent to the walls were defined as the peripheral region. At the beginning of the test, the mouse was placed in the center. In the subsequent 5 min, the number of grids crossed in central and peripheral regions, rearing, grooming, and defecation were counted.

3.2. Sucrose preference test—For the sucrose preference test, POKO (n = 7) and WT mice (n = 8) were acclimated to single-housing with the presence of two sipper tubes as the water source for 2 days before the test. For 12 h sucrose intake measurement from 6 pm to 6 am, food and water were withdrawn for 2 h from 4 – 6 pm. At the beginning of the test at 6 pm, premeasured 50 ml sipper tubes containing 2 % (W/V) sucrose and water were placed on the cage. At the end of the test at 6 am, liquid remaining in both sipper tubes was measured to determine: (1) sucrose consumption (ml); (2) water consumption (ml); and (3) total liquid consumption (sucrose + water).

3.3. Nomura water wheel and tail suspension tests for depressive like behavior—The time of immobility in the Nomura water wheel forced swimming test and in the tail suspension test were determined as described previously (Wu et al., 2010b, 2011). For the Nomura water wheel test, the turns of water wheel that the mice paddled instead of passively floating on the water surface were counted at 1, 2, and 5 min. For the tail suspension test, the tail of the mouse was taped to a horizontal bar 60 cm above the counter. The duration of immobility in each of the subsequent 6 min was measured.

4. Statistical analyses

Group means are presented with their standard errors. For accumulative changes of sleep parameters at 12 or 24 h, unpaired t-tests were performed to compare the differences of sleep stages between the WT and POKO groups. For changes of sleep stages over time, repeated measures two-way analysis was performed. For changes of neurobehavior, one-way analysis of variance was performed for comparison of 3 groups, followed by Tukey's post-hoc test. Unpaired t-tests were performed when only 2 groups were compared. The statistical analysis and graphics were produced by use of the Prism GraphPad software (San Diego, CA).

Results

1. Sleep architecture in POKO mice

1.1. Stage distribution—In undisturbed recording conditions, POKO mice had 703 ± 41.1 min of NREM (49.5%) 72.7 ± 6.7 min REM sleep (5.4%), and 664.1 ± 38.8 min wake (45.1%) in 24 h. In comparison with the WT littermate controls, they had more NREM sleep time and a higher percent of NREM, as well as reduced waking over 24 h ($p < 0.05$, Fig.1A). The changes were attributed to differences in the light span ($p < 0.05$, Fig.1B).

1.2. Sleep continuity and extent of sleep fragmentation—POKO mice had similar mean bout length for NREM and REM in the light span as the WT mice. However, there was a reduction of average waking bout length (900 s) compared with the WT mice (1400 s). There was no increase in the number of sleep stage transitions, indicating a similar level of sleep continuity without an increase of sleep fragmentation. In the dark span, the POKO mice had more REM bouts than the WT mice ($p < 0.05$, Fig.1C).

1.3. Differences across light-dark cycle—The circadian rhythm of sleep-wake cycle persisted in the POKO mice, though the amplitude of oscillation of NREM was higher than that in WT mice. The increase of NREM in the POKO mice was significant, as shown by the 2 h segmental plot, whereas that of wake was lower (Fig.2B). By contrast, there was no significant change of REM as shown in the 2 h segmental plot across the 24 h cycle (Fig. 2C).

1.4. Correlation of body weight with total sleep time—A linear correlation was identified between body weight and %NREM. This was seen in both WT mice ($r = 0.76$, $p < 0.05$) and POKO mice ($r = 0.86$, $p < 0.05$). There was no difference between the two groups (Fig.3A). The correlation between %REM and body weight was weaker in either group ($r = 0.57$ for WT and $r = 0.78$ for POKO, $p > 0.05$ for both), though the two regression lines were significantly different from each other ($p < 0.05$, Fig.3B). The %wake in 24 h and body weight showed a reverse correlation for both WT ($r = 0.73$, $p < 0.05$) and POKO ($r = 0.86$, $p < 0.05$), and there was no group difference (Fig.3C).

2. Neurobehavior of the POKO mice

2.1. Locomotor activity and anxiety-like behavior—When all POKO mice were grouped together to compare with their WT littermates (Fig.4A), the POKO group had fewer outer and inner grids crossed, and reduced rearing. This suggests a reduction of locomotor activity. They also had more defecation than the WT group, which might be associated with increased metabolic activity, or increased anxiety resulting from increased sympathetic output. There was no difference between male and female mice (data not shown). To determine the effect of body weight and adiposity on locomotor behavior, the POKO mice were further divided into extremely obese (body weight of 43 - 66 g) and relatively lean

(body weight of 23 - 39 g) groups, and compared with a third group of WT mice (body weight of 23 - 35 g). As shown in figure 4B, the “fat POKO” mice had fewer outer and inner grids crossed and fewer rearings, reflected by a significant reduction of activity in all planes in comparison with both the “lean POKO” ($p < 0.01$) and the WT ($p < 0.001$). The activity of WT mice showed a strong correlation with body weight ($r = 0.7$, $p < 0.0001$), as did the activity of the fat POKO mice with body weight ($r = 0.65$, $p < 0.05$). However, such correlation was not seen in the lean POKO mice ($r = 0.03$, $p > 0.05$) (Fig.4C). Besides the reduction of the level of locomotor activity in the horizontal (grids crossed) and vertical (rearing) planes, the fat POKO mice also had less grooming than the lean POKO mice, though there was no difference in defecation (Fig.4D).

2.2. Lack of change in forced immobility tests—There was no increase of immobility in either the Nomura water wheel test of forced swimming or tail suspension test (data not shown). There was no clear difference contributed by differences in body weight and adiposity. Along with a lack of difference in sucrose intake between the POKO and WT groups (data not shown), the results suggest that POKO mice did not exhibit depressive-like behavior.

Discussion

Sleep disturbance and obesity have combined effects to increase oxidative stress and cognitive dysfunction (Alzoubi et al., 2013). Here we show that POKO mice had increased sleep and reduced locomotor activity with increased periods of immobility, though there were no apparent changes in mood and depressive-like behavior. The changes of sleep pattern in these mice lacking leptin signaling were in the same direction of increased NREM and REM induced by leptin treatment of rats (Sinton et al., 1999), even though the treatment effect in the rats was acute whereas the changes in POKO mice were sustained. Leptin also has anxiolytic effects (Liu et al., 2010) and decreases exploratory behavior as well as feeding (Buyse et al., 2001). The results from POKO mice argue against a direct effect of leptin on sleep and neurobehavior in obesity, but rather suggest a greater influence of obesity itself in inducing hypersomnolence.

We have shown that homozygous POKO mice fail to respond to intracerebroventricular injection of leptin with phosphorylation and activation of Signal Transducer and Activator of Signaling (STAT)-3, and that these mice are obese and have reduced circadian amplitude of metabolic activities (Hsueh et al., 2013). This contrasts with mice with obesity resulting from defects downstream to leptin signaling, where leptin treatment continues to activate pSTAT3 in hypothalamic neurons (Pan et al., 2011). The obesity phenotype contrasts with the partial protective effect of endothelial leptin receptor knockout mice (Pan et al., 2012a) and astrocytic leptin receptor knockout mice (Jayaram et al., 2013). However, although the sleep stage distribution showed an increase of NREM with a reduction of wake time, the circadian rhythm of the sleep-wake cycle persisted in the POKO mice. The POKO mice showed reduced locomotor activity and appeared to have more periods of immobility, but there was no clear evidence that they exhibited increased forced immobility associated with depressive-like behavior. There was no anhedonia, in the absence of changes of sucrose intake, but rather hyperphagia (Hsueh et al., 2013). The increase of defecation is usually associated with either heightened metabolic activity or increased anxiety. We have shown that POKO mice have reduction of metabolic activities (Hsueh et al., 2013). Thus, the increase of defecation appears to support an increase of anxiety in association with an increased sympathetic tone.

Clinical research has shown daytime sleepiness in individuals with obesity associated with metabolic and circadian abnormality of biopotentials. Obese individuals commonly have

excessive daytime sleep (Vgontzas et al., 1998; Dixon et al., 2007). Although obesity and obstructive sleep apnea are associated with neuropsychological impairment in humans (Beebe et al., 2003; Sateia, 2003), mouse models of obesity seldom show signs of sleep apnea, even though astrogliosis and neuroinflammation may be evident in certain CNS regions (Li et al., 2008; Hsueh et al., 2009; Pan et al., 2012b; Jayaram et al., 2013). There are few non-human studies testing the interactions between sleep and changes in neurobehavior.

POKO mice provide a suitable model for determination of cognitive behavior and mood in association with changes of sleep in the absence of leptin signaling. Our results showed that the increased sleep in POKO mice correlated with weight gain. In the light span, there was increased NREM sleep and decreased wake time. In the dark span, there were increased REM bouts and more sleep state transitions. Sleep is suggested to alleviate anxiety and depression by influencing lighting and circadian rhythms (Fonken et al., 2009; Ramesh et al., 2012). The relatively mild neurobehavioral impairment of the POKO mice might suggest that extended sleep played a partially compensatory role.

In summary, POKO mice showed hypersomnolence with increased NREM sleep mainly in the light span, and mildly increased sleep fragmentation as shown by more REM bouts in the dark span. The increase of sleep correlated with body weight gain and yielded a mild neurobehavioral phenotype with a reduction of locomotor activity.

Acknowledgments

Grant support was provided by NIH (DK54880 and DK92245 to AJK, and NS62291 to WP).

References

- Alzoubi KH, Khabour OF, Salah HA, Abu Rashid BE. The combined effect of sleep deprivation and Western diet on spatial learning and memory: role of BDNF and oxidative stress. *J Mol Neurosci*. 2013; 50:124–133. [PubMed: 22956188]
- Banks WA, Clever CM, Farrell CL. Partial saturation and regional variation in the blood-to-brain transport of leptin in normal weight mice. *Am J Physiol*. 2000; 278:E1158–E1165.
- Banks WA, Kastin AJ, Huang W, Jaspan JB, Maness LM. Leptin enters the brain by a saturable system independent of insulin. *Peptides*. 1996; 17:305–311. [PubMed: 8801538]
- Beebe DW, Groesz L, Wells C, Nichols A, McGee K. The neuropsychological effects of obstructive sleep apnea: a meta-analysis of norm-referenced and case-controlled data. *Sleep*. 2003; 26:298–307. [PubMed: 12749549]
- Buyse M, Bado A, Dauge V. Leptin decreases feeding and exploratory behaviour via interactions with CCK(1) receptors in the rat. *Neuropharmacology*. 2001; 40:818–825. [PubMed: 11369035]
- Danguir J. Sleep patterns in the genetically obese Zucker rat: effect of acarbose treatment. *Am J Physiol*. 1989; 256:R281–R283. [PubMed: 2912222]
- Dixon JB, Dixon ME, Anderson ML, Schachter L, O'Brien PE. Daytime sleepiness in the obese: not as simple as obstructive sleep apnea. *Obesity (Silver Spring)*. 2007; 15:2504–2511. [PubMed: 17925477]
- Fonken LK, Finy MS, Walton JC, Weil ZM, Workman JL, Ross J, Nelson RJ. Influence of light at night on murine anxiety- and depressive-like responses. *Behav Brain Res*. 2009; 205:349–354. [PubMed: 19591880]
- Hsueh H, He Y, Kastin AJ, Tu H, Markadakis EN, Rogers RC, Fossier PB, Pan W. Obesity induces functional astrocytic leptin receptors in hypothalamus. *Brain*. 2009; 132:889–902. [PubMed: 19293246]
- Hsueh H, Wang Y, Cornelissen-Guillaume GG, Kastin AJ, Jang E, Halberg F, Pan W. Diminished leptin signaling can alter circadian rhythm of metabolic activity and feeding. *J Appl Physiol*. 2013 in press.

- Jayaram B, Pan W, Wang Y, Hsueh H, Mace A, Cornelissen-Guillaume GG, Mishra PK, Koza RA, Kastin AJ. Astrocytic leptin-receptor knockout mice show partial rescue of leptin resistance in diet-induced obesity. *J Appl Physiol*. 2013; 114:734–741. [PubMed: 23329815]
- Jenkins JB, Omori T, Guan Z, Vgontzas AN, Bixler EO, Fang J. Sleep is increased in mice with obesity induced by high-fat food. *Physiol Behav*. 2006; 87:255–262. [PubMed: 16360185]
- Laposky AD, Bradley MA, Williams DL, Bass J, Turek FW. Sleep-wake regulation is altered in leptin-resistant (db/db) genetically obese and diabetic mice. *Am J Physiol Regul Integr Comp Physiol*. 2008; 295:R2059–R2066. [PubMed: 18843095]
- Laposky AD, Shelton J, Bass J, Dugovic C, Perrino N, Turek FW. Altered sleep regulation in leptin-deficient mice. *Am J Physiol Regul Integr Comp Physiol*. 2006; 290:R894–R903. [PubMed: 16293682]
- Li AJ, Dinh TT, Ritter S. Hyperphagia and obesity produced by arcuate injection of NPY-saporin do not require upregulation of lateral hypothalamic orexigenic peptide genes. *Peptides*. 2008; 29:1732–1739. [PubMed: 18577407]
- Liu J, Garza JC, Bronner J, Kim CS, Zhang W, Lu XY. Acute administration of leptin produces anxiolytic-like effects: a comparison with fluoxetine. *Psychopharmacology (Berl)*. 2010; 207:535–545. [PubMed: 19823809]
- Louis RP, Lee J, Stephenson R. Design and validation of a computer-based sleep-scoring algorithm. *J Neurosci Methods*. 2004; 133:71–80. [PubMed: 14757347]
- Megirian D, Dmochowski J, Farkas GA. Mechanism controlling sleep organization of the obese Zucker rats. *J Appl Physiol*. 1998; 84:253–256. [PubMed: 9451643]
- Nesher E, Peskov V, Rylova A, Raz O, Pinhasov A. Comparative analysis of the behavioral and biomolecular parameters of four mouse strains. *J Mol Neurosci*. 2012; 46:276–284. [PubMed: 21598024]
- Pan W, Hsueh H, Cornelissen-Guillaume GG, Jayaram B, Wang Y, Tu H, Halberg F, Wu X, Chua SC Jr, Kastin AJ. Endothelial leptin receptor mutation provides partial resistance to diet-induced obesity. *J Appl Physiol*. 2012a; 112:1410–1418. [PubMed: 22323652]
- Pan W, Hsueh H, Jayaram B, Khan RS, Huang EYK, Wu X, Chen C, Kastin AJ. Leptin action on non-neuronal cells in the CNS: potential clinical implications. *Ann N Y Acad Sci*. 2012b; 1264:64–71. [PubMed: 22530983]
- Pan W, Hsueh H, Xu CL, Wu X, Bouret SG, Kastin AJ. Astrocytes modulate distribution and neuronal signaling of leptin in the hypothalamus of obese A^{vy} mice. *J Mol Neurosci*. 2011; 43:478–484. [PubMed: 21086065]
- Pan W, Kastin AJ. Diurnal variation of leptin entry from blood to brain involving partial saturation of the transport system. *Life Sci*. 2001; 68:2705–2714. [PubMed: 11400913]
- Pan W, Kastin AJ. Leptin: A biomarker for sleep disorders? *Sleep Med Rev*. 2013 in press.
- Panosian LA, Veasey SC. Daytime sleepiness in obesity: mechanisms beyond obstructive sleep apnea—a review. *Sleep*. 2012; 35:605–615. [PubMed: 22547886]
- Ramesh V, Nair D, Zhang SX, Hakim F, Kaushal N, Kayali F, Wang Y, Li RC, Carreras A, Gozal D. Disrupted sleep without sleep curtailment induces sleepiness and cognitive dysfunction via the tumor necrosis factor- α pathway. *J Neuroinflammation*. 2012; 9:91. [PubMed: 22578011]
- Sateia MJ. Neuropsychological impairment and quality of life in obstructive sleep apnea. *Clin Chest Med*. 2003; 24:249–259. [PubMed: 12800782]
- Sinton CM, Fitch TE, Gershenfeld HK. The effects of leptin on REM sleep and slow wave delta in rats are reversed by food deprivation. *J Sleep Res*. 1999; 8:197–203. [PubMed: 10476006]
- Veasey SC, Valladares O, Fenik P, Kapfhammer D, Sanford L, Benington J, Bucan M. An automated system for recording and analysis of sleep in mice. *Sleep*. 2000; 23:1025–1040. [PubMed: 11145318]
- Vgontzas AN, Bixler EO, Tan TL, Kantner D, Martin LF, Kales A. Obesity without sleep apnea is associated with daytime sleepiness. *Arch Intern Med*. 1998; 158:1333–1337. [PubMed: 9645828]
- Wisor JP, Clegern WC, Schmidt MA. Toll-like receptor 4 is a regulator of monocyte and electroencephalographic responses to sleep loss. *Sleep*. 2011; 34:1335–1345. [PubMed: 21966065]

- Wu X, Hsueh H, Kastin AJ, He Y, Khan RS, Stone KP, Cash MS, Pan W. Interleukin-15 affects serotonin system and exerts antidepressive effects through IL15Ralpha receptor. *Psychoneuroendocrinol.* 2011; 36:266–278.
- Wu X, Kastin AJ, He Y, Hsueh H, Rood JC, Pan W. Essential role of interleukin-15 receptor in normal anxiety behavior. *Brain Behav Immun.* 2010a; 24:1340–1346. [PubMed: 20600810]
- Wu X, Kastin AJ, Hsueh H, Pan W. The effects of IL2Rgamma knockout on depression and contextual memory. *Behav Brain Res.* 2010b; 213:319–322. [PubMed: 20438766]

A

B

C

Fig.1.

POKO mice (n = 7) showed hypersomnolence in comparison with their WT littermates (n = 8). They spent more time in NREM and less time in wake during 24 h (A). The changes were most apparent in the 12 h light span (B). During the dark span, there were increased REM bouts (C).

A

B

C

Fig.2. Segmental plots at 2 h intervals showed that POKO mice ($n = 7$) had more NREM (A) and less waking (B), but no significant change in REM (C) in comparison with the group of WT mice ($n = 8$).

A

B

C

Fig.3.

The %NREM sleep correlated with body weight in POKO mice ($r = 0.84$, $p < 0.05$) and to a lesser extent also in WT mice ($r = 0.71$, $p < 0.05$) (A). The %REM did not show strong correlation with body weight in either group (B). The %wake in 24 h and body weight showed a reverse correlation in POKO mice ($r = 0.84$, $p < 0.05$) as well as WT mice ($r = 0.71$, $p < 0.05$) (C).

A

B

C

D

Fig.4.

The effect of the POKO mutation on open field performance. (A) POKO mice had decreased locomotor activity and increased defecation in comparison with WT. (B) The fat POKO mice had less activity than the lean POKO and WT mice, shown by activity in all planes reflecting a summation of the total grids traveled (activity in the horizontal plane) and rearing (activity in the z-plane). (C) Correlation of ambulatory activity and body weight showed an inversed relationship in the WT and fat POKO groups, but was not apparent in the lean POKO group. (D) When the three groups were compared, the lean POKO mice showed less locomotor activity than the WT mice, whereas the fat POKO mice had a further reduction in comparison with the other two groups on most parameters. There was no difference in defecation between obese POKO and lean POKO. *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$.