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Upregulation of Gene Expression in Reward-Modulatory Striatal Opioid Systems by Sleep Loss

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Epidemiological studies have shown a link between sleep loss and the obesity 'epidemic,' and several observations indicate that sleep curtailment engenders positive energy balance via increased palatable-food 'snacking.' These effects suggest alterations in reward-modulatory brain systems. We explored the effects of 10 days of sleep deprivation in rats on the expression of striatal opioid peptide (OP) genes that subserve food motivation and hedonic reward, and compared effects with those seen in hypothalamic energy balance-regulatory systems. Sleep-deprived (Sleep-Dep) rats were compared with yoked forced-locomotion apparatus controls (App-Controls), food-restricted rats (Food-Restrict), and unmanipulated controls (Home-Cage). Detection of mRNA levels with *in situ* hybridization revealed a subregion-specific upregulation of striatal preproenkephalin and prodynorhin gene expression in the Sleep-Dep group relative to all other groups. Neuropeptide Y (NPY) gene expression in the hippocampal dentate gyrus and throughout neocortex was also robustly upregulated selectively in the Sleep-Dep group. In contrast, parallel gene expression changes were observed in the Sleep-Dep and Food-Restrict groups in hypothalamic energy-sensing systems (arcuate nucleus NPY was upregulated, and cocaine- and amphetamine-regulated transcript was downregulated), in alignment with leptin suppression in both groups. Together, these results reveal a novel set of sleep deprivation-induced transcriptional changes in reward-modulatory peptide systems, which are dissociable from the energy-balance perturbations of sleep loss or the potentially stressful effects of the forced-locomotion procedure. The recruitment of telencephalic food-reward systems may provide a feeding drive highly resistant to feedback control, which could engender obesity through the enhancement of palatable feeding.
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INTRODUCTION

The escalating social trend toward shortened average sleep duration may be contributing significantly to the obesity epidemic (Van Cauter and Knutson, 2008). Decreased sleep in humans is associated with weight gain, higher body mass index (BMI), greater body-fat percentage (Cappuccio *et al*, 2008; Patel and Hu, 2008), and impaired glucose metabolism (Hanlon and Van Cauter, 2011; Nedeltcheva *et al*, 2009). Remarkably, recent studies have shown that the positive energy-balance state incurred by sleep loss is accounted for by increased caloric intake outside of regular meals ('snacking'; Markwald *et al*, 2013), particularly on carbohydrate- or fat-enriched foods (Markwald *et al*, 2013; Nedeltcheva *et al*, 2009; Spiegel *et al*, 2004a; St-Onge *et al*, 2011; Weiss *et al*, 2010). There is much interest in identifying the physiological basis of these behavioral changes, and studies to date have focused mainly on peripheral endocrine systems (eg, leptin and ghrelin) that regulate feeding in conjunction with energy-balance fluctuations. In some human studies, leptin and ghrelin levels are altered in sleep loss in directions that promote food intake, and rodent studies have shown that sleep curtailment modulates hypothalamic peptides and other systems whose activity tracks these circulating hormones (Barf *et al*, 2012; Koban *et al*, 2006; Martins *et al*, 2010; Spiegel *et al*, 2004b).

Nevertheless, a recent study in humans showed that increased snacking in sleep loss could not be explained by changes in peripheral hunger or satiety hormones alone (Markwald *et al*, 2013), implicating systems that drive 'non-homeostatic' feeding; that is, excess feeding driven by motivational or cognitive processes. To date, however, the effects of sleep loss on neuromodulators governing the *hedonic* aspects of feeding, such as the opioid peptide (OP), enkephalin (ENK), are unknown. Stimulation of receptors for ENK within striatal subregions (particularly the nucleus

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accumbens (Acb)) augments the 'liking' of sweet tastes (Pecina and Berridge, 2005) and enhances the intake of sweet, and/or fat-rich foodstuffs (Woolley *et al*, 2006; Zhang *et al*, 1998)—the same types of food whose intake is augmented in sleep loss. Furthermore, endogenous ENK release in rat striatum accompanies the 'binge-like' intake of palatable food (Difeliceantonio *et al*, 2012), and striatal ENK gene expression is upregulated by palatable food-conditioned cues (Schiltz *et al*, 2007). ENK and related systems could therefore have an important role in the palatable or high-fat food snacking seen with sleep loss in humans, which appears to be a crucial factor promoting weight gain (Markwald *et al*, 2013; St-Onge *et al*, 2011).

Here, we investigated the effects of sleep deprivation in rats on gene expression in several feeding-related CNS peptide systems, chosen to provide points of contrast between telencephalic reward-modulatory substrates (including striatal OPs) and hypothalamic systems responsive to energy-balance status. Sleep deprivation was achieved using forced locomotion on a moving belt, automatically triggered during sleep episodes (Newman *et al*, 2009). We used two controls: a group to control for the sleep deprivation stimulus, in which rats were pair-yoked to sleep-deprived (Sleep-Dep) counterparts, with forced-locomotion epochs 'played back' during waking; and a food-restricted (Food-Restrict) group in which daily food allotments were calibrated to precisely match body weight changes of Sleep-Dep counterparts. The latter was done to tease apart effects of sleep loss from secondary effects arising from weight loss, which is commonly seen in rodent models of sleep deprivation (Newman *et al*, 2009; Rechtschaffen and Bergmann, 1995). We show a significant transcriptional upregulation of OPs in reward-related striatal regions following sleep loss, relative to both control manipulations. The implications of these findings for understanding reward-driven 'snacking' seen in sleep-restricted humans are discussed below.

MATERIALS AND METHODS

Subjects

Male Sprague–Dawley rats (Harlan Laboratories, Madison, WI) weighing 300–325 g were housed in pairs in clear cages with *ad libitum* access to food and water (except for certain experiments as described below) in a light- and temperature-controlled vivarium. They were maintained under a 12-h : 12-h light–dark cycle (lights on at 0700 hours). All facilities and procedures were in accordance with the guidelines regarding animal use and care from the US National Institutes of Health, and were supervised and approved by the Institutional Animal Care and Use Committee of the University of Wisconsin.

Conveyer-Over-Water (COW) Apparatus and Apparatus Control Procedure

The COW apparatus used here is similar in principle to the disk-over-water procedure, but uses motion detection rather than online EEG recording to trigger wake-inducing belt movements. Motion detection sleep deprivation with the COW previously been validated with EEG recording of sleep (Newman *et al*, 2009). A photograph of the COW apparatus is shown in Supplementary Figure S1 of Supplementary Information, and a detailed description is provided in Supplementary Materials and Methods. For apparatus control (App-Control) rats, the belt movements were played back from those recorded from a matched sleep-restricted animal, but the belt moved only when the control animal was awake. To minimize sleep disturbance in control rats, yet subject them to the same amount of belt rotation, the speed of the belt was speeded up by 50% and inter-stimuli intervals were decreased by one-third. Rats in the COWs were videotaped, and visual scoring of behavior was carried out daily on samples of video recording to ensure validity of motion detection-based sleep deprivation.

Food Restriction Procedure

Daily food allotment was adjusted to maintain a similar rate of weight loss as seen in the sleep-restricted rats. Rats in the Food-Restrict groups were housed in their home cages and never exposed to the COWs. The Food-Restrict and sleep-restricted groups were matched with regard to age and initial body weight. Rats' daily food allotments were adjusted so that the percentage of daily body weight loss matched the previous day's weight loss of the Sleep-Dep counterpart (see Supplementary Materials and Methods for further details).

Experimental Design

Two distinct experiments were carried out. Experiment 1 compared Sleep-Dep rats ('Sleep-Dep' group; $N=11$) with Food-Restrict rats ('Food-Restrict' group; $N=11$) that lost the same amount of weight. Experiment 2 compared Sleep-Dep rats ($N=15$) with forced-locomotion apparatus controls ('App-Controls' group; $N=15$). Home cage-housed rats ('Home-Cage' group) were included as controls in both experiments ($N=11$ for experiment 1; $N=17$ for experiment 2). In experiment 1, to maximize chances of a successful outcome, we used two 10-day sleep deprivation periods, with an in-between 10-day recovery period, based on the laboratory's past experience with such a protocol. In experiment 2, however, only one period of sleep deprivation was used because in experiment 1, we found this to be sufficient to robustly elevate feeding and we wanted to minimize the risk of subject drop-out because of mechanical problems with the COWs. We found no substantial differences between the two experiments with regard to the genes under study.

On the final day of the 10-day experimental period, rats of all groups were killed by decapitation under isoflurane anesthesia. Decapitations occurred between 1030 and 1200 hours; this was consistent across all groups for experiments 1 and 2. Their brains were rapidly dissected, flash frozen in $-20\,^{\circ}C$ 2-methylbutane, and stored at $-80\,^{\circ}C$ until cryostat sectioning. Trunk blood was also collected and processed at the time of killing; radioimmunoassays for plasma leptin and insulin were conducted according to standard techniques, as described in Supplementary Materials and Methods.

In Situ Hybridization

Brain slices were subjected to processing for *in situ* hybridization, according to standard methods (details are given in the Supplementary Materials and methods, including primers for probe generation in Supplementary Table S1). After processing, sections were exposed to a phosphorimager screen for 1–14 days, depending on intensity of the signal. The screen was scanned on a Typhoon scanner and quantification of average optical density in particular brain regions was performed using ImageQuant 5.2 software (Molecular Dynamics). Quantification of signal was standardized across rats using predetermined fixed-area shapes for each region of interest (ROI). Striatal ROIs are shown in Supplementary Figure S2. ROIs for neuropeptide Y (NPY) and cocaine- and amphetamine-regulated transcript (CART) are depicted in Figure 4.

Statistical Analyses

For the *in situ* hybridization data, the averaged pixel intensities for each region were analyzed by first performing a global two-factor mixed-design ANOVA (group × brain region). Following significance in this ANOVA, comparisons among the groups for each individual brain region was performed using one-factor (group) between-subjects ANOVAs.

RESULTS

Effects on OP Gene Expression in the Striatum

As shown in Figures 1 and 2, we observed a robust upregulation in proenkephalin (ENK) and prodynorphin (DYN) peptide gene expression in multiple striatal subregions in the Sleep-Dep group relative to the other groups (group × subregion interactions for ENK, experiment 1: $F(12, 203) = 10.2$, $P < 0.001$, ENK, experiment 2: $F(12, 308) = 3.7$, $P < 0.001$; DYN, experiment 1: $F(12, 175) = 1.9$, $P = 0.03$, experiment 2: $F(12, 308) = 6.2$; $P < 0.001$). Based on these results, subanalyses by striatal subregion for each gene separately were carried out and summarized in Supplementary Table S2. The most common effect observed was an increase in opioid precursor peptide mRNA levels in the Sleep-Dep group relative to all other groups, and the strong elevations in opioid gene expression seen in experiment 1 were replicated in experiment 2. The Acb core showed the strongest and most selective differentiation between the Sleep-Dep *vs* other groups both ENK and DYN, with increases in the Sleep-Dep group *vs* no effect in either the App-Control or Food-Restrict groups. Furthermore, the medial Acb shell, a region strongly implicated in μ-opioid-dependent reward modulation (Pecina and Berridge, 2005), displayed a highly selective upregulation of ENK expression in the Sleep-Dep group relative to the Food-Dep group. There was a small but significant increase in DYN in the medial Acb shell of App-Controls. Outside the Acb, the most common pattern was, again, for opioid gene expression to be significantly higher in the Sleep-Dep group relative to other groups. There were small but significant elevations of DYN in the ventrolateral and dorsal striatum of App-Control relative to Home-Cage rats; however, DYN upregulation was significantly greater in Sleep-Dep *vs* App-Control rats for these regions (see Supplementary Table S2).

The dissociation between weight loss *vs* sleep loss can be appreciated in the scatterplots of Figure 3. Within the same range of weight loss, the distribution of optical density values (the index of gene expression levels), particularly for ENK, is largely non-overlapping for the Food-Restrict and Sleep-Dep groups; moreover, it can be seen that the Food-Restrict group completely overlaps the Home-Cage group.

Neither ENK nor DYN expression was changed in any group in a control site, primary somatosensory cortex. Moreover, *c-fos* expression was not changed in any striatal area except DLS, where there was a small suppression in the App-Control group relative to the Sleep-Dep group ($F(3, 66) = 3.1$, $P < 0.04$). This observation indicates that the upregulation of ENK and DYN was not the nonspecific outcome of a global upregulation of all striatal gene expression. Nor were striatal opioid effects a reflection of generalized upregulation in all central feeding-modulatory systems, because expression of hypothalamic melanin concentrating hormone (MCH) was not changed in any group, and effects on hypocretin/orexin (H/O) were inconsistent: an upregulation in the Sleep-Dep group of experiment 1 ($F(2, 25) = 3.9$; $P < 0.04$), but not experiment 2 ($F(2, 44) = 1.6$; NS). MCH and H/O results are summarized in Supplementary Table S3.

Effects on NPY and CART Expression

As shown in Figure 4, sleep deprivation markedly augmented levels of mRNA for the NPY gene and suppressed levels for CART; these effects varied by anatomical region. For NPY, we analyzed the (1) the arcuate nucleus, (2) a sector of somatosensory cortex representative of all neocortex, and (3) dentate gyrus of the hippocampus where we noted a strikingly circumscribed signal in the Sleep-Dep rats (Figure 4, and Figure 3 of Supplementary Information). For NPY, there was a highly significant group × brain region interaction for experiment 1 ($F(4, 87) = 16.7$; $P < 0.001$) and experiment 2 ($F(4, 126) = 6.4$; $P < 0.001$). There were also significant effects for CART in an omnibus ANOVA, which incorporated the two brain regions in which CART was assayed, the arcuate nucleus and a hypothalamic ROI dorsal to the arcuate termed 'dorsal hypothalamus' for simplicity (group × brain region in experiment 1: $F(2, 50) = 18.3$; $P < 0.001$; in experiment 2: $F(2, 74) = 4.8$; $P = 0.01$).

Based on these interactions and multiple strong main effects, we conducted further analyses with one-way ANOVAs in the individual brain regions. Effects can be seen in the digital images of Figure 4, and are summarized in Supplementary Table S4. In the arcuate nucleus, sleep deprivation and food restriction (but not the App-Control manipulation) robustly upregulated NPY expression and downregulated CART expression. For CART, sleep loss-induced downregulation was also seen in dorsal hypothalamus. These results agree with literature indicating that hypothalamic NPY and CART are regulated in a reciprocal manner in negative energy balance (Hillebrand *et al*, 2002).

Levels of NPY mRNA in the dentate gyrus (Figure 4, Supplementary Figure S3) and in neocortex (the ROI was an

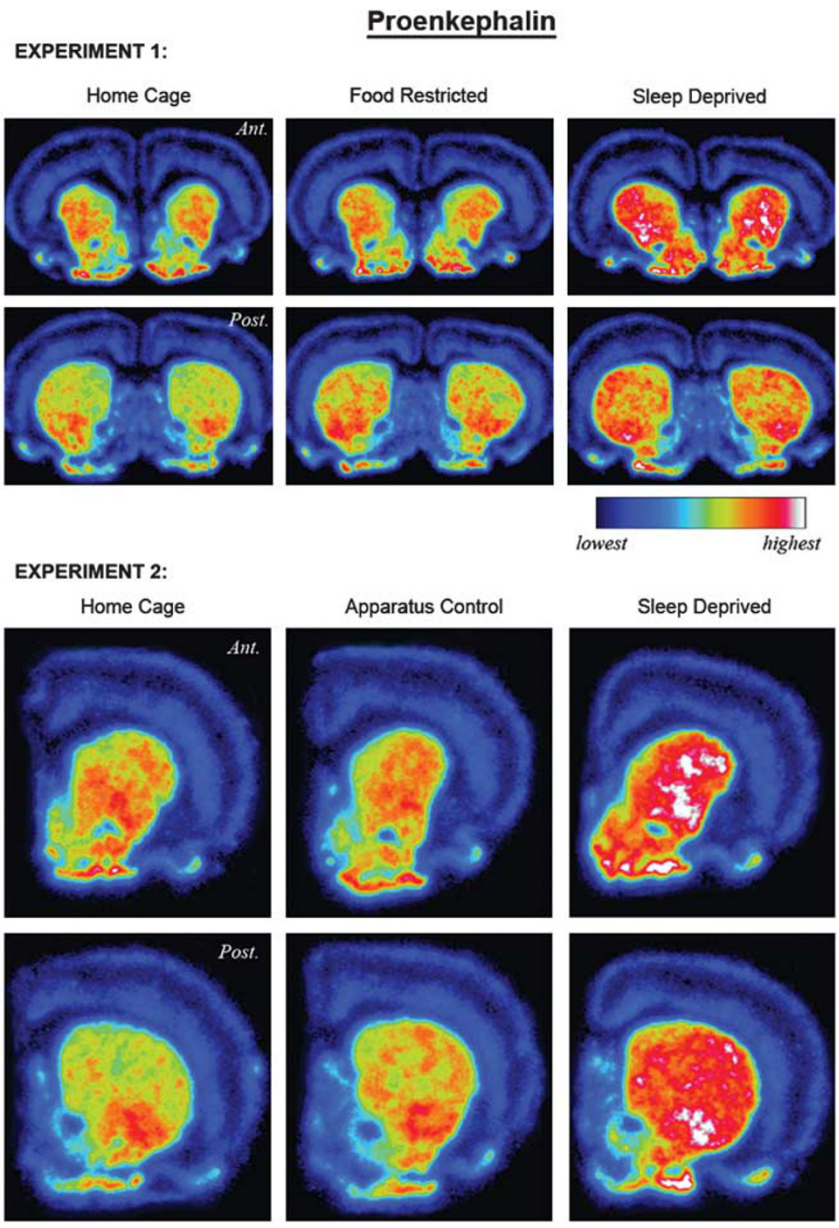

Figure 1 Pseudocolor images of coronal sections through two levels of the striatum (Ant., anterior; Post., posterior) from representative rats in each treatment group for each of the two experiments, showing proenkephalin expression. Color key depicts intensity range of labeling.

area in somatosensory cortex, but the effect was seen throughout neocortex) were upregulated in Sleep-Dep rats (dentate gyrus, experiment 1: $F(2, 30) = 29.6$; $P < 0.001$; experiment 2: $F(3, 65) = 31.6$; $P < 0.001$). For the neocortex, the effect was significant in experiment 2 ($F(3, 65) = 30.9$; $P < 0.001$), but missed significance in experiment 1 ($F(2, 29) = 2.2$, NS). These effects were completely restricted to the Sleep-Dep rats; no effects on NPY expression were seen in other groups.

Figure 5 shows scatterplots of gene expression *vs* weight loss in all groups for arcuate-localized NPY and hippocampal NPY. Arcuate nucleus gene expression levels are completely overlapping for the Food-Restrict and Sleep-Dep groups, but hippocampal gene expression values are completely non-overlapping, despite comparable weight loss in the two groups.

Sleep

Sleep epochs were reduced by $>85\%$ (see Supplementary Figure S4) during the 10-day sleep deprivation periods. There was little change in the percentage of sleep epochs for the App-Control rats. Accuracy of the machine scoring approached 90% relative to the human reviewers.

Changes in Food Intake, Body Weight, Plasma Insulin, and Leptin

Sleep-Dep and Food-Restrict rats exhibited almost identical degrees of mean daily weight loss throughout the 10-day experimental periods of experiment 1; mean daily weight change (g/day $\pm$ SEM) was -3.7 ± 0.2 for the Sleep-Dep group, and -3.9 ± 0.2 for the Food-Restrict group. The

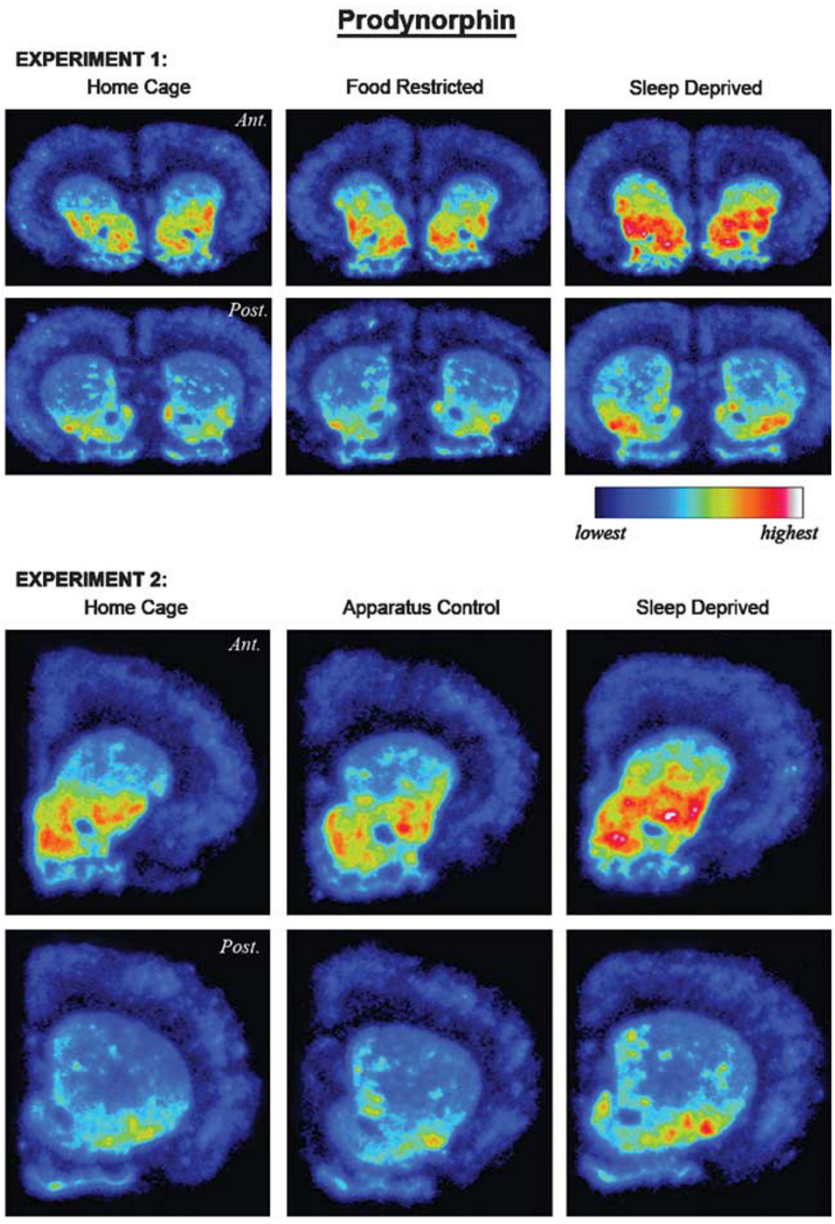

Figure 2 Pseudocolor images of coronal sections through two levels of the striatum (Ant., anterior; Post., posterior) from representative rats in each treatment group for each of the two experiments, showing prodynorphin expression. Color key depicts intensity range of labeling.

Sleep-Dep group of experiment 2 rats lost weight at about the same rate (-2.85 ± 0.2) as seen in experiment 1. App-Control rats gained weight throughout the 10-day experimental period (0.70 ± 0.2) at about the same rate as Home-Cage controls of experiment 1 (0.92 ± 0.2), but at a lesser rate than Home-cage controls of experiment 2 (2.0 ± 0.2). Furthermore, as shown previously (eg, Newman *et al*, 2009; Rechtschaffen and Bergmann, 1995), food intake escalated over the days of sleep deprivation (see Supplementary Figure S5).

Confirming prior results (Barf *et al*, 2012; Spiegel *et al*, 2004a, b), leptin levels were depressed in the Sleep-Dep rats, beyond the leptin suppression seen in Food-Restrict rats or the mild suppression seen in App-Controls. Insulin was also suppressed in the Sleep-Dep groups (experiment 1: insulin, $F(2, 29) = 8.3$, $P = 0.001$; leptin, $F(2, 29) = 29.4$, $P < 0.001$); experiment 2: insulin, $F(2, 44) = 6.7$, $P = 0.0028$; leptin, $F(2, 44) = 51.4$, $P < 0.0001$; see Supplementary Figure S6).

DISCUSSION

Summary

In this study, we found robust, sleep loss-associated transcriptional upregulation of telencephalic peptides that modulate higher-order motivation or learning processes—specifically, ENK and DYN throughout widespread areas of the striatum, and NPY in hippocampus. On an anatomical subregion basis, these effects were separable from weight loss or generalized perturbations of either hunger or the forced-locomotion sleep deprivation procedure. In contrast, in energy balance-regulatory systems of the hypothalamic

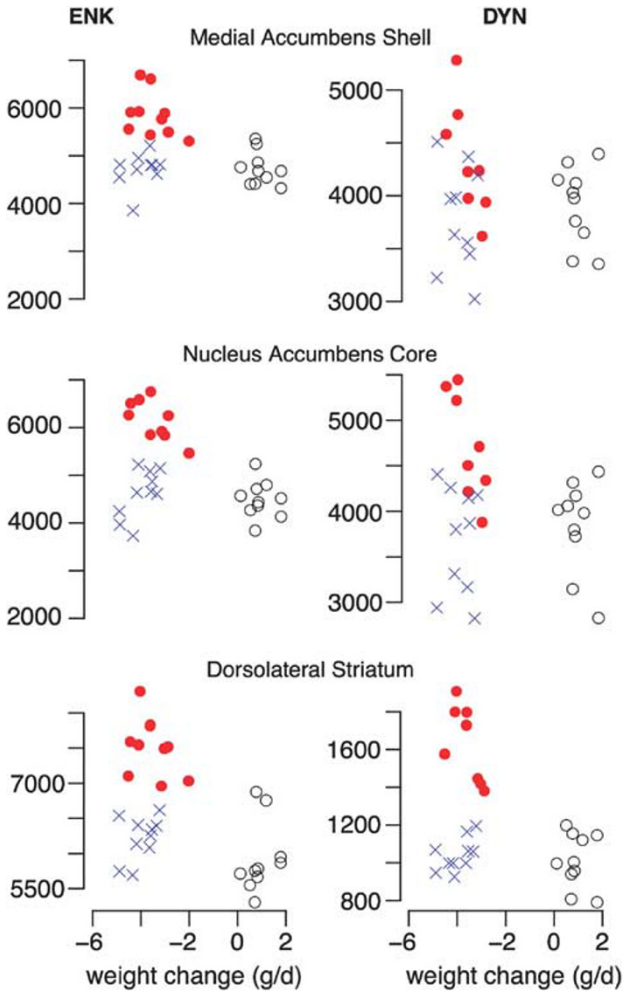

Figure 3 Scatterplots displaying gene expression levels × weight change for ENK and DYN in experiment 1, for individual subjects. The Y axes show optical density (arbitrary units), an index of gene expression levels. X axes show mean weight change over the second of two 10-day sleep restriction periods (see Materials and Methods for further details about the sleep deprivation protocol). Red circles, Sleep-Dep group; blue X's, Food-Restrict group; unfilled black circles, Home-Cage group.

arcuate nucleus, both sleep loss and food restriction altered transcriptional activity in parallel. The effects of sleep deprivation on striatal opioid systems, which are known to regulate palatable feeding, may help explain the 'junk-food snacking' seen with sleep curtailment in humans (Markwald *et al*, 2013; St-Onge *et al*, 2011; Weiss *et al*, 2010).

Comparing the Effects of Sleep Deprivation and Food Deprivation

The present results confirm prior work indicating that sleep loss in rodents engenders an endocrinological profile consistent with negative energy balance, in which rats lose weight and peripheral fat mass, and leptin levels decline along with concordant changes in feeding-regulatory peptide systems of the medial basal hypothalamus (Barf *et al*, 2012; Koban and Swinson, 2005; Koban *et al*, 2006; Newman *et al*, 2009; Rechtschaffen and Bergmann, 1995). In this sense, transcriptional changes in arcuate nucleus NPY and CART were not qualitatively different in sleep loss *vs* food restriction. Indeed, the degree to which arcuate nucleus-localized NPY gene expression was upregulated, and arcuate-localized CART downregulated, was similar between the two manipulations.

In numerous striatal subregions, however, the sleep loss-associated upregulation of ENK and DYN gene expression was not seen in Food-Restrict rats that lost the same amount of weight or in App-Control rats subjected to sleep deprivation stimuli during waking. Striatal ENK is modulated by motivational contingencies such as the expectation of scheduled feeding or contextual stimuli associated with palatable food, but not by food restriction (Schlitz *et al*, 2007; Will *et al*, 2007), and stimulation of striatal μ-opioid receptors (the cognate receptors for ENK) strongly enhances palatable feeding and hedonic taste reactivity (Baldo and Kelley, 2007; Pecina and Berridge, 2005; Woolley *et al*, 2006; Zhang *et al*, 1998; Zhang and Kelley, 2002). A recent study showed that surges of endogenously released striatal ENK act as signals to overeat palatable foods (Difeliceantonio *et al*, 2012). Accordingly, with regard to food motivation, fluctuations in striatal ENK expression have been hypothesized to track short-term motivational contingencies associated with feeding (Kelley *et al*, 2005; Will *et al*, 2007). This model proposes that striatal ENK downregulation is the 'off-switch' that signals recent completion of feeding. Importantly, ENK upregulation in Sleep-Dep rats was present despite the escalation of daily food intake, suggesting perhaps that sleep deprivation renders the striatal ENK system insensitive to negative feedback control (ie, the feeding 'switch' is stuck in the 'on' position). Striatal DYN expression was also upregulated in the Sleep-Dep rats, indicating that medium-spiny neurons are affected by sleep loss in both the 'direct' and 'indirect' striatal output pathways, for which DYN and ENK are cellular markers (Steiner and Gerfen, 1998). The upregulation of DYN further distinguishes the effects of food restriction and sleep deprivation; food restriction produces a small suppression of DYN expression in the Acb core (Haberny and Carr, 2005; Will *et al*, 2007; also see present work). Again, these observations support the idea that the upregulation of striatal opioid gene expression in the Sleep-Dep rats was independent of their energy-balance state. Indeed, it is possible that shorter sleep deprivation periods may affect striatal opioid systems without changing hypothalamic energy-sensing systems, a question that merits further study. The current results show, however, that sleep loss affects the transcriptional control of a telencephalic reward system in a manner dissociable from weight loss *per se*. Finally, the fact that sleep loss produced effects on striatal opioid systems beyond those seen with food restriction or yoked, forced locomotion in the COWs would suggest that the transcriptional upregulation of opioid systems cannot be explained as a consequence of stress alone; nevertheless, it will be interesting in future studies to directly compare the present regimen of sleep loss with other types and intensities of laboratory stressors (eg, restraint, shock-paired contexts) with regard to opioid expression.

In contrast to striatal opioid systems, transcriptional changes in H/O and MCH either did not occur or were inconsistent in this study. Although we partly replicated the

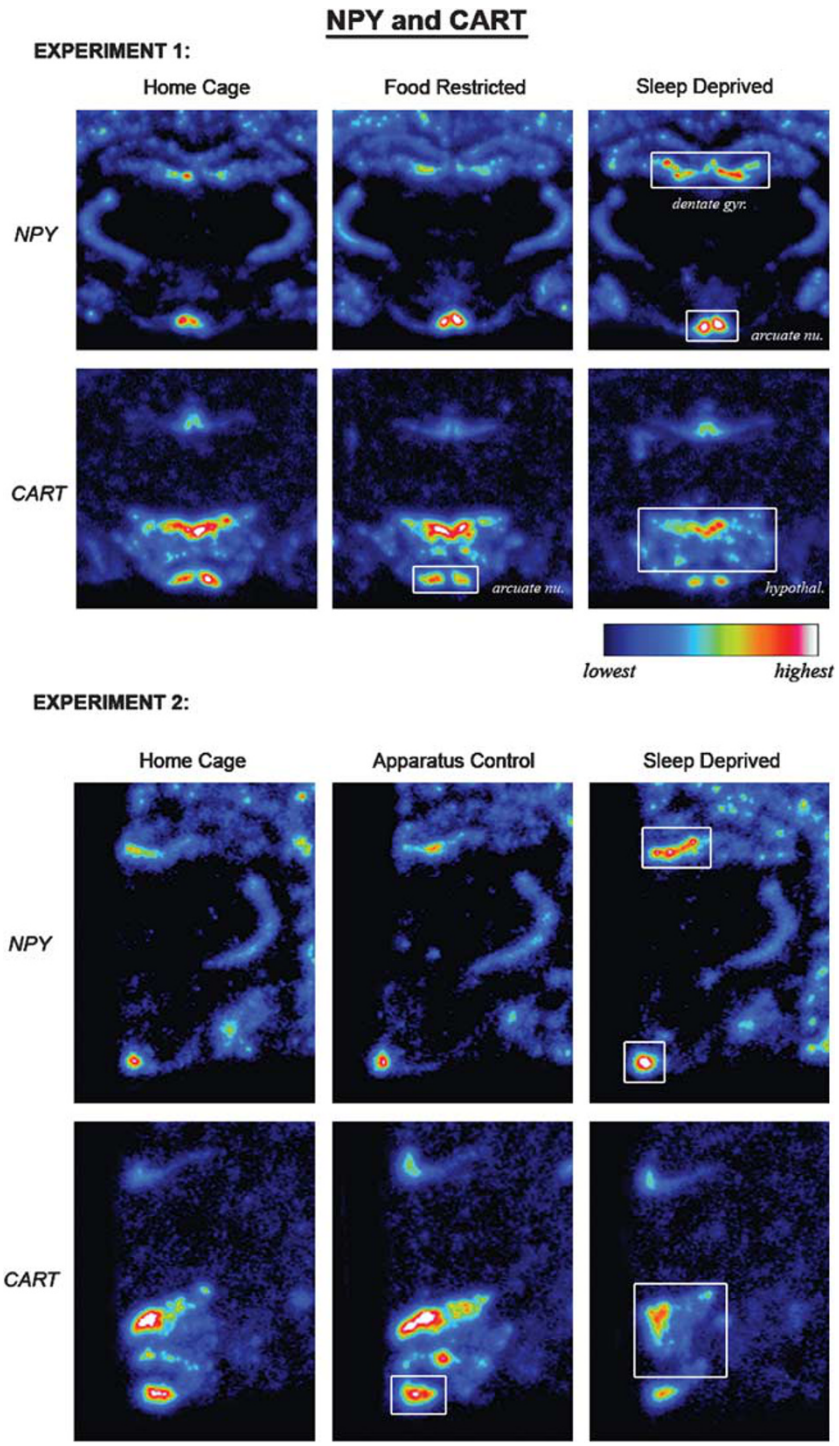

Figure 4 Pseudocolor images of coronal sections through the tuberal level of the hypothalamus from representative rats in each treatment group for each of the two experiments, showing NPY and CART expression. Color key depicts intensity range of labeling. Examples of regions of analysis for NPY in the hippocampal dentate gyrus and hypothalamic arcuate nucleus, and CART within and outside the arcuate nucleus, are shown with rectangles.

previously reported upregulation of H/O mRNA by sleep deprivation (Martins *et al*, 2010), the effect was inconsistent across the two experiments, suggesting that a global change in H/O expression is not an obligatory accompaniment of feeding escalation in the present sleep deprivation paradigm.

Sleep Deprivation, NPY Expression, and Hippocampal Plasticity

Among the most selective of the transcriptional changes incurred by sleep loss was the robust upregulation of NPY expression in allo- and neocortex. Neocortical NPY is

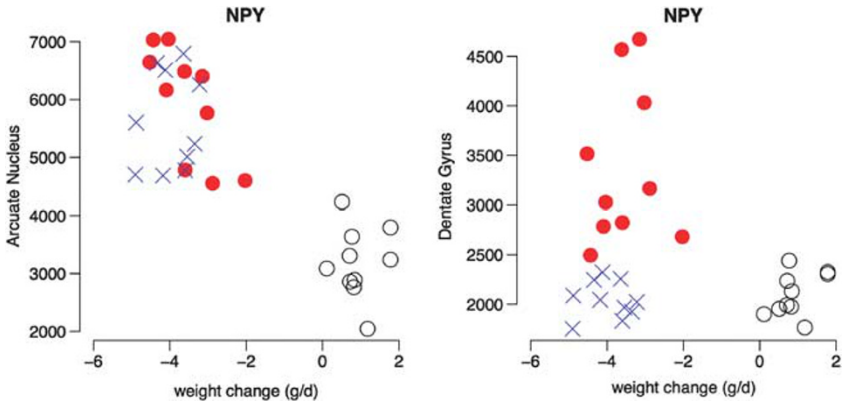

Figure 5 Scatterplots displaying gene expression levels × weight change for NPY in experiment 1, for individual subjects. Axes show optical density by weight change; see Figure 3 figure legend for further details. Red circles, Sleep-Dep group; blue X's, Food-Restrict group. Scores for the Home-Cage controls are shown with unfilled black circles.

localized in interneurons (Hendry *et al*, 1984; Karagiannis *et al*, 2009), and hippocampal NPY is found both in GABA interneurons (Sperk *et al*, 2007) and granule cells (McQuiston *et al*, 1996). The role of NPY in hippocampus is not fully understood; however, several studies suggest a role in cellular defenses against excitatory stress. For example, NPY gene expression is upregulated by metabotropic glutamate receptor stimulation (Schwartzer and Sperk, 1998) and NPY in the dentate gyrus inhibits glutamate release (Whittaker *et al*, 1999) has anticonvulsive actions (Patrylo *et al*, 1999). NPY also reduces depolarization-induced Ca^{2+} influx into dentate granule cells (McQuiston *et al*, 1996), which could be responsible for NPY-induced inhibition of perforant pathway LTP (Whittaker *et al*, 1999). Hippocampal NPY may thus serve a neuroprotective role during conditions of cellular stress, but at the same time inhibit neuroplasticity relevant to learning. Finally, NPY produces a strong neuroproliferative effect in the dentate gyrus (Howell *et al*, 2005; Howell *et al*, 2007), which may represent a line of defense against the suppression of neurogenesis seen in sleep loss (Guzman-Marin *et al*, 2003). Further studies are needed to test these possibilities. In a general sense, however, the transcriptional regulation of dentate gyrus-NPY seen here may have implications for understanding sleep loss-induced changes in hippocampal-based learning and cognition.

Clinical Implications

Perhaps the clearest clinical implication of opioid upregulation engendered by sleep deprivation relates to understanding the increased preference for and 'snacking' on palatable fat- or carbohydrate-enriched foods (Markwald *et al*, 2013; St-Onge *et al*, 2011; Weiss *et al*, 2010), which appears to be a crucial casual factor for positive energy balance in human sleep loss (Markwald *et al*, 2013). In fact, recent results indicate that this 'snacking' is not entirely explained by changes in peripheral hunger- or satiety-modulating hormones (Markwald *et al*, 2013), implicating central reward-modulatory systems such as the ENK system identified here. Furthermore, poor sleep is associated with reduced dietary restraint (Markwald *et al*, 2013) and binge eating (Trace *et al*, 2012), both of which may stem from altered central opioid function; for example, central μ-opioid signaling is implicated in food bingeing (Nathan and Bullmnore, 2009). Similarly, striatal ENK changes could also contribute to bingeing in specific disorders such as night eating syndrome and sleep-related eating disorder, both of which are characterized by sleep disruption (Vinai *et al*, 2012).

Another important consideration is that striatal ENK and DYN were upregulated in concert. DYN signaling is associated with aversive or depression-like effects in animal studies (Nestler and Carlezon, 2006), and striatal DYN levels are elevated in post-mortem brains of suicide victims and in animal models of stress and drug withdrawal (Horner *et al*, 2009; Hurd *et al*, 1997; Shippenberg *et al*, 2007; Shirayama *et al*, 2004). Hence, coordinated upregulation of ENK and DYN could produce a mixture of depressed mood and increased preference for palatable food, as is often observed in eating disorders characterized by dysfunctional 'emotional eating.' A related possibility is that sleep deprivation-induced upregulation of ENK could have a normalizing influence on mood in pathological states associated with already-elevated DYN levels. A specific case could be the palliative effect of acute sleep deprivation in depression (Benedetti and Colombo, 2010; Giedke and Schwarzler, 2002; Gillin *et al*, 2001; Wu *et al*, 2009). Although speculative, these hypotheses lead to testable predictions, particularly with the advent of PET imaging techniques capable of detecting *in vivo* opioid signaling.

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Supplementary Information accompanies the paper on the Neuropsychopharmacology website (http://www.nature.com/npp)