Changes in brain glycogen after sleep deprivation vary with genotype

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Submitted 30 October 2002; accepted in final form 25 April 2003

Franken, Paul, Phung Gip, Grace Hagiwara, Norman F. Ruby, and H. Craig Heller. Changes in brain glycogen after sleep deprivation vary with genotype. Am J Physiol Regul Integr Comp Physiol 285: R413–R419, 2003—Sleep has been functionally implicated in brain energy homeostasis in that it could serve to replenish brain energy stores that become depleted while awake. Sleep deprivation (SD) should therefore lower brain glycogen content. We tested this hypothesis by sleep depriving mice of three inbred strains, i.e., AKR/J (AK), DBA/2J (D2), and C57BL/6J (B6), that differ greatly in their sleep regulation. After a 6-h SD, these mice and their controls were killed by microwave irradiation, and glycogen and glucose were quantified in the cerebral cortex, brain stem, and cerebellum. After SD, both glycogen and glucose were quantified in the cerebral cortex, brain stem, and cerebellum of AK and D2 mice. In contrast, after SD, glucose content increased in all three structures in AK mice and did not change in D2 mice. The increase in glycogen after SD in B6 mice persisted under conditions of food deprivation that, by itself, lowered cortical glycogen. Furthermore, the strains that differ most in their compensatory response to sleep loss, i.e., AK and D2, did not differ in their glycogen response. Thus glycogen content per se is an unlikely end point of sleep’s functional role in brain energy homeostasis.

Compared with wakefulness, non-rapid-eye-movement sleep (NREMS) is accompanied by a decrease in brain metabolism (reviewed in Ref. 26). These observations have led to several hypotheses concerning a functional link between sleep and energy homeostasis in the brain. We have advanced a hypothesis integrating both functional and regulatory aspects regarding sleep (3). As to sleep function, we proposed that cerebral glycogen stores, the most important energy store in the brain (19), are progressively depleted during wakefulness and have to be replenished during NREMS. As to sleep regulation, we implicated adenosine as a molecular feedback signal for sleep need (reviewed by Refs. 3, 33, 35). We proposed that glycogen depletion enhances extracellular adenosine release, thereby promoting sleep onset, sleep continuity, and NREMS delta power [i.e., EEG power in the 1- to 4-Hz range, a marker of NREMS need (5, 6, 12)]. A sleep-regulatory role for adenosine has gained considerable support (reviewed by Ref. 33). A functional role for sleep in regulating glycogen content has received less attention. Recently, two studies directly tested this hypothesis by comparing brain glycogen between sleep-deprived and control rats. The results were inconclusive. In one study, a decrease in glycogen was observed throughout most of the brain of adult rats sleep deprived for 12 h or longer (21), thus supporting our hypothesis. In a developmental study from our own laboratory, glycogen was found to decrease after sleep deprivation (SD) in the cerebellum but not in the cortex of 24- to 50-day-old rats (15). Moreover, in 59-day-old rats (the oldest age group studied), glycogen increased in the cortex after a 12-h SD and did not change in the cerebellum. The present study aimed at further investigating the relationship between sleep loss and brain glycogen content in strains of adult mice that differ in responses to sleep deprivation.

Large differences in sleep regulation have been observed among inbred strains of mice (12, 13). Of special relevance here is the finding that the homeostatic response in NREMS delta power to a 6-h SD differed, being most pronounced in AKR/J (AK), intermediate in C57BL/6J (B6), and smallest in DBA/2J (D2) mice, suggesting that the rate at which sleep need accumulates varies with genetic background (12). Genetic dissection of this sleep trait in recombinant-inbred lines of mice derived from B6 and D2 revealed two quantitative-trait loci (QTL) marking genomic regions that potentially contain genes underlying this trait (12). One of the two QTL implicated brain glycogen phosphorlase (Pygb) as a candidate gene. As an example of a possible cellular mechanism that could underlie the strain differences in NREMS regulation, a functional Pygb polymorphism might alter the rate at which brain glycogen depletes during wakefulness.

Here we test the hypothesis that sleep loss is associated with a decrease in brain glycogen content. Within this general hypothesis, two specific hypotheses will be addressed. One states that in mice in which NREMS need accumulates at a faster rate (i.e., AK mice), glycogen should be depleted at a faster rate as...
well. Thus, when kept awake for the same duration, AK mice are expected to have a larger decrease in glycogen compared with D2 mice. A second hypothesis concerns the brain region specificity of the SD effects on glycogen content. Several hypotheses about sleep function suggest the cerebral cortex as the main target of the restorative effects of sleep (25). Furthermore, delta oscillations that are characteristic of the NREMS EEG and of which the amplitude reliably reflects sleep-wake history (5, 12, 18) originate from the cortex (40).

For these reasons we expect a significant glycogen decrease to occur specifically in the cortex (3). To test these predictions we sleep deprived AK, B6, and D2 mice for 6 h and determined glycogen content in the cortex, cerebellum, and brain stem. The SD duration used in our study was the same used to identify strain differences in sleep homeostasis (12, 13).

Control experiments. Four control mice were placed in a similar chamber and left behaviorally inactive (1.5% -2.5% in air). To avoid hypothermia during the anesthesia, the box was placed on a heating pad so that temperature inside the box was maintained at 32°C. In all experiments mice were killed by inhaled halothane and the box was placed on a heating pad to ensure the rapid attainment of halothane levels of at least 85% and to avoid hypothermia (14). With a 0.50- to 0.65-s radiation exposure (2,450 MHz at 3.5 kW nominal power), brain temperatures of at least 85°C were achieved, ensuring the rapid inactivation of enzymes involved in glycogen metabolism. The cerebral cortex, brain stem, and cerebellum were dissected, weighed, and stored at -70°C. In the initial experiments (the food deprivation, the halothane exposure, and the first 6-h SD session in B6 mice), only cortex was dissected. In the second experiment, the whole brain of D2 mice was dissected, and cerebellum and brain stem were added to the cortices.

Tissue samples were dissected: halothane exposure, which increases brain glycogen content (14), and halothane exposure, which increases glycogen content (7, 27, 28, 41).

Materials and Methods

Subjects and housing. Inbred strains (AK, D2, and B6) of male mice were obtained from the Jackson Laboratory, and out-bred Swiss-Webster (SW) mice were obtained from Simonsen Laboratories. Mice used in the SD experiments were between 10.5 and 14 wk of age. Mice were kept in pairs under a 12:12-h light-dark cycle (lights on at 0800) and with food and water available ad libitum. As in the study in which sleep regulation was examined in these strains (12, 13), the ambient temperature used was 25°C. Mice were given at least 14 days to adjust to these conditions before the experiment (except for the halothane experiment where habituation was 2 days).

SD experiment. The SD experiment data were collected in eight sessions with 5–6 control mice and 5–6 sleep-deprived mice per session. The following numbers of mice (n) were used for the control and SD groups, respectively: AK, n = 23 and 21; B6, n = 11 and 12; D2, n = 12 and 12. At lights on, mice to be sleep deprived were transferred to a separate room. SD was achieved by gentle handling for the duration of 6 h. After the SD, animals were killed along with the controls.

Control experiments. In the food deprivation experiment, eight 8-wk-old B6 mice were starved for 24 h. Food was removed 6 h after lights on (at 1400), and the next day, again at 1400, animals were killed. The control group of the SD experiment served as controls. Four of the eight mice were sleep deprived (see above) for the last 6 h of the food deprivation. For the halothane experiment, four 5-wk-old SW mice were put into an acrylic box and exposed for 2 h (1200 to 1400) to halothane at the lowest dose that kept the animals behaviorally inactive (1.5–2.5% in air). To avoid hypothermia during the anesthesia, the box was placed on a heating pad so that temperature inside the box was maintained at 32°C. Four control mice were placed in a similar chamber and left undisturbed in another room.

Glycogen and glucose determination. The methods for the determination of glycogen and glucose have been described in detail elsewhere (15). In all experiments mice were killed by microwave irradiation between 1400 and 1430 using a Gerling-Moore 4101 microwave. With a 0.50- to 0.65-s radiation exposure (2,450 MHz at 3.5 kW nominal power), brain temperatures of at least 85°C were achieved (14). The cerebral cortex, brain stem, and cerebellum were dissected, weighed, and stored at -70°C. In the initial experiments (the food deprivation experiment, the halothane exposure experiment, and the first 6-h SD session in B6 mice), only cortex was dissected. In the second experiment, the whole brain of D2 mice was dissected, and cerebellum and brain stem were added to the cortices.

Tissue samples were dissected and stored in 10% wt/vol in 0.1 N NaOH and 0.01% SDS, sonicated, and centrifuged for 15 min at 16,000 g at 4°C. Glycogen and glucose were assayed enzymatically according to Passoneau and Lauderdale (30). Glycogen and glucose were both expressed as micromoles per gram tissue.

Statistics. The dependent variables in this study were levels of brain glycogen and glucose. In the main experiment, the fixed effects of the factors SD (baseline, SD), genetic background (strain: AK, B6, D2), and brain structure (cortex, brain stem, cerebellum) on these dependent variables were assessed. For factor structure, three glycogen and three glucose values were obtained within the same individual. We can represent our data in terms of a mixed-effects model (32)

\[
Y_{ij} = X_{ij} \beta + V_i + e_{ij}
\]

where \(Y_{ij}\) is the jth response measurement on mouse i, with \(j = 1, 2, 3\); \(X_{ij}\) are the design variables for representing the fixed-effects contrasts, i.e., SD, strain, and structure, and their interactions; \(\beta\) is a vector of the fixed-effects parameters; \(V_i\) is a random effect for representing the response level in the ith mouse [in the model we assume that the \(V_i\) are independently and identically distributed (iid) across mice, having a normal distribution with mean zero and variance \(\sigma_V^2\); and \(e_{ij}\) represents iid measurement errors, assumed to be \(N(0, \sigma^2)\).

The random effect \(V_i\) accounts for the correlation in the repeated measurements on mice. It also allows a potentially large source of variation to be removed in the comparison of levels of the structure variable (and interactions) and gives a generalization of the paired t-tests for these contrasts. Because our design is balanced, the mixed-effects ANOVA table that results when we fit this model by restricted maximum likelihood (REML, Proc Mixed in SAS/STAT v8.02; SAS Institute, Cary, NC) is identical to what one obtains from a repeated-measures ANOVA (Proc GLM in SAS/STAT; see Ref. 10 and T. Hastie, personal communication) used in the present analyses.

After having established significant \((P < 0.05)\) main effects and/or interactions (see Results), post hoc comparisons were performed to assess for which genotype and brain structure the SD induced a significant deviation from control (unpaired, 2-sided t-tests). These changes from baseline are illustrated as percent change in Fig. 2. Additional post hoc analyses concern strain and brain structure differences within the control group and within the SD group used two-way ANOVAs (see Figs. 1 and 2, respectively). Subsequent pairwise testing among genotype and brain structure was performed using Tukey’s multiple-range tests (for comparisons among genotypes) or paired, two-sided t-tests (for comparisons between brain regions). The within-subject comparison among brain structures in the control group was illustrated in Fig. 1 by expressing the dependent variables as pairwise differences between structures. For the two pilot experiments, i.e., the food-deprivation and halothane experiments, group comparisons were made using unpaired, two-sided t-tests. Glycogen and glucose levels obtained in the food deprivation experiment were contrasted to the control values of the B6 mice of the SD experiment.
RESULTS

Control group. Significant differences in strain and brain structure were present in both glycogen and glucose content (Fig. 1). Glycogen levels in the cerebral cortex and cerebellum were significantly higher in AK mice than in D2 and B6 mice, whereas brain stem levels did not significantly differ among strains (Fig. 1). Brain glucose levels were also higher in AK mice, although significant differences between AK and B6 mice were limited to the cortex (Fig. 1). Across strains, cortical glycogen levels were highest and cerebellar levels lowest (Fig. 1). Glucose levels showed an opposite trend: cortical levels were generally lower than in brain stem and cerebellum (Fig. 1).

SD. SD affected glycogen levels in a strain- and brain structure-specific way (3-way ANOVA: factor SD, $F_{1,64} = 3.3, P = 0.07$; strain, $F_{2,64} = 16.6, P < 0.0001$; structure, $F_{2,128} = 115.8, P < 0.0001$; interactions: SD-strain, $F_{2,64} = 7.3, P = 0.001$; SD-structure, $F_{2,128} = 16.4, P < 0.0001$; strain-structure, $F_{4,128} = 5.3, P = 0.0005$; SD-strain-structure, $F_{4,128} = 2.0, P = 0.10$). Strains differed dramatically in their glycogen response: sleep-deprived B6 mice had a robust (+42%) and significant increase in cortical glycogen levels ($P < 0.0001$, unpaired, 2-sided t-test; Fig. 2) and no change in the other two structures compared with the control group; sleep-deprived AK and D2 mice had no change in cortical glycogen levels but significant decreases in brain stem ($P = 0.0002$ and $P < 0.0001$) and cerebellum ($P = 0.01$ and $P < 0.0001$ in AK and D2, respectively; unpaired 2-sided t-tests). As a result of these strain- and structure-specific SD effects, glycogen content in B6 mice was significantly higher than in D2 mice in all three structures and even tended to be higher than in AK mice in the cortex and brain stem ($P = 0.08$ and $0.10$, respectively; unpaired 2-sided t-tests, Fig. 2), even though, in the control group, glycogen content in B6 mice did not differ from that in D2 mice and was significantly lower than that in AK mice in cortex and cerebellum (Fig. 1).

The SD did not seem to affect the strain- and brain structure-specific differences in glucose content (i.e., factor SD did not interact with strain or structure; 3-way ANOVA: factor SD, $F_{1,64} = 3.0, P = 0.09$; strain, $F_{2,64} = 13.7, P < 0.0001$; structure, $F_{2,128} = 15.8, P < 0.0001$; interactions: SD-strain, $F_{2,64} = 1.1, P = 0.34$; SD-structure, $F_{2,128} = 2.5, P = 0.08$; strain-structure, $F_{4,128} = 4.1, P = 0.004$; SD-strain-structure, $F_{4,128} = 0.8, P = 0.51$). Nevertheless, glucose levels in AK mice were consistently increased in all three brain areas (Fig. 2), thereby further augmenting the differences observed in the control group between AK on the one hand and D2 and B6 on the other (Fig. 1).

In B6 mice the SD-induced changes in glycogen paralleled those in glucose, with an increase in cortex and no change in the other two structures (Fig. 2). In AK and D2 mice, however, changes in glycogen did not seem to covary with changes in glucose content.

Food deprivation. A 24-h starvation is associated with a decrease in brain glycogen (14). As a negative control, we reproduced this finding in B6 mice. Twenty-four hours of food deprivation reduced cortical levels of both glycogen and glucose compared with the levels observed in the B6 control group of the SD experiment (Table 1). Four B6 mice were sleep deprived during the last 6 h of the 24-h food deprivation. Also under these conditions, SD elicited a significant increase in cortical glycogen compared with the animals that were only food deprived (Table 1). The magnitude of this SD-induced change (+39%) was comparable to that ob-
served in the ad libitum-fed B6 mice (+42%; Fig. 2). As a result, glycogen values in mice that were both food and sleep deprived no longer differed from the control group (Table 1). Even though glucose content also increased with the SD (P = 0.001, unpaired, 2-sided t-test), it did not reach control levels (Table 1).

**Halothane anesthesia.** Halothane and other anesthetics increase brain glycogen in rats and mice (7, 27, 28, 41). As a positive control we studied the effect of a 2-h halothane exposure on cortical glycogen and glucose content in SW mice, the mouse strain in which the effect of halothane has been demonstrated (7). Control SW mice had cortical glycogen and glucose values comparable to that of B6 and D2 mice (Table 1, Fig. 1).

**Table 1. Food deprivation and anesthesia affect glycogen and glucose in the cerebral cortex**

<table>
<thead>
<tr>
<th>Food Deprivation in B6 Mice</th>
<th>Glycogen (μmol/g tissue)</th>
<th>Glucose (μmol/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 11)</td>
<td>3.36 ± 0.09</td>
<td>1.42 ± 0.08</td>
</tr>
<tr>
<td>FD (n = 4)</td>
<td>2.29 ± 0.09*</td>
<td>0.69 ± 0.02*</td>
</tr>
<tr>
<td>FD + SD (n = 4)</td>
<td>3.19 ± 0.35†</td>
<td>1.00 ± 0.05*</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Halothane Anesthesia in SW Mice</th>
<th>Glycogen (μmol/g tissue)</th>
<th>Glucose (μmol/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 4)</td>
<td>2.94 ± 0.34</td>
<td>1.18 ± 0.05</td>
</tr>
<tr>
<td>Halothane (n = 4)</td>
<td>4.94 ± 0.33‡</td>
<td>1.46 ± 0.03‡</td>
</tr>
</tbody>
</table>

Values are means ± SE in μmol/g tissue. Mice strains: B6, C57BL/6J; SW, Swiss-Webster. Food deprivation (FD) lowered cortical glycogen and glucose content. FD in combination with sleep deprivation (SD) reverted glycogen back to control levels (1-way ANOVA factor treatment (control, FD, FD + SD): glycogen, F<sub>2,16</sub> = 11.4, P = 0.0008; glucose, F<sub>2,16</sub> = 19.3, P < 0.0001; *P < 0.05 vs. control; †P < 0.05 vs. FD; Tukey's range test). B6 control data same as in Table 1. Halothane anesthesia increased cortical levels of both glycogen and glucose (‡P < 0.01 vs. control; unpaired 2-sided t-tests).

After 2-h halothane exposure, glycogen and glucose content increased significantly (Table 1), as expected.

**DISCUSSION**

The present study and our previous work (15) show that changes in brain glycogen after SD are strongly influenced by age, brain region, and genotype. Most striking was our finding that glycogen increased by 40% in the cortex of B6 mice after SD, while the same treatment had no effect on cortical glycogen in either AK or D2 mice. This finding, along with our previous work in rats, contradicts our hypothesis that SD would decrease glycogen in the cortex (3). These results differ from those obtained by Kong et al. (21), who found a widespread decrease in brain glycogen, including the cortex, of adult rats after SD of 12 h. This discrepancy cannot be due to a difference in radiation intensity used in the two studies, as has been suggested (21). Accurate and precise measurements of brain glycogen require rapid inactivation of glycolytic enzymes, which is achieved by rapidly raising brain temperature to 85°C with microwave irradiation (9). Due to its larger mass, a rat brain requires 1.2 s of radiation at 10 kW to reach this temperature (21), whereas in a mouse this can be achieved in half that time (0.6 s) at a power level of 3.5 kW. The glycogen values obtained in the present study are well within the ranges reported by others in mice and rats (e.g., Ref. 43), including those obtained by Kong et al. (21) using a higher microwave intensity. In addition, we reproduced the increase in brain glycogen observed after halothane exposure (7, 27, 28, 41) and the decrease in brain glycogen after food deprivation (14).

Several factors varied among the present study, our previous study (15), and the study of Kong et al. (21) that could have contributed to the discrepancies in the
results obtained. Given the marked differences in the glycogen response to SD among the mouse strains reported here, the strain of rats used (Sprague-Dawley in Ref. 21 vs. Long-Evans in Ref. 15) could be an important factor. Developmental age also plays an important role in this response (15), and the age of rats used in one study is unknown (21). Although in both rat studies 12-h SD was used, in neither of them could the efficacy of SD be verified polysomnographically, and there may have been differences in sleep during the SD that could have affected glycogen content. Regardless of these technical issues, there does not appear to be a consistent relationship between waking duration and brain glycogen content. SD is, however, invariably followed by a compensatory sleep response that is proportional to the SD duration (11–13, 18, 44).

Glycogen is the single largest energy reserve of the brain (19). Nevertheless, glycogen content in the brain is low compared with other tissues (29). Therefore, while brain glycogen stores may be regarded as an emergency fuel source under conditions of extreme stress (e.g., hypoglycemia and cerebral ischemia), these stores could meet total brain energy demand for a very limited time only (an estimated 4–6 min; Ref. 49). The more likely view is that brain glycogen takes part in normal physiological processes (23, 38, 42, 43). Thus brain glycogen undergoes continuous turnover, can be mobilized rapidly and locally, and its level is defined by the balance between breakdown and replenishment (Ref. 42; reviewed in Ref. 8). Glycogenolysis and glycogen synthesis are mediated by brain glycogen phosphorylase and brain glycogen synthase activity, respectively. Excitatory neurotransmitters, such as norepinephrine (NE) and vasoactive intestinal peptide (VIP), potentiate glycogenolysis (reviewed in Ref. 24), while conditions of reduced neuronal activity such as NREMS (20), hibernation (1, 22), and anesthesia (7, 27, 28 and the present results) have been found to increase glycogen stores (reviewed by 41). Nevertheless, during sustained moderate neuronal activation, steady-state levels of glycogen can be maintained (38).

Consistent with the concepts outlined above, we hypothesized that sustained periods of wakefulness would be accompanied by a promotion of glycogenolysis that outweighs glycogen synthesis, resulting in progressive depletion of brain glycogen. SD should therefore have been followed by a decrease in glycogen, especially in the cerebral cortex (3) where the delta oscillations characteristic of the NREMS EEG originate (40), and the amplitude of which reliably reflects sleep-wake history (5, 6, 12, 18). The present and our previous study (15), however, demonstrate that the SD-induced changes in brain glycogen are not a simple function of time spent awake. Contrary to our predictions, cortical glycogen content increased after SD in B6 mice and 59-day-old rats (15). This increase was robust in mice and persisted even under conditions of food restriction. In the other two strains, glycogen content significantly decreased in the cerebellum and brain stem but did not change in the cortex, reminiscent of the results obtained in 50-day-old and younger rats (15). Furthermore, the two strains in which the SD-elicited compensatory sleep rebound differed the most (AK and D2; Refs. 12 and 13), did not differ in their glycogen response to SD. Clearly, the dynamics of the changes in brain glycogen in relation to sleep and wakefulness are complex.

The neurotransmitters NE and VIP can induce glycogenolysis within minutes (24), but both neurotransmitters initiate a second, longer-term process that results in glycogen resynthesis. At least in vitro, glycogen levels can surpass the levels reached before the NE or VIP administration within 9 h (39). Thus, depending on the precise time course of glycogenolysis and resynthesis and the duration of the SD, both increases and decreases in brain glycogen can be anticipated. This is further underscored by the finding that 6-h SD elicited a twofold increase in protein targeting-to-glycogen mRNA and a 2.5-fold increase in glycogen synthase activity in the mouse brain (31). Both proteins are involved in glycogen synthesis (2, 34), and these findings support the cortical glycogen increase observed in the B6 mice in the present study and in the 59-day-old rats in our previous study (15). In the fruitfly Drosophila melanogaster, a decrease in glycogen levels was observed after 3-h SD, whereas after 6-h SD, glycogen levels surpassed baseline (47), reminiscent of the bimodal effects of NE and VIP described above.

Other factors associated with the SD protocol might have contributed to the results obtained. One possible confound that unavoidably accompanies any SD protocol is stress. SD can result in an increase of plasma corticosterone (17, 45), a physiological stress marker in rodents. Little is known about how corticosterone affects brain glycogen content, although in vitro corticosterone seems glycogenolytic (46). At least in the liver, glucocorticoid administration appears to result in a protein synthesis-dependent induction of glycogen synthesis 3–4 h later (4). Preliminary data from our laboratory indicate that the corticosterone response to SD may modulate the SD-induced changes in brain glycogen; whereas 34-day-old rats show a decrease in cerebellar glycogen and no change in cortical glycogen after SD (15), adrenalectomized littermates with corticosterone replacement showed an increase in cortical glycogen and no change in the cerebellum (16). These different glycogen responses to SD correspond so well with the strain differences observed in the present study that genotype differences in corticosterone induction during SD or genotype differences in the mechanism by which corticosterone induces glycogen synthesis might contribute to the glycogen increase observed in B6 mice and the decreases observed in AK and D2 mice. B6 mice are widely regarded as behaviorally more stress resistant than D2 mice, and some studies do report a decreased corticosterone response to stress in B6 mice (36; but also see Ref. 37). We have to determine whether SD is followed by different corticosterone inductions in the three inbred strains presented here. Of course, many other factors might underlie the strain differences in the SD-induced changes.
in glycogen content, and differences in corticosterone serve to illustrate one possible pathway.

**Perspectives**

Our findings demonstrate that brain glycogen content per se is an unlikely end point of sleep’s functional role in brain energy homeostasis. With few exceptions, SD evokes a compensatory sleep response in mice (13, 18), whereas brain glycogen is increased, decreased, or unchanged depending on genetic background and brain structure. Also, the difference in sleep rebound between the AK and D2 strains (12, 13) is not reflected at the level of brain glycogen.

The momentary level of glycogen is the product of glycogenolysis and glycogen synthesis, but the dynamic interaction between initial and secondary effects of neuromodulators such as NE, VIP, and corticosterone over the course of a SD makes it difficult to make predictions. Sleep might play a role in balancing glycogen turnover rather than directly regulating its content. Future studies should focus more on the time course of the changes in glycogen and the activity of enzymes implicated in the control of brain glycogen before, during, and after SD. The marked strain differences in the glycogen response after SD provide a tool to genetically dissect the regulation of brain energy stores in relation to sleep loss.

We are indebted to Prof. T. Hastie for extensive help with the statistical analysis.

**DISCLOSURES**

This work was supported by National Institutes of Health Grants HL-64148 and HD-37315.

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