ELSEVIER

Brain Research Bulletin 75 (2008) 591–597

BRAIN RESEARCH BULLETIN

www.elsevier.com/locate/brainresbull

Research report

Cortical metabolic rates as measured by 2-deoxyglucose-uptake are increased after waking and decreased after sleep in mice

V.V. Vyazovskiy [a,b], C. Cirelli [b], G. Tononi [b,*], I. Tobler [a,**]

[a] *Institute of Pharmacology and Toxicology, University of Zurich, Winterthurerstr. 190, CH-8057 Zürich, Switzerland*
[b] *Department of Psychiatry, University of Wisconsin-Madison, 6001 Research Park Boulevard, Madison, WI 53719, USA*

Received 10 August 2007; received in revised form 11 October 2007; accepted 12 October 2007
Available online 20 November 2007

Abstract

A recent hypothesis suggests that a major function of sleep is to renormalize synaptic changes that occur during wakefulness as a result of learning processes [G. Tononi, C. Cirelli, Sleep and synaptic homeostasis: a hypothesis, Brain Res. Bull. 62 (2003) 143–150; G. Tononi, C. Cirelli, Sleep function and synaptic homeostasis, Sleep Med. Rev. 10 (2006) 49–62]. Specifically, according to this synaptic homeostasis hypothesis, wakefulness results in a net increase in synaptic strength, while sleep is associated with synaptic downscaling. Since synaptic activity accounts for a large fraction of brain energy metabolism, one of the predictions of the hypothesis is that if synaptic weight increases in the course of wakefulness, cerebral metabolic rates should also increase, while the opposite would happen after a period of sleep. In this study we therefore measured brain metabolic rate during wakefulness and determined whether it was affected by the previous sleep–wake history. Three groups of mice in which behavioral states were determined by visual observation were subjected to 6 h of sleep deprivation (SD). Group 1 was injected with 2-deoxyglucose (2-DG) 45 min before the end of SD, while Group 2 and Group 3 were injected with 2-DG after an additional period (2–3 h) of waking or sleep, respectively. During the 45-min interval between 2-DG injection and sacrifice all mice were kept awake. We found that in mice that slept ∼2.5 h the 2-DG-uptake was globally decreased, on average by 15–20%, compared to the first two groups that were kept awake. On average, Group 2, which stayed awake ∼2 h more than Group 1, showed only a small further increase in 2-DG-uptake relative to Group 1. Moreover, the brain regions in which 2-DG-uptake increased the least when waking was prolonged by ∼2 h showed the most pronounced decrease in DG-uptake after sleep. The data are consistent with the prediction that sleep may reset cerebral metabolic rates to a lower level.

Keywords: Sleep regulation; Sleep homeostasis; Synaptic homeostasis; Deoxyglucose; Mice; Brain metabolism

1. Introduction

A fundamental difference between wakefulness and sleep is the extent to which the brain is engaged in the acquisition and processing of information. During spontaneous wakefulness, animals face environmental challenges that require an adequate behavioral response. Continuous behavioral adjustments would necessarily involve learning, associated with neuronal plasticity [36]. It has recently been proposed that waking is associated with net synaptic potentiation while sleep results in synaptic downscaling [31,32]. Such regulation would ensure the restoration of the brain's ability to learn by preventing synaptic saturation, and would maintain an overall balance of space and energy resources. The latter is especially important since the brain is the most metabolically expensive tissue in the body [2,3] and synaptic activity is responsible for the bulk of the brain's energy consumption (around 75%), largely for synaptic currents/repolarization and for the propagation of action potentials [3]. It is reasonable to assume therefore, that the higher the synaptic weight impinging on a neuron, and hence the higher its firing rate, the larger the need for metabolic substrates. If, as predicted by the synaptic homeostasis hypothesis [31,32], synaptic weight increases in the course of normal wakefulness,

Abbreviations: 2-DG, 2-deoxyglucose; Cg, cingulate cortex; CC, corpus callosum; RSG, retrosplenial granular cortex; Cpu, caudate putamen; EEG, electroencephalogram; EMG, electromyogram; NREM sleep, non-rapid eye movement sleep; PB, Probst bundle; PET, positron emission tomography; REM sleep, rapid eye movement sleep; SEM, standard error of the mean; SD, sleep deprivation; SWA, slow wave activity.

* Corresponding author. Tel.: +1 608 263 60 63.
** Corresponding author. Tel.: +41 44 635 59 57.
E-mail addresses: gtononi@wisc.edu (G. Tononi), tobler@pharma.unizh.ch (I. Tobler).

0361-9230/$ – see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.brainresbull.2007.10.040

brain metabolism should also increase. Consistent with this prediction, a PET study reported that in humans absolute blood flow values were ~18% higher at the end of the waking day than after a night of sleep, and this was the case almost everywhere in the brain [5].

In this paper we report experiments which tested the hypothesis which brain metabolism, as measured by 2-DG autoradiography, changes depending on sleep–wake history. For this purpose, we took advantage of the data obtained in a recent 2-DG study [34] aimed at quantifying regional effects of unilateral whisker stimulation during sleep deprivation (SD). In that study, mice were divided into three groups: Group 1 was injected with 2-DG immediately at the end of 6 h of SD, while Group 2 and Group 3 were injected with 2-DG after an additional period (2–3 h) of waking or sleep, respectively. In the original study, which focused on relative differences in 2-DG-uptake between stimulated and unstimulated cortical areas, we noticed that the level of 2-DG-uptake for several cortical and subcortical regions seemed to be lower in Group 3, which slept, relative to Groups 1 and 2, which were always awake before sacrifice. In the present study, we analyzed in detail absolute 2-DG-uptake values (*n*Ci/g) from these three experimental groups. We expected that the animals which were awake prior to the 2-DG injection would have more 2-DG-uptake than animals which were asleep prior to the 2-DG injection.

2. Materials and methods

2.1. Animals

The details of the methodology are described elsewhere [34]. The experiments were performed in accordance with the European Communities' Council Directive of 24 November 1986 (86/609/EEC) and were approved by the Cantonal Veterinary Office of Zurich. All efforts were made to minimize the number of mice used and their suffering. Adult male B1 mice ($n = 25$) were used at the age of 14.4 ± 0.3 weeks. The choice of B1 mice, which have partial or total callosal dysgenesis, was due to the original aim of our study [34], where we investigated the local changes in brain metabolism after unilateral whisker stimulation. These mice show no significant abnormalities in behavior or sleep architecture. Moreover, they exhibit the typical physiological increase in sleep intensity after a period of waking, thereby making it possible to generalize the data obtained with this strain to other mouse strains and other species. Prior to and during the experiment the mice were kept individually in Macrolon cages (36 cm × 20 cm × 35 cm), with food and water available ad libitum, and maintained on a 12 h light–12 h dark cycle (daylight type fluorescent tubes, 58 W, approximately 30 lx) at 22–24 °C ambient temperature.

2.2. Experimental protocol and data acquisition

In the present study we used 2-DG as a metabolic marker. After its phosphorylation by hexokinase to [^{14}C]deoxyglucose-6-phosphate, this marker is essentially trapped in the tissue because, unlike glucose-6-phosphate, it cannot be isomerized to fructose-6-phosphate, a step required for its further metabolism. The stability of 2-DG in the brain tissue, however, is less than previously assumed [30], since it has been shown that 10 min after the injection 2-DG is lost from the brain at a significant rate [11,12]. In any case, the direct comparison of metabolic rates assessed by the 2-DG and ^{14}C-glucose consumption revealed close correspondence between the two methods for many brain regions [9,10], and 2-DG appears to be a more sensitive marker of regional functional activation after sensory stimulation compared to ^{14}C-glucose [7–9]. Furthermore, the use of 2-DG enabled us to compare our results with previous reports [25,30,35].

We exploited a semi-quantitative technique of 2-DG autoradiography [34,35]. The original quantitative 2-DG-autoradiography method developed by Sokoloff et al. [30] requires the collection of arterial blood samples throughout the experimental period after an intravenous pulse of [^{14}C]DG to determine arterial blood content of 2-DG and glucose concentration. These measurements, together with their respective kinetic constants of phosphorylation, are subsequently used to determine local cerebral glucose utilization in mmol/100 g/min. As previously done [19,35], we used instead a semi-quantitative technique which does not require blood sampling but relies on optical densitometry of the 2-DG autoradiograms. This approach is based on the observation that regional optical density in the autoradiography images of the brain is proportional to the local concentration of the [^{14}C] isotope, which is directly related to regional metabolic rates [30]. In some cases semi-quantitative techniques require the normalization of the optical density values to an internal control (e.g. the mean value for the entire brain). In other cases, like ours, in order to obtain absolute values of radioactivity the optical density values were converted into units of *n*Ci/g of grey matter with the aid of radiological standards that are co-exposed with the brain sections [19].

On the day before the experiments mice were weighed and placed in new cages without food 3 h after dark onset (33.2 ± 0.9 g, $n = 25$). Food deprivation results in higher values of 2-DG-uptake [34]. At light onset of the following day all mice were subjected to 6 h SD by introducing a variety of objects into the cage (pieces of wood, PVC boxes with small holes, metal grids and tissues). The weight determined 1–2 h before ending the SD was 29.8 ± 0.8 g.

All mice were injected with 2-DG 45 min before the brains were collected. It has been shown that 45 min after 2-DG administration its plasma concentration reaches very low levels, while tissue concentration reaches a maximum [30]. For the injection the mice were transferred to another room and placed in novel cages. None of the mice slept during the 45-min exposure to 2-DG. The mice were subdivided into three groups. Group 1 ($n = 9$) was injected with 2-DG 45 min before the end of SD, and brains were collected immediately after the 6 h of SD and thus had been continuously awake for the last 6 h. SD always started at light onset and was performed by introducing novel objects into the cage when the animal assumed a sleep posture, or exhibited behavioral signs of drowsiness. The animals were not touched or handled directly, and were not disturbed when they were spontaneously awake, feeding or drinking. On average, the interval between two consecutive interventions varied from 3–5 to 20–30 min. SD was used to further enhance natural sleep pressure, and thus ensure a consolidated bout of sleep prior to sacrifice in Group 3 (see below). Group 2 ($n = 8$) was sleep deprived for an additional 2.3 ± 0.2 h. Mice belonging to Group 3 ($n = 8$) prior to 2-DG injection were left undisturbed for 3.5 h after the 6 h of SD in their home cages, where they were observed constantly. Behavior was scored three times every 5 min to determine whether the mice slept. Sleep was scored when the animals adopted a sphinx-like posture, or were curled up and the eyes were closed. Neither age nor weight differed significantly between the groups (two-way ANOVAs, interaction 'group' × 'tested variable' (age, weight), $p > 0.3$).

The preparation of the brains and autoradiogram analysis was performed as in previous reports [19,34]. Briefly, a solution of [1-^{14}C]-2-deoxy-D-glucose in sterile water, radioactivity content 0.1 mCi/ml, concentration 304.1 μg/ml, radiochemical purity 98.4% (Moravek Biochemical, Inc., 577 Mercury Lane, Brea California 92821, USA) was injected (i.p. dose 16.5 μCi/100 g) 45 min prior to perfusion with formaldehyde solution. All sections were exposed to Roentgen films (Microvision-Ci, Röntgen Bender AG) together with a set of radiological standards, poly[^{14}C]metacrylate microscales (RPA 504 (39.4–1075.1 *n*Ci/g), Amersham Biosciences Europe GmbH), calibrated for brain grey matter equivalent to ^{14}C concentration (*n*Ci/g of tissue) for 3 weeks at 5 °C. After exposure the sections were Nissl stained (cresyl violet).

The semi-quantitative technique of autoradiography required us to take several steps in the data collection and processing to enable us to compare the absolute values of 2-DG-uptake among groups. First, the optical density values from the digitized images were converted to the concentration of 2-DG (*n*Ci/g of brain tissue) with aid of calibration curves computed individually for each film from a set of radiological standards that were exposed together with the brain sections (see above). Second, all animals were of the same strain, from the same breeding colony, and were precisely matched based on their weight and age. Furthermore, the amount of injected 2-DG was computed based on the individual weight of each animal. Finally, the 2-DG injected in all animals

was from the same stock, injections and perfusions were performed by the same person, and perfusions were performed under strict control of the amount and flow rate of the perfusate.

2.3. Data analysis and statistics

Image analysis was performed with a microcomputer imaging device (MCID) image analysis system (Imaging Research, St. Catharines, Ont.). Eight coronal sections (20 μm thick) between 0.5 mm rostral and 2 mm caudal to bregma were analyzed for every mouse. Regions of interest were outlined on an image of Nissl-stained section according to the mouse brain atlas [22], and included cortical areas: motor (M1, four sections), cingulate (Cg, four sections), retrosplenial (RSG, four sections), and parietal (S1 and S2, eight sections) of the left and right hemisphere; subcortical areas: white matter (corpus callosum or Probst bundle: CC or PB, respectively, eight sections) and striatum (CPu, four sections). 2-DG-uptake (*n*Ci/g of brain tissue) was assessed in the corresponding autoradiography image. We selected a variety of cortical and subcortical brain regions with different cytoarchitecture, blood supply and function, to determine whether any change observed as a result of sleep–wake history was specific for the grey versus white matter, cortical versus subcortical structures, or more generally, whether it was widespread or local. An overall value of brain metabolism for each mouse was obtained by measuring the 2-DG-uptake in the entire brain section for each of the eight sections used per animal. 2-DG-uptake (*n*Ci/g of brain tissue) was assessed in the corresponding autoradiography image. The original design of this study [34] included whisker stimulation and whisker trimming on one side. Previously, we found that these procedures induced a left–right asymmetry only in layer IV of the barrel cortex. In the present study, we reasoned that including only the stimulated or non-stimulated side might introduce a bias, and therefore, to rule out potential unilateral effects, we averaged data from the left and right hemisphere within an individual for each region.

Regional 2-DG-uptake was compared across groups. The effect of the preceding sleep–wake history was tested by two-way ANOVA with factors 'region' and 'group'. When ANOVAs reached significance, group differences were determined by post hoc Tukey's multiple comparison tests.

3. Results

3.1. Sleep–wake history differs among the three groups

During the entire duration of the experiment the behavior of all animals was under constant visual observation. Mice in Groups 1 and 2 had been continuously awake for 6 h and 8.3 ± 0.1 h, respectively, while mice in Group 3 slept on an average for 2.3 ± 0.2 h prior to sacrifice. The amount of sleep observed in Group 3 prior to the 2-DG injection varied between 106.7 and 180.0 min, while most of the remaining time was spent in quiet wakefulness (active waking occupied only 10.8 ± 2.9 min). All mice were awake during the 45-min period between the 2-DG injection and sacrifice. Behavioral observations confirmed that waking behavior during this time was indistinguishable among the three groups. Thus, the regional changes in 2-DG-uptake described below do not merely reflect differences in behavioral state during the uptake period.

3.2. Sleep–wake history affects regional 2-DG-uptake

Relative values of 2-DG-uptake (% of total uptake) have been reported elsewhere [34]. In this study we focused instead on the absolute values that were not the main focus of our previous work. The different approach was necessary since normalization of the data to the total uptake would have eliminated the differences between the groups. In agreement with previous studies [28,30], we found regional differences in the absolute level of 2-DG-uptake, as indicated by the significant difference in the two-way ANOVA using the factor "Region", independent of the factor "Group" ($p < 0.01$). As summarized in Table 1 and Fig. 1, in all groups the highest values of 2-DG-uptake were invariably observed in the medial cortical areas (retrosplenial granular, RSG), while the lowest 2-DG-uptake was measured in the white matter, as shown previously [16,28]. When 2-DG-uptake was compared across groups, the most significant result was the high intensity of 2-DG labelling in mice which were sacrificed after 6 h of wakefulness (Group 1) or when wakefulness was prolonged to ∼8.3 h (Group 2) compared to the animals which after the 6-h waking period were left undisturbed and slept ad libitum for at least ∼1.5 h (Group 3). Two-way ANOVA revealed that the difference across groups was not region-specific (factor 'Group' and 'Region', $p < 0.001$, interaction, $p > 0.9$). Post hoc Tukey's test showed that the absolute values (*n*Ci/g) of 2-DG-uptake for most regions differed among the groups, with Group 3 reaching the lowest values (Fig. 1). As can be seen from Fig. 1 and Table 1, the values in Group 3 were lower in all areas and sections, but due to the relatively small number of animals significance was reached only where variance was sufficiently low. Specifically, 2-DG-uptake values in Group 3 were significantly below those of Group 1 in RSG, motor cortex (M1), and white matter (corpus callosum, CC), and below those of Group 2 in M1, cingulate cortex (Cg) and CC.

Table 1
Regional 2-deoxyglucose-uptake

Region	6 h SD (Group 1)	Waking (Group 2)	Sleep (Group 3)
M1 (four sections)	417.4 (9.7)	424.1 (19.8)	351.5 (18.8) a,b
S1 (eight sections)	413.9 (15.4)	421.3 (22.0)	354.1 (20.2)
S2 (eight sections)	413.5 (16.7)	431.7 (26.1)	369.2 (23.4)
Cg (four sections)	436.4 (10.3)	463.9 (23.8)	389.2 (20.5) b
RSG (four sections)	498.2 (19.1)	495.9 (33.8)	405.5 (21.4) a
CPu (four sections)	389.6 (10.5)	408.0 (24.9)	350.6 (20.2)
CC/PB (eight sections)	269.6 (8.3)	275.4 (13.8)	227.9 (10.9) a,b
Entire section (eight sections)	350.0 (8.7)	371.3 (19.9)	313.7 (17.4) b

Regional 2-DG-uptake (average of left and right hemisphere) in Group 1 ($n = 9$ mice), Group 2 ($n = 8$) and Group 3 ($n = 8$). Values (*n*Ci/g) are mean (±S.E.M. in parenthesis) calculated by averaging four to eight sections (as indicated in column 1) per animal. Letters indicate significant differences between groups (a: Group 1 vs. Group 3; b: Group 2 vs. Group 3). $p \leq 0.05$, Tukey's multiple comparison test after significance in one-way ANOVA, factor 'group').

3.3. Sleep leads to an overall decrease in 2-DG-uptake in cortical and subcortical brain regions

To obtain an overall measure of 2-DG-uptake we measured the 2-DG labelling for each of the eight sections collected for each mouse, averaged them to yield a single value of total 2-DG-uptake per animal, and finally averaged these values within all the individuals of each experimental group. Three representative sections, one per Group are shown in Fig. 2A. Mean values

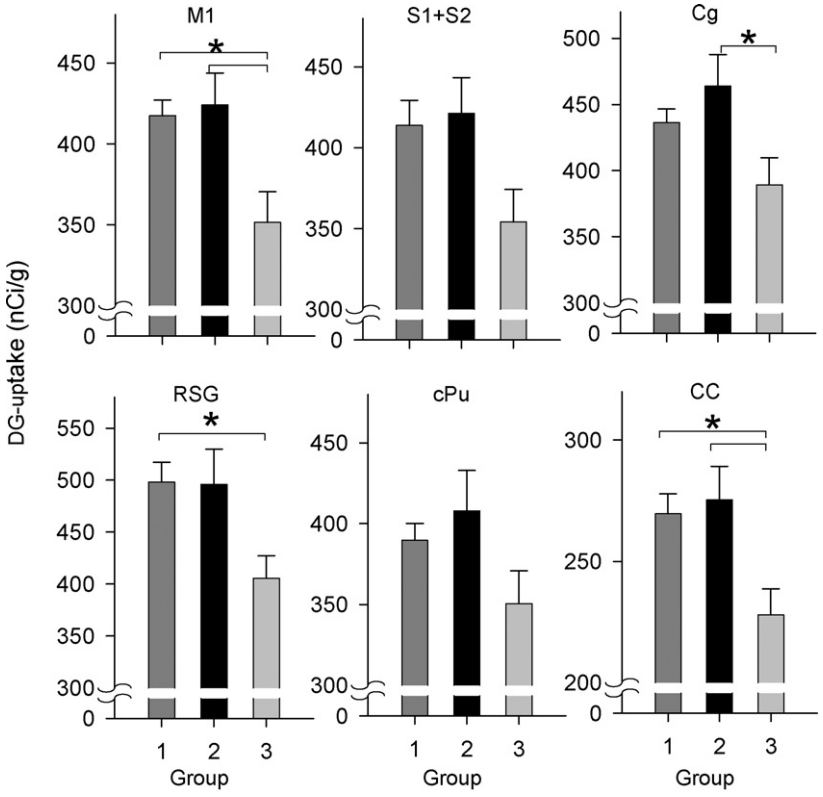

Fig. 1. Absolute values of 2-DG-uptake (*n*Ci/g) in cortical and subcortical regions. Values are mean (± S.E.M.) for Groups 1–3 ($n = 9$, 8 and 8, respectively, see Section 2 for details). Asterisks indicate significant differences between groups ($p < 0.05$, Tukey's multiple comparison test). Primary (S1) and secondary (S2) somatosensory areas were averaged. The remaining regions were primary motor (M1), cingulate (Cg) and retrosplenial (RSG) cortex, striatum (CPu) and white matter (CC).

for the three groups are shown in Fig. 2B. The level of 2-DG-uptake of Group 1 and Group 2, which had been kept awake for 6 and 8.3 h, respectively, were comparable (though slightly higher in Group 2). Values for Group 3, on the other hand, were lower (Group 1: 350.0 ± 8.7; Group 2: 371.3 ± 19.9; Group 3: 313.7 ± 17.4 *n*Ci/g; Table 1). The difference was significant for the Group 2/Group 3 comparison ($p < 0.05$, unpaired *t*-test) and approached but did not reach statistical significance for the Group 1/Group 3 comparison ($p = 0.07$, unpaired *t*-test). As indicated in Fig. 2C, which shows the mean 2-DG values for each of

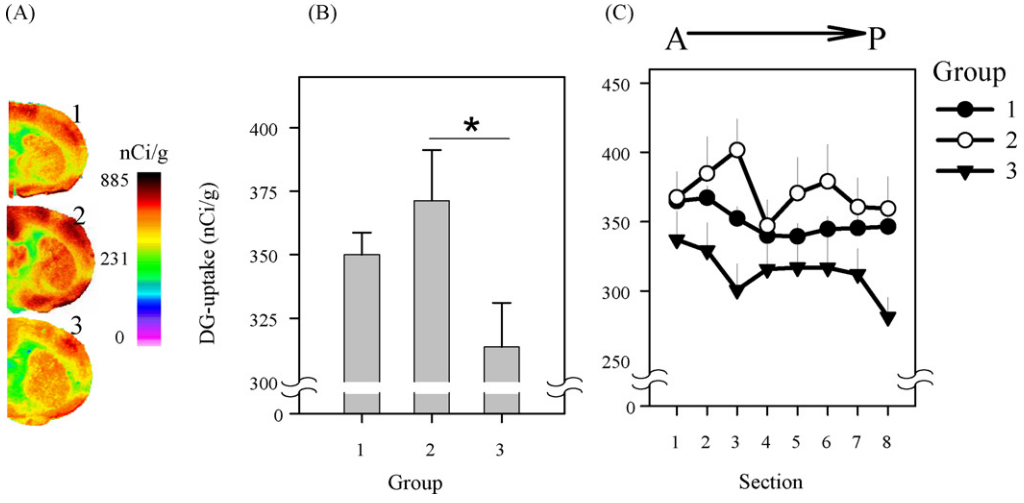

Fig. 2. (A) Pseudocoloured digitized autoradiograms of coronal sections (right hemisphere) at the level of the barrel cortex from a representative mouse of each experimental group. Levels of 2-DG-uptake are colour-coded according to scale (*n*Ci/g). (B) Total 2-DG-uptake values (mean ± S.E.M.) for Groups 1–3. Asterisk indicates significant difference between groups ($p < 0.05$, unpaired *t*-test). (C) Total 2-DG-uptake computed for eight consecutive equidistant sections taken from A (~0.5 mm rostral to bregma) to P (~2 mm caudal to bregma). Values are mean ± S.E.M.

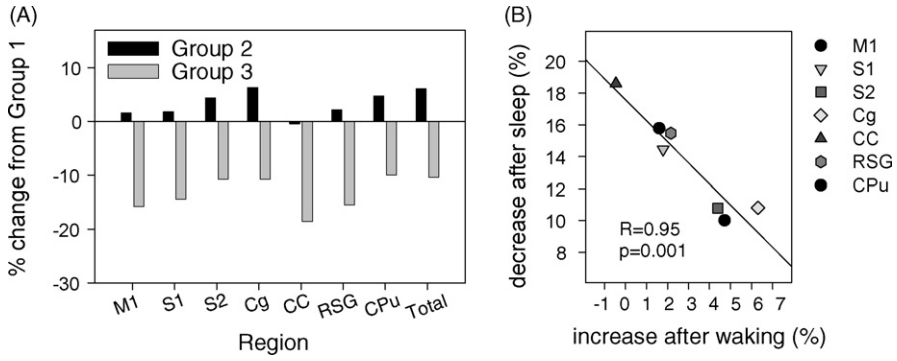

Fig. 3. (A) Mean regional 2-DG-uptake in Groups 2 and 3 shown as percent change relative to Group 1. Abbreviations for the brain regions are as in Fig. 1. (B) The relationship between the changes in 2-DG-uptake after waking and after sleep across brain regions. Scatter plot of the percent difference in 2-DG-uptake between mean 2-DG-uptake values of Groups 1 and 3 (*Y*-axis, decrease after sleep) plotted as a function of the difference between mean 2-DG-uptake values of Groups 1 and 2 (*X*-axis, increase after waking). Each symbol represents one of seven brain regions (abbreviations for the brain regions are as in Fig. 1). Straight line depicts linear regression. Pearson correlation coefficient (*R*) and the corresponding *p*-value are shown within the panel.

the eight equidistantly taken sections (from ∼0.5 mm rostral to ∼2 mm caudal to bregma), the reduced 2-DG-uptake in Group 3 seemed to encompass most of the brain.

As shown in Fig. 3A, the overall decrease in 2-DG-uptake in Group 3 relative to Group 2 was about 15–20%. Interestingly, in those regions where 2-DG-uptake increased from Group 1 to Group 2, the decrease from Group 1 to Group 3 was less pronounced. In contrast, those brain areas, such as RSG and CC, in which 2-DG values showed a smaller further increase from Group 1 to Group 2, showed a more pronounced decrease in 2-DG-uptake after sleep. This resulted in a significant negative correlation between the increase in 2-DG-uptake after waking and decrease after sleep across the brain regions (Fig. 3B).

4. Discussion

In the present study, we analyzed data obtained from a previous investigation [34] to ask whether cerebral metabolic rates, quantified as absolute values of 2-DG-uptake, are higher after a period of wakefulness and lower after a period of sleep. This question stemmed from the hypothesis that wakefulness is associated with a net increase in synaptic weight, whereas sleep is associated with synaptic downscaling [31,32]. Indeed, we found that absolute values of 2-DG-uptake were higher in mice which were awake for several hours prior to sacrifice, compared to mice which were allowed to sleep ad libitum.

These data indicate that sleep–wake history is a major determinant of cerebral metabolic rate. Importantly, our results cannot be accounted for by differences in vigilance state during the 2-DG-uptake period, because all mice were injected while they were awake, and all stayed awake during the 45-min interval between injection and sacrifice. This potential confounding factor needed to be addressed specifically because there is extensive evidence that cerebral metabolic rates depend on the levels of neuronal activity [2,3] and therefore might be influenced by behavior as well as vigilance state. For example, barbiturate anaesthesia, which is associated with inhibition of catecholamine release [14] and marked suppression of neuronal activity [1] is also characterized by lowered cerebral blood flow [13,17] and reduced 2-DG utilization [30]. Furthermore, during NREM sleep, 2-DG-uptake is reduced in animals [16,25,26], and a global deactivation of the neocortex and thalamus during sleep has been demonstrated with PET studies in humans (reviewed in [20]). Consistently, there is evidence that mRNA and protein levels of genes involved in cerebral energy metabolism (e.g. mitochondrial enzymes and glucose transporters) are lower during sleep than during waking [6,21,23].

An interesting observation was that the effect of the preceding sleep–wake history on 2-DG-uptake was apparent not only in the cerebral cortex (e.g. RSG) but also in the white matter (the corpus callosum). A previous study in monkeys found that 2-DG-uptake decreased during sleep relative to waking by 16–46% in different cortical areas, and by ∼20% in the corpus callosum [16]. The magnitude of changes in our study falls within the same range, although in our case we measured 2-DG-uptake always in the awake brain, but after a different sleep–wake history. Theoretical calculations suggested that most energy consumed by the nervous tissue is related to postsynaptic events related to glutamate signalling [3]. Since the white matter is devoid of synaptic connections, the levels of 2-DG-uptake in this tissue should therefore reflect the energy needed for the propagation of the action potentials [29]. Our data suggest that the need in energy substrates after sleep is reduced both in the regions rich of synaptic connections, such as the cerebral cortex, as well as in the regions, such as the corpus callosum, crucial for neuronal crosstalk and dominated by axons. We speculate that the lower 2-DG consumption we found after sleep in the cortex and in white matter might indicate that fewer action potentials are generated as a result of postsynaptic glutamate release, and fewer action potentials propagate between the two cortical hemispheres. Both these changes are consistent with a generalized downscaling of synaptic strength during sleep [31,32].

We found a significant negative correlation across regions between the magnitude of increase in 2-DG-uptake after an additional waking interval and its decrease after sleep. In other words, those brain areas where 2-DG-uptake increased further when the waking period was prolonged showed less of a decrease after sleep. In contrast, those areas in which 2-DG values showed

a smaller further increase from Group 1 to Group 2 showed a more pronounced decrease in 2-DG-uptake after sleep. This may seem surprising, because one might have expected that the brain regions that upon further waking have on average greater increases in glucose utilization (e.g. secondary somatosensory and cingulate cortex and CPu) would show a stronger build-up of sleep need, and thus a bigger decline in glucose metabolism during sleep. However, it is also possible that those regions (e.g. primary motor, somatosensory and retrosplenial cortex) that only show small additional increase in 2-DG-uptake did so because their metabolic rate was closer to saturation, and thus more in need to decrease during sleep. This interpretation is consistent with the synaptic homeostasis hypothesis, because if high metabolic rates indeed are a result of high net synaptic weight, downscaling during sleep would be more efficient. Furthermore, such data are consistent with the exponential saturating increase during waking of the homeostatic Process S, a marker of sleep pressure [4] and with the regional differences in sleep intensity [34]. Furthermore, learning prevents further LTP in the rat motor cortex [27] and hippocampus [36], while the induction of LTD is facilitated, indicating that in these areas the ability to further increase synaptic strength during waking may have reached saturation.

We wish to emphasize that this study has several limitations, because it was not originally designed to test the hypothesis that sleep–wake history affects absolute cerebral metabolic rates. The original aim of the study was to investigate how the regional pattern of 2-DG-uptake is related to the regional EEG differences between hemispheres and within each hemisphere. The group size was small because our previous study was based on relative values of 2-DG-uptake that significantly reduced variability. Also, we employed a semi-quantitative version of the 2-DG autoradiography method, which proved to be reliable in assessing regional differences in 2-DG-uptake [35]. The original quantitative technique of 2-DG autoradiography [30], however, could provide more accurate measures of cerebral glucose consumption because it takes into account not only local tissue concentrations of 2-DG-uptake, as measured in the present study, but also the dynamics of its clearance from the blood. Future studies using the quantitative 2-DG autoradiography technique are needed to confirm our results in a larger group of mice, and possibly in other species as well. Furthermore, mice in this study were not implanted for polysomnography, to avoid potential tissue damage due to the electrodes, and we relied on continuous visual observation to score the behavioral state of the animals. It cannot be excluded that short sleep attempts remain unnoticed. Moreover, REM sleep and NREM sleep could not be reliably scored based on behavioral criteria, thereby precluding predictions about the specific contribution of different sleep states to synaptic downscaling, as well as the role of sleep consolidation. Quantitative [6-^{14}C]glucose-autoradiography in cats has shown that absolute cerebral metabolic rate increases significantly during REM sleep in several regions encompassing the limbic system, hypothalamus and pontine reticular formation (~20–40% increase compared to waking) [18], while in the cerebral cortex absolute values of glucose utilization are generally similar between waking and REM sleep [18]. In future experiments the EEG could be recorded from one hemisphere, while the other hemisphere could be used for 2-DG measurements. It should be mentioned, however, that total sleep time in mice includes significantly larger (~80–85%) portion of NREM sleep compared to REM sleep [15]. Moreover, our recent data indicate that synaptic downscaling, measured as a decrease in the slope of cortical evoked responses, is positively correlated with the amount of NREM sleep, but not with that of REM sleep (Vyazovskiy, Cirelli, Tononi, unpublished).

With these caveats in mind, however, the results of this study are consistent with the predictions of the synaptic homeostasis hypothesis. Other recent experimental observations also support this hypothesis. Specifically, we found in synaptoneurosomes that suggested molecular markers of synaptic potentiation in vivo, such as the number of glutamatergic AMPA receptors containing the GluR1 subunit, increase after waking and decrease after sleep, while suggested markers of synaptic depression, such as the dephosphorylation of GluR1 at Ser845, do the opposite [24]. Consistently, we also found that electrophysiological markers of LTP – the slope and amplitude of cortical evoked potentials in freely behaving awake rats – increase after a period of wakefulness and decrease after a period of sleep [33]. Since synaptic activity is a major determinant of brain metabolic rates, the differences in 2-DG-uptake that occur as a result of preceding sleep–wake history may indeed reflect a net increase in synaptic weight during waking. Furthermore, they also suggest that an important function of sleep may be to decrease the energy requirements that built up as a result of synaptic potentiation during normal waking. Thus, synaptic downscaling during sleep would maintain the homeostasis of brain metabolism that is necessary for its normal functioning and plasticity.

Conflicts of interest

This was not an industry-supported study. Drs V.V. Vyazovskiy, C. Cirelli, G. Tononi and I. Tobler have indicated no financial conflicts of interest.

Acknowledgements

The study was supported by the Swiss National Science Foundation grants 3100A0-100567, 320000-112674 and PBZHB-106264, and NIH director Pioneer Award to GT. We thank Drs J.M. Fritschy, E. Welker and S. Palchykova for their help with the experiment and data analysis and C. Sidler and C. Musetti for technical assistance.

References

[1] B. Antkowiak, Different actions of general anesthetics on the firing patterns of neocortical neurons mediated by the GABA(A) receptor, Anesthesiology 91 (1999) 500–511.
[2] D. Attwell, A. Gibb, Neuroenergetics and the kinetic design of excitatory synapses, Nat. Rev. Neurosci. 6 (2005) 841–849.
[3] D. Attwell, S.B. Laughlin, An energy budget for signaling in the grey matter of the brain, J. Cereb. Blood Flow Metab. 21 (2001) 1133–1145.
[4] A.A. Borbély, A two process model of sleep regulation, Hum. Neurobiol. 1 (1982) 195–204.

[5] A.R. Braun, T.J. Balkin, N.J. Wesenten, R.E. Carson, M. Varga, P. Baldwin, S. Selbie, G. Belenky, P. Herscovitch, Regional cerebral blood flow throughout the sleep–wake cycle. An $H_2(15)O$ PET study, Brain 120 (Pt 7) (1997) 1173–1197.
[6] C. Cirelli, C.M. Gutierrez, G. Tononi, Extensive and divergent effects of sleep and wakefulness on brain gene expression, Neuron 41 (2004) 35–43.
[7] R.C. Collins, D.W. McCandless, I.L. Wagman, Cerebral glucose utilization: comparison of [^{14}C]deoxyglucose and [6-^{14}C]glucose quantitative autoradiography, J. Neurochem. 49 (1987) 1564–1570.
[8] N.F. Cruz, K.K. Ball, G.A. Dienel, Functional imaging of focal brain activation in conscious rats: impact of [^{14}C]glucose metabolite spreading and release, J. Neurosci. Res. (2007).
[9] G.E. Duncan, W.E. Stumpf, Brain activity patterns: assessment by high-resolution autoradiographic imaging of radiolabeled 2-deoxyglucose and glucose uptake, Prog. Neurobiol. 37 (1991) 365–382.
[10] R.A. Hawkins, A.M. Mans, D.W. Davis, M.R. DeJoseph, Comparison of [^{14}C]glucose and [^{14}C]deoxyglucose as tracers of brain glucose use, Am. J. Physiol. 254 (1988) E310–E317.
[11] R.A. Hawkins, A.L. Miller, Loss of radioactive 2-deoxy-D-glucose-6-phosphate from brains of conscious rats: implications for quantitative autoradiographic determination of regional glucose utilization, Neuroscience 3 (1978) 251–258.
[12] R.A. Hawkins, A.L. Miller, Deoxyglucose-6-phosphate stability in vivo and the deoxyglucose method, J. Neurochem. 49 (1987) 1941–1960.
[13] K.S. Hendrich, P.M. Kochanek, J.A. Melick, J.K. Schiding, K.D. Statler, D.S. Williams, D.W. Marion, C. Ho, Cerebral perfusion during anesthesia with fentanyl, isoflurane, or pentobarbital in normal rats studied by arterial spin-labeled MRI, Magn. Reson. Med. 46 (2001) 202–206.
[14] K. Hirota, M. Kudo, T. Kudo, M. Kitayama, T. Kushikata, D.G. Lambert, A. Matsuki, Barbiturates inhibit K(+)-evoked noradrenaline and dopamine release from rat striatal slices—involvement of voltage sensitive Ca(2+) channels, Neurosci. Lett. 291 (2000) 175–178.
[15] R. Huber, T. Deboer, I. Tobler, Effects of sleep deprivation on sleep and sleep EEG in three mouse strains: empirical data and simulations, Brain Res. 857 (2000) 8–19.
[16] C. Kennedy, J.C. Gillin, W. Mendelson, S. Suda, M. Miyaoka, M. Ito, R.K. Nakamura, F.I. Storch, K. Pettigrew, M. Mishkin, L. Sokoloff, Local cerebral glucose utilization in non-rapid eye movement sleep, Nature 297 (1982) 325–327.
[17] J.P. Lowry, M. Fillenz, Real-time monitoring of brain energy metabolism in vivo using microelectrochemical sensors: the effects of anesthesia, Bioelectrochemistry 54 (2001) 39–47.
[18] R. Lydic, H.A. Baghdoyan, L. Hibbard, E.V. Bonyak, M.R. DeJoseph, R.A. Hawkins, Regional brain glucose metabolism is altered during rapid eye movement sleep in the cat: a preliminary study, J. Comp. Neurol. 304 (1991) 517–529.
[19] F. Magara, E. Welker, D.P. Wolfer, I. Drescher-Lindh, H.P. Lipp, Increased asymmetries in 2-deoxyglucose uptake in the brain of freely moving congenitally acallosal mice, Neuroscience 87 (1998) 243–254.
[20] P. Maquet, Functional neuroimaging of normal human sleep by positron emission tomography, J. Sleep Res. 9 (2000) 207–231.
[21] E.V. Nikonova, C. Vijayasarathy, L. Zhang, J.R. Cater, R.J. Galante, S.E. Ward, N.G. Avadhani, A.I. Pack, Differences in activity of cytochrome *c* oxidase in brain between sleep and wakefulness, Sleep 28 (2005) 21–27.
[22] G. Paxinos, K.B.J. Franklin, The Mouse Brain in Stereotaxic Coordinates, xxv, 1 v., 2nd ed., Academic Press, San Diego, 2001.
[23] J.M. Petit, I. Tobler, I. Allaman, A.A. Borbély, P.J. Magistretti, Sleep deprivation modulates brain mRNAs encoding genes of glycogen metabolism, Eur. J. Neurosci. 16 (2002) 1163–1167.
[24] M. Pfister-Genskow, C. Cirelli, G. Tononi, Molecular evidence for synaptic potentiation during waking and synaptic downscaling during sleep, Sleep 30 (2007) A5–A6.
[25] P. Ramm, B.J. Frost, Regional metabolic activity in the rat brain during sleep–wake activity, Sleep 6 (1983) 196–216.
[26] P. Ramm, B.J. Frost, Cerebral and local cerebral metabolism in the cat during slow wave and REM sleep, Brain Res. 365 (1986) 112–124.
[27] M.S. Rioult-Pedotti, D. Friedman, J.P. Donoghue, Learning-induced LTP in neocortex, Science 290 (2000) 533–536.
[28] W.J. Schwartz, F.R. Sharp, Autoradiographic maps of regional brain glucose consumption in resting, awake rats using (^{14}C) 2-deoxyglucose, J. Comp. Neurol. 177 (1978) 335–359.
[29] L. Sokoloff, Energetics of functional activation in neural tissues, Neurochem. Res. 24 (1999) 321–329.
[30] L. Sokoloff, M. Reivich, C. Kennedy, M.H. Des Rosiers, C.S. Patlak, K.D. Pettigrew, O. Sakurada, M. Shinohara, The [^{14}C]deoxyglucose method for the measurement of local cerebral glucose utilization: theory, procedure, and normal values in the conscious and anesthetized albino rat, J. Neurochem. 28 (1977) 897–916.
[31] G. Tononi, C. Cirelli, Sleep and synaptic homeostasis: a hypothesis, Brain Res. Bull. 62 (2003) 143–150.
[32] G. Tononi, C. Cirelli, Sleep function and synaptic homeostasis, Sleep Med. Rev. 10 (2006) 49–62.
[33] V.V. Vyazovskiy, U. Faraguna, C. Cirelli, G. Tononi, Electrophysiological evidence for synaptic potentiation during waking and synaptic downscaling during sleep, Sleep 30 (2007) A21–A22.
[34] V.V. Vyazovskiy, E. Welker, J.M. Fritschy, I. Tobler, Regional pattern of metabolic activation is reflected in the sleep EEG after sleep deprivation combined with unilateral whisker stimulation in mice, Eur. J. Neurosci. 20 (2004) 1363–1370.
[35] E. Welker, S.B. Rao, J. Dorfl, P. Melzer, H. van der Loos, Plasticity in the barrel cortex of the adult mouse: effects of chronic stimulation upon deoxyglucose uptake in the behaving animal, J. Neurosci. 12 (1992) 153–170.
[36] J.R. Whitlock, A.J. Heynen, M.G. Shuler, M.F. Bear, Learning induces long-term potentiation in the hippocampus, Science 313 (2006) 1093–1097.