Sleep fragmentation alters brain energy metabolism without modifying hippocampal electrophysiological response to novelty exposure

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SUMMARY
Sleep is viewed as a fundamental restorative function of the brain, but its specific role in neural energy budget remains poorly understood. Sleep deprivation dampens brain energy metabolism and impairs cognitive functions. Intriguingly, sleep fragmentation, despite normal total sleep duration, has a similar cognitive impact, and in this paper we ask the question of whether it may also impair brain energy metabolism. To this end, we used a recently developed mouse model of 2 weeks of sleep fragmentation and measured 2-deoxy-glucose uptake and glycogen, glucose and lactate concentration in different brain regions. In order to homogenize mice behaviour during metabolic measurements, we exposed them to a novel environment for 1 h. Using an intrahippocampal electrode, we first showed that hippocampal electroencephalograph (EEG) response to exploration was unaltered by 1 or 14 days of sleep fragmentation. However, after 14 days, sleep fragmented mice exhibited a lower uptake of 2-deoxy-glucose in cortex and hippocampus and lower cortical lactate levels than control mice. Our results suggest that long-term sleep fragmentation impaired brain metabolism to a similar extent as total sleep deprivation without affecting the neuronal responsiveness of hippocampus to a novel environment.

INTRODUCTION
During wakefulness, brain energy metabolism is coupled tightly with neuronal activity to adapt energy supply to neuronal needs, a mechanism also known as ‘neurometabolic coupling’, where astrocytes play a key role, particularly through the release of lactate which serves as energy substrate when glutamate is released during neuronal activation (Escartin and Rouach, 2013; Magistretti, 2006; Magistretti et al., 1999). It was shown recently that this mechanism, known as ‘Astrocyte-Neurone Lactate Shuttle’ (ANLS), plays a crucial role in long-term memory formation (Suzuki et al., 2011). Moreover, we showed that most transcripts coding for ANLS-related proteins are up-regulated in astrocytes following 6 h of sleep deprivation (SD) (Petit et al., 2013), suggesting that sleep disturbances might impact neurometabolic coupling at the molecular level.

When wakefulness is prolonged artificially (SD), a decrease of 10–20% in the glucose uptake is observed in all brain regions in humans (Thomas et al., 2000; Wu et al., 2006) as well as in rodents (Eversen et al., 1994). This body of data indicates that neurometabolic coupling is probably impaired when sleep is acutely disturbed, particularly in the hippocampus, where this may explain difficulties with spatial memory formation in sleep-deprived subjects (Diekelmann and Born, 2010; Peigneux et al., 2004) and rodents (Graves, 2003; Ruskin et al., 2006).

In vivo, neurometabolic coupling mechanisms include an increase in astrocytic glucose uptake (Chuquet et al., 2010), neuronal lactate uptake (Wyss et al., 2011) as well as an increase in glycogen breakdown and turnover (Swanson et al., 1992). Given the high prevalence of chronic sleep disorders involving sleep fragmentation (SF) and their impact on cognition, including working memory in humans (Bonnet
and Arand, 2003), as well as spatial memory in rodents (Nair et al., 2011; Ramesh et al., 2012), it is of prime interest to investigate the impact of SF on brain energy metabolism. For that purpose, we used a recently developed chronic SF protocol in mice (Baud et al., 2013, 2015) and studied its impact on brain energy metabolism during ‘novelty exposure’ (NE, introduction of the animal into a novel environment) to normalize the behaviour of mice in all groups during metabolic measurements. In addition, this experimental condition induces a specific electrophysiological signature in the hippocampus (Staiger et al., 2002).

In the present study, we first assessed the extent of neuronal activation during NE by measuring c-fos expression in different brain areas. Next, we coupled 1 or 14 days of SF followed by 1 h of NE and recorded the electrophysiological activation in the hippocampal CA1 using hippocampal depth electroencephalograph (EEG). Finally, we measured regional 2-deoxy-glucose uptake as well as lactate, glucose and glycogen contents in different brain areas.

MATERIALS AND METHODS

Animals

Adult male C57BL6/J mice (aged 7–9 weeks; Charles River, Saint-Germain-Nuelles, France) were single-housed under a 12-h light/dark cycle [light on at 05:00 hours corresponding to zeitgeber time (ZT) = 0] in standard conditions (23 ± 1 °C) during at least 7 days prior to and for the duration of experiments. All procedures were carried out according to the European Community’s Council directives (86/609/EEC) and with authorization of the veterinary service of the canton of Vaud.

Sleep fragmentation

We used a homemade device (named ‘CaResS’) developed to perform automatic instrumental SD and SF (Baud et al., 2013; Petit et al., 2013). Briefly, in the sleep fragmented group (F), SF was enforced by intermittent cage activation at a rate of 60 arousals h⁻¹ (20 s every min) during 24 h for 1 (D1) or 14 days (D14). Control groups (motor control, MC) underwent enforced locomotion for 40 min h⁻¹ during the active period only (dark, 17:00–05:00 hours) to match locomotor activity of the F groups while not interfering with sleep. Based on previous experiments, a sleep restriction of 18 and 12% is expected at D1 and D14, respectively, in the F group compared to the MC group (Baud et al., 2013).

Novelty exposure

For 2-DG uptake, brain metabolites determination and hippocampal EEG recordings, mice from SF and MC groups were exposed to novelty for 60 min (NE group) by placing them individually in a new enriched environment (see Fig S1). All NE experiments began between 14:00 and 16:00 hours (corresponding to ZT9 and ZT11).

Hippocampal EEG (hEEG)

To determine whether hippocampal activation during NE could be altered by SF, we implanted mice chronically with a single hippocampal depth electrode situated into the CA1 region of dorsal hippocampus (see Supporting information). Mice were exposed to novelty in differently arranged environments on D1 (n = 5 per group) and D14 (MC, n = 4 and F, n = 3). The hEEG signal was analysed during the first 30 min of NE in reference to 30 min baseline prior to NE [more details in the Supporting information (section II.1)]. The hEEG recordings of individual mice were shown as a density plot along the time axis (x-axis) and the frequency axis (y-axis) (Fig. 1a,b). The average power spectrum of hEEG during NE (expressed as percentage of the baseline) was calculated on days 1 and 14 of SF (Fig. 1c,d). Power changes over time were calculated for predefined frequency bands (see Figs S1f–I and S3). After

Figure 1. Hippocampal electroencephalograph (hEEG) before and during novelty exposure in sleep fragmented mice (F) and in control mice (MC). (a,b) Example of a density plot obtained in a control mouse (a) and in an F mouse (b) during 30 min of baseline and 30 min of exploration in a novel environment (NE). Colour code represents log₁₀ of frequency power. Note the immediate but transient increase in beta-2 power (β2) and the immediate and persistent increase in theta (θ) and gamma (γ) power during NE. (c,d) Group average of power normalized by baseline values for D1 (c) and D14 (d). Dotted line shows the 100% baseline. Grey rectangles represent frequency bins where values differed significantly from baseline (P < 0.05) for the SF (group) effect and NE effect. Note the increase in delta, theta, alpha, beta1 and 2, and gamma power in the MC group (black) and F group (red) during exploration without any significant difference between groups for these frequencies on D1 or D14. (e,h) Shift of theta frequency peak (maximum power) between baseline and NE averaged per group. Note a significant increase on D1 (e) and D14 (h) by approximately 3–4 Hz. This is also visible on the sample density plots (a,b). (f,g) Time-course of high theta (7–10 Hz) to low theta (4–Hz) ratio during baseline and NE. Note the significant and sustained shift of the ratio towards higher frequencies. Dotted horizontal lines represent average baseline value and are colour-coded by group. (i,j) Beta-2 power increase during NE normalized by baseline (100%, horizontal line). Note significant increase during the 1–10 min interval on D1 and D14 and the 11–20 min interval on D14 (P ≤ 0.017 for all). (k,l) Gamma power increase during NE normalized by baseline (100%, horizontal line). This significant increase is present throughout exploration on D1 and D14 (P ≤ 0.017). P-values in insert represent result of two-way analysis of variance (ANOVA) for SF (group) effect and NE (exposure) effect. Asterisk indicates within-group effect where values differed significantly from baseline (NE effect) in both groups (MC and F) on post-hoc paired t-test. For time-series, a Bonferroni correction was used for comparison of cumulative values over three time intervals (P < 0.017). No between-group effect was observed (P > 0.05 on two-way ANOVA).
killing, brains were sliced and a Nissl staining was performed to confirm that the electrode was implanted in the CA1 region.

**Regional 2-deoxy-glucose uptake**

After 1 (MC, \( n = 7 \) and F, \( n = 8 \)) or 14 days (MC, \( n = 7 \), F, \( n = 7 \)), mice were injected intraperitoneally with \(^{14}\)C-2-deoxy-d-glucose (2-DG) (5 \( \mu \)Ci in 50 \( \mu \)L; Hartmann Analytic, Braunschweig, Germany) and placed in the NE for 1 h. After decapitation, trunk blood was collected, brain was dissected out and frozen on carbonic ice and stocked at \(-80^\circ\text{C}\) until cutting. Serial 20-\(\mu\)m coronal brain slices were collected every 100 \(\mu\)m on slides, dehydrated immediately on a hot plate at 60 \(^\circ\text{C}\), and then processed for autoradiography analysis (see Supporting information for details). Relative
Novelty exposure following sleep fragmentation

After 1 or 14 days both F and MC groups were exposed to a novel environment for 60 min. Locomotor activity measured during NE was unaltered in the F group compared to MC (P > 0.05, t-test, data not shown). None of the animals entered sleep during NE, as attested by video-monitoring. Blood glucose at the time of killing was not different in the F group compared to the MC group (8.8 ± 0.7 versus 10.8 ± 0.8 mm, P > 0.05, t-test).

hEEG

Spectral analysis of the hEEG before and during NE displayed a specific pattern of activation, which was similar in the F and MC groups of mice (Fig. 1a,b). We used two-way analyses of variance (ANOVA) to delineate the effects of NE on repeated measures within and between groups MC and F. Given the small numbers of animals (n = 3–5 per group) we performed a retrospective power analysis, and found that the experiment had sufficient power to distinguish the effects of treatment (F versus MC, see Supporting information). Change in frequency composition during NE was similar in the two groups, without any statistically significant difference. First, for the first 10 min of NE, power in all frequency bands increased but was specifically marked and transient in the beta range (beta 1 and 2, two-way ANOVA with P < 0.05 for group effect, P < 0.01 for NE effect, post-hoc paired t-test on D1 and D14 with P < 0.017 for time bin 1–10 min, and not significant for bin 11–20 min (except beta-2 on D1) and bin 21–30). Secondly, the power remained increased for the two-first 10-min bins in the delta, theta and alpha ranges (two-way ANOVA with P > 0.05 for group effect, P < 0.001 for NE effect, post-hoc paired t-test on D1 and D14 with P < 0.017 for time bin 1–10 min and bin 11–20 min). Finally, power increase in gamma (ANOVA, P > 0.05 for group effect, P < 0.001 for NE effect, post-hoc paired t-test on D1 and D14 with P < 0.01 for all time), shift in peak theta frequency (ANOVA, P > 0.05 for group effect, P < 0.025 for NE effect), as well as high theta/low theta ratio (ANOVA, P > 0.05 for group effect, P < 0.001 for NE effect, post-hoc paired t-test min on D1 and D14 with P < 0.01 for all time) remained increased throughout the 30 min of exploration.

Regional 2-deoxy-glucose uptake

Brain autoradiograms obtained from MC and F mice subjected to NE for 60 min on D1 or D14 of the SF protocol revealed that SF decreased relative regional glucose uptake normalized by values of the corpus callosum in several ROI. On D1, a two-way ANOVA analysis with the factors group and region showed that the region effect and the group effect were significant (P < 0.0001 and P = 0.01, respectively), reflecting a tendency to lower metabolic activity in the F group when all regions are considered together. Post-hoc testing for each individual region showed close to significant
lower metabolic rates in the somatosensory cortex (CxS) and the whole brain of the F group (−10 and −8%, \(P = 0.1\) for both, Tukey post-hoc, Fig. 2).

On D14, a two-way ANOVA analysis with the factors group and region showed that the region effect was highly significant \((P < 0.0001)\), that the group effect was highly significant \((P < 0.0001)\) and that the interaction was also significant \((P = 0.01)\), meaning that the magnitude of the effect of SF (group effect) depended on the region analysed. Post-hoc analysis revealed that when considering the whole brain, a global impairment in glucose uptake was present (−10%, \(P < 0.01\), Tukey’s post-hoc, Fig. 2). Among cortical regions, the motor cortex showed a significant decrease (−9%, \(P = 0.02\), Tukey’s post-hoc, Fig. 2) while the CxS was not affected significantly (\(P > 0.05\), Tukey’s post-hoc). Subcortical regions such as the thalamus showed a similar trend, but was not significant (−10%, \(P = 0.06\), Tukey’s post-hoc, Fig. 2). Of all the regions tested, the hippocampus exhibited the greatest decrease in 2-DG uptake (−14%, \(P < 0.01\), Tukey’s post-hoc, Fig. 2).

**Brain glycogen, glucose and lactate levels**

No change was observed in microwave-irradiated tissue collected from MC or F groups on D14 after 60 min of exploration. Glycogen levels were similar in the cortex, hippocampus and thalamus in all groups (\(t\)-test, \(P > 0.05\) for all structures, Fig. 3a). Glucose levels showed a tendency to be lower without reaching statistical significance (\(t\)-test, \(P > 0.05\) for all structures, Fig. 3b). Lactate levels were decreased significantly in the cortex (−22%, \(P < 0.05\), \(t\)-test, Fig. 3c), but not in other structures.

**DISCUSSION**

We showed in a preliminary experiment that NE could be used as a paradigm of neuronal activation, as indicated by the induction of c-fos in a variety of cortical and subcortical
Our hippocampal EEG recordings indicated that NE induced a specific electrophysiological pattern that was not altered by chronic SF (Fig. 1). Indeed, a transient increase in beta-2 power and a sustained increase in theta and gamma power occurred in both groups. This electrophysiological ‘signature’ of NE was described previously using local field potential (LFP) recordings in a different mouse strain (Berke et al., 2008) and, with few differences, also in humans (Park et al., 2014). ‘Beta-2 tagging’ in the hippocampus might reflect novelty detection for unfamiliar environments (Berke et al., 2008) and objects (França et al., 2014) in rodents. It is not observed upon subsequent exposure to the same environment, but reappears in each animal upon exposure to two different novel environments 1 day or 2 weeks apart, as in our study. After novelty detection, spatial navigation encoding takes place in the hippocampus and relies upon increased network synchronization in the theta and gamma frequencies, as observed here. This leads ultimately to the individualization of hippocampal place cells coding for a unique spatial location (O’Neill et al., 2010). Although we did not perform intracellular or LFP recordings, our hEEG results suggest that SF does not impair the novelty detection or the online memory encoding mechanisms taking place during NE. Delta power changes observed here were present to a minor degree in previous reports, due probably to technical differences such as the choice of electrodes (single electrode versus tetrodes) (Berke et al., 2008).

In spite of a similar electrophysiological pattern induced by NE, SF mice displayed lower levels of 2-DG uptake in the hippocampus compared to MC mice (Fig. 2a). As ‘baseline’ values of 2-DG uptake before NE were not obtained (due to the concern for inhomogeneous brain states), its decrease after NE can be interpreted in two ways which are not mutually exclusive. First, baseline glucose uptake levels were different before NE and this pre-existing difference remained after NE. Secondly, the altered 2-DG uptake could correspond to impairment in the neurometabolic coupling mechanisms evoked by NE-induced neuronal activation. Regardless of the exact mechanism, our experiment indicates that 1 or 14 days of SF impact the brain glucose uptake. Interestingly, metabolic vulnerability of the hippocampus following 14 days of SF is in line with metabolic mapping studies after SD in humans (Thomas et al., 2000) and rodents (Everson et al., 1994). Furthermore, whereas encoding of new information takes place during wakefulness (online processing), consolidation of spatial memory requires sleep. Specifically, during subsequent slow wave sleep, synchronization of the thalamic-ocortical spindles with the hippocampal sharp-wave ripples accompanies replays of firing sequences established during wakefulness and stimulates the transfer of the memory trace to the neocortex (offline post-processing) (Diekelmann and Born, 2010; Walker and Stickgold, 2004). It is therefore conceivable that SF impact on glucose uptake during NE might alter hippocampal offline post-processing and spatial memory formation (Nair et al., 2011; Tartar et al., 2009), even if online encoding is intact. When considering the entire telencephalon, we observed a chronic global impairment in glucose uptake in the same order of magnitude as found in humans after 1 night of SD (decrease by 8–10%; Thomas et al., 2000; Wu et al., 2006) and in rodents after 14 days of SD (Everson et al., 1994). This suggests that sleep quality may be as important as sleep quantity with regard to energy restoration and that potential compensatory mechanisms do not mitigate the metabolic need for continuous sleep in the chronic setting.

In addition to the 2-DG uptake measurements that reflect dynamic uptake of glucose integrated over the entire duration of novelty exposure, we assayed hippocampal contents in glycogen, glucose and lactate as a ‘metabolic snapshot’ at the end of the NE. No change was observed in the levels of these metabolites in the SF group (Fig. 3). This indicates that the decrease in 2-DG uptake observed in hippocampus of SF mice did not impact glucose homeostasis durably. Accordingly, it was shown that extracellular levels of hippocampal lactate and glucose displayed opposite and significant changes over the first 10 min of a spatial working memory task with a subsequent return to baseline levels at the end of the task (Newman et al., 2011). The decrease in lactate content, together with a lesser and not significant decrease in 2-DG uptake observed in the CxS, suggest that SF impacts this cortical area differentially (Fig. 3c). As our lactate measurements are not specific of a cellular or extracellular compartment, the decrease in lactate content possibly reflects an increase in its utilization and/or a decrease in its production at baseline, during NE or both. This is in line with results obtained in humans where SD impaired the lactate increase triggered by cognitive stimulation of the prefrontal cortex (Urrila et al., 2004). Glycogen levels after NE remained unaffected by SF in all brain structures considered here. This is in agreement with glycogen stability observed after SD in mice (Franken et al., 2003; Petit et al., 2010), and suggests that glycogen homeostasis is maintained during SF.

In conclusion, our results show that 14 days of chronic SF impaired energy metabolism in cerebral cortex and hippocampus. Interestingly, this impairment was not accompanied by a concomitant decrease in ‘online’ neuronal activity, but might impact ‘offline’ information processing in the hippocampus and cortex. These findings bring new elements to explain how chronic SF observed in many sleep disorders may impact cognitive functions in humans.

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CONFLICT OF INTEREST
The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS
MB, JMP and PJM designed the study. AN and JMP collected and analysed the preliminary study data. MB and JMP collected the main study data. MB, JMP, JP analysed the main study data. MB and JMP wrote the paper.

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SUPPORTING INFORMATION
Additional Supporting Information may be found online in the supporting information tab for this article:
Figure S1. Photograph of the set-up used in the novelty exposure experiments.
Figure S2. Effect of novelty exposure on C-fos, Egr3, Na-K alpha2 et Glut1 mRNA levels in somato-sensory cortex.
(CxSS) and hippocampus (Hpc). The mRNA levels were expressed relative to beta-Actin mRNA levels in each structure. Statistical comparisons were performed with a t-test. Significance levels: *P < 0.05, **P < 0.01 and ***P < 0.001; NE group (n = 7) and CTL group (n = 6).

**Figure S3.** Delta, theta, alpha and beta-1 power increase during NE normalized by baseline (100%, horizontal dotted line). The t = 0 corresponds to the time the animal was placed in the novel environment. The P-values in insert represent results of 2-way ANOVA for SF (group) effect and NE (exposure) effect. Asterisk indicates statistical significance for NE effect over time bin on post-hoc paired t-test with Bonferroni correction (P < 0.017).

**Figure S4.** Statistical power analysis. The hEEG experiment statistical power versus difference of group means is expressed in percentage of baseline for 6 predefined frequency bands on day 1 and day 14. In addition, power was simulated for day 14 with n = 7 per group assuming the same mean and variance (dark green). Note that detectable effect falls around 20% range for all frequency bands except delta (60%) where variance was high, and is slightly improved by increasing the n.

**Table S1.** Primers used in preliminary study were designed using the web-based software Primer Blast (NCBI) and synthesized by Microsynth (Microsynth, Switzerland). Whenever possible, amplicons extended over an exon/exon junction to avoid amplification of any residual genomic DNA contamination.

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