Published in final edited form as:

Neuroscience. 2006 August 11; 141(1): 371–378. doi:10.1016/j.neuroscience.2006.03.028.

GENE EXPRESSION IN THE RAT CEREBRAL CORTEX: COMPARISON OF RECOVERY SLEEP AND HYPNOTIC-INDUCED SLEEP

Jonathan P. Wisor, **Stephen R. Morairty**, **Nhung T. Huynh**, **Teresa L Steininger**, and **Thomas S. Kilduff**

Biosciences Division SRI International 333 Ravenswood Avenue Menlo Park, CA 94025

Abstract

Most hypnotic medications currently on the market target some aspect of GABAergic neurotransmission. Although all such compounds increase sleep, these drugs differentially affect the activity of the cerebral cortex as measured by the electroencephalogram (EEG). Whereas benzodiazepine medications such as triazolam tend to suppress slow wave activity (SWA) in the cortex, the $GABA_B$ ligand gamma-hydroxybutyrate greatly enhances SWA and the non-benzodiazepine, zolpidem, which binds to the ω1 site on the $GABA_A$ receptor/Cl^- ionophore complex, is intermediate in this regard. Our previous studies have demonstrated that a small number of genes exhibit increased expression in the cerebral cortex of the mouse and rat during recovery sleep (RS) after sleep deprivation: *egr-3*, *fra-2*, *grp78*, *grp94*, *ngfi-b*, and *nr4a3*. Using these genes as a panel of biomarkers associated with sleep, we asked whether hypnotic medications induce similar molecular changes in the cerebral cortex to those observed when both sleep continuity and SWA are enhanced during RS. We find that, although each drug increases the expression of a subset of genes in the panel of biomarkers, no drug fully replicates the molecular changes in the cortex associated with RS. Furthermore, high levels of SWA in the cortex are correlated with increased expression of *fra-2* whereas the expression of *grp94* is correlated with body temperature. These results demonstrate that sleep-related changes in gene expression may be affected by physiological covariates of sleep and wakefulness rather than by vigilance state *per se*.

Keywords

Taqman analysis; sleep deprivation; glucose-regulated proteins; heat shock proteins; immediate early genes; *grp78*; *grp94*; *fra-2*; *egr-3*; *ngfi-b*; *nr4a3*; gamma-hydroxybutyrate; zolpidem; triazolam

Time spent asleep, and the depth and intensity of sleep, vary in proportion to the duration of prior wakefulness (Tobler and Borbely, 1986, Franken et al., 1991, Borbely and Achermann, 2000). In rodents, this relationship is exemplified by changes in the distribution of vigilance states (e.g., increases in sleep time), in indices of sleep continuity (decreases in the frequency of brief awakenings and increases in sleep bout duration), and in measures of

Address correspondence to: Dr. Thomas S. Kilduff, Molecular Neurobiology Laboratory, SRI International, 333 Ravenswood Avenue, Menlo Park, CA 94025, Voice: 650-859-5509; FAX: 650-859-3153, thomas.kilduff@sri.com.

sleep intensity such as slow wave activity (SWA; spectral power in the delta range; 0.5-4.0 Hz) of the electroencephalographam (EEG) during non-rapid eye movement sleep (NREMS) following sleep deprivation (SD) (Pappenheimer et al., 1975, Aalto and Kiianmaa, 1984, Trachsel et al., 1989, Tobler et al., 1997, Franken et al., 1999, Franken et al., 2000, Huber et al., 2000, Wisor et al., 2002). Analogous changes occur in the human sleep EEG subsequent to SD (Borbely et al., 1981, Akerstedt and Gillberg, 1986, Aeschbach et al., 1996, Borbely and Achermann, 1999). Several prominent models of sleep regulation posit that these physiological parameters are driven by neurochemical changes that accumulate during sustained wakefulness (Tobler, 1995, Borbely and Achermann, 2000, Franken et al., 2001). The neurochemical events that underlie the accumulation of sleep need during wakefulness and the discharge of sleep need during sleep are little understood, but are likely to involve changes in gene expression. Indeed, there are many well-characterized substances that enhance EEG SWA during NREMS including adenosine, prostaglandin D_2, growth hormone releasing hormone (GHRH), tumor necrosis factor-α (TNF-α), and interleukin-1. Furthermore, if signaling mediated by these substances is blocked, sleep need and EEG SWA are attenuated (for review, see Krueger et al., 2001; Opp, 2002).

Changes in gene expression have been documented in specific brain regions during recovery sleep (RS; O'Hara et al., 1993, Terao et al., 2003a, b, Cirelli et al., 2004, Terao et al., 2006). We have previously identified a small number of genes whose expression is increased in both the mouse (Terao et al., 2003a, b) and rat (Terao et al., 2006) cerebral cortex during RS in the inactive (light) phase of the LD12:12 cycle. Although the relationship between increased expression of these genes in the cortex and the putative restorative function of sleep is unclear, the fact that similar molecular changes were seen in two mammalian species during RS indicates that they might be associated with the consolidation of sleep and/or increased EEG delta power that occurs during RS.

Drugs that act on the benzodiazepine (BZ) site of the $GABA_A$/BZ receptor/Cl^- channel complex have been among the most widely prescribed hypnotic medications worldwide for the last 20 years because they induce changes in sleep parameters and EEG characteristics that, in some cases, are similar to those that occur during normal sleep (Lancel, 1999, Roth et al., 2001). The first generation BZ triazolam and drugs that bind to the BZ site, along with barbiturates and neurosteroids, act as allosteric modulators to enhance neuronal inhibition by gamma-aminobutyric acid (GABA), resulting in sleep consolidation (Lancel, 1999). However, agonists at the BZ site have distinct effects on sleep and the EEG. While BZ compounds such as triazolam act to increase NREMS, they also decrease SWA in the EEG during NREMS in both humans and rats and increase activity in higher frequency bands (Achermann and Borbely, 1987). Triazolam has been replaced in clinical practice by non-BZ compounds such as zolpidem that bind to the ω1 site on the $GABA_A$/BZ/Cl^- complex and do not suppress SWA to the extent that BZs do. Despite their clinical utility and our understanding of their mechanism of action at the cellular level, it is not understood whether ligands at the $GABA_A$/BZ/Cl^- complex activate the normal sleep regulatory mechanisms.

In the present study, we address this issue by investigating the effects of GABA receptor ligands on cortical gene expression and compare the results with those changes seen

following RS. We hypothesize that comparison of gene expression changes induced by clinically effective hypnotic agents to those occurring in RS may identify common molecular pathways associated with sleep regulation. As a first step to test this hypothesis, we evaluated whether three hypnotic medications that have different modes of influence on GABAergic activity in the CNS — a $GABA_A$ agonist (zolpidem), a $GABA_B$ agonist (GHB) and a $GABA_A$ modulator (triazolam) — affect the expression of the panel of genes known to increase expression during RS in the mouse and rat cerebral cortex. We find that each of these drugs induces only a subset of the molecular changes in the cortex associated with RS.

METHODS

Sleep Physiology

Thirty male WKY rats (Charles River Laboratories, Wilmington, MA) aged 2-3 months were surgically prepared for electroencephalographic (EEG) and electromyographic (EMG), body temperature (T_b) and locomotor activity. Rats were maintained in a light/dark 12:12 cycle at 23±1° C and subjected to a 24-hr baseline observation starting at lights-off (ZT12) before experimentation. On the experimental day, rats were randomly assigned to one of five groups (n=6/group): (1) sleep deprived (SD) for 6 h from lights-off (ZT12) to ZT18 and then injected at ZT18 with saline vehicle (i.p.) followed by 2 h of RS (SD/RS group); (2) saline vehicle (i.p.) at ZT18; (3) GHB (300 mg/kg, i.p.) at ZT18; (4) triazolam (1.6 mg/kg, i.p.) at ZT18; or (5) zolpidem (20 mg/kg, i.p.) at ZT18. All rats were killed by decapitation 2 h later at ZT20. Experimental manipulations were performed in dim red light (< 10 lux). SD was achieved by a combination of cage tapping, introduction of novel objects into the cage, and stroking the vibrissae or fur with an artist's brush. The doses of GHB, triazolam and zolpidem were chosen based on a combination of the published literature (Trachsel et al., 1992, Edgar et al., 1997, Vanover et al., 1999) and the experience of our laboratory with these compounds. Animal use procedures were approved by the Institutional Animal Care and Use Committee and conformed to international guidelines on the ethical use of animals. The minimum number of animals was used to obtain statistically meaningful results and all attempts were made to mitigate any suffering.

EEG data were digitized at 100 Hz with a 1–30 Hz band pass filter and EMG was integrated using a 10–100 Hz band pass filter. Vigilance states were classified in 10-s epochs as wakefulness (W), rapid eye movement sleep (REMS), or NREMS by visual inspection on the basis of conventional criteria. For the SD/RS group, EEG and EMG data were scored for the hours ZT12 to ZT20 on both the baseline day and the day of SD. EEG and EMG data were unavailable from one rat in the SD/RS group, but tissues were collected from this animal and used for gene expression analysis. For the other four treatment groups, data were visually scored from injection (ZT18) to decapitation (ZT20); this interval ranged from 90 to 150 minutes and did not differ significantly among treatment groups. Data from the corresponding time of day during the baseline recording day were also visually scored for these treatment groups. At the end of the treatment period (ZT20), brains were rapidly removed and dissected into multiple regions which were flash-frozen on dry ice as described previously (Terao et al., 2006).

Five sleep timing variables were derived from the state classification data on the experimental night (and, where applicable, on the baseline night): (1) the latency to consolidated sleep, defined as the time interval between injection and the first continuous minute classified as sleep; (2) NREMS and REMS time, calculated as a percentage of time in the interval between injection and sacrifice; (3) average NREMS bout duration, calculated across all episodes of NREMS ≥ 10 sec; (4) number of brief awakenings (NBA), defined as episodes of wakefulness ≤ 20 sec preceded and followed by sleep episodes of at least 20 sec, and expressed as the ratio of NBA to the total number of minutes of sleep (the sum of NREMS and REMS). Two additional physiological variables were also determined: spectral power in the delta frequency (1-4 Hz) range during NREMS and body temperature (T_b). NREMS delta power was computed using Hartley's modified fast Fourier transform for all groups. Artifacts were eliminated from analysis by visual inspection and an artifact-marking algorithm. Delta power values were averaged across all artifact-free NREMS epochs ('average NREMS delta power') and summed across all epochs ('cumulative NREMS delta power'). T_b was reported both as the average raw value irrespective of sleep/wake state and as a change relative to the baseline night irrespective of sleep/wake state.

Assessment of Gene Expression by Real-time Polymerase Chain Reaction (PCR)

Quantitation of mRNA levels was carried out using a fluorescence detection method using real-time amplification kinetics (Taqman; ABI Prism 7700 Sequence Detection System, Perkin-Elmer Corp., Foster City, CA) as described previously (Terao et al., 2000, Terao et al., 2003a, b). Total RNA was prepared using the Atlas™ Pure Total RNA Labeling System (CLONTECH, Palo Alto, CA). To remove any genomic DNA contamination, total RNA was treated with RNase-free DNase I (Epicentre, Madison, WI) in the presence of anti-RNase (Ambion, Austin, TX). First-strand cDNA was prepared from the cerebral cortex of six rats from each of the five experimental conditions (30 individual cDNA syntheses) using the Advantage™ RT-for-PCR kit (CLONTECH, Palo Alto, CA). For each reaction, a "target" cDNA of interest and the reference cDNA (*glyceraldehyde-3-phosphate dehydrogenase*; *g3pdh*) were simultaneously PCR-amplified in duplicate in 96 well plates along with 8 concentrations of a mouse cortex RNA standard (Terao et al., 2003a, b). Relative expression levels were thus determined for the glucose-regulated protein mRNAs *grp78* and *grp94* and the immediate early genes *egr-3* and *fra-2*, all of which have previously been demonstrated to be upregulated in the mouse (Terao et al., 2003a, b) and rat (Terao et al., 2006) cerebral cortex during RS, as well as *ngfi-b* and *nr4a3*, which are upregulated in the rat cerebral cortex in RS during the light phase of the LD cycle (Terao et al., 2006). The primers and probes used have been described previously (Terao et al., 2003a, b, Terao et al., 2006).

Statistical analysis

Data were initially analyzed by ANOVA using Statview 5.0.1 (SAS Institute, Cary, NC) with alpha set at 0.05 to determine whether significant effects occurred in any of the parameters. EEG spectra were subjected to two-way ANOVA, with day (treatment vs. baseline) as a repeated factor - to control for individual differences in EEG spectra under baseline conditions - and treatment as a grouping factor. All other dependent variables that did not vary systematically across treatment groups under baseline conditions were

subjected to one-way ANOVA with treatment as a grouping factor. Significant effects identified by ANOVA were followed by Fisher's PLSD *post hoc* test to identify which experimental groups differed from the vehicle controls.

RESULTS

Sleep Physiology

Hypnograms of sleep states from ZT12 to ZT18 during the baseline (Figure 1A) and the treatment day (Figure 1B) for the SD/RS group illustrate a dramatic loss of sleep on the experimental day relative to the baseline. The SD/RS group spent 63±7 min asleep during ZT18-20 after SD from ZT12-18, an increase of 29% over the corresponding time on the baseline day (49±7 min).

Nine sleep and physiological parameters varied significantly among treatment groups in the post-treatment conditions including sleep timing parameters (latency to consolidated sleep, % NREMS and % REMS), measures of sleep continuity (NREMS bout duration, NBAs/min of sleep), sleep intensity (average NREMS delta power and total NREMS delta power) and T_b (mean and change from baseline). Each treatment had a distinct constellation of effects on sleep timing (Figure 2). ANOVA indicated a significant effect of treatment on sleep latency across the five treatment groups ($p=0.025$); *post hoc* tests revealed significantly shorter sleep latency in the GHB, RS and triazolam groups relative to vehicle-injected controls (Figure 2A). ANOVA also indicated significant effects of treatment on the percent of time in NREMS ($p=0.042$); *post hoc* tests revealed significant increases in NREMS in the GHB- and triazolam-treated groups relative to vehicle-injected controls (Figure 2B). The trend toward increased NREMS in the RS group did not reach significance, likely due to the sleep disruption that occurs in rodents during the first hour after cessation of SD. Significant effects of treatment on sleep continuity as reflected by increased NREMS bout duration (ANOVA $p=0.027$; Figure 2C) and decreased frequency of brief awakenings/min sleep (ANOVA $p=0.015$; Figure 2E) were observed in the triazolam- and zolpidem-treated groups relative to vehicle-injected controls. REMS was significantly suppressed by GHB (ANOVA $p=0.004$; Figure 2D) but not affected by other treatments.

Figure 3 depicts the NREMS EEG spectra in the 1-20 Hz range after treatment and during the corresponding time period on the baseline night. The treatment x EEG frequency analysis revealed that EEG power was significantly affected (ANOVA, $P < 0.001$). EEG was most strongly affected in the delta range, and was significantly increased by GHB (2-4 Hz) and zolpidem (1-4 Hz), and in RS (2-6 Hz), relative to baseline. In addition, power in higher EEG frequencies was significantly suppressed by triazolam (4-10 Hz) and zolpidem (7-15 Hz). GHB induced the most rapid and sustained increase in EEG delta power during NREMS. Both the average (Figure 4A) and cumulative (Figure 4B) delta power across the entire post-injection recording interval were strongly affected by treatment (ANOVA $p\leq0.002$). *Post hoc* tests revealed significantly higher average EEG delta power in the GHB, RS and zolpidem-treated groups relative to vehicle-injected controls (Figure 4A), but significantly higher cumulative delta power occurred only in the GHB and RS groups (Figure 4B).

Figure 4C presents the raw T_b data. ANOVA indicated significant variation in T_b across the treatment groups (p=0.003); *post hoc* tests revealed significantly lower T_b values in the GHB and zolpidem groups compared to vehicle. T_b change relative to baseline was strongly influenced by treatment (p<0.001; Figure 4D). Decreases in T_b in the GHB- and zolpidem-treated groups were significantly different from vehicle-injected controls, which exhibited virtually no change in T_b (0.09±0.06 °C) relative to baseline values in this group.

Candidate Gene Expression

The relative mRNA expression levels in the cerebral cortex for the six gene panel following RS and hypnotic treatment are shown in Figure 5.

egr-3—ANOVA revealed a highly significant treatment effect on *egr-3* expression in the cortex (p=0.001); *post hoc* tests revealed an increase in expression only in the RS group (178%). *Egr-3* expression in other treatment groups did not differ from vehicle.

fra-2—ANOVA revealed a highly significant treatment effect on *fra-2* expression in the cortex (p=0.002); *post hoc* tests revealed an increase in expression (88%) in the GHB-treated group.

grp78—ANOVA revealed a highly significant treatment effect on *grp78* expression in the cortex (p=0.007); *post hoc* tests revealed increased expression (94%) only in the RS group.

grp94—ANOVA revealed a significant treatment effect on *grp94* expression (p=0.041); *post hoc* tests revealed significantly increased expression in the cortex of the RS (51%), triazolam (43%), and zolpidem (44%) groups relative to vehicle controls.

ngfi-b—ANOVA failed to find a significant effect of treatment in the cerebral cortex (p=0.075).

nr4a3—ANOVA revealed a nearly significant treatment effect on *nr4a3* expression in the cortex (p=0.052), but *post hoc* tests failed to identify any group that differed from vehicle treatment.

Correlations Between Sleep/Physiological Parameters and Gene Expression in the Cerebral Cortex

Of the possible correlations between the nine sleep/physiological variables depicted in Figures 2 and 4 and the six transcript levels reported in Figure 5, only two relationships were found to be statistically significant. A significant correlation was found between EEG delta power and *fra-2* expression whether this relationship was assessed for cumulative delta power (Figure 6A; r^2=0.26, p=0.004) or average delta power (r^2=0.23, p=0.007; data not shown). A significant correlation was found between T_b and *grp94* expression whether this relationship was assessed for change in T_b on the treatment night relative to the baseline night (Figure 6B; r^2=0.27, p=0.003) or T_b on the experimental night (r^2=0.17, p=0.024; data not shown).

DISCUSSION

Activation or potentiation of GABAergic transmission induces acute changes in sleep timing and the sleep EEG similar to those that accompany RS following SD, including decreased latency to sleep, increased sleep consolidation and increased SWA during NREMS. Consequently, sleep after acute hypnotic administration and RS might be accompanied by similar changes in gene expression. To test this hypothesis, we compared the effects of a $GABA_A$ agonist (zolpidem), a $GABA_B$ agonist (GHB) and a $GABA_A$ modulator (triazolam) on the expression of six genes known to be induced during RS. To maximize the likelihood of detecting hypnotic effects using these agents, we conducted the present study during the dark phase, the normal active period for the rat, in contrast to our previous studies of RS which occurred during the light phase (Terao et al., 2003a, b, Terao et al., 2006).

The experimental manipulations were effective in altering various parameters of sleep timing and related physiological variables. Following 6 h SD at a time of day when rats are normally active and have relatively little sleep, the RS group exhibited a shorter latency to sleep and increased delta power relative to vehicle-injected rats that had not been sleep deprived. Among the pharmacological treatments, GHB had the most striking effects including decreased sleep latency, increased NREMS time, decreased REMS time, increased delta power and decreased T_b. Both triazolam and zolpidem had robust effects on sleep continuity, as manifested by increased bout duration and decreased NBAs, but their other effects differed. Triazolam increased NREMS as a percentage of time and decreased sleep latency without affecting NREMS delta power or T_b. In contrast, zolpidem increased NREMS delta power and decreased T_b without affecting NREMS time or sleep latency.

Triazolam, zolpidem and GHB have all been previously shown to increase NREM sleep time and decrease T_b in rats at the doses administered in the current study (Godschalk et al., 1977, Snead, 1990, Edgar et al., 1997, Takahashi et al., 1999). Our results generally concur with these previous findings. The lack of uniformity in other variables (augmentation of delta power, suppression of 6-15 Hz EEG power) indicates some independence in the regulation of each of these sleep-related measures from NREMS timing. Further evidence for independence of the regulation of sleep-related phenomena is demonstrated by differences in the temporal dynamics of NREMS delta power among mouse strains, which vary independently of total daily NREMS time (Franken et al., 2001). Pharmacological treatments can dissociate sleep-like EEG waveforms from behavioral and postural components of sleep (Coenen and van Luijtelaar, 1991). For example, GHB administration in mice at a dose (250 mg/kg, i.p.) similar to that of the current study resulted in abnormal postures and “hypersynchronous EEG” (Meerlo et al., 2004). In the current study, GHB administration (200 mg/kg, i.p.) to WKY rats resulted in an EEG with increased delta power that was otherwise indistinguishable from baseline EEG in the 1-20 Hz range and did not prevent responsiveness to environmental stimuli or cause abnormal sleep postures. Dose-dependent effects of GHB and other drugs on EEG and behavior are likely strain- and species-specific.

As indicated in Figure 5, significant variation occurred across the experimental groups for all genes in the panel except *ngfi-b* and *nr4a3*. Our results demonstrate that induction of

grp78, *grp94* and *egr-3* mRNAs during RS (Figure 5) occurs in the dark phase of the LD cycle, when sleep propensity is relatively low, as well as during the light phase (Terao et al., 2006), when sleep propensity is high. The lack of agreement with our previous studies showing induction of *fra-2*, *nr4a3* and *ngfi-b* in RS (Terao et al., 2006) may be due to fact that experiments in the present study were performed during the opposite phase of the light-dark cycle. Rats spend less time asleep during the dark than during the light phase. Consequently, the difference in sleep history between control rats and SD rats was more modest in the current study than in our previous studies (Terao et al., 2003a, b, Terao et al., 2006), as was the effect of SD on subsequent sleep parameters: the increases in sleep time and sleep consolidation that occur when SD is applied during the light phase of the LD cycle were not observed in the current study. Furthermore, vehicle injection of control rats, a necessary control for handling effects in drug-treated rats, was followed by a brief period of wakefulness, which may have influenced gene expression. A longer duration of SD might therefore be necessary to significantly induce *fra-2*, *nr4a3* and *ngfi-b* during the dark phase. Interpretation of the current results is also limited by the fact that only one duration of SD and one dose of each hypnotic were used in this experiment. Each compound is likely to have a dose-response relationship at the level of gene expression as well as at the level of sleep physiology.

The effects of hypnotics on gene expression only partly correspond to the effects of RS on cerebral gene expression. The partial overlap among treatments in affecting gene expression may be due to their partially overlapping effects on sleep timing and physiology. For instance, both GHB and RS resulted in robust increases in NREMS EEG delta power relative to vehicle controls, while triazolam did not. In contrast, both triazolam and GHB increased NREMS time while SD did not significantly affect sleep duration. Discrepancies in hypnotic-induced gene expression may thus result from distinct effects of treatments on physiology. To address this possibility, we determined the correlation coefficients for sleep and other physiological variables and gene expression across all treatment groups and found a significant relationship between NREMS delta power and *fra-2* expression in the cortex (Figure 6A), but not between delta power and any of the other genes assessed by real-time PCR analysis. The relationship between delta power and *fra-2* expression is evident in the graphical relationships of group means for these two variables: the treatment that resulted in the highest amplitude delta power (GHB; Figure 4A) also caused the most robust increase in *fra-2* expression (GHB; Figure 5B). In contrast, *grp94* expression in the cortex was correlated with T_b rather than delta power (Figure 6B). Since *grp94* is a temperature-sensitive (i.e., heat shock) transcript, this finding may not be surprising.

Technologies that assess the expression of large numbers of genes simultaneously have demonstrated widespread changes in gene expression in the cerebral cortex during sustained wake and during sleep subsequent to sustained wake (Cirelli and Tononi, 2000, Cirelli et al., 2004, Terao et al., 2006). Changes in gene expression in conjunction with sleep or waking may represent molecular components of a regulatory process underlying accumulation and discharge of sleep need. Yet the identification of such genes is complicated by the fact that sustained waking results in a constellation of physiological changes before and during subsequent sleep (Lavie, 2001, Rechtschaffen and Bergmann, 2002, Castellani et al., 2003,

Van Dongen et al., 2005), any of which might influence gene expression. Thus, a sleep-dependent change in gene expression may be a result of a change in the amplitude of SWA, a change in the frequency of transitions between wake and sleep states, a change in T_b or other aspects of systems physiology, or a combination of the above parameters.

As sleep-related patterns of gene expression continue to be documented, it will be critical to distinguish among changes in gene expression that are directly related to the homeostatic regulation of sleep (i.e., accumulation of sleep need during wake and its discharge during sleep), changes that occur as a result of sustained neuronal activity of sleep regulatory CNS circuitry, and changes that occur as a result of physiological covariates associated with pharmacological or behavioral manipulations of sleep such as alterations in T_b. Further experiments should characterize gene expression changes following treatments that induce similar changes in these physiological covariates in order to identify those changes that are epiphenomenal (e.g., the effects of hypnotics administered at doses that increase delta power to an equal degree or have equivalent thermoregulatory effects). Changes in ambient temperature might also be applied to minimize the thermoregulatory effects of experimental manipulations of the sleep/wake cycle. Ultimately, gene expression data must be paired with functional data, such as knockout or conditional transgenic mouse models to ascertain the roles of specific loci in determining sleep need.

ACKNOWLEDGEMENTS

Supported by NIH R01 HL59658

REFERENCES

Aalto J, Kiianmaa K. Circadian rhythms of water and alcohol intake: effect of REM-sleep deprivation and lesion of the suprachiasmatic nucleus. Alcohol. 1984; 1:403–407. [PubMed: 6537227]

Achermann P, Borbely AA. Dynamics of EEG slow wave activity during physiological sleep and after administration of benzodiazepine hypnotics. Hum Neurobiol. 1987; 6:203–210. [PubMed: 2896653]

Aeschbach D, Cajochen C, Landolt H, Borbely AA. Homeostatic sleep regulation in habitual short sleepers and long sleepers. Am J Physiol. 1996; 270:R41–53. [PubMed: 8769783]

Akerstedt T, Gillberg M. Sleep duration and the power spectral density of the EEG. Electroencephalogr Clin Neurophysiol. 1986; 64:119–122. [PubMed: 2424728]

Borbely AA, Achermann P. Sleep homeostasis and models of sleep regulation. J Biol Rhythms. 1999; 14:557–568. [PubMed: 10643753]

Borbely, AA.; Achermann, P. Sleep homeostasis and models of sleep regulation. In: Kryger, MH., et al., editors. Principles and Practice of Sleep Medicine. W.B. Saunders; Philadelphia: 2000. p. 377-390.

Borbely AA, Baumann F, Brandeis D, Strauch I, Lehmann D. Sleep deprivation: effect on sleep stages and EEG power density in man. Electroencephalogr Clin Neurophysiol. 1981; 51:483–495. [PubMed: 6165548]

Castellani JW, Stulz DA, Degroot DW, Blanchard LA, Cadarette BS, Nindl BC, Montain SJ. Eighty-four hours of sustained operations alter thermoregulation during cold exposure. Med Sci Sports Exerc. 2003; 35:175–181. [PubMed: 12544652]

Cirelli C, Gutierrez CM, Tononi G. Extensive and divergent effects of sleep and wakefulness on brain gene expression. Neuron. 2004; 41:35–43. [PubMed: 14715133]

Cirelli C, Tononi G. Gene expression in the brain across the sleep-waking cycle. Brain Res. 2000; 885:303–321. [PubMed: 11102586]

Coenen AM, van Luijtelaar EL. Pharmacological dissociation of EEG and behavior: a basic problem in sleep-wake classification. Sleep. 1991; 14:464–465. [PubMed: 1759100]

Edgar DM, Seidel WF. Modafinil induces wakefulness without intensifying motor activity or subsequent rebound hypersomnolence in the rat. J Pharmacol Exp Ther. 1997; 283:757–769. [PubMed: 9353396]

Edgar DM, Seidel WF, Gee KW, Lan NC, Field G, Xia H, Hawkinson JE, Wieland S, Carter RB, Wood PL. CCD-3693: an orally bioavailable analog of the endogenous neuroactive steroid, pregnanolone, demonstrates potent sedative hypnotic actions in the rat. J Pharmacol Exp Ther. 1997; 282:420–429. [PubMed: 9223583]

Franken P, Chollet D, Tafti M. The homeostatic regulation of sleep need is under genetic control. J Neurosci. 2001; 21:2610–2621. [PubMed: 11306614]

Franken P, Lopez-Molina L, Marcacci L, Schibler U, Tafti M. The transcription factor DBP affects circadian sleep consolidation and rhythmic EEG activity. J Neurosci. 2000; 20:617–625. [PubMed: 10632591]

Franken P, Malafosse A, Tafti M. Genetic determinants of sleep regulation in inbred mice. Sleep. 1999; 22:155–169. [PubMed: 10201060]

Franken P, Tobler I, Borbely AA. Sleep homeostasis in the rat: simulation of the time course of EEG slow-wave activity. Neurosci Lett. 1991; 130:141–144. [PubMed: 1795873]

Godschalk M, Dzoljic MR, Bonta IL. Slow wave sleep and a state resembling absence epilepsy induced in the rat by gamma-hydroxybutyrate. Eur J Pharmacol. 1977; 44:105–111. [PubMed: 407087]

Huber R, Deboer T, Tobler I. Effects of sleep deprivation on sleep and sleep EEG in three mouse strains: empirical data and simulations. Brain Res. 2000; 857:8–19. [PubMed: 10700548]

Krueger JM, Obal FJ, Fang J, Kubota T, Taishi P. The role of cytokines in physiological sleep regulation. Ann N Y Acad Sci. 2001; 933:211–221. [PubMed: 12000022]

Lancel M. Role of $GABA_A$ receptors in the regulation of sleep: initial sleep responses to peripherally administered modulators and agonists. Sleep. 1999; 22:33–42. [PubMed: 9989364]

Lavie P. Sleep-wake as a biological rhythm. Annu Rev Psychol. 2001; 52:277–303. [PubMed: 11148307]

Meerlo P, Westerveld P, Turek FW, Koehl M. Effects of gamma-hydroxybutyrate (GHB) on vigilance states and EEG in mice. Sleep. 2004; 27:899–904. [PubMed: 15453548]

Obal F Jr. Krueger JM. Biochemical regulation of non-rapid-eye-movement sleep. Front Biosci. 2003; 8:520–550.

O'Hara BF, Young KA, Watson FL, Heller HC, Kilduff TS. Immediate early gene expression in brain during sleep deprivation: preliminary observations. Sleep. 1993; 16:1–7. [PubMed: 8456228]

Opp, MR. Cytokines and sleep promotion- A potential mechanism for disorders of excessive daytime sleepiness. In: Pack, A., editor. Sleep Apnea: pathogenesis, diagnosis and treatment. Marcel Dekker; 2002. p. 327-351.

Rechtschaffen A, Bergmann BM. Sleep deprivation in the rat: an update of the 1989 paper. Sleep. 2002; 25:18–24. [PubMed: 11833856]

Pappenheimer JR, Koski G, Fencl V, Karnovsky ML, Krueger J. Extraction of sleep-promoting factor S from cerebrospinal fluid and from brains of sleep-deprived animals. J Neurophysiol. 1975; 38:1299–1311. [PubMed: 1221075]

Rechtschaffen A, Bergmann BM. Sleep deprivation in the rat: an update of the 1989 paper. Sleep. 2002; 25:18–24. [PubMed: 11833856]

Roth T, Hajak G, Ustun TB. Consensus for the pharmacological management of insomnia in the new millennium. Int J Clin Pract. 2001; 55:42–52. [PubMed: 11219318]

Snead OC 3rd. gamma-Hydroxybutyric acid-induced seizures bear no relation to core temperature. Epilepsia. 1990; 31:253–258. [PubMed: 2344842]

Takahashi H, Yoshimoto M, Higuchi H, Shimizu T, Hishikawa Y. Different effects of L-type and T-type calcium channel blockers on the hypnotic potency of triazolam and zolpidem in rats. Eur Neuropsychopharmacol. 1999; 9:317–321. [PubMed: 10422892]

Terao A, Greco MA, Davis RW, Heller HC, Kilduff TS. Region-specific changes in immediate early gene expression in response to sleep deprivation and recovery sleep in the mouse brain. Neuroscience. 2003a; 120:1115–1124. [PubMed: 12927216]

Terao A, Steininger TL, Hyder K, Apte-Deshpande A, Ding J, Rishipathak D, Davis RW, Heller HC, Kilduff TS. Differential increase in the expression of heat shock protein family members during sleep deprivation and during sleep. Neuroscience. 2003b; 116:187–200. [PubMed: 12535952]

Terao A, Peyron C, Ding J, Wurts SW, Edgar DM, Heller HC, Kilduff TS. Prepro-hypocretin (prepro-orexin) expression is unaffected by short-term sleep deprivation in rats and mice. Sleep. 2000; 23:867–874. [PubMed: 11083595]

Terao A, Wisor JP, Peyron C, Apte-Deshpande A, Wurts SW, Edgar DM, Kilduff TS. Gene expression in the rat brain during sleep deprivation and recovery sleep: an affymetrix GeneChip study. Neuroscience. 2006; 137:593–605. [PubMed: 16257491]

Tobler I. Is sleep fundamentally different between mammalian species? Behav Brain Res. 1995; 69:35–41. [PubMed: 7546316]

Tobler I, Borbely AA. Sleep EEG in the rat as a function of prior waking. Electroencephalogr Clin Neurophysiol. 1986; 64:74–76. [PubMed: 2424723]

Tobler I, Deboer T, Fischer M. Sleep and sleep regulation in normal and prion protein-deficient mice. J Neurosci. 1997; 17:1869–1879. [PubMed: 9030645]

Trachsel L, Edgar DM, Seidel WF, Heller HC, Dement WC. Sleep homeostasis in suprachiasmatic nuclei-lesioned rats: effects of sleep deprivation and triazolam administration. Brain Res. 1992; 589:253–261. [PubMed: 1393593]

Trachsel L, Tobler I, Borbely AA. Effect of sleep deprivation on EEG slow wave activity within non-REM sleep episodes in the rat. Electroencephalogr Clin Neurophysiol. 1989; 73:167–171. [PubMed: 2473885]

Van Dongen HP, Vitellaro KM, Dinges DF. Individual differences in adult human sleep and wakefulness: Leitmotif for a research agenda. Sleep. 2005; 28:479–496. [PubMed: 16171293]

Vanover KE, Edgar DM, Seidel WF, Hogenkamp DJ, Fick DB, Lan NC, Gee KW, Carter RB. Response-rate suppression in operant paradigm as predictor of soporific potency in rats and identification of three novel sedative-hypnotic neuroactive steroids. J Pharmacol Exp Ther. 1999; 291:1317–1323. [PubMed: 10565857]

Wisor JP, O'Hara BF, Terao A, Selby CP, Kilduff TS, Sancar A, Edgar DM, Franken P. A role for cryptochromes in sleep regulation. BMC Neurosci. 2002; 3:20. [PubMed: 12495442]

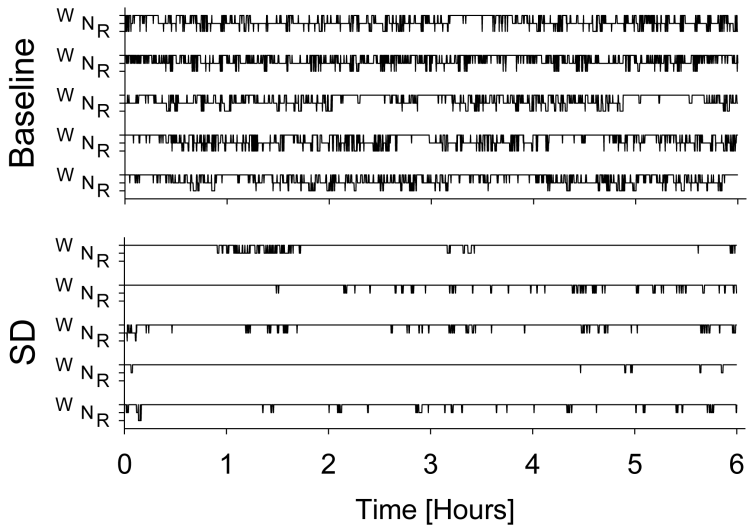

Figure 1.

Hypnograms illustrating the distribution of sleep states during ZT12-18 on the baseline and SD nights for the 5 rats in the SD group. SD was enforced by introduction of novel objects into the cage and gentle handling when necessary. W, wakefulness; N, NREM sleep; R, REM sleep.

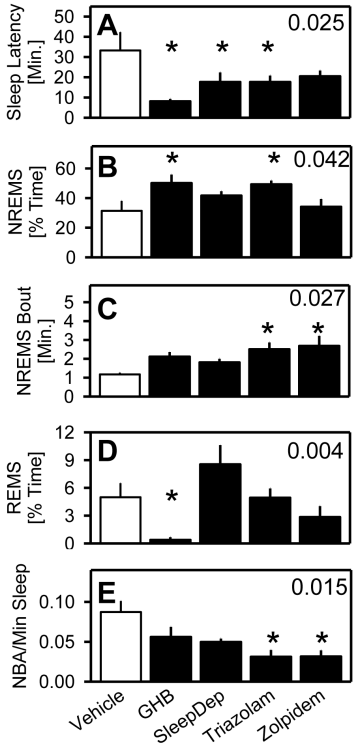

Figure 2.

Sleep timing variables in the interval between injection and sacrifice (group means ± SEM). A, latency from injection to the first continuous minute of sleep. B, NREMS as a percentage of time. C, average NREMS bout duration. D, REMS as a percentage of time. E, number of brief awakenings (NBA) per minute of sleep. Numbers in each panel refer to p values for treatment effect as determined by ANOVA. *Significantly different from vehicle-injected group as determined by Fisher's least significant difference test (PLSD).

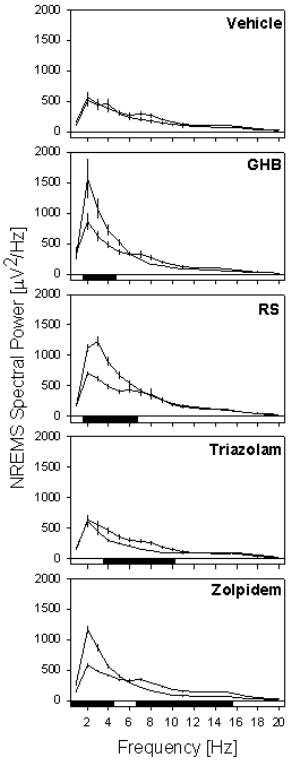

Figure 3.

Effects of treatment on NREMS EEG spectral power in the 1-20 Hz range. Thick lines represent post-treatment data; thin lines represent data from the analogous time interval on the baseline night. Black bars at the base of each graph indicate those frequencies at which post-treatment power was significantly different from baseline power as determined by Fisher's PLSD test.

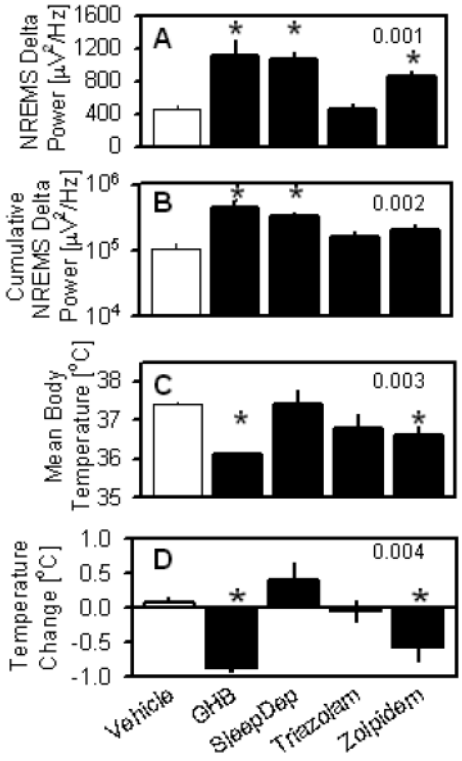

Figure 4.

Physiological variables in the interval between injection and decapitation (group means ± SEM). A, delta power averaged across all NREMS epochs. B, delta power summed across all NREMS epochs. C, body temperature. D, change in body temperature relative to the analogous time interval on the baseline night. Numbers in each panel refer to p values for treatment effect as determined by ANOVA. *Significantly different from vehicle-injected group as determined by Fisher's PLSD test.

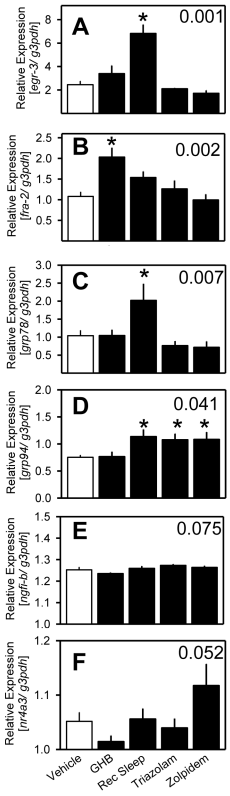

Figure 5.

Relative gene expression (group means ± SEM) in the cerebral cortex, as assessed by Taqman real-time polymerase chain reaction. A, *egr-3*. B, *fra-2*. C, *grp78*. D, *grp94*. E, *ngfi-b*. F, *nr4a3*. Numbers in each panel refer to p values for treatment effect as determined by ANOVA. *Significantly different from vehicle-injected group as determined by Fisher's PLSD test.

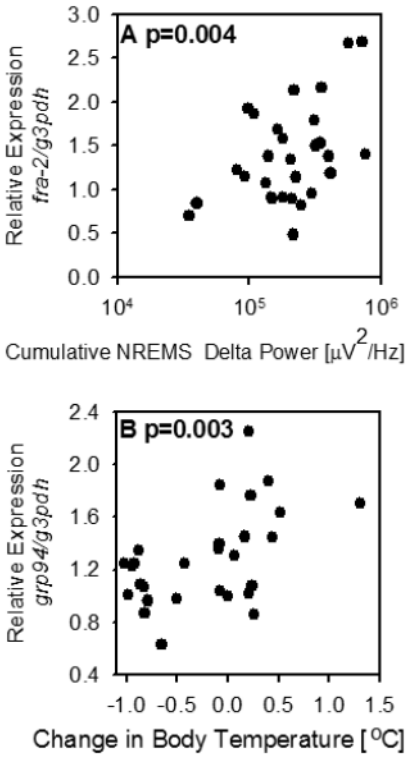

Figure 6.

Correlational relationships between delta power and *fra-2* expression (A) and between body temperature and *grp94* expression (B) were statistically significant. Numbers in each panel indicate p values for Pearson's correlation coefficient.