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Sleep deprivation-induced impairment of memory consolidation is not mediated by glucocorticoid stress hormones

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Abstract

The general consensus is that sleep promotes neuronal recovery and plasticity, whereas sleep deprivation (SD) impairs brain function, including cognitive processes. Indeed, a wealth of data has shown a negative impact of SD on learning and memory processes, particularly those that involve the hippocampus. The mechanisms underlying these negative effects of sleep loss are only partly understood, but a reoccurring question is whether they are in part caused by stress hormones that may be released during SD. The purpose of the present study is therefore to examine the role of glucocorticoid stress hormones in SD-induced memory impairment. Male C57BL/6J mice were trained in an object-location memory paradigm, followed by 6 hr of SD by mild stimulation. At the beginning of the SD mice were injected with the corticosterone synthesis inhibitor metyrapone. Memory was tested 24 hr after training. Blood samples taken in a separate group of mice showed that SD resulted in a mild but significant increase in plasma corticosterone levels, which was prevented by metyrapone. However, the SD-induced impairment in object-location memory was not prevented by metyrapone treatment. This indicates that glucocorticoids play no role in causing the memory impairments seen after a short period of SD.

KEYWORDS
cognition, sleep disturbance, sleep restriction

1 | INTRODUCTION

Sleep is a universal phenomenon and a highly conserved trait through the course of evolution. Even though its functions remain largely unknown, sleep is often thought to be important for regulating neuronal plasticity and synaptic strength, which, in turn, are essential for brain functions such as information processing, learning and memory (Benington & Frank, 2003; Kreutzmann, Havekes, Abel, & Meerlo, 2015; Raven, Van der Zee, Meerlo, & Havekes, 2018; Tononi & Cirelli, 2006). Numerous studies in both humans and animals have demonstrated that a lack of sleep impairs the processing and storage of new information in the brain (Havekes, Meerlo, & Abel, 2015; Kreutzmann et al., 2015; Raven et al., 2018). More specifically, studies have shown that sleep deprivation (SD) impairs memory processes, particularly when involving the hippocampus (Graves, Heller, Pack, & Abel, 2003; Havekes et al.,

2016; Raven, Meerlo, Van der Zee, Abel, & Havekes, 2019; Vecsey et al., 2009), a brain region crucial for learning and memory. For example, even a short period of 6 hr of SD impairs object-location memory and contextual fear-conditioning memory, both of which are highly hippocampus dependent. In contrast, learning and memory tasks that are hippocampus independent, such as tone-cued fear conditioning, were unaffected by SD (Graves et al., 2003). Nevertheless, the mechanisms through which SD impairs hippocampal function and disturbs the formation and consolidation of new memories is only partly understood.

One commonly proposed mechanism is that SD acts as a stressor and that stress hormones released during SD may directly influence hippocampal function through pathways involved in neuronal plasticity and memory storage. Indeed, SD can be a mild stressor and can lead to mild activation of classical neuroendocrine systems, particularly the hypothalamic-pituitary-adrenal (HPA) axis (Meerlo, Sgoifo, & Suchecki, 2008). In more detail, SD could initiate the release of corticotropin-releasing hormone (CRH) from the hypothalamus, which stimulates adrenocorticotropic hormone (ACTH) release from the pituitary. Subsequently, ACTH induces the liberation of glucocorticoids from the adrenal cortex (i.e., cortisol in humans or corticosterone [CORT] in rats and mice). In fact, slightly elevated levels of glucocorticoid stress hormones after SD have been reported in both humans (Chapotot, Buguet, Gronfier, & Brandenberger, 2001; Leproult, Copinschi, Buxton, & Van Cauter, 1997; Spiegel, Leproult, & Van Cauter, 1999) and laboratory rodents (Mirescu, Peters, Noiman, & Gould, 2006; Mongrain et al., 2010; Roman, Hagewoud, Luiten, & Meerlo, 2006; Takatsu-Coleman et al., 2013; Tartar et al., 2009). A number of rodent studies experimentally prevented glucocorticoid signalling during SD and showed this manipulation could not prevent cognitive deficits (Ruskin, Dunn, Billiot, Bazan, & LaHoste, 2006; Tiba, Oliveira, Rossi, Tufik, & Suchecki, 2008). However, in these studies SD was conducted prior to training to assess effects on learning capacity and not SD after training, during the critical phase of memory consolidation, which may involve different mechanisms. In other words, these studies do not exclude the possibility that glucocorticoids during SD after training are responsible for deficits in memory consolidation. For this reason, it was important to perform the current study in which we selectively blocked corticosterone release during SD after learning.

2 | METHODS

2.1 | Animals and housing

Eighty male C57BL/6 mice (Janvier Laboratories) were ordered at 6 weeks of age and pair-housed on arrival. Mice were individually housed 1 week before the start of our experiments when the animals were 12–16 weeks old. The experimental room was kept under constant temperature (22°C ± 5°C) and a 12 hr light/12 hr dark cycle (lights on 09:00–21:00 hours). Poly-carb clear cages with stainless-steel wired lids were provided with nesting material, a paper roll and sawdust as bedding. A chow diet and water

Statement of significance

In the current manuscript we addressed the long-standing discussion of whether the detrimental effects of sleep deprivation on memory function are mediated through glucocorticoids released during sleep deprivation. First, we show that metyrapone can successfully be used to block the synthesis of corticosterone during sleep deprivation. Second, we show that 6 hr of sleep deprivation impairs memory performance, which could not be prevented by metyrapone treatment. Together these studies indicate that glucocorticoids play no role in causing the memory impairments seen after a short period of sleep deprivation.

were available ad libitum. All procedures were approved by the National Central Authority for Scientific Procedures on Animals (CCD) and the Institutional Animal Welfare Body (IvD, University of Groningen, the Netherlands).

2.2 | Experimental set-up

In a first experiment we validated the use of the glucocorticoid synthesis inhibitor metyrapone to block the release of CORT during SD. Mice received a systemic injection of metyrapone or saline at the beginning of SD and after 3 hr blood samples were collected for assessment of plasma CORT levels. We chose to assess CORT levels after 3 hr of SD, instead of the 6-hr SD applied in other experiments (including our second experiment). We did this because in some of our studies CORT levels after 6-hr SD are low and no longer significantly different from controls, although this does not exclude the possibility that CORT levels are higher early on in the SD session (Meerlo, Koehl, van der Borght, & Turek, 2002; Palchykova, Winsky-Sommerer, Meerlo, Durr, & Tobler, 2006).

In a second experiment, we tested whether blocking CORT release during SD by metyrapone can prevent the memory impairments that are normally associated with SD. Mice were trained in an object-location memory task (OLM), received a systemic injection of metyrapone or saline immediately after training, and were then subjected to 6 hr of SD. Memory for object location was tested the next day; that is, 24 hr after training and 18 hr after the end of SD (see Figure 1).

2.3 | Drug preparation and administration

Metyrapone (2-methyl-I,2-di-3-pyridyl-1-propanone [ALDRICH®]) was used to reduce glucocorticoid synthesis via inhibition of steroid 11-β-hydroxylase. Previous studies have already demonstrated its potency in reducing memory recall by blocking glucocorticoid synthesis (Careaga, Tiba, Ota, & Suchecki, 2015; Clay et al., 2011). Metyrapone was dissolved in a vehicle solution containing
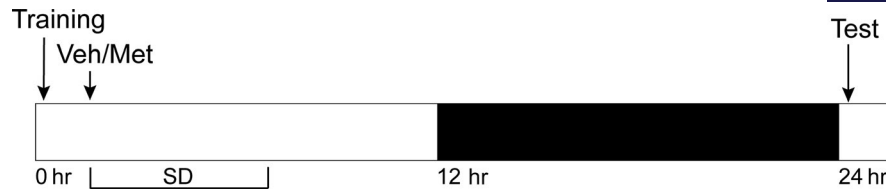
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FIGURE 1 Experimental design for the behavioural task. Animals were trained for object-location memory at the onset of the light phase. Directly after training, animals were sleep deprived for 6 hr or left undisturbed and injected with either vehicle (veh) or metyrapone (met). Memory was tested 24 hr after training. SD, sleep deprivation

physiological saline and 5% ethanol. The solutions were made fresh on each experimental day and kept at 4°C until use. Mice were injected with metyrapone (90 mg/kg) or vehicle subcutaneously at the start of SD.

2.4 | Corticosterone assay

To measure CORT levels in experiment 1, animals were sacrificed by decapitation and trunk blood was collected in a cup containing ethylenediaminetetraacetic (9 g/100 ml) acid (EDTA; Hagewoud, Whitcomb, et al., 2010; van der Borght et al., 2006). Subsequently, the samples were centrifuged at 2,600 g at 4°C and the supernatant was collected in polycarbonate cups. Plasma CORT levels were measured by a double antibody radioimmunoassay method for rodents, using an immuChem™ kit (MP Biomedicals LLC). CORT levels of samples were measured in duplicate.

2.5 | Sleep deprivation procedure

In the first experiments, animals were sleep deprived for 3 hr starting at the beginning of the light phase. In the second experiment, mice were sleep deprived during the first 6 hr of the light phase, directly after training in the OLM. In both experiments, mice were sleep deprived using the gentle stimulation method (Havekes et al., 2016; Prince & Abel, 2013; Raven et al., 2019; van der Borght et al., 2006). In brief, animals were kept awake by tapping or shaking the cage. Their bedding was disturbed only in cases when mice did not respond to tapping or shaking. Notably, we did not use any objects, cages, clean bedding or other arousing stimuli to keep the animals awake. This SD method has been validated previously using EEG recordings (Meerlo, de Bruin, Strijkstra, & Daan, 2001).

2.6 | Object-location memory

The OLM is a hippocampus-dependent spatial memory task (Bruno et al., 2011; Oliveira, Hawk, Abel, & Havekes, 2010; Vanmierlo et al., 2011). The rectangular arena was made of PVC and had a length of 40 cm, width of 30 cm, and was 50 cm high. The four walls of the arena consisted of grey-coloured PVC and the bottom consisted of transparent PVC. In this task, four pairs of two identical objects

were used (one pair per trial). These objects were two blue aluminum cylinders (height 12 cm and diameter 3.5 cm), two orange aluminum cylinders with tapering tops (height 12 cm and diameter at widest point 3.5 cm), two green glass cylinders (height 12 cm and diameter 2.5 cm) or two pink round vases (height 10 cm and diameter ranging from 3.5 cm at the bottom to 1.5 at the top). Inside the arena, two spatial cues were presented at the short walls on opposite sides of the rectangular arena. One cue consisted of black and white striping and the other cue consisted of a black and white checkerboard pattern. The animals were unable to move the objects or sit on the objects.

In the present study, the task consisted of two trials of free exploration with a time interval in-between. The first trial (T1) was the learning or acquisition trial, in which two identical objects (objects A1 and A2) were placed symmetrically on a horizontal line in the arena, approximately 7.5 cm from the wall. At the start of T1, the animals were always placed in the front of the arena facing the wall and were allowed to explore the objects for 10 min, after which they were put back into their home cage. The second trial (T2) was the test trial and took place after a predetermined delay interval of 24 hr. In this trial, one of the objects was displaced along a straight line to a position that was 15 cm away from the previous location, whereas the other object was placed at a similar location to that during T1 (objects B and A3, respectively). The object that was moved (either left or right), the direction of movement (front or back) and the objects themselves were all counterbalanced to avoid place and object preferences. The mice were again allowed to explore this new spatial arrangement for 10 min. Between animals and trials the objects were cleaned with a 70% ethanol solution to avoid the presence of olfactory cues. Prior to testing animals were habituated to handling, the experimenter, the testing arena and injections.

The readout parameters of the OLM refer to the exploration time for each object during T1 and T2 (Akkerman, Blokland, et al., 2012; Akkerman, Prickaerts, Steinbusch, & Blokland, 2012). The exploration time of each object was scored manually by the experimenter, using a computer. Exploration was defined as follows: directing the nose to the object at a distance of no more than 1 cm and/or touching the object with the nose. Leaning toward an object was not considered to be exploratory behaviour. The exploration times (in seconds) of each object during T1 are presented as "a1" and "a2". The time spent exploring the familiar and the displaced object in T2 are represented as "a3" and "b", respectively. Using this information, the following variables were calculated: T1 [e1 (=a1 + a2)], the total exploration time during T2 [e2

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(=a3 + b)] and the discrimination index [d2 (=b − a3/e2)]. The d2 index is a relative measure of discrimination corrected for total exploration time and can range from −1 to 1. A significant difference from zero (i.e., chance level) indicates that the mice remembered the object locations from T1, and a difference from the vehicle condition signifies an actual effect of the drug on memory performance.

2.7 | Statistical analysis

Statistical analysis was carried out using IBM SPSS Statistics 25 software (IBM). Behavioural and CORT data were analysed using a two-way ANOVA. The Bonferroni procedure was used for post hoc analysis when necessary. Differences were considered statistically significant when $p < .05$ and all data are plotted as mean ± standard error of the mean (SEM).

3 | RESULTS

3.1 | Plasma corticosterone

To assess whether brief SD results in elevated CORT levels, and whether this could be prevented by metyrapone, we measured blood samples after 3 hr of SD. As can be seen in Figure 2, a short period of SD resulted in a mild but significant increase in CORT levels. Two-way ANOVA revealed a significant interaction between SD (sleep deprived/non-sleep-deprived) and metyrapone injection (metyrapone/vehicle), indicating that the effect of SD on CORT levels depended on whether the mice were injected with
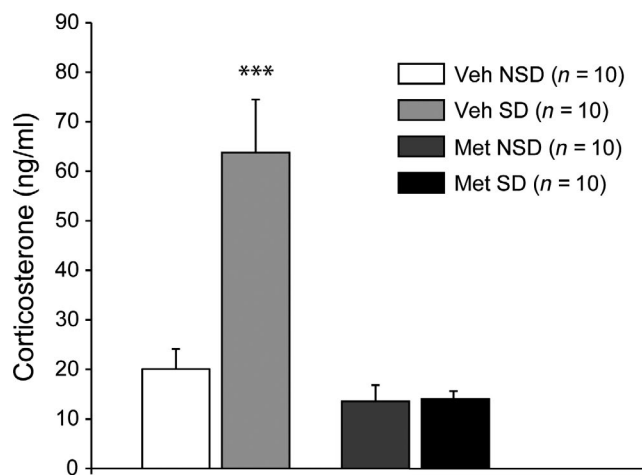

FIGURE 2 Plasma corticosterone levels in mice subjected to sleep deprivation (SD) or non-sleep-deprived control animals (NSD). Animals in each group (n = 10) received an injection of metyrapone (met) or vehicle (veh) and were sleep deprived (SD) or left undisturbed (NSD). SD caused a mild increase in CORT levels measured after 3 hr SD, which was prevented by the injection of metyrapone at the start of SD. ***Significant difference from all other conditions (***$p < .001$, Bonferroni procedure)

metyrapone or vehicle ($F_{1,40} = 13.0$, $p = .001$). Post hoc analyses showed that SD mice injected with vehicle had higher CORT levels than non-SD vehicle-treated mice (Bonferroni, $p < .001$; Figure 2). CORT levels in the SD-metyrapone-treated mice were significantly lower than the levels in the SD-vehicle-treated mice (Bonferroni, $p < .001$; Figure 2) and not different from the non-SD vehicle-treated mice (Bonferroni, $p = 1.000$; Figure 2). This finding indicates that the SD-induced elevation of CORT was successfully blocked by metyrapone.

3.2 | Object-location memory

In the next study we used the OLM to investigate whether SD affects hippocampus-dependent memory and whether this effect is mediated by glucocorticoid stress hormones. There were no significant differences in overall exploration time for T1 (e1) or T2 (e2) between the positions, as indicated by a one-way ANOVA (T1 (e1): $F_{3,39} = 0.908$, $p = .447$; T2 (e2): $F_{3,39} = 1.571$, $p = .213$; data not shown). These behavioural findings indicate that overall exploratory behaviour did not differ between positions and therefore did not influence any potential difference in performance. One-sample t tests comparing the d2 index to zero showed that both sleep-deprived groups (SD vehicle and SD metyrapone) did not differ from chance level performance (zero), but both non-sleep-deprived group (non-SD vehicle and non-SD metyrapone) did significantly differ from zero ($p < .001$; Figure 3). The latter observation shows that the mice did recognize the new position under both non-sleep-deprived conditions, irrespective of metyrapone treatment. To test whether SD-induced memory deficits are mediated via glucocorticoid stress hormones, a two-way ANOVA was conducted using SD (sleep deprived/non-sleep-deprived) and metyrapone treatment (metyrapone/vehicle) as between-subject factors. No significant SD × metyrapone interaction effect was found ($F_{1,40} = 0.101$, $p = .753$; Figure 3), indicating that the negative effects of SD on memory consolidation could not be prevented by the inhibition of corticosterone. It also indicates that lowering corticosterone levels during non-SD memory does not affect normal memory consolidation (at least not at the behavioural level). Subsequent analysis showed a main effect for SD ($F_{1,40} = 19.104$, $p < .001$; Figure 3), indicating that SD impairs memory consolidation irrespective of metyrapone treatment.

4 | DISCUSSION

In the current study, we examined the role of glucocorticoids in SD-induced hippocampus-dependent memory impairment. First, we assessed whether metyrapone successfully blocked CORT synthesis during SD. The results show that vehicle-treated sleep-deprived mice had mildly increased levels of CORT compared to non-sleep-deprived mice and that metyrapone successfully prevented the SD-induced increase of CORT synthesis. Furthermore, results from the behavioural task revealed that SD impaired memory consolidation irrespective of
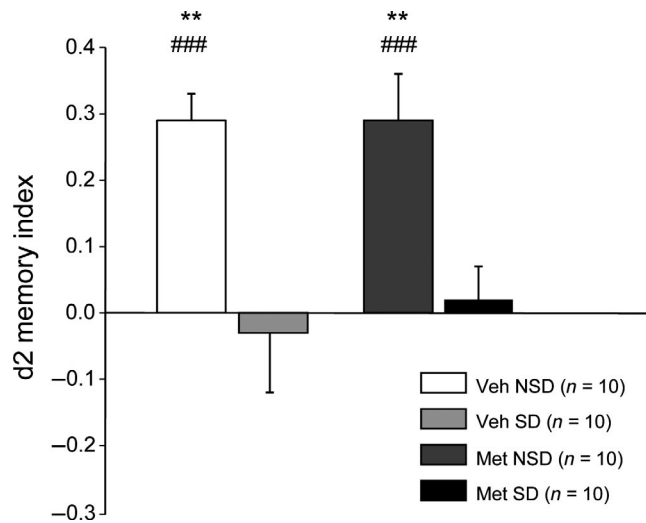
FIGURE 3 Effects of sleep deprivation and metyrapone treatment on spatial memory consolidation as measured with the object-location task. Animals in each group (n = 10) received an injection of metyrapone (met) or vehicle (veh) and were sleep deprived (SD) or left undisturbed (NSD). NSD, non-sleep-deprived; SD, sleep deprived; Veh, vehicle. #Significant difference from chance level performance (zero; ###p < .001, one-sample t tests); *Significant difference from both NSD conditions (**p < .01, two-way ANOVA)

metyrapone treatment, indicating that the behavioural deficits associated with SD are not results of elevated CORT levels.

Many studies have reported that SD can have a stimulatory effect on the HPA axis, associated with elevated CORT levels (Hagewoud, Havekes, et al., 2010; van der Borght et al., 2006). Most often these elevations are mild and sometimes they are even absent. In some of our earlier work in rodents, plasma levels of corticosterone were not significantly different between sleep-deprived animals and non-sleep-deprived controls (Hagewoud, Havekes, et al., 2010; van der Borght et al., 2006). In the majority of studies using comparable designs to ours, glucocorticoid levels are only measured at the end of SD (i.e., after 5–6 hr SD) (Hagewoud, Whitcomb, et al., 2010; van der Borght et al., 2006; Vecsey et al., 2009). This does not exclude the possibility that corticosterone may have been elevated in the early phase of SD, potentially affecting ongoing memory formation. For this reason, in the current study CORT was measured after 3 hr of SD. Indeed, after 3 hr of SD plasma corticosterone levels were significantly elevated. The plasma CORT levels of around 60 ng/ml we found in mice after SD during their normal resting phase are fairly low compared with levels reported after conventional stressors such as immobilization, which can be as high as 300–400 ng/ml (Palchykova et al., 2006). Even the performance of a learning task, such as the acquisition of an object task comparable to the one we used in our study, may induce CORT levels up to nearly 200 ng/ml (Palchykova et al., 2006), which is still considerably higher than the levels we found after SD.

In addition, for CORT to exert any effect on learning and memory, it needs to pass the blood–brain barrier and occupy glucocorticoid receptors (GRs). However, because mineralocorticoid receptors

(MRs) have a 10-fold greater affinity for glucocorticoids than GRs, GRs are only occupied at circadian peak levels. Therefore, it is highly unlikely that CORT, at the levels we currently found after SD, affects memory processes (Roozendaal, 2002; Veniant et al., 2009). Of note, we are not implying that there is thus no connection between CORT and learning, as a large body of literature has previously shown this. However, (a) for CORT to affect memory function much higher levels must be reached compared to those we found in our SD studies, and (b) these studies mostly observe improvements of memory function instead of impairment.

The current findings are in line with other studies showing that stress hormones cannot explain the deficits in hippocampal function that result from SD (Ruskin et al., 2006; Tiba et al., 2008; Vecsey et al., 2009). For example, rats subjected to 4 days of REM SD had a diminished capacity for subsequent learning in a fear-conditioning task, which could not be prevented by blockade of glucocorticoid release by means of metyrapone injections (Tiba et al., 2008). In another study, prolonged SD for 3 days induced acquisition deficits in the Morris water maze, which could not be prevented by blocking CORT release through removal of the adrenals (Ruskin et al., 2006). Furthermore, previous research has shown that prolonged SD hampers hippocampus-dependent plasticity processes, such as neurogenesis, independent of stress hormones (Guzman-Marin, Bashir, Suntsova, Szymusiak, & McGinty, 2007; Meerlo, Mistlberger, Jacobs, Heller, & McGinty, 2009; Mueller et al., 2008). Importantly, whereas previous studies showed that blocking CORT release cannot prevent memory deficits that result from SD prior to acquisition (Roozendaal, 2002; Veniant et al., 2009), in the present study we show that blocking CORT release also does not prevent the memory deficits that result from SD after acquisition during the critical phase of memory storage. Although together these studies support the finding that glucocorticoids are probably not responsible for producing the hippocampus-dependent memory impairments seen after a short period of SD, it is not excluded that more severe and prolonged restriction or disruption of sleep and sleep disorders may be associated with higher levels of stress hormones that can affect brain function and performance.

Sleep loss is a very debilitating phenomenon, especially in our modern society with heavy workloads and around the clock lifestyles. Our memory function seems to be particularly affected by sleep loss and, although several studies have shown this effect to be induced through pathways involved in hippocampal synaptic plasticity, the question remained of whether glucocorticoids could be a mediating factor. Here, we investigated the effects of temporarily blocking glucocorticoids during SD on hippocampus-dependent memory storage. The present study is the first to show that hippocampus-dependent memory consolidation is attenuated by a single, short period of SD, which is not mediated by stress hormones.

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CONFLICT OF INTEREST

No conflicts of interest declared.

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