Sleep deprivation in the dark period

does not impair memory in OF1 mice

SVITLANA PALCHYKOVA, RAPHAËLLE WINSKY-SOMMERER

and IRENE TOBLER*

Institute of Pharmacology and Toxicology, University of Zurich,

Zurich, Switzerland

Running title: Sleep and memory consolidation

Submitted to Chronobiology International
Date of submission: February 27, 2009

*Corresponding author:
Prof. Irene Tobler Ph.D.
Institute of Pharmacology and Toxicology
University of Zurich
Winterthurerstr. 190
CH-8057 Zurich
Phone: +41 (0)44 635 59 57
Fax: +41 (0)44 635 57 07
email: tobler@pharma.uzh.ch

This work was supported by the Swiss National Science Foundation grant nr. 3100A0-100567/1 and EU grant STREP-518189.

ABSTRACT

There is increasing evidence that sleep facilitates memory acquisition and consolidation. Moreover, the sleep-wake history preceding memory acquisition and retention as well as circadian timing may be important. We showed previously that sleep deprivation (SD) following learning in OF1 mice impaired their performance in an object recognition task. The learning task was scheduled at the end of the 12-h dark period and the test 24 h later. To investigate the influence of the prominent circadian sleep-wake distribution typical for rodents, we now scheduled the learning task at the beginning of the dark period. Wakefulness following immediately after the learning task, was attained either by gentle interference (SD; *n = 20*) or by spontaneous wheel running (RW; *n = 20*). Two control groups were used. One had no RW throughout the experiment (*n = 23*), while the other group's wheel was blocked immediately after acquisition (*n = 16*), thereby preventing its use until test. Recognition memory, defined as the difference in exploration of a novel and familiar objects, was assessed 24 h later during the test phase. Motor activity and RW use were continuously recorded.

Remarkably, performance in the object recognition task was not influenced by the protocols; the waking period following acquisition did not impair memory, independently of the method inducing wakefulness, i.e. sleep deprivation or spontaneous running. Thus, all groups explored the novel object significantly longer than the familiar ones during the test phase. Interestingly, neither the amount of rest lost during the SD interventions nor the amount of rest preceding acquisition influenced performance. However, the total amount of rest obtained

by the control and SD mice subjected to acquisition at “dark offset” correlated positively (r = 0.66) with memory at test, while no such relationship occurred in the corresponding groups tested at dark onset. Neither the amount of running nor intermediate rest correlated with performance at test in the RW group.

We conclude that interfering with sleep during the dark period does not affect object recognition memory consolidation.

KEY WORDS: sleep deprivation, recognition memory, running wheel, mice, polyphasic sleep-wake rhythm

INTRODUCTION

There is compelling evidence that sleep is beneficial for learning, memory consolidation and retrieval in both animals and humans. In contrast, an interval of sleep deprivation (SD) following upon learning, leads to performance impairment (reviewed by Stickgold and Walker 2007). Moreover, only sleep occurring within an early, specific time window following upon the learning phase facilitates memory consolidation effectively (e.g. rats: Pearlman 1973; Smith and Butler 1982; Smith et al. 1991; Smith and Rose 1996; Smith et al. 1998; Bjorness et al. 2005; Hairston et al. 2005; Fu et al. 2007; mice: Graves et al. 2003). We have shown previously that mice subjected to 6 h SD at light onset, following immediately upon acquisition, displayed a significant object recognition deficit 24 h later, while memory was not impaired when the same SD was performed with a 6-h delay (Palchykova et al. 2006b). Learning and memory have been shown to be influenced by circadian timing (rodents: Holloway and Wansley 1973; Stephan and Kovacevic 1978; Chaudhury and Colwell 2002; Eckel-Mahan et al. 2008; Aplisia: Lyons et al. 2005). Mice showed a faster acquisition of contextual fear when they were trained during the light period compared to the dark period, and their recall was markedly superior after the light-phase training (Chaudhury and Colwell 2002; Eckel-Mahan et al. 2008). The better performance coincided with the circadian peak of hippocampal mitogen-activated protein kinase activity (Eckel-Mahan et al. 2008). Both studies replicated the results under continuous darkness, thereby confirming a circadian influence on acquisition and retention. In humans, studies investigating the effect of circadian timing of sleep on learning

are scarce (reviewed by Schmidt et al. 2007), because the designs are challenging, e.g. a forced desynchrony or constant routine protocol.

Also the sleep-wake history preceding learning influences memory. Thus, 6 h SD preceding acquisition of a water maze task impaired spatial memory in rats tested 24 h later, although it had not affected the learning curve during acquisition (Guan et al. 2004), and a 10 h SD preceding a spontaneous alternation test impaired spatial working memory in mice (Pierard et al. 2007). In human subjects a night of SD prior to learning, caused a deficit in the ability to encode new episodic memories, resulting in a worsening in retention (Yoo et al. 2007).

The daily sleep pattern of rodents is polyphasic and exhibits a strong circadian amplitude (e.g. Neuhaus and Borbely 1978; Franken et al. 1992; Tobler 1995). These frequent sleep episodes provide numerous opportunities to consolidate memories acquired during the wakefulness intervals. We investigated whether the sleep episodes which occur normally also in the dark period, play a role in facilitating memory consolidation. Task learning was scheduled at the beginning of the dark period, the main activity period of the mice, and SD was performed immediately thereafter. To control for non-specific effects of SD on memory consolidation, another means of keeping the animals awake was provided, by exploiting the tendency of rodents to run spontaneously when they are provided with a wheel (RW) (e.g. Deboer and Tobler 2000; Vyazovskiy et al. 2006b). This design enabled the comparison of induced (SD) or spontaneous (RW) wakefulness with undisturbed sleep. Furthermore, the well-known variability

in running-wheel use allowed us to explore, whether the amount of waking/running would interfere with memory consolidation. The experiments were performed in OF1 mice, whose sleep-wake pattern is known (Kopp et al. 2002). Moreover, they typically run for long intervals when provided with a RW (own observations), and we had used this strain previously in a similar object recognition task (Palchykova et al. 2006b).

It is well known that rats, mice and hamsters display preferential exploration of novel items when choosing between novel and familiar objects (Ennaceur and Delacour 1988; Dodart et al. 1997; Palchykova et al. 2006a). Such differential item exploration is generally believed to be a manifestation of recognition memory. We applied a one-trial object recognition task, which is based on this inherent curiosity of rodents towards novelty.

MATERIALS AND METHODS

Animals

Adult male outbred OF1 mice (*n* = *79*; weighing 41 ± 4 g) were kept individually in Macrolon cages (36 x 20 x 35 cm), some equipped with running wheels (RW; Vyazovskiy et al. 2006b) and some without a wheel. The mice were maintained on a 12:12 h light-dark cycle (~ 30 lux) superimposed on constant red light (< 0.5 lux) at 23°C ambient temperature with food and water available *ad libitum.* Behavioral tests were performed in young adult mice (age 13.2 ± 0.2 wks, ±SEM) under dim red light (< 1 lux), beginning after dark onset. At least 3 weeks were allowed for adaptation to the housing conditions. All experimental procedures

were performed according to Portaluppi et al. 2008 and were approved by the Cantonal Veterinary Office of Zurich.

Activity recordings

Motor activity (via infra-red, IR, sensors with a resolution of 0-200 counts per minute) and RW revolutions were recorded continuously throughout the experiment, except during the SD procedure. Data were integrated over consecutive one-minute epochs and stored on a computer (Tobler et al. 1996). The amount of rest (number of one-minute epochs with activity counts equal zero) was determined based on activity data. The lack of sleep recordings, in order to avoid interference with behavior during the object exploration, should not be an important limitation, since rest behavior provides an approximation of the amount of sleep in mice (Vyazovskiy et al. 2006a), although it overestimates sleep duration.

Behavioral task

The object recognition task comprised an acquisition, delay and test phase. All mice were individually familiarized with the experimental context without objects (grey plexiglas open field, 75 x 75 x 37 cm) for 15 min at dark onset on the two days immediately preceding the acquisition phase (Palchykova et al. 2006b). Real-time video recordings were obtained during acquisition and test.

To test memory for a complex scene, all mice were given the opportunity to explore a triplet of novel objects placed as a triangle (Fig. 1a) approximately 10

cm from the walls of the open field for five times 5 min (acquisition phase). No specific spatial cues were provided within the field. During the 15 min between exposures, the mouse was returned to its home-cage (the procedure for 6 mice lasted 2 h). After acquisition the mice were subjected to the protocol described below (Fig. 1b and c). A 24-h retention interval (delay phase) was chosen to avoid confounding circadian effects on performance (e.g. Rusak and Zucker 1975; Chaudhury and Colwell 2002) and to provide sufficient time for recovery from SD (Kopp et al. 2002). During the test phase, the mice were exposed to a new triplet of objects for 5 min: two objects encountered previously during the acquisition phase were replaced by an identical copy (familiar), and presented together with a novel object.

Experimental groups

The mice were divided into four groups (Fig. 1b). One of the two groups with continuous access to a wheel was allowed to run during the entire retention interval (RW group; *n = 20*), while in the second RW group the wheel was blocked immediately after acquisition and remained blocked until test (RWbl group; *n = 16*). The blocked RW group was used to control for the effect of the animal's running history on performance during the test phase. The third group had no wheel and served as control (*n = 23*). The fourth group was kept awake immediately after acquisition (duration of SD, *n = 20:* 5.4 ± 0.3 h; range: 194 - 522 min; see Fig. 4a, sum of hatched and black bars). A maximum of 6 individuals were sleep deprived simultaneously. During the SD the mice were

observed continuously: whenever the mice assumed a sleep posture, familiar nesting material (only tissues; no novel objects) was introduced into the cage or the mice were aroused by mild acoustic stimulation (tapping on the cage). Special care was taken not to interfere with feeding and drinking behavior. The duration of SD was matched on an individual basis to the main running bout occurring after acquisition in the mice belonging to the RW group ("RW bout" duration was 4.6 ± 0.5 h; range: 120 - 600 min; Fig. 4b). Since running was frequently interrupted by 1-2 min of other activities in the cage, including rest, the "RW bout" was defined for every individual as time elapsed between the end of the acquisition phase and the first episode of consolidated rest. Consolidation had to fulfil two criteria: at least 8 consecutive one-minute epochs with no RW revolutions and the corresponding IR-activity could not exceed zero. The criteria were not fulfilled by 8 of the 20 mice, which due to dispersed running obtained longer rest episodes (Fig. 4b). The order of testing was randomized between RW, SD, RWbl and control mice. However, the RW group was always tested before the SD group in order to match the SD interval to the RW bout length.

For comparison, several variables of our previous study were reanalyzed (Palchykova et al. 2006b). For illustration of both designs see Figure 1b and c. The duration of SD did not differ between the two studies (SD during light vs dark: 6.0 ± 0 min vs 5.4 ± 0.3 h, unpaired t-test, n.s.)

Data analysis

Interactions with the objects were quantified by visual off-line scoring of the video tapes by a trained observer (Bevins and Besheer 2006; Palchykova et al. 2006a; Palchykova et al. 2006b). The mean of the two objects was used, when exploration at acquisition or test did not differ significantly between them (Wilcoxon or paired *t*-test). Performance during the test phase was defined as the difference in exploration of the novel object and the mean of two familiar objects.

Significant ANOVA ($P < 0.05$) was followed by the post-hoc tests (unpaired or paired *t*-test, Wilcoxon or Kruskal-Wallis test if the data were not normally distributed; Tukey-Kramer test to correct for multiple comparisons; SAS software). Correlations were computed by Pearson product-moment correlation.

RESULTS

All four groups explored the novel object significantly longer than the two familiar objects during the test phase 24 h after acquisition, thereby clearly discriminating between the novel and familiar objects (Fig. 2a). Remarkably, the discrimination did not differ significantly between the groups. Therefore, scheduling the intervention during the first hours after dark onset, had no effect on performance (Fig. 2a), while SD at light onset had led to a significant performance deterioration (Fig. 2b).

Rest-activity behavior prior to acquisition

Since it is likely that the sleep-wake history preceding acquisition contributes to optimal learning, in a first step we determined the amount of rest the control mice obtained in this interval (Fig. 3a). Indeed, in the 10 h pre-acquisition, control mice subjected to acquisition at dark onset rested significantly more than the mice which had learned the task at the end of the dark period. Notably, this difference did not affect performance at the test phase 24 h later (pre-acquisition rest vs performance: r = 0.11, n.s., *n = 31*; one mouse, an outlier, was excluded).

Amount of rest lost did not affect memory

Another factor which could contribute to a difference in object discrimination is the considerably lower loss of sleep during the dark period SD (175.1 ± 13.1 min; range: 74 – 309 min; Fig. 4a) compared to SD during the light period (261.9 ± 3.4 min). However, excluding those individuals subjected to SD in the dark whose loss of rest was below the group median (175.8 min; Fig. 4a asterisks) showed that the remaining mice nevertheless remembered the objects.

Rest, before and after acquisition, influenced performance

It is possible that rest occurring during the consolidation period may have been sufficient to enable successful performance at test despite the SD interventions. However, the contribution of rest following acquisition on performance was not significant (23-h interval: r = 21; *n = 60*, pooled control and SD groups). Therefore, it is likely that the combined history before and after learning was the

determining factor influencing the performance differences between the two SDs. Indeed, the impact of rest on performance was remarkably different, depending on the time of day of acquisition and intervention. Thus, a strong positive correlation was observed between "total" rest (sum of 10 h preceding and 23 h following acquisition) and performance both in control and SD mice subjected to acquisition at dark offset, while no such relationship occurred in the corresponding groups tested at dark onset (Fig. 3b).

Influence of self-induced sleep deprivation by spontaneous running

The RW mice used the wheel almost exclusively during the dark period. In the remaining 10-h of the dark period following acquisition, the mean distance the mice ran was 10.1 ± 0.9 km (range: 4.0 – 21.9 km). Despite this spontaneous SD, the RW mice discriminated the objects at test (Fig. 2a). The amount of running after acquisition did not influence performance during the test phase ($r = 0.03$, $n = 20$). The rest episodes occurring within the "RW bout" (see methods section) may have contributed to memory consolidation (46.8 ± 6.7 min; range: 13 – 138 min; Fig. 4b). However, these RW bout interruptions consisted largely of short episodes lasting only 1 or 2 minutes, and excluding those 8 mice which did not fulfill the RW bout definition criteria (Fig. 4b asterisks) had no effect on the successful performance of the remaining mice.

We next investigated whether the amount of rest occurring during the entire 23-h retention interval following acquisition, influenced performance at test (RW group rested 1.5 h less than either the control or RWbl group). This was not

the case (RW: r = 0.05, *n* = *20*; RWbl: r = 0.22, *n* = *16*, n.s.). Moreover, neither rest obtained in the 10-h pre-acquisition interval nor total rest before and after acquisition correlated with performance in the RW mice (not shown).

Importance of rest immediately following acquisition for performance?

In rats the first 3 h but not 6 h following acquisition of object memory were sensitive to protein synthesis inhibitors (Rossato et al. 2007). We therefore determined the amount of rest obtained by our mice in this 3-h interval (the SD mice did not sleep during this interval). The control and RWbl groups rested for ~90-98 min, which was significantly more than the RW group (39 ± 7 min, $P < 0.0001$, Tukey-Kramer; 'group': $F = 41.16$, $P < 0.0001$). Performance during the test phase did not correlate with the amount of rest obtained in the first 3 h after acquisition (control: r = 0.10, *n* = *23*; RW: r = 0.02, *n* = *20*; RWbl: r = 0.14, *n* = *16*; pooled: r = 0.13, *n* = *59*, n.s.).

Object exploration during acquisition

The four groups did not differ during acquisition: they all showed the expected reduction in object exploration, thereby indicating a similar familiarization with the objects (not shown). Exploration of the three objects computed over the 5 sessions was significantly lower in the RW group compared to the RWbl group (Table 1; 'group': $F = 5.12$, $P = 0.0028$). Nevertheless, both groups performed equally well during test.

To investigate whether the time of day of acquisition or test influenced the results, we compared performance of the corresponding control groups (acquisition at dark onset and at dark offset). No group difference was observed (Table 1 and Fig. 2).

DISCUSSION

Our main finding shows that interfering with sleep during the dark period in the first hours after acquisition has no detrimental effect on recognition memory. The mice remembered the familiar objects and discriminated them successfully from the novel one, despite the almost total absence of rest immediately after acquisition. Moreover, performance of the SD and RW groups was as good as that of the two control groups (Fig. 2b). This finding is in remarkable contrast to the impairment of recognition memory in our previous study, where a similar interval of SD was scheduled at light onset (Figs. 1b and 2a). The different circadian timing of the studies entailed large differences in spontaneous activity and in the amount of sleep the animals lost during the SD, but did not affect performance during acquisition (Table 1).

Several factors could have contributed to the differences in performance at test between the two studies. First, there is evidence that there is a circadian component to learning and memory (Holloway and Wansley 1973; Stephan and Kovacevic 1978; Chaudhury and Colwell 2002; Lyons et al. 2005; Eckel-Mahan et al. 2008). Circadian differences in performance in a passive avoidance retention task were abolished in SCN lesioned rats, while the sham-operated and

control rats displayed optimal retention only 24 h after acquisition (Stephan and Kovacevic 1978). Moreover, the ability of mice to form and maintain hippocampus-dependent contextual fear memory also undergoes a circadian oscillation (Chaudhury and Colwell 2002; Eckel-Mahan et al. 2008). The control mice of our two studies showed similar object recognition, despite the difference in timing of training and test. In contrast, the timing of SD was critical for performance. Only in the mice sleep deprived during the light period and only when SD was scheduled immediately after acquisition, an object memory deficit became evident. Second, memories are not established in their definitive form during acquisition, but only after a consolidation period (for review Abel and Lattal 2001; Dudai 2002). Sleep is most intense in the first hours after light onset (Tobler et al. 1997; Huber et al. 2000; Franken et al. 2001), therefore, consolidation may be optimal during this period, regardless of the timing of acquisition. However, this notion is not supported by the detrimental effect SD had on contextual fear memory, when acquisition and a 5-h SD were timed during the second half of the light period and testing occurred, as in our case, 24 h later (Graves et al. 2003).

A third contributing factor may have been the degree of interference with the natural sleep pattern. Thus, interference may be less detrimental in the SD dark condition, when the animals are predominantly awake and active, spending less time asleep. This interpretation is supported by the RW mice, which kept themselves awake spontaneously. It is unlikely that the small amount of rest the RW mice obtained within the first long running wheel bout underlies the

successful performance, since the SD mice, obtaining no rest at all, remembered the objects. Moreover, the amount of rest obtained by the RW group within the first 3 or 23 h immediately following acquisition did not correlate with performance at test. In addition, it has been shown that spontaneous wheel running as well as forced treadmill activity can facilitate the processes underlying learning and memory consolidation (Samorajski et al. 1985; van Praag et al. 1999; O'Callaghan et al. 2007).

The larger amount of sleep lost by the mice subjected to SD during the light period, compared to the dark period, could be responsible for the performance difference between the groups. Interestingly, those mice which lost the largest amount of sleep during the SD dark (Fig. 4a; loss of rest was 218.7 ± 14.3 min, *n=10*), still remembered the objects. The minimal amount of sleep needed during the light period to allow successful consolidation is unknown.

The preceding sleep-wake history might be critical for acquisition and consolidation of memories. Thus, spatial memory was impaired in rats when a 6 h SD preceded acquisition in a water maze task (Guan et al. 2004), and in humans, a retention deficit was found when a night without sleep was scheduled before encoding of new episodic memories (Yoo et al. 2007). In our experiment memory seemed to be more labile in those mice which learned the task at dark offset, i.e. after a prolonged waking period. In contrast, when the learning task was scheduled after a period of sleep, as was the case in the acquisition at dark onset, memory seems to be more stable. This notion is consistent with the hypothesis that sleep facilitates downscaling or reducing synaptic strength which

was increased during preceding wakefulness (Tononi and Cirelli 2006). Thus, slope and amplitude of cortical evoked responses, which are believed to reflect the strength of population excitatory postsynaptic currents (Rall 1967), were shown to increase after wakefulness and decrease after sleep in the rat (Vyazovskiy et al. 2008). However, our correlation analyses showed that only total amount of rest before and after acquisition was positively correlated with performance at test (Fig. 3b; acquisition at dark offset).

The SD intervention *per se* may induce stress. However, the interference necessary to keep mice awake during the dark period was considerably less than in the mice sleep deprived at light onset. Moreover, corticosterone levels in mice subjected to SD at light onset (Palchykova et al. 2006b) or at dark onset (not shown) were not enhanced compared to matched time of day controls. Since long-term wheel running increased corticosterone levels in B6 mice (Droste et al. 2003; Otawa et al. 2007), it is possible that the HPA axis contributed to the successful learning in our RW mice, despite their lack of sleep.

In summary, interfering with sleep during the dark period does not affect object recognition memory consolidation, while it is detrimental for consolidation when sleep is disturbed during the light period. We conclude that the animal's history prior to learning as well as the timing of sleep relative to the learning task may play a role in the processes underlying learning and memory consolidation.

ACKNOWLEDGMENTS

We thank Dr. Alexander Borbély and Dr. Vladislav Vyazovskiy for discussions and comments on the manuscript. This work was supported by the Swiss National Science Foundation grant nr. 3100A0-100567/1 and EU grant STREP-518189.

REFERENCES

Abel T and Lattal KM. (2001). Molecular mechanisms of memory acquisition, consolidation and retrieval. *Curr. Opin. Neurobiol.* 11:180-187.
Bevins RA and Besheer J. (2006). Object recognition in rats and mice: a one-trial non-matching-to-sample learning task to study 'recognition memory'. *Nat. Protocols* 1:1306-1311.
Bjorness TE, Riley BT, Tysor MK, Poe GR. (2005). REM restriction persistently alters strategy used to solve a spatial task. *Learn. Mem.* 12:352-359.
Chaudhury D and Colwell CS. (2002). Circadian modulation of learning and memory in fear-conditioned mice. *Behav. Brain. Res.* 133:95-108.
Deboer T and Tobler I. (2000). Running wheel size influences circadian rhythm period and its phase shift in mice. *J. Comp. Physiol.* 186:969-973.
Dodart JC, Mathis C, Ungerer A. (1997). Scopolamine-induced deficits in a two-trial object recognition task in mice. *Neuroreport* 8:1173-1178.
Droste SK, Gesing A, Ulbricht S, Muller MB, Linthorst AC, Reul JM. (2003). Effects of long-term voluntary exercise on the mouse hypothalamic-pituitary-adrenocortical axis. *Endocrinology* 144:3012-3023.
Dudai Y. (2002). Molecular bases of long-term memories: a question of persistence. *Curr. Opin. Neurobiol.* 12:211-216.
Eckel-Mahan KL, Phan T, Han S, Wang H, Chan GC, Scheiner ZS, Storm DR. (2008). Circadian oscillation of hippocampal MAPK activity and cAMP: implications for memory persistence. *Nat. Neurosci.* 11:1074-1082.
Ennaceur A and Delacour J. (1988). A new one-trial test for neurobiological studies of memory in rats. 1: Behavioral data. *Behav. Brain Res.* 31:47-59.
Franken P, Tobler I, Borbely AA. (1992). Sleep and waking have a major effect on the 24-hr rhythm of cortical temperature in the rat. *J. Biol. Rhythms* 7:341-352.
Franken P, Chollet D, Tafti M. (2001). The homeostatic regulation of sleep need is under genetic control. *J. Neurosci.* 21:2610-2621.
Fu J, Li P, Ouyang X, Gu C, Song Z, Gao J, Han L, Feng S, Tian S, Hu B. (2007). Rapid eye movement sleep deprivation selectively impairs recall of fear extinction in hippocampus-independent tasks in rats. *Neuroscience* 144:1186-1192.
Graves LA, Heller EA, Pack AI, Abel T. (2003). Sleep deprivation selectively impairs memory consolidation for contextual fear conditioning. *Learn. Mem.* 10:168-176.

Guan Z, Peng X, Fang J. (2004). Sleep deprivation impairs spatial memory and decreases extracellular signal-regulated kinase phosphorylation in the hippocampus. *Brain Res.* 1018:38-47.
Hairston IS, Little MT, Scanlon MD, Barakat MT, Palmer TD, Sapolsky RM, Heller HC. (2005). Sleep restriction suppresses neurogenesis induced by hippocampus-dependent learning. *J. Neurophysiol.* 94:4224-4233.
Holloway FA and Wansley R. (1973). Multiphasic retention deficits at periodic intervals after passive-avoidance learning. *Science* 180:208-210.
Huber R, Deboer T, Tobler I. (2000). Effects of sleep deprivation on sleep and sleep EEG in three mouse strains: empirical data and simulations. *Brain Res.* 857:8-19.
Kopp C, Petit JM, Magistretti P, Borbely AA, Tobler I. (2002). Comparison of the effects of modafinil and sleep deprivation on sleep and cortical EEG spectra in mice. *Neuropharmacology* 43:110-118.
Lyons LC, Rawashdeh O, Katzoff A, Susswein AJ, Eskin A. (2005). Circadian modulation of complex learning in diurnal and nocturnal Aplysia. *Proc. Natl. Acad. Sci. U. S. A.* 102:12589-12594.
Neuhaus HU and Borbely AA. (1978). Sleep telemetry in the rat. II. Automatic identification and recording of vigilance states. *Electroencephalogr. Clin. Neurophysiol.* 44:115-119.
O'Callaghan RM, Ohle R, Kelly AM. (2007). The effects of forced exercise on hippocampal plasticity in the rat: A comparison of LTP, spatial- and non-spatial learning. *Behav. Brain Res.* 176:362-366.
Otawa M, Arai H, Atomi Y. (2007). Molecular aspects of adrenal regulation for circadian glucocorticoid synthesis by chronic voluntary exercise. *Life Sci.* 80:725-731.
Palchykova S, Crestani F, Meerlo P, Tobler I. (2006a). Sleep deprivation and daily torpor impair object recognition in Djungarian hamsters. *Physiol. Behav.* 87:144-153.
Palchykova S, Winsky-Sommerer R, Meerlo P, Durr R, Tobler I. (2006b). Sleep deprivation impairs object recognition in mice. *Neurobiol. Learn. Mem.* 85:263-271.
Pearlman C. (1973). REM sleep deprivation impairs latent extinction in rats. *Physiol. Behav.* 11:233-237.
Pierard C, Liscia P, Philippin JN, Mons N, Lafon T, Chauveau F, Van Beers P, Drouet I, Serra A, Jouanin JC, Beracochea D. (2007). Modafinil restores memory performance and neural activity impaired by sleep deprivation in mice. *Pharmacol. Biochem. Behav.* 88:55-63.
Portaluppi F, Touitou Y, Smolensky MH. (2008). Ethical and methodological standards for laboratory and medical biological rhythm research. *Chronobiol. Int.* 25:999-1016.
Rall W. (1967). Distinguishing theoretical synaptic potentials computed for different soma-dendritic distributions of synaptic input. *J. Neurophysiol.* 30:1138-1168.
Rossato JI, Bevilaqua LR, Myskiw JC, Medina JH, Izquierdo I, Cammarota M. (2007). On the role of hippocampal protein synthesis in the consolidation and reconsolidation of object recognition memory. *Learn. Mem.* 14:36-46.
Rusak B and Zucker I. (1975). Biological rhythms and animal behavior. *Annu. Rev. Psychol.* 26:137-171.
Samorajski T, Delaney C, Durham L, Ordy JM, Johnson JA, Dunlap WP. (1985). Effect of exercise on longevity, body weight, locomotor performance, and passive-avoidance memory of C57BL/6J mice. *Neurobiol. Aging* 6:17-24.

Schmidt C, Collette F, Cajochen C, Peigneux P. (2007). A time to think: circadian rhythms in human cognition. *Cogn. Neuropsychol.* 24:755-789.
Smith C and Butler S. (1982). Paradoxical sleep at selective times following training is necessary for learning. *Physiol. Behav.* 29:469-473.
Smith C, Tenn C, Annett R. (1991). Some biochemical and behavioural aspects of the paradoxical sleep window. *Can. J. Psychol.* 45:115-124.
Smith C and Rose GM. (1996). Evidence for a paradoxical sleep window for place learning in the Morris water maze. *Physiol. Behav.* 59:93-97.
Smith CT, Conway JM, Rose GM. (1998). Brief paradoxical sleep deprivation impairs reference, but not working, memory in the radial arm maze task. *Neurobiol. Learn. Mem.* 69:211-217.
Stephan FK and Kovacevic NS. (1978). Multiple retention deficit in passive avoidance in rats is eliminated by suprachiasmatic lesions. *Behav. Biol.* 22:456-462.
Stickgold R and Walker MP. (2007). Sleep-dependent memory consolidation and reconsolidation. *Sleep Med.* 8:331-343.
Tobler I. (1995). Is sleep fundamentally different between mammalian species? *Behav. Brain. Res.* 69:35-41.
Tobler I, Gaus SE, Deboer T, Achermann P, Fischer M, Rulicke T, Moser M, Oesch B, McBride PA, Manson JC. (1996). Altered circadian activity rhythms and sleep in mice devoid of prion protein. *Nature* 380:639-642.
Tobler I, Deboer T, Fischer M. (1997). Sleep and sleep regulation in normal and prion protein-deficient mice. *J. Neurosci.* 17:1869-1879.
Tononi G and Cirelli C. (2006). Sleep function and synaptic homeostasis. *Sleep Med. Rev.* 10:49-62.
van Praag H, Christie BR, Sejnowski TJ, Gage FH. (1999). Running enhances neurogenesis, learning, and long-term potentiation in mice. *Proc Natl Acad Sci U S A* 96:13427-13431.
Vyazovskiy VV, Kopp C, Wigger E, Jones ME, Simpson ER, Tobler I. (2006a). Sleep and rest regulation in young and old oestrogen-deficient female mice. *J. Neuroendocrinol.* 18:567-576.
Vyazovskiy VV, Ruijgrok G, Deboer T, Tobler I. (2006b). Running wheel accessibility affects the regional electroencephalogram during sleep in mice. *Cereb. Cortex* 16:328-336.
Vyazovskiy VV, Cirelli C, Pfister-Genskow M, Faraguna U, Tononi G. (2008). Molecular and electrophysiological evidence for net synaptic potentiation in wake and depression in sleep. *Nat. Neurosci.* 11:200-208.
Yoo SS, Hu PT, Gujar N, Jolesz FA, Walker MP. (2007). A deficit in the ability to form new human memories without sleep. *Nat. Neurosci.* 10:385-392.

FIGURE LEGEND

Figure 1. Experimental design

(a): Scheme of the object recognition task. During the acquisition phase, mice were exposed to a triplet of novel objects for five 5-min intervals interspersed by 15 min during which the animal was returned to its home-cage. Acquisition was followed by a 24-h delay phase. During the test phase, the mice were exposed to a new triplet of objects for 5 min: two objects encountered previously during the acquisition phase were replaced by an identical copy and presented together with a novel object.

(b): *Acquisition at dark onset* (groups 1-4). All mice were subjected to an acquisition phase at the beginning of the dark period and their recognition memory was assessed 24 h later during the test phase. The first group was kept awake by gentle interference for 5.4 h during the dark period, starting immediately after acquisition, and left undisturbed thereafter (SD group; *n = 20*). The second group, which was provided with a wheel throughout the experiment, was allowed to run during the retention interval (RW group; *n = 20*). The third group had no wheel and served as an undisturbed control (*n = 23*). In the fourth group, the RW was blocked immediately after acquisition (RWbl group; *n = 16*).

(c): *Acquisition at dark offset* (groups 1-2). The acquisition phase of the object recognition task was scheduled at the end of the dark period and recognition memory was assessed 24 h later. One group (1) was kept awake for 6 h by gentle interference during the light period, immediately following upon acquisition

(SD group, *n* = *9*). The control group (2) was returned to the home-cage after acquisition and left undisturbed (*n* = *9*).

Figure 2. Performance during the test phase, 24 h after acquisition

Time spent exploring a novel and the familiar objects (mean of two objects ± SEM in seconds) during the test phase.

(a): *Effect on memory when SD was performed during the dark period.* Control (*n* = *23*), blocked running wheel (RWbl; *n* = *16*), sleep deprived (SD; *n* = *20*) and running wheel (RW; *n* = *20*) mice explored the novel object significantly longer than the familiar one (* $P < 0.01$, ** $P < 0.005$, Wilcoxon after ANOVA 'object': $F = 39.25$, $P < 0.0001$).

(b): *Effect on memory when SD was performed during the light period.* Control mice explored the novel object significantly longer than the familiar one, while no difference in object exploration was observed in the SD group.

Figure 3. Amount of rest preceding and following acquisition.

(a): Rest (defined as one-minute epochs with infra-red activity =0; mean ± SEM) during the 10 h preceding acquisition in the control groups subjected to acquisition either at dark offset (*n* = *9*) or dark onset (*n* = *22*). * $P < 0.0001$: difference between the groups (Kruskal-Wallis).

(b): Correlations between the amounts of rest obtained during 10 h preceding and 23 h following acquisition and performance during the test phase for the pooled control and SD groups. Acquisition was either at dark onset or dark offset.

Figure 4. Individual amount of rest following acquisition

(a): Duration of the sleep deprivation (SD) procedure (black bars), loss of rest during SD (dashed bars; amount of rest during the baseline interval corresponding to the SD period, 10-day mean) in individuals belonging to the SD group (*n = 20*) sorted according to their performance during the test phase (difference in exploration of the novel and the mean of two familiar objects; the best performance score equals 20). Asterisks indicate those individuals which lost <175.8 min of rest during SD. Discrimination between the novel vs. familiar objects of remaining *n = 10* mice*: P* < 0.038, Wilcoxon.

(b): Length of the "RW bout" following acquisition (black bars; see methods section for definition), total amount of rest within the "RW bout" (white bars) and amount of consecutive 1- and 2-min epochs of rest within the "RW bout" (grey bars) of individuals belonging to the RW group (*n = 20*) sorted according to their performance during the test phase. Asterisks indicate mice which obtained more than 8 consecutive 1-min rest epochs within the "RW bout". Discrimination between the novel vs. familiar objects of remaining *n = 12* mice*: P* < 0.009, Wilcoxon.

Table 1. Duration of object exploration during acquisition (seconds)

Groups	Acquisition at dark *onset*		Acquisition at dark *offset*	
	N	**Total exploration**	**N**	**Total exploration**
Control	23	127.3 ± 13.6	9	161.5 ± 19.5
RWbl	16	170.2 ± 15.1	-	-
SD	20	126.0 ± 15.8	9	157.5 ± 20.0
RW	20	91.1 ± 8.7$^{\#}$	-	-

Duration of object exploration (mean ± SEM in seconds) of undisturbed controls, mice with a blocked running wheel (RWbl), and a sleep deprived (SD) and RW group during acquisition. N = number of animals. Total exploration during acquisition is defined as the sum of exploration of the three novel objects over the 5 consecutive sessions (see methods for details). Acquisition occurred either at dark onset or dark offset. RW *vs.* RWbl: $^{\#}$ $P < 0.001$, Tukey.

Figure 1

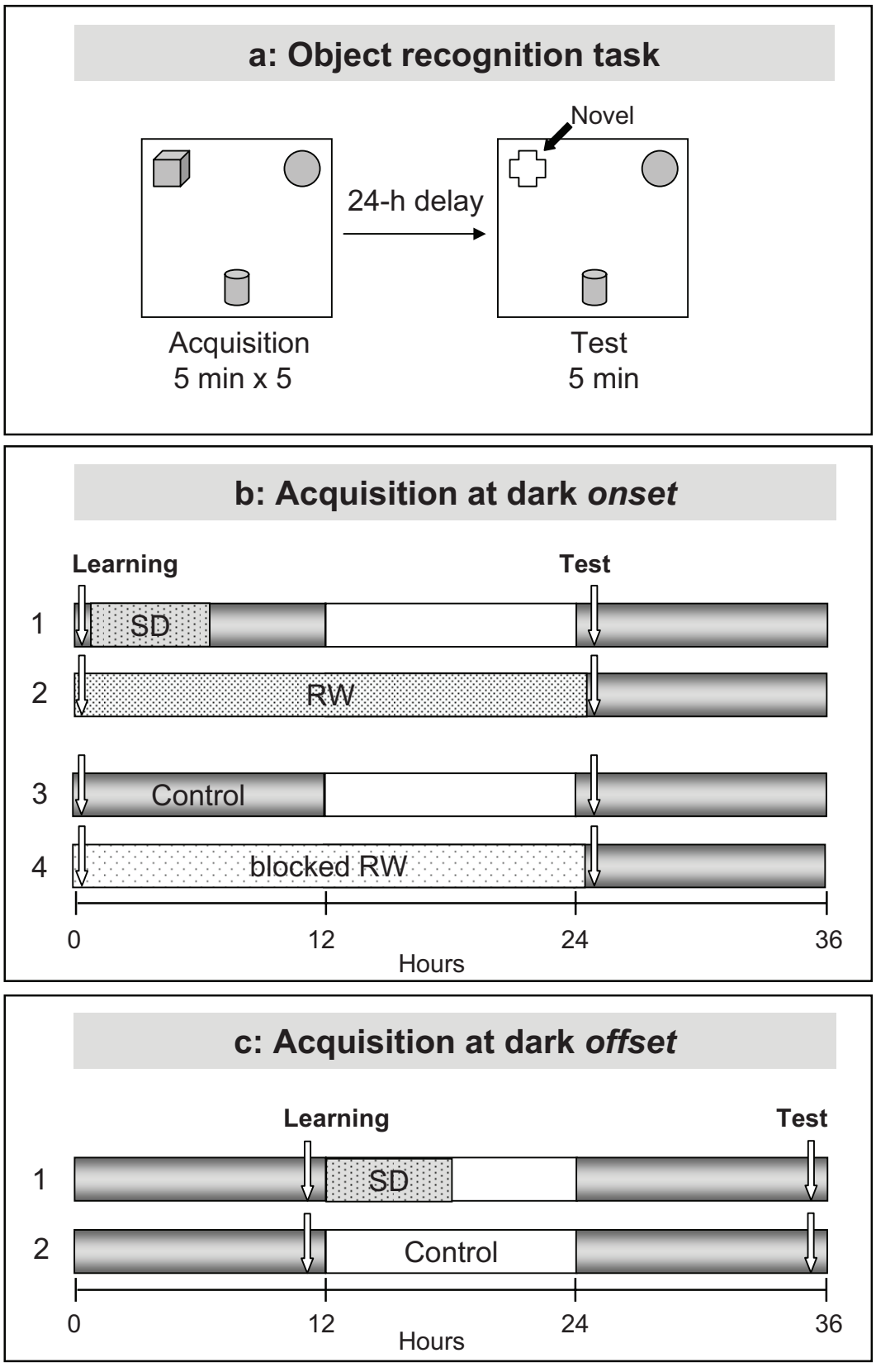

a: Object recognition task
Novel
24-h delay
Acquisition
5 min x 5
Test
5 min
b: Acquisition at dark onset
Learning
Test
1
SD
2
RW
3
Control
4
blocked RW
0
12
24
36
Hours
c: Acquisition at dark offset
Learning
Test
1
SD
2
Control
0
12
24
36
Hours

Figure 2

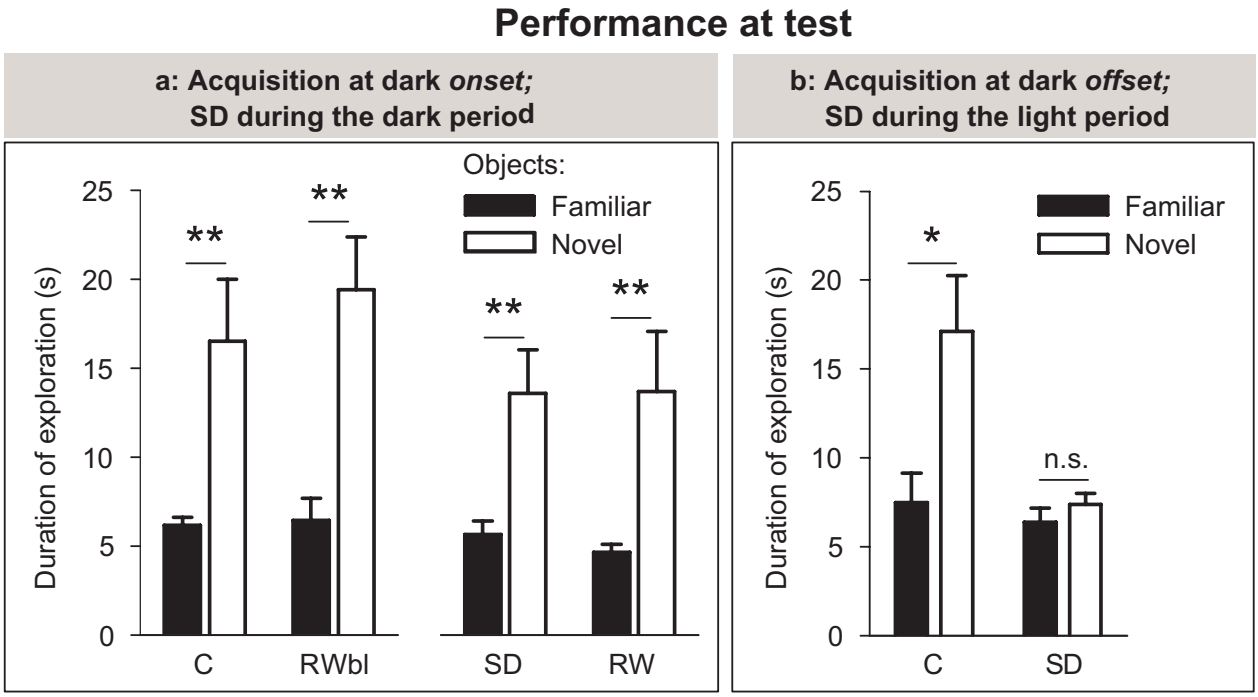

Performance at test
a: Acquisition at dark onset; SD during the dark period
b: Acquisition at dark offset; SD during the light period
Duration of exploration (s)
25
20
15
10
5
0
Objects:
Familiar
Novel
**
**
**
**
C
RWbl
SD
RW
*
n.s.
C
SD

Figure 3

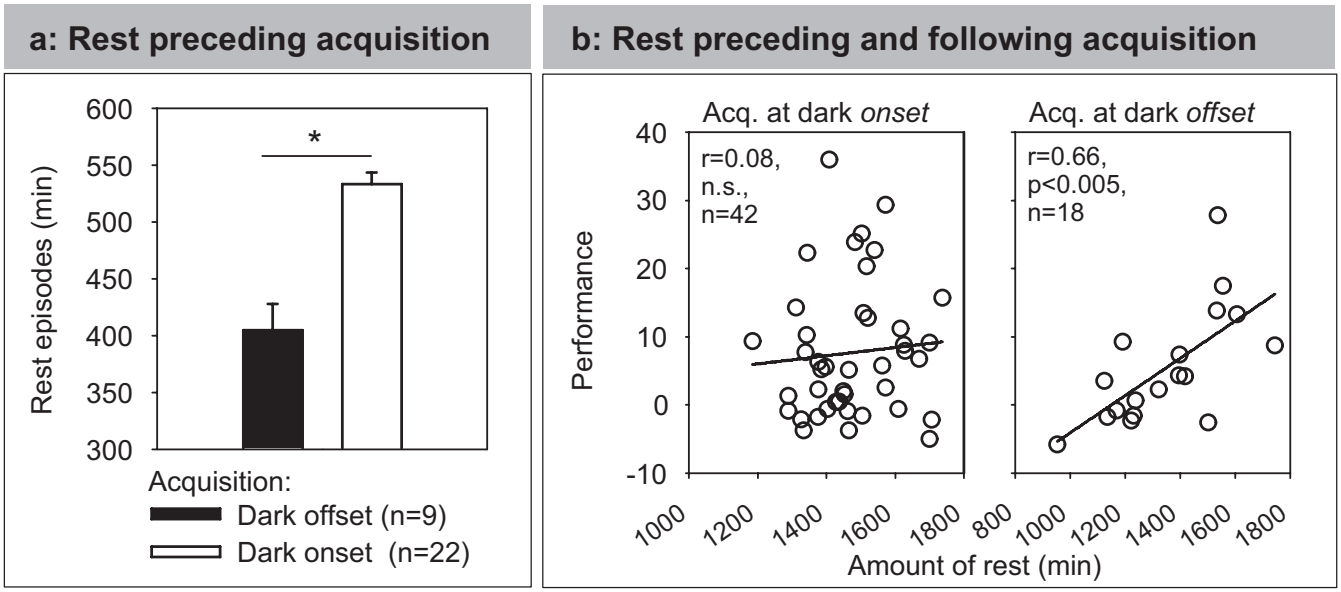
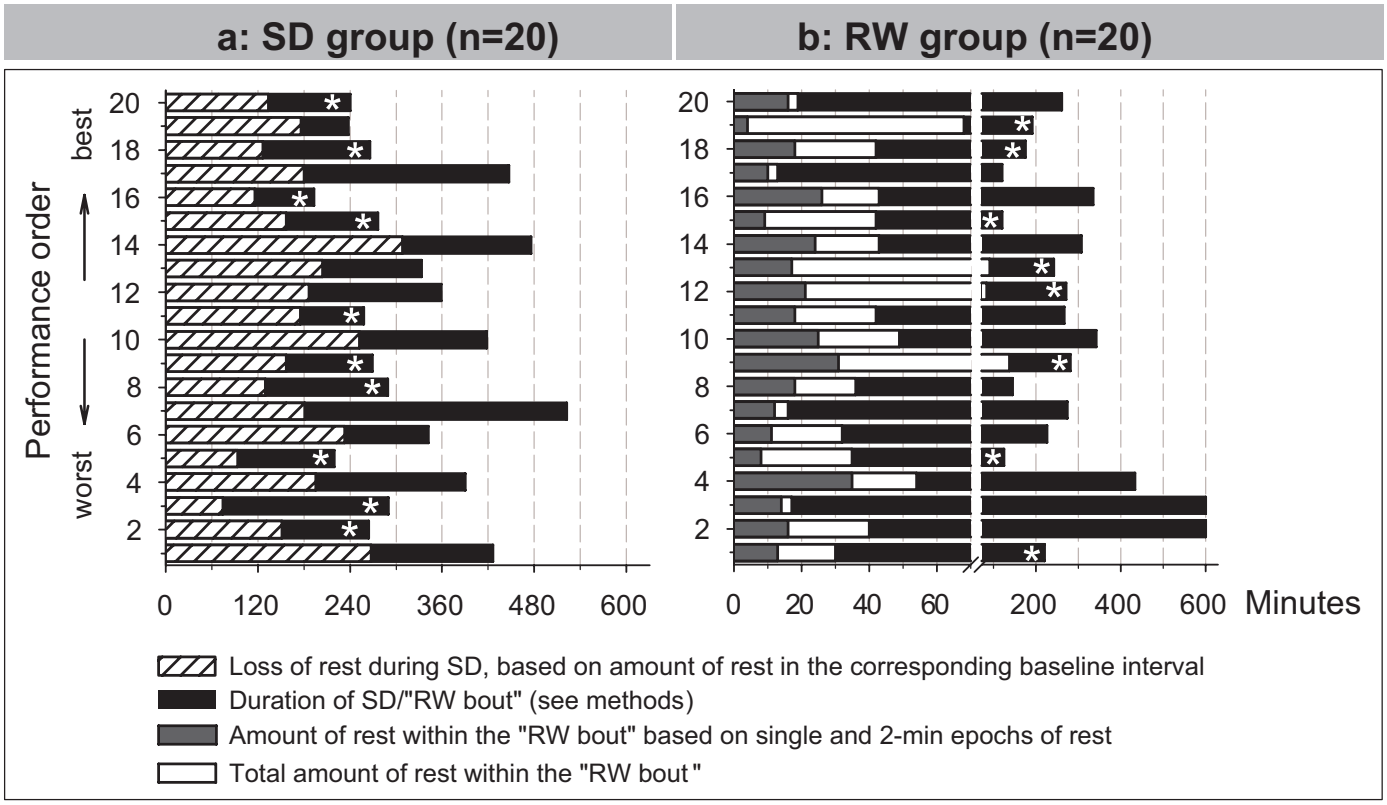

a: Rest preceding acquisition
b: Rest preceding and following acquisition
Rest episodes (min)
600
550
500
450
400
350
300
*
Acquisition:
Dark offset (n=9)
Dark onset (n=22)
Acq. at dark onset
Acq. at dark offset
Performance
40
30
20
10
0
-10
r=0.08,
n.s.,
n=42
r=0.66,
p<0.005,
n=18
1000
1200
1400
1600
1800
800
1000
1200
1400
1600
1800
Amount of rest (min)

Figure 4


a: SD group (n=20)
b: RW group (n=20)
Performance order
best
worst
20
18
16
14
12
10
8
6
4
2
0
120
240
360
480
600
0
20
40
60
200
400
600
Minutes
Loss of rest during SD, based on amount of rest in the corresponding baseline interval
Duration of SD/"RW bout" (see methods)
Amount of rest within the "RW bout" based on single and 2-min epochs of rest
Total amount of rest within the "RW bout"