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Restoring serotonergic homeostasis in the lateral hypothalamus rescues sleep disturbances induced by early-life obesity

Abbreviated title: **Early-life obesity programs sleep via serotonin**

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Abstract

Early life obesity predisposes to obesity in adulthood, a condition with broad medical implications including sleep disorders; the latter can exacerbate metabolic disturbances and disrupt cognitive and affective behaviors. In this study, we examined the long-term impact of transient peripubertal diet-induced obesity (ppDIO, induced between 4 and 10 weeks of age) on sleep-wake behavior in male mice. Electroencephalographic and electromyographic recordings revealed that ppDIO increases sleep during the active phase but reduces resting-phase sleep quality. This impaired sleep phenotype persisted for up to one year although animals were returned to a non-obesogenic diet from postnatal week 11 onwards. To better understand the mechanisms responsible for the ppDIO-induced alterations in sleep, we focused on the lateral hypothalamus (LH). Mice exposed to ppDIO did not show altered mRNA expression levels of orexin and melanin-concentrating hormone, two peptides that are important for sleep-wake behavior and food intake. On the other hand, the LH of ppDIO-exposed mice had reduced contents of serotonin, a neurotransmitter involved in both sleep-wake and satiety regulation. Interestingly, an acute peripheral injection of the satiety-signaling peptide YY 3-36 (PYY_{3-36}) increased serotonin turnover in the LH and ameliorated the ppDIO-induced sleep disturbances, suggesting the therapeutic potential of this peptide. These findings provide new insights into how sleep-wake behavior is programmed during early life and how peripheral and central signals are integrated to coordinate sleep.

Key words:

Sleep disorders, puberty, obesity, serotonin, PYY

54 **Significance Statement**

55 Adult physiology and behavior are strongly influenced by dynamic reorganization of the brain
56 during puberty. The present work shows that obesity during puberty leads to persistently
57 dysregulated patterns of sleep and wakefulness by blunting serotonergic signaling in the lateral
58 hypothalamus. It also shows that pharmacological mimicry of satiety with peptide YY_{3-36} can
59 reverse this neurochemical imbalance and acutely restore sleep composition. These findings add
60 insight into how innate behaviors such as feeding and sleep are integrated and suggest a novel
61 mechanism through which diet-induced obesity during puberty imposes its long-lasting effects on
62 sleep-wake behavior.
63

Introduction

Many aspects of adult physiology and behavior, including the timing, duration and depth of sleep, are determined by extensive reorganization of neural circuits during the peripubertal period (Spear, 2000; Hagenauer and Lee, 2013; Nelson et al., 2013). Rodent studies have suggested that even mild, transient environmental perturbations during this sensitive phase exert persistent effects on health and well-being (Vendruscolo et al., 2010; Patchev et al., 2014; Iniguez et al., 2015). Food preferences develop early in humans (Spear, 2000) and increase the propensity to become obese (Serdula et al., 1993; Spear, 2000), thereby raising the risk for acquiring serious conditions such as cardiovascular disease, type 2 diabetes and, affective and cognitive disorders (Alfaradhi and Ozanne, 2011; Kelsey et al., 2014; Balakumar et al., 2016). Importantly, obesity also triggers disturbed sleep-wake behavior, with poor sleep due to frequent nighttime awakening and daytime sleepiness being a common complaint in obese subjects (Vgontzas et al., 2008). It is also pertinent to note that sleep disturbances are strongly linked to impaired mood (Steiger and Kimura, 2010) and cognition (Havekes et al., 2015; Tarokh et al., 2016). The recognition of an association between sleep, metabolism and physical and mental health has spurred considerable interest recently (Dresler et al., 2014); however, little is known regarding the mechanisms underpinning these interactions or indeed about where and when they are initiated. Obese humans and mice both display sleep disturbances (Jenkins et al., 2006; Guan et al., 2008; Perron et al., 2015). Importantly, polysomnograms of electroencephalographic (EEG) and electromyographic (EMG) activity in both species allow distinction between shallow-to-deep, non-rapid eye movement (NREM) sleep (characterized by synchronized cortical neuronal activity, reflected in high delta power EEG) and REM sleep (low-conscious state despite high cortical activity) (Tobler, 1995). Moreover, quality of sleep, which often correlates negatively with obesity and ageing (Vgontzas et al., 2008; Steiger and Kimura, 2010) and which is reflected in high delta power during uninterrupted NREM sleep (Borbely, 1977), can be equally well assessed in humans and rodents.

Feeding rhythms are tightly coupled to sleep-wake bouts, and both behaviors occur in a circadian fashion (Challet, 2013). In this regard, sleep curtailment promotes food intake such as

intermittent “snacking” and raises the risk of obesity in humans and rodents (Markwald et al., 2013; Wang et al., 2014; Chaput and Dutil, 2016). On the other hand, food deprivation in rodents causes arousal and facilitates food-seeking behavior (Borbely, 1977). The effects of fasting are however abolished by ablation of orexin neurons in the lateral hypothalamus (LH) (Yamanaka et al., 2003a), a finding consistent with recent work demonstrating that signals converging in the LH contribute to the coordination of feeding and sleep-wake behavior (Bonnavion et al., 2016; Stuber and Wise, 2016). Complementary work demonstrated that neuropeptide secretion by the LH is subject to modulation by arousal- and satiety-promoting serotonergic (5-HTergic) inputs from the dorsal raphe nucleus (DR) (Yamanaka et al., 2003b; Saper et al., 2010; Ito et al., 2013; Chowdhury and Yamanaka, 2016). Together, these previous observations prompted the question of whether peripubertal obesity predisposes to impaired sleep quality in later life and if so, how altered LH function might serve as a conduit of the effects of early life challenges to metabolic status. Besides confirming our hypothesis that peripubertal diet-induced obesity (ppDIO) generates long-lasting impairments in sleep quality, we demonstrated that ppDIO-induced sleep disturbances are accompanied by reductions in the serotonin content of the LH. In addition, we show that a single peripheral injection of the satiety-signaling gut peptide, peptide YY (PYY_{3-36}), significantly ameliorates the aberrations in sleep patterns produced by ppDIO.

Materials and Methods

Animals. Male C57BL/6N mice, bred at the Max Planck Institute of Biochemistry (Martinsried, Germany), were obtained at 21 days of age and housed (4 mice/cage) under standard conditions. One group of mice (ppDIO; N = 65) was given *ad libitum* access to a high fat diet (HFD; 4.73 kcal/g, D12451, Research Diets, Inc., New Brunswick, NJ) for 6 weeks (postnatal weeks 4-10). A control group of mice (N = 68) were maintained on standard laboratory chow (2.85kcal/g, diet #1324, Altromin GmbH & Co. KG, Lage, Germany) throughout the experiment. All procedures on animals were approved by the Government of Upper Bavaria's Commission for the Care and Use of Laboratory Animals, Germany.

Diets. Composition of diets were as follows: High fat diet used in ppDIO paradigm from weeks 4-10 (HFD, 4.73 kcal/g) 45 % from fat, 20 % from protein and 35 % from carbohydrates; standard laboratory diet fed to controls and to ppDIO mice from 11 weeks of age onwards (2.84 kcal/g) 12.6 % from fat, 26.7 % from protein, 60.7 % from carbohydrates.

Animal housing. Mice were kept on a 12:12 light/dark cycle (lights on at 08:00, ZT 0) under constant temperature (24 ± 1°C) and relative humidity (50 ± 10%) in a sound-attenuated chamber. The animals were individually housed in custom-made Lucite® cages (transparent Lucite® walls, 35cm height; replaceable, grey Lucite® floor, 26cm x 26cm) from the time of surgeries and maintained under the same environmental conditions as before.

Surgery. Control and ppDIO mice, aged 8, 22 and 50 weeks, were implanted with four epidural EEG gold-wire electrodes under isoflurane/oxygen anesthesia. As previously described (Romanowski et al., 2010; Kumar et al., 2015), bilateral electrodes were placed on the frontal lobes (L: ± 1.7 mm, AP: + 1.5 mm from bregma) and parietal lobes (L: ± 3 mm from bregma, AP: + 1 mm from lambda), respectively. In addition, each mouse received 2 intramuscular (neck) gold-wire electrodes for EMG recordings. The lead wires of the EEG and EMG electrodes were connected to

an electric swivel through a flexible tether, as previously described (Kumar et al., 2015). The first baseline EEG and EMG recordings were made after a 14-d post-surgical recovery period in freely-behaving mice.

Sleep recording and analysis. Polysomnographic recordings were obtained using a LabVIEW-based acquisition system (National Instruments, Austin, TX, USA), customized for use in mice (EGErAVigilanz, SEA, Cologne, Germany) (Kumar et al., 2015). EEG and EMG signals were amplified (10,000×), and both filtered EEGs (0.25-64 Hz) and EMGs (175-1,000Hz, root mean square rectified) were digitized using a high-speed analog-to-digital converter (NI-USB-6343-X-series, National Instruments, Austin, TX, USA) at a sampling rate of 128 Hz.

Sleep data were processed by fast Fourier transform algorithmic analysis, and semiautomatic classification of particular sleep-wake vigilance states was achieved using the LabVIEW-based acquisition system (National Instruments). As described previously (Kimura et al., 2010), vigilance states were defined from polygraphic traces in consecutive 4 s epochs as wakefulness, rapid eye movement sleep (REM) and non-REM (NREM) sleep; these states were confirmed by visual inspection of the somnograms. Images of dynamic changes in spectral power densities were produced by averaging absolute spectrum data encompassing all vigilance states from individual animals (thirty consecutive 4-s epochs, i.e., per 2-min duration); 3-D plots were subsequently created using Origin® 8 software (OriginLab Corporation, Northampton, MA), as described previously (Jakubcakova et al., 2011). Slow-wave activity (SWA) was calculated from the absolute power of the frequency range between 0.5–4 Hz during NREM sleep per 0.25 Hz bins, and normalized to the individually averaged total EEG power of all vigilance states. Epochs containing artifacts were eliminated from the power spectral analysis. Sleep architecture was analyzed by quantifying the number of transitions from WAKE to NREMS, NREMS to REMS, REMS to WAKE and NREMS to WAKE in 6 h bins.

Brain tissue collection and processing. Mice were deeply anesthetized (isoflurane) and sacrificed by decapitation at ZT8 or ZT13 (± 15 minutes) following PYY_{3-36}/vehicle injections. Brains were carefully removed from the skull and snap-frozen in isopentane/dry ice and stored at -80°C

166 until further processing.

167 For *in situ* hybridization histochemistry (see below), 10 µm-thick coronal sections from areas
168 containing the lateral hypothalamus (LH) were cryo-cut at -20°C and mounted on SuperFrost Plus
169 slides (Menzel GmbH, Braunschweig, Germany).

170 For neurotransmitter and neuropeptide measurements (see below), cylindrical cubes (0.5 mm^3
171 tissue punches) containing the LH or dorsal raphe (DR) were obtained according to Palkovits
172 (Palkovits, 1973) (coordinates for LH: AP -1.3 mm, DV -4.8 mm, L ± 1.0 mm from bregma, and for
173 DR: AP -4.3 mm, DV -2.8 mm, L ± 0.5 mm from bregma).

174

175 ***In situ* hybridization histochemistry.** 10 µm cryo-cut sections of brains obtained from mice at
176 ZT8 were hybridized with ^{35}S-dATP-labeled oligonucleotide probes for *orexin*
177 (5'AGCAGCGTCACGGCGGCCCAGGGAACCTTTGTAG), *MCH*
178 (5'CAACATGGTCGGTAGACTCTTCCCAGCATACACCTGAGCATGTCAA), and *GAD67* (5'
179 CAGTCAACCAGGATCTGCTCCAGAGACTCGGGGTGGTCAGACAGCTCCA), as described
180 previously (Patchev et al., 2007). Autoradiograms were generated by exposure to
181 chemiluminescent film (BioMax MR; Kodak, Rochester, NY, USA) and digitized; signal intensity
182 (relative mRNA levels) was quantified by computer-assisted optical densitometry (ImageJ,
183 http://rsb.info.nih.gov/ij/) against a ^{14}C reference (range: 0-35 nCi/mg, ARC 0146, American
184 Radiolabeled Chemicals Inc, St. Louis, MO, USA). Signal intensities are given as nCi/mg.

185

186 **Monoamine assay.** Monoamines were extracted from the LH and DR using 0.1 M perchloric acid
187 (1:20 for LH and 1:10 for DR). The extracts were assayed for serotonin, dopamine, DOPAC, HVA,
188 3-MT and 5-HIAA using reverse-phase high-performance liquid chromatography (RP-HPLC) with
189 electrochemical detection (UltiMate3000 / CoulochemIII, ThermoFischer, USA), using citrate-
190 phosphate buffer (containing 8.5 % acetonitrile, pH 3) as the mobile phase, as described
191 previously (Anderzhanova et al., 2013). Monoamines were separated on an analytical column
192 (C18, 150 mm×3 mm, 3 µm, YMC Triart, YMC Europe GmbH, Germany) at a flow rate of 0.5
193 ml/min and detected at -175mV. The detection limit for all compounds of interest was between
194 0.032-0.050 nM. Peak areas were used to calculate monoamine concentrations by reference to an

external standard curve. Monoamine tissue contents are reported as µmol/mg protein [protein concentration of extracts determined by the Lowry Assay method (Lowry et al., 1951)]. To evaluate the acute effects of PYY_{3-36} treatment, 5-HT turnover was calculated as the ratio between the tissue levels of 5-HT and its major metabolite 5-HIAA (Le Moal and Simon, 1991).

Radioimmunoassay. A radioimmunoassay (RIA) kit (Phoenix Pharmaceuticals, Phoenix, AZ) was used to determine orexin A concentrations in bilateral perchloric acid-extracted LH tissue extracts (see "*Monoamine assay*", above). The limit of detection of the RIA was 10 pg/ml and inter- and intra-assay coefficients of variation were < 15 %.

Peptide YY_{3-36} administration. Subgroups of control and ppDIO mice were given a single i.p. injection of either PYY_{3-36} (H-6042, Bachem AG, Bubendorf, Switzerland) or vehicle (0.9 % NaCl) when 10 or 52 weeks of age. Previous (Akanmu et al., 2006) and our own pilot studies showed that the dose chosen (60 µg/kg) reduces food ingestion and induces NREMS in naïve adult rodents. Here, food intake was monitored at 1, 3 and 24 h post-injection and sleep EEG/EMG recordings were made for 24 h post-injection. Brain punches obtained from a separate group of control and ppDIO mice treated with vehicle or PYY_{3-36} for 1 h were used for determining neurotransmitter contents by RP-HPLC (see above).

Experimental design and statistical analysis. Primarily, 6 individual groups were set for studying and comparing the effects of transient HFD exposure during puberty (ppDIO *vs*. control group) on sleep parameters at three different time spans (10, 24, and 52 weeks of age). Weekly changes in body weight (g), hourly changes in 24-h sleep (percent per hour), the amount of time spent in sleep during ZT 13-18 (percent per 6 h), differences in SWA during baseline (in percent of total power) and after sleep deprivation (relative changes from baseline SWA in percent) were analyzed by two-way ANOVA, with the between factors age and diet. A multivariate ANOVA (MANOVA) was applied to evaluate the effects of age, diet (both between factors), and time (within factor) on vigilance transitions (counts) in 6 h-bins. Neurochemical parameters such as orexin mRNA expression or protein levels and tissue neurotransmitter contents, which might have been affected by the ppDIO

paradigm, were analyzed between only 2 age groups (10 and 52 weeks) using two-way ANOVA, with the between factors age and diet. To explore a therapeutic potentiality, the effects of peripherally administered PYY_{3-36} on food intake and sleep amount were compared with the counterpart group that received vehicle in10 and 52 weeks of age by two-way ANOVA (PYY_{3-36} vs vehicle treatment served as within factors, and diet or age as between factors). The effects of PYY_{3-36} *vs.* vehicle on neurotransmitter turnover were tested only in 10 week-old groups, and analyzed by two-way ANOVA, the included factors were diet (between) and treatment (within). The above ANOVA and MANOVA analyses were followed by Sidak's multiple comparisons test and Bonferroni *post-hoc* comparisons, respectively; a value of $P < 0.05$ was considered significant. To perform these statistical comparisons, GraphPad Prism (Version 6.01, GraphPad, San Diego, CA) and SPSS (SPSS 18, Chicago, IL) software were used. All depicted data are mean ± SEM.

Results

Transient exposure to an obesogenic diet during puberty results in persistent sleep impairments

The ppDIO paradigm [transient exposure of mice to an obesogenic high-fat diet (HFD) between the ages of 4 and 10 weeks] significantly increased body weight (BW) (Fig. 1A; $P < 0.01$, Sidak's post-hoc test; weight gain between 4 and 10 weeks of age in control mice: 15.49 ± 0.35 g *vs.* 18.04 ± 2.28 g in ppDIO animals). Reinstatement of the standard, non-obesogenic, diet resulted in an initial loss (weeks 11-14) of BW in ppDIO mice, but this was followed by consistently higher BW that were maintained for up to one year (Fig. 1A; diet effect: $F_{1,29} = 7.201$, $P < 0.05$, age effect: $F_{47,1363} = 485.4$, $P < 0.01$, two-way ANOVA). These BW profiles were matched by significant differences in BW-adjusted energy intake between ppDIO and control mice with the factors age and diet making significant contributions (inset in Fig. 1A; diet effect: $F_{1,6} = 8.932$, $P < 0.05$, age effect: $F_{46,276} = 447.2$, $P < 0.01$, diet-age interaction: $F_{46,276} = 17.91$, $P < 0.01$, two-way ANOVA). Notably, whereas the obesogenic diet led to an expected increase in caloric intake ($P < 0.01$, Sidak's post-hoc test) because of its high energy density and known hedonic properties, ppDIO mice showed an initial decrease in energy consumption when returned to standard diet (Fig. 1A inset; $P < 0.01$, weeks 11 -14, Sidak's post-hoc test, ppDIO *vs.* control mice).

Next, using standardized EEG/EMG recording-based classification of vigilance states, we here observed typical circadian sleep-wake profiles in control and ppDIO mice aged between 10 and 52 weeks (Fig. 1B). However, 10-week old ppDIO mice showed an increase in NREM sleep time between Zeitgeber time (ZT) 13-18 when control mice were displaying high levels of activity (wakefulness; $P < 0.01$, Sidak's post-hoc test, NREM sleep in 10-week old controls: 10.8 ± 1.51 % *vs.* in ppDIO mice: 21.0 ± 1.43 %); the aberrant NREM sleep in ppDIO animals was particularly conspicuous at ZT17 (Fig. 1B, upper left panel; Fig. 1C, left panel; $P < 0.05$, Sidak's post-hoc test). A similar trend for increased NREM sleep during ZT13-18 was observed in 24-week old ppDIO (Fig. 1B, middle left panel; Fig. 1C, left panel; NREM sleep in 24-week old controls: 18.3 ± 2.47 % *vs.* ppDIO mice: 24.1 ± 2.75 %). At 52 weeks of age, NREM sleep duration between ZT13 and

ZT18 was significantly greater in the ppDIO group than in control mice (Fig. 1B, lower left panel; Fig. 1C, left panel; $P < 0.05$, Sidak's post-hoc test; NREM sleep in controls: 20.8 ± 2.18 % *vs.* ppDIO mice: 28.7 ± 2.77 %). However, whereas both ppDIO and control mice displayed age-related increases in NREM sleep during ZT13-18 (Fig. 1C, left panel; age effect: $F_{2,61} = 9.53$, $P < 0.01$, two-way ANOVA), the ppDIO group showed consistently greater amounts of NREM sleep as compared to the controls (diet effect: $F_{1,61} = 20.40$, $P < 0.01$, two-way ANOVA).

At 10, 24 and 52 weeks of age, control and ppDIO mice showed highly similar circadian REM sleep profiles (Fig. 1B, right panels). However, consistent with the corresponding patterns of NREM sleep, ppDIO animals spent more time in REM sleep during ZT13-ZT18, as compared to controls (Fig. 1C, right panel; diet effect: $F_{1,61} = 7.29$, $P < 0.01$, two-way ANOVA; Sidak's post-hoc comparisons: n.s.). Nevertheless, the REM/NREM sleep ratios did not differ between control and ppDIO mice at any of the ages studied (data not shown).

Sleep architecture, judged by the frequency of transitions between different states of vigilance, differed significantly between 10-week old control and ppDIO mice. Specifically, stability of sleep was poorer in the ppDIO group; in these animals, NREM sleep was frequently interrupted by bouts of wakefulness (Fig. 2A, upper panels, $P < 0.01$, Bonferroni post-hoc comparisons). However, the difference in sleep-wake architecture between control and ppDIO mice was diminished when animals were 1-year old (Fig. 2A, lower panels); this could be ascribed to age-dependent increases in the number of NREM→WAKE and WAKE→NREM transitions in control mice (Fig. 2A, lower panels, diet-age-time interaction: NREM→WAKE transitions: $F_{1,46} = 9.266$, $P < 0.01$; WAKE→NREM transitions: $F_{1,46} = 9.599$, $P < 0.01$, MANOVA). In contrast, no differences in switches from NREM→REM and REM→WAKE were found between ppDIO and control mice, regardless of age (data not shown). These observations show that the disruptive effects of ppDIO on sleep continuity are greater in peripubertal *vs.* mature mice.

Sleep demand (homeostatic sleep pressure) accumulates during periods of wakefulness and can be assessed by measurements of slow-wave activity (SWA) during NREM sleep; SWA, which peaks at the onset of sleep and decreases as sleep progresses, provides an index of sleep depth and serves as an objective indicator of sleep quality (Borbely et al., 1989). As shown in

representative EEG power spectrograms (Fig. 2B), the power density within the lower frequency bands (ca. 0.5 – 8 Hz) during NREM sleep bouts was weaker in mice that had been exposed to ppDIO, as compared to age-matched control animals. Indeed, markedly reduced SWA was observed in ppDIO mice from as early as 10 weeks of age (Fig. 2C, upper panel; diet effect: $F_{1,24}$ = 4.869, $P < 0.05$, two-way ANOVA). Those signs of impaired sleep quality were evident for at least 42 weeks after termination of the ppDIO paradigm when mice were aged 52 weeks (Fig. 2C, lower panel; diet-time interaction: $F_{11,176}$ = 1.895, $P < 0.05$,two-way ANOVA), being most pronounced at the onset of the resting phase (ZT2, $P < 0.05$, Sidak's post-hoc test).

We next investigated whether ppDIO has a detrimental impact upon sleep homeostasis during recovery from sleep deprivation (6 h of forced wakefulness during the resting phase). Sleep deprivation similarly increased SWA (Fig, 2D) as well as the amount of rebound sleep NREM and REM sleep time in both, ppDIO and control mice (data not shown). Notably, the magnitude of "catch-up" sleep and the relative change in SWA after sleep deprivation did not differ significantly between peripubertal (10-week old) and adult (52-week old) ppDIO *vs.* control mice (data not shown and Fig. 2D); these data indicate that homeostatic sleep pressure is not influenced by ppDIO.

Hypothalamic correlates of ppDIO-associated changes in sleep

Given the coincidence in the timing of body weight gain and altered sleep patterns (increased amount of nocturnal sleep and decreased sleep quality) in ppDIO mice, we next asked whether changes in the activity of the lateral hypothalamus (LH) might be responsible for the impact of ppDIO on feeding and sleep. It is pertinent to note here that, the LH serves as an integratory hub for both behaviors (Bonnavion et al., 2016). Briefly, hunger and satiety signals are chiefly relayed to the LH from the arcuate nucleus (Arc) (Elias et al., 1998; Schwartz et al., 2000), and feeding is ultimately stimulated following activation of orexin-, melanin-concentrating hormone (MCH)- and γ-aminobutyric acid (GABA)-producing neurons in the LH (Qu et al., 1996; Lubkin and Stricker-Krongrad, 1998; Jennings et al., 2013). Moreover, while LH orexin and GABA have been shown to facilitate wakefulness (Adamantidis et al., 2007; Herrera et al., 2016), MCH is known to promote NREM and REM sleep (Jego et al., 2013; Konadhode et al., 2013). At ZT8, orexin mRNA levels in

the LH did not differ between young (10-week old) and adult (52-week old) ppDIO mice (Fig.3A, left panel). However, there was an age-related reduction in the LH content of orexin (Fig. 3A, middle panel, age effect: $F_{1,35}$ = 13.02, P < 0.01, two-way ANOVA), a phenomenon that was more pronounced in control animals (P < 0.01, Sidak's post-hoc test). Neither ppDIO nor age influenced mRNA expression of glutamic acid decarboxylase 67 [GAD67, the rate-limiting enzyme in GABA biosynthesis: 10-week old mice, 0.41 ± 0.02 (control) *vs.* 0.41 ± 0.01 nCi/mg (ppDIO); 52-week old mice, 0.39 ± 0.02 (control) *vs.* 0.39 ± 0.01 nCi/mg (ppDIO)] or MCH mRNA levels (Fig. 3A, right panel) in the LH.

Since the LH is densely innervated by serotonergic (5-HT) and dopaminergic (DA) fibers from the dorsal raphe (DR) and ventral tegmental area (VTA), respectively, and 5-HT and DA are implicated in the induction of satiety and wakefulness (Hoebel et al., 1989; Schwartz et al., 1989; Dzirasa et al., 2006; Nonogaki, 2012; Ito et al., 2013), we next analyzed 5-HT and DA levels in the LH. Whereas the LH content of DA was not affected by ppDIO exposure [10-week old mice, 9.35 ± 1.49 (control) *vs.* 9.34 ± 0.99 μmol/μg protein (ppDIO); 52-week old mice, 8.39 ± 1.27 (control) *vs.* 7.00 ± 1.19 μmol/μg protein (ppDIO)], that of 5-HT was significantly reduced in ppDIO mice aged 10 and 52 weeks (Fig. 3B, left panel; diet effect: $F_{1,34}$ = 10.61, P < 0.01, two-way ANOVA; Sidak's multiple comparisons post-hoc test: 10-week old controls *vs.* ppDIO mice: P < 0.05; 52-week old control *vs.* ppDIO mice: n.s.). However, the DR content of 5-HT did not differ between ppDIO and control mice (Fig. 3C, diet effect: $F_{1,28}$ = 2.28, P > 0.05; age effect: $F_{1,28}$ = 0.63, P > 0.05), suggesting that reduced 5-HTergic tone in the LH contributes to the aberrations in the sleep-wake patterns observed in ppDIO mice.

Rescue of excessive nocturnal sleep in ppDIO mice by peptide YY_{3-36}

Given that the satiety-inducing effects of 5-HT in the LH are most likely modulated by satiety signals from the periphery (Elias et al., 1999), we hypothesized that ppDIO may interfere with the magnitude or efficacy of the latter. To test this, we mimicked satiety by injection of the potent and rapidly-acting satiety peptide PYY_{3-36} (60 μg/kg, i.p.) (Batterham et al., 2002, 2003; Karra et al., 2009) at ZT12 (Fig. 3B, right panel) and measured 5-HT [and its metabolite 5-hydroxyindoleacetic

348 acid (5-HIAA)] and DA [and its metabolites 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic
349 acid (HVA) and 3-methoxytyramine (3-MT)] levels in the LH of control and ppDIO mice 1 h after
350 injecting PYY_{3-36}. While PYY_{3-36} produced only a tendential increase in DA turnover (data not
351 shown), the peptide significantly increased 5-HT turnover in the LH (Fig. 3B, right panel; treatment
352 effect: $F_{1,26} = 5.609$, $P < 0.05$, two-way ANOVA) of ppDIO mice ($P < 0.05$, Sidak's post-hoc test), as
353 compared to controls.

354 Strikingly, the acute application of PYY_{3-36} reversed the sleep impairments observed in
355 ppDIO mice (Fig. 3D). Specifically, PYY_{3-36} increased NREM sleep time in control mice during the
356 first hour post-injection, independently of age (treatment effect in controls: $F_{1,17} = 9.792$, $P < 0.01$,
357 two-way ANOVA; Sidak's post-hoc test: 10-week old controls: n.s., 52-week old controls: $P < 0.05$);
358 in contrast, PYY_{3-36} reduced NREM sleep time in ppDIO mice (Fig. 3D; treatment effect in ppDIO
359 mice: $F_{1,15} = 12.75$, $P < 0.01$, two-way ANOVA; Sidak's post-hoc test: 10-week old ppDIO mice: $P <$
360 0.05, 52-week old ppDIO mice: n.s.). Thus, dietary history (standard chow *vs.* ppDIO, diet-
361 treatment interaction: $F_{3,32} = 7.309$, $P < 0.01$, two-way ANOVA) clearly plays a role in determining
362 sleep duration. Further, PYY_{3-36} reversed the aberrant distribution of NREM sleep in adult animals
363 that had experienced the ppDIO paradigm; however this single treatment did not elicit prolonged
364 effects on sleep quality (SWA and state-transition frequency) during the subsequent light period.

Discussion

Neural circuits undergo continuous remodeling under the influence of inherent developmental programs and external environmental stimuli. Reorganization reaches a peak during puberty, with recent research suggesting that peripubertal experiences can determine lifetime behavior and health trajectories (Blakemore, 2012). Sleep, a complex behavior regulated through intricate interactions between different brain areas, neurotransmitters, neuropeptides and metabolic signals, and modulated by a variety of external cues and Zeitgebers, plays an essential role in maintaining physiological and behavioral homeostasis (Roenneberg and Merrow, 2007; Saper et al., 2010; Shukla and Basheer, 2016).

The present study demonstrates that transient exposure to an obesogenic diet during peripuberty (ppDIO) triggers profound and long-lasting alterations in sleep depth, duration, and distribution. Similar aberrations in sleep have been previously reported after DIO in adult rodents (Jenkins et al., 2006; Guan et al., 2008). However, whereas the disturbed sleep phenotype that follows DIO during adulthood is reversible by induction of weight loss (e.g. through re-exposure to standard diet) (Guan et al., 2008; Perron et al., 2015), the present results show that the effects of transient DIO during the peripubertal period on body weight and sleep disturbances persist throughout life. Analysis revealed that ppDIO impairs sleep quality by increasing sleep fragmentation and lowering SWA followed by a compensatory increase in sleep during the nocturnal (active) phase. The sleep phenotype of ppDIO mice resembles that seen in obese humans who report daytime sleepiness and the frequent occurrence of arousal during normal sleep time (Vgontzas et al., 2008); thus, these observations may have wider implications because sleep quality is known to impact on cognitive and affective functions (Steiger and Kimura, 2010; Roth, 2015).

Our search for mechanisms that might explain the impaired sleep phenotype expressed by ppDIO mice was based on the postulate that ppDIO induces robust rewiring of neuronal circuits that regulate and link metabolism and sleep regulation. We initially considered the orexinergic system as a likely target of ppDIO, because orexin neurons in the LH innervate brain regions involved in the regulation of both arousal and feeding (Sakurai, 2005; Adamantidis and de Lecea,

2008). Previous studies suggested an inverse relationship between orexin mRNA expression and peptide levels in the LH and the amount of NREM sleep in adult DIO mice (Kohsaka et al., 2007; Tanno et al., 2013). Since we did not detect altered orexin expression in ppDIO mice, we next asked, does ppDIO modulate MCH and/or GABA expression in the LH? The LH is known to contain both, MCH and GABA neurons, which exert reciprocal control over metabolism and sleep. Specifically, enhanced GABA activity in the LH induces arousal from NREM sleep and elicits feeding (Jennings et al., 2015; Herrera et al., 2016), while activation of MCH neurons in the LH increases meal size and counters the wake-promoting actions of orexin (Kowalski et al., 2004; Jego et al., 2013; Konadhode et al., 2013; Apergis-Schoute et al., 2015). However, lack of altered expression of MCH and GAD67 (catalyzes decarboxylation of glutamate to GABA) mRNA in ppDIO mice suggested that neuromodulators other than MCH/GAD67 might be responsible for the impaired sleep displayed by these animals. In addition to MCH/GAD67 and orexin, the LH contains a separate population of GABAergic [vesicular GABA transport (Vgat)-expressing] neurons, which support the actions of orexin by producing arousal and facilitating feeding (Jennings et al., 2015; Herrera et al., 2016). Moreover, glutamatergic, and dynorphin-expressing neurons are present in the LH, which are reported to oppose orexinergic function in reward-related tasks (Muschamp et al., 2014; Stamatakis et al., 2016); although the role of these latter neurons in sleep-wake regulation has yet to be defined, their possible involvement in obesity-associated sleep disturbances cannot be excluded.

Since 5-HT is suggested to dissociate feeding cues from wakefulness (Sakurai, 2005; Nonogaki, 2012), we subsequently examined whether ppDIO modulates the amount of 5-HT reaching the LH from the DR nucleus. While increased 5-HTergic tone is generally associated with wakefulness, it also induces satiety specifically by acting on the LH (Hoebel et al., 1989; Le Feuvre et al., 1991; Nonogaki, 2012; Ito et al., 2013). Although similar 5-HT levels were found in the DR of control and ppDIO mice, we observed markedly lower amounts of 5-HT in the LH of ppDIO *vs.* control animals. Since 5-HT is a potent signal of satiety (Schwartz et al., 1989; Le Feuvre et al., 1991; Kumar et al., 2007; Burke et al., 2014), the reduced 5-HTergic tone observed in the LH of both, peripubertal and adult ppDIO mice implies that ppDIO impairs the transduction of satiety signals, leading to persistent obesity. Further, given that 5-HT promotes wakefulness (Ito et al.,

2013), the reduced 5-HT-mediated signaling of satiety may plausibly be linked to the sleep phenotype of ppDIO animals.

Peripheral cues of satiety are centrally integrated into the regulatory mechanisms responsible for sleep-wake behavior (Shukla and Basheer, 2016). Since systemic levels of the gut-derived satiety peptide PYY_{3-36} are reduced in obese humans (Batterham et al., 2003; Karra et al., 2009), we hypothesized that mimicking a state of satiety with exogenous PYY_{3-36} would restore normal sleep-wake profiles in ppDIO mice. In agreement with previous findings in diet-induced obese adult rats (Reidelberger et al., 2008), acute peripheral administration of PYY_{3-36} was found to similarly suppress feeding in control and ppDIO mice. As predicted (Akanmu et al., 2006), PYY_{3-36} promoted sleep time in control animals. In contrast, injection with the same dose of PYY_{3-36} reduced the abnormally enhanced sleep displayed by ppDIO mice during the active phase of the light-dark cycle. Interestingly, the pharmacological intervention was accompanied by a contemporaneous increase in 5-HT turnover in the LH. Based on this result, we propose that PYY_{3-36} rescues the sleep impairment induced by ppDIO by augmenting 5-HT tone in the LH (see Fig. 4 for a hypothetical model). At present, the mechanism by which PYY_{3-36} affects 5-HT signaling in the LH is unknown. However, since PYY_{3-36} can cross the blood-brain barrier and act directly on Arc neurons (Nonaka et al., 2003), it is plausible that PYY_{3-36} signals are polysynaptically conveyed to the LH (Blevins et al., 2008) (green terminal in Fig. 4). Indeed, PYY_{3-36} likely suppresses the release of the orexigenic neuropeptide Y (NPY) after binding to Y2 receptors in the Arc (Grandt et al., 1992); note that NPY neurons in the Arc project to the LH (Elias et al., 1999) and that intra-LH injections of NPY were previously found to blunt 5-HT signaling locally (Shimizu and Bray, 1989). Pending the results of experiments to unravel the complex pathways involved, we presently suggest that PYY_{3-36}-induced increases in 5-HT release in the LH results from the relief of NPY inhibition of 5-HT activity. While it is also plausible that PYY_{3-36} directly modulates the activity of 5-HT neurons by binding to Y2 receptors in the DR (Dumont et al., 1996; Nectow et al., 2017), the latter mechanism seems unlikely since the current study found no enhancement of DR 5-HT turnover by peripherally administered PYY_{3-36}.

An additional important finding in this study was that PYY_{3-36} reversed the “excessive daytime sleepiness” displayed by ppDIO mice, suggesting that the therapeutic potential of PYY_{3-36}

452 to reset diet-induced sleep disturbances may be worthy to exploration. Furthermore, our results
453 demonstrate that the window of time for interventions aimed at reversing the detrimental effects of
454 ppDIO is wide, possibly extending over the whole lifespan.

455 In summary, this study shows that transient DIO during peripubertal development
456 predestinates mice for lifetime obesity and dysregulated sleep-wake behavior by affecting the 5-
457 HT-mediated relay of peripheral signals of satiety to the LH. This work also demonstrates that
458 pharmacological mimicry of satiety, by an acute injection of PYY_{3-36}, can reverse the neurochemical
459 imbalance induced by peripubertal obesity and re-establish normophysiological sleep-wake cycles.
460 Interestingly, the pharmacological intervention is efficient even if introduced months after the onset
461 of adult obesity.

462 **References**

463

464 Adamantidis A, de Lecea L (2008) Sleep and metabolism: shared circuits, new connections.
465 Trends Endocrinol Metab 19:362–370.

466 Adamantidis AR, Zhang F, Aravanis AM, Deisseroth K, de Lecea L (2007) Neural substrates of
467 awakening probed with optogenetic control of hypocretin neurons. Nature 450:420–424.

468 Akanmu MA, Ukponmwan OE, Katayama Y, Honda K (2006) Neuropeptide-Y Y2-receptor agonist,
469 PYY3-36 promotes non-rapid eye movement sleep in rat. Neurosci Res 54:165–170.

470 Alfaradhi MZ, Ozanne SE (2011) Developmental programming in response to maternal
471 overnutrition. Front Genet 2:27.

472 Anderzhanova EA, Bachli H, Buneeva OA, Narkevich VB, Medvedev AE, Thoeringer CK, Wotjak
473 CT, Kudrin VS (2013) Strain differences in profiles of dopaminergic neurotransmission in the
474 prefrontal cortex of the BALB/C vs. C57Bl/6 mice: consequences of stress and afobazole. Eur
475 J Pharmacol 708:95–104.

476 Apergis-Schoute J, Iordanidou P, Faure C, Jego S, Schone C, Aitta-Aho T, Adamantidis A,
477 Burdakov D (2015) Optogenetic evidence for inhibitory signaling from orexin to MCH neurons
478 via local microcircuits. J Neurosci 35:5435–5441.

479 Balakumar P, Maung-U K, Jagadeesh G (2016) Prevalence and prevention of cardiovascular
480 disease and diabetes mellitus. Pharmacol Res 113:600–609.

481 Batterham RL, Cohen MA, Ellis SM, Le Roux CW, Withers DJ, Frost GS, Ghatei MA, Bloom SR
482 (2003) Inhibition of food intake in obese subjects by peptide YY3-36. N Engl J Med 349:941–
483 948.

484 Batterham RL, Cowley MA, Small CJ, Herzog H, Cohen MA, Dakin CL, Wren AM, Brynes AE, Low
485 MJ, Ghatei MA, Cone RD, Bloom SR (2002) Gut hormone PYY(3-36) physiologically inhibits
486 food intake. Nature 418:650–654.

487 Blakemore S-J (2012) Imaging brain development: the adolescent brain. Neuroimage 61:397–406.

488 Blevins JE, Chelikani PK, Haver AC, Reidelberger RD (2008) PYY(3-36) induces Fos in the
489 arcuate nucleus and in both catecholaminergic and non-catecholaminergic neurons in the
490 nucleus tractus solitarius of rats. Peptides 29:112–119.

491 Bonnavion P, Mickelsen LE, Fujita A, de Lecea L, Jackson AC (2016) Hubs and spokes of the
492 lateral hypothalamus: cell types, circuits and behaviour. J Physiol.

493 Borbely AA (1977) Sleep in the rat during food deprivation and subsequent restitution of food. Brain
494 Res 124:457–471.

495 Borbely AA, Achermann P, Trachsel L, Tobler I (1989) Sleep initiation and initial sleep intensity:
496 interactions of homeostatic and circadian mechanisms. J Biol Rhythms 4:149–160.

497 Burke LK, Doslikova B, D'Agostino G, Garfield AS, Farooq G, Burdakov D, Low MJ, Rubinstein M,
498 Evans ML, Billups B, Heisler LK (2014) 5-HT obesity medication efficacy via POMC activation
499 is maintained during aging. Endocrinology 155:3732–3738.

500 Challet E (2013) Circadian clocks, food intake, and metabolism. Prog Mol Biol Transl Sci 119:105–
501 135.

502 Chaput J-P, Dutil C (2016) Lack of sleep as a contributor to obesity in adolescents: impacts on
503 eating and activity behaviors. Int J Behav Nutr Phys Act 13:103.

504 Chowdhury S, Yamanaka A (2016) Optogenetic activation of serotonergic terminals facilitates
505 GABAergic inhibitory input to orexin/hypocretin neurons. Sci Rep 6:36039.

506 Dresler M, Spoormaker VI, Beitinger P, Czisch M, Kimura M, Steiger A, Holsboer F (2014)
507 Neuroscience-driven discovery and development of sleep therapeutics. Pharmacol Ther
508 141:300–334.

509 Dumont Y, Fournier A, St-Pierre S, Quirion R (1996) Autoradiographic distribution of
510 [125I]Leu31,Pro34]PYY and [125I]PYY3-36 binding sites in the rat brain evaluated with two
511 newly developed Y1 and Y2 receptor radioligands. Synapse 22:139–158.

512 Dzirasa K, Ribeiro S, Costa R, Santos LM, Lin S-C, Grosmark A, Sotnikova TD, Gainetdinov RR,
513 Caron MG, Nicolelis MAL (2006) Dopaminergic control of sleep-wake states. J Neurosci

26:10577–10589.

Elias CF, Aschkenasi C, Lee C, Kelly J, Ahima RS, Bjorbaek C, Flier JS, Saper CB, Elmquist JK (1999) Leptin differentially regulates NPY and POMC neurons projecting to the lateral hypothalamic area. Neuron 23:775–786.

Elias CF, Saper CB, Maratos-Flier E, Tritos NA, Lee C, Kelly J, Tatro JB, Hoffman GE, Ollmann MM, Barsh GS, Sakurai T, Yanagisawa M, Elmquist JK (1998) Chemically defined projections linking the mediobasal hypothalamus and the lateral hypothalamic area. J Comp Neurol 402:442–459.

Grandt D, Teyssen S, Schimiczek M, Reeve JRJ, Feth F, Rascher W, Hirche H, Singer M V, Layer P, Goebell H (1992) Novel generation of hormone receptor specificity by amino terminal processing of peptide YY. Biochem Biophys Res Commun 186:1299–1306.

Guan Z, Vgontzas AN, Bixler EO, Fang J (2008) Sleep is increased by weight gain and decreased by weight loss in mice. Sleep 31:627–633.

Hagenauer MH, Lee TM (2013) Adolescent sleep patterns in humans and laboratory animals. Horm Behav 64:270–279.

Havekes R, Meerlo P, Abel T (2015) Animal studies on the role of sleep in memory: from behavioral performance to molecular mechanisms. Curr Top Behav Neurosci 25:183–206.

Herrera CG, Cadavieco MC, Jego S, Ponomarenko A, Korotkova T, Adamantidis A (2016) Hypothalamic feedforward inhibition of thalamocortical network controls arousal and consciousness. Nat Neurosci 19:290–298.

Hoebel BG, Hernandez L, Schwartz DH, Mark GP, Hunter GA (1989) Microdialysis studies of brain norepinephrine, serotonin, and dopamine release during ingestive behavior. Theoretical and clinical implications. Ann N Y Acad Sci 575:171–173.

Iniguez SD, Riggs LM, Nieto SJ, Wright KN, Zamora NN, Cruz B, Zavala AR, Robison AJ, Mazei-Robison MS (2015) Fluoxetine exposure during adolescence increases preference for cocaine in adulthood. Sci Rep 5:15009.

Ito H, Yanase M, Yamashita A, Kitabatake C, Hamada A, Suhara Y, Narita M, Ikegami D, Sakai H, Yamazaki M, Narita M (2013) Analysis of sleep disorders under pain using an optogenetic tool: possible involvement of the activation of dorsal raphe nucleus-serotonergic neurons. Mol Brain 6:59.

Jakubcakova V, Flachskamm C, Deussing JM, Kimura M (2011) Deficiency of corticotropin-releasing hormone type-2 receptor alters sleep responses to bacterial lipopolysaccharide in mice. Brain Behav Immun 25:1626–1636.

Jego S, Glasgow SD, Herrera CG, Ekstrand M, Reed SJ, Boyce R, Friedman J, Burdakov D, Adamantidis AR (2013) Optogenetic identification of a rapid eye movement sleep modulatory circuit in the hypothalamus. Nat Neurosci 16:1637–1643.

Jenkins JB, Omori T, Guan Z, Vgontzas AN, Bixler EO, Fang J (2006) Sleep is increased in mice with obesity induced by high-fat food. Physiol Behav 87:255–262.

Jennings JH, Rizzi G, Stamatakis AM, Ung RL, Stuber GD (2013) The inhibitory circuit architecture of the lateral hypothalamus orchestrates feeding. Science 341:1517–1521.

Jennings JH, Ung RL, Resendez SL, Stamatakis AM, Taylor JG, Huang J, Veleta K, Kantak PA, Aita M, Shilling-Scrivo K, Ramakrishnan C, Deisseroth K, Otte S, Stuber GD (2015) Visualizing hypothalamic network dynamics for appetitive and consummatory behaviors. Cell 160:516–527.

Karra E, Chandarana K, Batterham RL (2009) The role of peptide YY in appetite regulation and obesity. J Physiol 587:19–25.

Kelsey MM, Zaepfel A, Bjornstad P, Nadeau KJ (2014) Age-related consequences of childhood obesity. Gerontology 60:222–228.

Kimura M, Muller-Preuss P, Lu A, Wiesner E, Flachskamm C, Wurst W, Holsboer F, Deussing JM (2010) Conditional corticotropin-releasing hormone overexpression in the mouse forebrain enhances rapid eye movement sleep. Mol Psychiatry 15:154–165.

Kohsaka A, Laposky AD, Ramsey KM, Estrada C, Joshu C, Kobayashi Y, Turek FW, Bass J (2007)

566 High-fat diet disrupts behavioral and molecular circadian rhythms in mice. Cell Metab 6:414–
567 421.

568 Konadhode RR, Pelluru D, Blanco-Centurion C, Zayachkivsky A, Liu M, Uhde T, Glen WBJ, van
569 den Pol AN, Mulholland PJ, Shiromani PJ (2013) Optogenetic stimulation of MCH neurons
570 increases sleep. J Neurosci 33:10257–10263.

571 Kowalski TJ, Farley C, Cohen-Williams ME, Varty G, Spar BD (2004) Melanin-concentrating
572 hormone-1 receptor antagonism decreases feeding by reducing meal size. Eur J Pharmacol
573 497:41–47.

574 Kumar D, Dedic N, Flachskamm C, Voule S, Deussing JM, Kimura M (2015) Cacna1c (Cav1.2)
575 Modulates Electroencephalographic Rhythm and Rapid Eye Movement Sleep Recovery.
576 Sleep 38:1371–1380.

577 Kumar S, Szymusiak R, Bashir T, Rai S, McGinty D, Alam MN (2007) Effects of serotonin on
578 perifornical-lateral hypothalamic area neurons in rat. Eur J Neurosci 25:201–212.

579 Le Feuvre RA, Aisenthal L, Rothwell NJ (1991) Involvement of corticotrophin releasing factor
580 (CRF) in the thermogenic and anorexic actions of serotonin (5-HT) and related compounds.
581 Brain Res 555:245–250.

582 Le Moal M, Simon H (1991) Mesocorticolimbic dopaminergic network: functional and regulatory
583 roles. Physiol Rev 71:155–234.

584 Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol
585 reagent. J Biol Chem 193:265–275.

586 Lubkin M, Stricker-Krongrad A (1998) Independent feeding and metabolic actions of orexins in
587 mice. Biochem Biophys Res Commun 253:241–245.

588 Markwald RR, Melanson EL, Smith MR, Higgins J, Perreault L, Eckel RH, Wright KPJ (2013)
589 Impact of insufficient sleep on total daily energy expenditure, food intake, and weight gain.
590 Proc Natl Acad Sci U S A 110:5695–5700.

591 Muschamp JW, Hollander JA, Thompson JL, Voren G, Hassinger LC, Onvani S, Kamenecka TM,

592 Borgland SL, Kenny PJ, Carlezon WAJ (2014) Hypocretin (orexin) facilitates reward by
593 attenuating the antireward effects of its cotransmitter dynorphin in ventral tegmental area.
594 Proc Natl Acad Sci U S A 111:E1648-55.

595 Nectow AR, Schneeberger M, Zhang H, Field BC, Renier N, Azevedo E, Patel B, Liang Y, Mitra S,
596 Tessier-Lavigne M, Han M-H, Friedman JM (2017) Identification of a Brainstem Circuit
597 Controlling Feeding. Cell 170:429–442.e11.

598 Nelson AB, Faraguna U, Zoltan JT, Tononi G, Cirelli C (2013) Sleep patterns and homeostatic
599 mechanisms in adolescent mice. Brain Sci 3:318–343.

600 Nonaka N, Shioda S, Niehoff ML, Banks WA (2003) Characterization of blood-brain barrier
601 permeability to PYY3-36 in the mouse. J Pharmacol Exp Ther 306:948–953.

602 Nonogaki K (2012) Serotonin conflict in sleep-feeding. Vitam Horm 89:223–239.

603 Palkovits M (1973) Isolated removal of hypothalamic or other brain nuclei of the rat. Brain Res
604 59:449–450.

605 Patchev A V, Fischer D, Wolf SS, Herkenham M, Gotz F, Gehin M, Chambon P, Patchev VK,
606 Almeida OFX (2007) Insidious adrenocortical insufficiency underlies neuroendocrine
607 dysregulation in TIF-2 deficient mice. FASEB J Off Publ Fed Am Soc Exp Biol 21:231–238.

608 Patchev A V, Rodrigues AJ, Sousa N, Spengler D, Almeida OFX (2014) The future is now: early life
609 events preset adult behaviour. Acta Physiol (Oxf) 210:46–57.

610 Paxinos G, Franklin KB (2001) The mouse brain in stereotaxic coordinates, 2nd ed. Academic
611 Press Inc.

612 Perron IJ, Pack AI, Veasey S (2015) Diet/Energy Balance Affect Sleep and Wakefulness
613 Independent of Body Weight. Sleep 38:1893–1903.

614 Qu D, Ludwig DS, Gammeltoft S, Piper M, Pelleymounter MA, Cullen MJ, Mathes WF, Przypek R,
615 Kanarek R, Maratos-Flier E (1996) A role for melanin-concentrating hormone in the central
616 regulation of feeding behaviour. Nature 380:243–247.

617 Reidelberger RD, Haver AC, Chelikani PK, Buescher JL (2008) Effects of different intermittent

peptide YY (3-36) dosing strategies on food intake, body weight, and adiposity in diet-induced obese rats. Am J Physiol Regul Integr Comp Physiol 295:R449-58.

Roenneberg T, Merrow M (2007) Entrainment of the human circadian clock. Cold Spring Harb Symp Quant Biol 72:293–299.

Romanowski CPN, Fenzl T, Flachskamm C, Wurst W, Holsboer F, Deussing JM, Kimura M (2010) Central deficiency of corticotropin-releasing hormone receptor type 1 (CRH-R1) abolishes effects of CRH on NREM but not on REM sleep in mice. Sleep 33:427–436.

Roth T (2015) Effects of excessive daytime sleepiness and fatigue on overall health and cognitive function. J Clin Psychiatry 76:e1145.

Sakurai T (2005) Roles of orexin/hypocretin in regulation of sleep/wakefulness and energy homeostasis. Sleep Med Rev 9:231–241.

Saper CB, Fuller PM, Pedersen NP, Lu J, Scammell TE (2010) Sleep State Switching. Neuron 68:1023–1042.

Schwartz DH, McClane S, Hernandez L, Hoebel BG (1989) Feeding increases extracellular serotonin in the lateral hypothalamus of the rat as measured by microdialysis. Brain Res 479:349–354.

Schwartz MW, Woods SC, Porte DJ, Seeley RJ, Baskin DG (2000) Central nervous system control of food intake. Nature 404:661–671.

Serdula MK, Ivery D, Coates RJ, Freedman DS, Williamson DF, Byers T (1993) Do obese children become obese adults? A review of the literature. Prev Med (Baltim) 22:167–177.

Shimizu H, Bray GA (1989) Effects of neuropeptide Y on norepinephrine and serotonin metabolism in rat hypothalamus in vivo. Brain Res Bull 22:945–950.

Shukla C, Basheer R (2016) Metabolic signals in sleep regulation: recent insights. Nat Sci Sleep 8:9–20.

Spear LP (2000) The adolescent brain and age-related behavioral manifestations. Neurosci Biobehav Rev 24:417–463.

644 Stamatakis AM, Van Swieten M, Basiri ML, Blair GA, Kantak P, Stuber GD (2016) Lateral
645 Hypothalamic Area Glutamatergic Neurons and Their Projections to the Lateral Habenula
646 Regulate Feeding and Reward. J Neurosci 36:302–311.

647 Steiger A, Kimura M (2010) Wake and sleep EEG provide biomarkers in depression. J Psychiatr
648 Res 44:242–252.

649 Stuber GD, Wise RA (2016) Lateral hypothalamic circuits for feeding and reward. Nat Neurosci
650 19:198–205.

651 Tanno S, Terao A, Okamatsu-Ogura Y, Kimura K (2013) Hypothalamic prepro-orexin mRNA level is
652 inversely correlated to the non-rapid eye movement sleep level in high-fat diet-induced obese
653 mice. Obes Res Clin Pract 7:e251-7.

654 Tarokh L, Saletin JM, Carskadon MA (2016) Sleep in adolescence: Physiology, cognition and
655 mental health. Neurosci Biobehav Rev 70:182–188.

656 Tobler I (1995) Is sleep fundamentally different between mammalian species? Behav Brain Res
657 69:35–41.

658 Vendruscolo LF, Gueye AB, Darnaudery M, Ahmed SH, Cador M (2010) Sugar overconsumption
659 during adolescence selectively alters motivation and reward function in adult rats. PLoS One
660 5:e9296.

661 Vgontzas AN, Bixler EO, Chrousos GP, Pejovic S (2008) Obesity and sleep disturbances:
662 meaningful sub-typing of obesity. Arch Physiol Biochem 114:224–236.

663 Wang Y, Carreras A, Lee S, Hakim F, Zhang SX, Nair D, Ye H, Gozal D (2014) Chronic sleep
664 fragmentation promotes obesity in young adult mice. Obesity (Silver Spring) 22:758–762.

665 Yamanaka A, Beuckmann CT, Willie JT, Hara J, Tsujino N, Mieda M, Tominaga M, Yagami K ichi,
666 Sugiyama F, Goto K, Yanagisawa M, Sakurai T (2003a) Hypothalamic orexin neurons regulate
667 arousal according to energy balance in mice. Neuron 38:701–713.

668 Yamanaka A, Muraki Y, Tsujino N, Goto K, Sakurai T (2003b) Regulation of orexin neurons by the
669 monoaminergic and cholinergic systems. Biochem Biophys Res Commun 303:120–129.

670 **Figure legends**

671

672 **Fig. 1. Peripubertal exposure to obesogenic food induces lifetime obesity and increased**
673 **nocturnal sleep. (A)** Body weights in ppDIO (N = 16) and control mice (N = 15) are given as mean
674 gram per week ± SEM. The six-week HFD exposure during peripuberty (postnatal weeks 4 to 10)
675 led to significantly enhanced weight gain in ppDIO mice as compared to controls, even after HFD
676 was replaced with the standard diet ($P < 0.05$). A dashed grey line indicates the end of HFD
677 exposure. Inset shows weekly body weight-adjusted energy intake in ppDIO and control mice,
678 shown as mean kcal/g body weight ± SEM. **(B)** Circadian dynamics of 24-hour NREM (*left panels*)
679 and REM sleep (*right panels*) in ppDIO and control mice during aging. Data points represent time
680 spent in NREM or REM sleep per hour in percentage ± SEM. Significant differences in sleep
681 patterns between 2 groups appeared during the first half of the dark period when the active phase
682 started ($P < 0.05$). White and black bars above the x-axis refer to the daily light-dark cycle. The
683 respective numbers of animals studied under each condition are shown in corresponding bars in
684 panel C. **(C)** Comparison of the amount of sleep during Zeitgeber time (ZT) 13-18 in different age
685 groups. Each bar represents averaged percentage time (± SEM) spent in NREM (*left panel*) or
686 REM sleep (*right panel*) between ZT13 and 18. Time spent in NREM sleep increased significantly
687 during aging (age effect; $P < 0.01$) and was consistently higher in ppDIO mice (diet effect; $P <$
688 0.01). Similarly, REM sleep was elevated in ppDIO mice (diet effect; $P < 0.01$). *$P < 0.05$

689

690 **Fig. 2. Comparison of changes in sleep architecture and homeostasis in control mice and**
691 **mice with a ppDIO history. (A)** Number (± SEM) of transitions from NREM sleep to wakefulness
692 (*left panel*) and from wakefulness to NREM sleep (*right panel*) in 10-week old (*upper panel*, ppDIO:
693 N = 17; control: N= 11) and 52-week old *(lower panel;* ppDIO: N = 10; control: N = 11) ppDIO and
694 control mice during 6 h bins. At 10 weeks of age, ppDIO mice displayed significantly more frequent
695 transitions as compared to control mice ($P < 0.01$), indicative of impaired sleep stability; such a
696 difference was not detected in mice aged 52 weeks (diet-age-time interaction: NREM→WAKE
697 transitions: $F_{1,46} = 9.266$, $P < 0.01$; WAKE→NREM transitions: $F_{1,46} = 9.599$, $P < 0.01$). **(B)**

Exemplary spectrograms with corresponding hypnograms of a 10-week old control (*upper panel*) and ppDIO mouse (*lower panel*) across a 24-hour baseline recording. As compared to controls, ppDIO mice displayed lower intensity of EEG power in the lower frequency bands during NREM sleep. **(C)** Hourly changes in SWA during NREM sleep under baseline conditions (% ± SEM; *left panel;* 10 week old ppDIO: N = 17; 10 week old control: N = 9; 52 week old ppDIO: N = 7; 52 week old control: N = 7*)* and **(D)** SWA responses to sleep deprivation through forced wakefulness (relative to the baseline, % ± SEM) in 10 week old (*upper panel;* ppDIO: N = 10; control: N = 4*)* and 52 week old (*lower panel;* ppDIO: N = 7; control: N = 7*)* mice. As compared to baseline SWA (a marker of sleep depth) in controls, SWA was significantly lower in 10 week old ($P < 0.05$) and 52 week old ppDIO mice ($P < 0.05$). However, homeostatic sleep pressure, tested here by forced waking, was accumulated to a similar extent in ppDIO and control mice. *$P < 0.05$.

Fig. 3. Reduced hypothalamic serotonin levels in ppDIO mice are rescued by PYY3-36 treatment. (A) Orexin (*left panel*) and MCH mRNA (*right panel*) and orexin A peptide (*middle panel*) levels in the LH of ppDIO and control mice aged 10 or 52 weeks. The inset (*left panel*) is an image of a brain section from a ppDIO mouse showing *in situ* hybridization signal for orexin mRNA and the corresponding anatomical reference image [adapted from Paxinos and Franklin(2001)]. **(B)** Comparison of serotonin (5-HT) contents in the LH of ppDIO and control mice (*left panel*). The content of 5-HT in the LH was significantly reduced in ppDIO-exposed animals ($P < 0.05$). An acute injection of PYY_{3-36} enhanced 5-HTergic tone (5-HT turnover) in the LH of 10-week old ppDIO mice (*middle panel*, $P < 0.05$). **(C)** 5-HT contents in the DR of ppDIO and control mice. Age and diet did not have any significant effect on this measure. **(D)** Treatment with PYY_{3-36} rectified the altered nocturnal sleep patterns for at least 1 h in ppDIO mice ($P < 0.05$); data are shown as mean percentage ± SEM in 10- (*left panel*) and 52- (*middle panel*) week old ppDIO and control mice. Treatment with PYY_{3-36} resulted in a significant reduction of food intake in all experimental groups (*right panel*). The dashed line (100%) represents the amount of food consumed by vehicle-treated ppDIO and control mice; measurements were made at 1 h post-injection of PYY_{3-36} or vehicle. Numbers of animals in each group are indicated in the bars. *$P < 0.05$ between diet groups, § $P <$ 0.05 between age groups.

Fig. 4. Schematic representation of how restoration of serotonergic (5-HTergic) tone in the lateral hypothalamus (LH) by peripherally administered PYY_{3-36} reverses the detrimental effects of ppDIO on sleep-wake behavior.

Sample hypnograms *(uppermost panel)* and hypothetical representations of 5-HT neurotransmission at synaptic terminals in the LH *(lower panel).* ***Left-hand panel:*** Normal sleep-wake patterns depend on adequate 5-HTergic innervation of LH neurons involved in the regulation of feeding and energy metabolism as well as sleep; among others, orexin neurons are a likely target of 5-HT originating in neurons located in the dorsal raphe. ***Middle panel:*** Exposure to ppDIO results in persistently blunted 5-HTergic stimulation of the LH and increased amount of inappropriately timed sleep, i.e. reduced wakefulness during the normal active phase, especially during the first half of the dark period (green frame). ***Right-hand panel:*** Exogenous (peripheral) administration of PYY_{3-36} to mimic a state of satiety rapidly delivers 5-HT to the LH and restores normal sleep-wake behavior in adult ppDIO mice. Since ppDIO likely results in reduced presynaptic levels of 5-HT, it is proposed that the effects of PYY_{3-36} stimulate the release and re-uptake of 5-HT in the LH. The effect of PYY_{3-36} on 5-HT levels in the LH is likely conveyed by intermediary neurons (identity presently unknown) rather than by diffusion of PYY_{3-36} to the LH, as indicated by the green terminal.