5. TWO-LEVER SPATIAL REVERSAL LEARNING IS ROBUST TO THE EFFECTS OF TOTAL SLEEP DEPRIVATION

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5.1. Abstract

Sleep deprivation affects cognitive functions that depend on the prefrontal cortex (PFC) such as cognitive flexibility, and the consolidation of newly learned information. The identification of cognitive processes that are either robustly sensitive or robustly insensitive to the same experimental sleep deprivation procedure, will allow us to better focus on the specific effects of sleep on cognition, and increase understanding of the mechanisms involved. In this chapter we investigate if sleep deprivation differentially affects the two separate cognitive processes of acquisition and consolidation of a spatial reversal task.

After training on a spatial discrimination between two levers in a Skinner box, male Wistar rats were exposed to a reversal of the previously learned stimulus-response contingency. We first evaluated the effect of sleep deprivation on the acquisition of reversal learning. Performance on reversal learning after 12h of sleep deprivation (n=12) was compared to performance after control conditions (n=12). The second experiment evaluated the effect of sleep deprivation on the consolidation of reversal learning; the first session of reversal learning was followed by 3h of nap prevention (n=8) or undisturbed control conditions (n=8). The experiments had sufficient statistical power (0.90 and 0.81 respectively) to detect differences with medium effect sizes.

Neither the acquisition, nor the consolidation, of reversal learning was affected by acute sleep deprivation. Together with previous findings, these results help to further delineate the role of sleep in cognitive processing.
5.2. Introduction

A large number of studies on the interaction between sleep and cognitive performance have led to the suggestion that sleep is most important for two cognitive domains: Firstly, cognitive functions that depend on the integrity of the prefrontal cortical system, e.g. cognitive flexibility, appear to be particularly sensitive to sleep deprivation (Jones & Harrison, 2001; Muzur et al., 2002). Secondly, sleep is also thought to be important for the consolidation of at least some types of newly acquired memories (Walker & Stickgold, 2006).

Studies in humans and primates indicated that performance on tests of cognitive flexibility such as extradimensional set-shifting and reversal learning is selectively dependent on different parts of the PFC (Owen et al., 1991; Dias et al., 1996; Rahman et al., 1999; Robbins, 2007). These findings hold as well for in rodents (Brown & bowman, 2002), confirming the functional homology of rodent and primate PFC areas (Kolb, 1984; Uylings et al., 2003). In reversal learning, a previously acquired response-outcome contingency is reversed: the previously unrewarded response becomes the one that is rewarded and vice versa. Reversal learning involves the orbitofrontal cortex (OFC) in both rats and primates (Dias et al., 1996; 1997; McAlonan & Brown, 2003; Schoenbaum et al., 2006; Boulougouris et al., 2007).

The functionality of the prefrontal cortex has been suggested to be particularly sensitive to sleep (Muzur et al., 2002). Prefrontal regions of the human brain show prominent deactivation during sleep (Hobson & Pace-Schaott, 2002) and during sleep deprivation (Thomas et al., 2000). Although a few studies address the negative consequences of sleep restriction on PFC-dependent behaviour in rodents, none reported on the effects of a single exposure to total sleep deprivation on tests of cognitive flexibility.

E.g. Walsh et al. (2011) reported that in rats, spatial reversal learning of a hidden platform in a Morris water maze is not sensitive to the selective deprivation of 6h of rapid eye movement (REM) sleep, by means of the multiple inverted flowerpot method. In contrast, spatial reversal learning on a Y-maze is sensitive to the effects of repetitive 5h sleep deprivation during the preceding discrimination training in mice (Hagewoud et al., 2010b). In a third study, sleep was experimentally interrupted as a model of the sleep fragmentation that is typical of sleep apnoea (McCoy et al., 2007). The manipulation did not affect reversal learning of a compound discrimination between stimuli consisting of an odour and a digging medium (McCoy et al., 2007). On the other hand, the manipulation did affect performance on the extra-dimensional set-shifting version of this task (McCoy et al., 2007), where a shift in attention to a previously irrelevant perceptual dimension (e.g. digging medium when the preceding discrimination was based on odour) is required. Extra-dimensional set-shifting involves different areas of the PFC (see above) than reversal learning, and is considered to be a more complex form of cognitive flexibility. It has been hypothesized that the complexity of a task determines its sensitivity to sleep curtailment. Still, a
complete lack of sensitivity of reversal learning to sleep curtailment cannot be concluded from the work of McCoy and colleagues (2007), because their fragmentation procedure induced only partial sleep-deprivation. Following up on the work of McCoy and colleagues (2007) and Walsh and colleagues (2011), we here aim to evaluate whether reversal learning is still insensitive to sleep curtailment if deprivation is total instead of partial.

While previous studies used reversal learning paradigms mainly to investigate cognitive flexibility, it is of particular interest for sleep studies that these paradigms also offer the opportunity to study memory consolidation. Numerous studies in humans support a role of sleep in this process (e.g. Stickgold et al., 2001; Rauchs et al., 2005; Yoo et al; 2007; Van Der Werf et al; 2009b). Also in rodents, memory consolidation appears to profit from sleep (Hagewoud et al., 2010b; Palchykova et al., 2006; Rabat et al., 2006; Tiba et al; 2008). No previous rodent studies however investigated whether sleep is involved in the consolidation of newly acquired reversal learning skills. We therefore also evaluated in the present study whether total sleep deprivation affects the consolidation of reversal learning skills.

We have previously implemented a paradigm for serial reversal learning in rats, that is based on spatial discrimination between two levers in a Skinner box (Boulougouris et al., 2007, De Bruin et al., 2000; Van der Meulen et al., 2003; Van der Meulen et al., 2006; Van der Plasse et al., 2008). A distinct advantage of a Skinner box-based paradigm is that the task is automated and does not involve the frequent handling of the animal by the experimenter that is inherent to the protocols used by McCoy et al. (2007) and Walsh et al. (2011). It is not difficult to imagine that frequent handling of sleep-deprived rats might influence their activational state.

Spatial reversal learning has been shown to depend on OFC (Boulougouris et al., 2007), although involvement of the medial PFC has also been suggested (De Bruin et al., 2000). We expected that sleep deprivation would affect the early phase of learning, when flexible responding is called for and prefrontal involvement is high (De Bruin et al., 2000; Boulougouris et al., 2007). To test this hypothesis, we investigated whether 12h of sleep deprivation during the inactive phase affected first-time reversal learning.

Consolidation of the new task contingencies can be observed in the reversal learning paradigm that we used as a continuous improvement in performance from session to session. Rats are typically slow to switch their response to the other lever and only reach 50% correct responses in one session of 64 trials. After an interval of several hours, they resume the next session at a similar level of performance. Our previous observations suggest that a large majority of the rats sleep in between the subsequent sessions of the reversal learning (M Voorhaar & MGP Feenstra, unpublished results).

In humans, memory consolidation can already be promoted by a single six minute nap subsequent to learning (Lahl et al., 2008). Indeed, napping has repeatedly been shown to be beneficial to cognitive performance (Milner &
Cote, 2009). We expected that sleep deprivation would affect the consolidation of early reversal learning, which could be quantified from a relative decrease in performance at the start of the second session. To test this hypothesis, we investigated whether 3h of sleep deprivation during the active phase immediately following reversal learning affected its consolidation.

For both sleep deprivation experiments, we used a novel procedure (Leenaars et al., 2011, Chapter 2). Our previous experiments show this method to block all sleep very effectively for 12h during the light phase, without causing extra-physiological levels of corticosterone release or locomotor activity. As we have not described the use of our method for 3h of nap prevention during the active dark phase previously, we briefly report activity measurements to support the validity of the method in the methods section.

5.3. Materials and Methods

Behavioural experiments were performed in a total of 40 male Wistar rats (Harlan, Horst, the Netherlands; weight upon arrival 200-225g). They were housed under reversed light-dark conditions (lights OFF at 10:00 (dim red light), lights ON at 22:00 for the deprivation experiment, half an hour earlier for the no nap experiment) in groups of 4 in type-IV macrolon cages (60*38*20cm), in a room with controlled temperature (20°C ±2°C) and humidity (60% ±20%).

All rats were left undisturbed for at least one week after arrival for acclimatization, and habituated to daily handling for at least another week prior to starting experiments. Food restriction was started three days before first behavioural testing. Water was unrestricted.

The experiments were approved by the experimental animal committee of the Royal Netherlands Academy of Arts and Sciences and performed in accordance with Dutch legislation (Wet Op de Dierproeven, 1996) and European guidelines.

5.3.1. Behavioural training

Approximately three days before the onset of behavioural training, food was restricted to 12g/rat/day. During task-performance, rats could earn an additional 3g of food. On the days that no behavioural tests were performed, rats were fed 15g/rat/day. This food restriction has previously been shown to keep rats motivated to perform a task, even after sleep deprivation (Leenaars et al., 2011). All rats increased their body weight during these experiments.

Behavioural experiments were conducted in eight Skinner boxes (Med-associates, St. Albans, US) controlled by MED-PC software (Med-associates). Each box was equipped with an operant panel containing two levers, a cue-light above each lever and a food tray with tray light between the levers on one wall. The opposite wall contained a house light. Every rat was
appointed one Skinner box in which daily (on workdays) training and testing of that specific rat took place, at the onset of the active phase (dark-onset).

After rats had been shaped to associate lever pressing on both levers with food reward (Bio-Serve dustless precision pellets, 45 mg, BioServ, Frenchtown, US) within 5-6 shape sessions, aided by hand-shaping (the experimenter rewarding approximate responses during shaping sessions, via a remote control, until the rat started pressing the lever) when no spontaneous increases in lever pressing occurred, they were trained on a fixed-ratio-3 (FR3); within a single training session; they gradually learned to press the levers 3 times for a reward. The FR3 was implemented to prevent accidentally touching the lever to be registered as a response.

Following FR3-training, rats were exposed to spatial discrimination sessions on a daily basis. In these discrimination sessions (discrimination session 1 to 5; D1 - D5) only one out of the two levers was rewarded for 64 trials. Rats were assigned to groups for which either the left or the right lever was rewarded throughout all discrimination sessions. A flow chart of a trial within the spatial discrimination task is provided in Figure 25. Rats generally finished discrimination sessions in less than 15 minutes. Reward on the left or the right lever was counterbalanced over rats from sleep-deprived and control experimental conditions.

After the third discrimination session, rats were placed in the sleep deprivation devices for habituation. During the remainder of the experimental week, they were housed in the deprivation devices between behavioural testing. The first reversal (R1) consisted of two or three sessions (R1.1 - R1.3 with 3h intervals), in which a response on the previously unrewarded lever now led to a reward. Rats were returned to the deprivation devices for 3h between these sessions.

Two experiments were performed to study the effects of SD. In experiment 1, rats were exposed to 12 hour of total sleep deprivation before the first session of the first reversal (R1.1), or served as an undisturbed control. In experiment 2, rats were exposed to 3h of total sleep deprivation between the first and the second session of the first reversal (between R1.1-R1.2), or served as an undisturbed control. In experiment 1 (12h sleep deprivation before reversal learning), a third session was added if rats did not reach the learning criterion of 29 correct responses in the second half of R1.2 (>90% correct, R1.3: n=4 for the SD group, n=2 for the control group, data not shown). In experiment 2, rats were always exposed to the third session (R1.3).
A house-light continuously illuminated the skinnerbox throughout the task.

On the following day, a second reversal (R2), back to the originally rewarded lever was offered, again in 2 sessions (R2.1 and R2.2) separated by a three-hour break in the deprivation devices. All rats reached the learning criterion in the second half of R2.2. Reversal sessions were exactly the same as the discrimination sessions (64 trials), except that for each rat, the opposite lever was rewarded.
As rats gradually learn to press the formerly unrewarded lever during these sessions, data were analyzed for eight subsequent time-points (blocks of eight trials) within each session.

5.3.2. Experiment 1, Twelve hours of total sleep deprivation

Sleep deprivation was accomplished with gradually increasing mild forced locomotion, as described previously (Leenaars et al., 2011; Chapter 2). Briefly, sleep deprivation devices consisted of a rotating drum (Ø 39cm, height 37cm, Figure 1), divided into two semicircular compartments by a stationary central wall. The bottom moves bi-directionally and at varying speed, and both speed and the number of directional alternations are gradually increased over time to maintain deprivation efficacy in spite of increasing sleep pressure.

Rats were housed in the sleep deprivation boxes during a full test week, from Monday after their daily testing onwards. Sleep deprivation was always started on a Wednesday evening (22:00; ±11.5h after finishing the last discrimination session). Deprived rats (n=12) were tested at the onset of their active phase (dark onset at 10:00 on Thursday morning). After testing, rats were placed back in the deprivation devices. Control rats (n=12) were housed in the deprivation devices during the same periods, but never exposed to mild forced locomotion.

5.3.2.1. Behavioural activity and sleep deprivation

To confirm effectiveness of the sleep deprivation protocol, activity levels were measured by counting of infrared displacement in two-minute intervals as described previously (Leenaars et al., 2011). Behavioural activity was measured for 24h before sleep deprivation, during the protocols and for 12 hour of subsequent recovery. When rats were being tested in the Skinner boxes, no activity data were collected. Passive movement (when the rat does not move while the bottom plate changes direction) is also registered by the activity meters. We subtracted passive movement values from the activity values before further calculations. Passive movement had been estimated previously (Leenaars et al., 2011). Actigrams are presented for uncorrected data.

Representative actigrams for a sleep-deprived and a control rat are shown in Figure 26. Hourly total activity values were calculated. These values are presented for the 12 hour deprivation period and for the subsequent 12 hours of recovery, as well as for the corresponding period in undisturbed control rats. Averaged hourly totals for the deprivation period and the subsequent recovery are shown in Figure 27a. Totals for the 12 hour deprivation period during the light phase and for the 12 hours of recovery afterwards are shown in Figure 27b. Sleep deprived rats are more active during the deprivation period than during the subsequent recovery period, while control rats show a normal circadian rhythm, and are more active during the dark phase than during the light phase. During light-phase deprivation, deprived rats are more active than control rats, while during
dark-phase recovery, previously deprived rats are less active than control rats. Statistical details are provided with Figure 27.

**Figure 26** Representative actigrams of one rat on the sleep deprivation protocol and of one on the matching movement control

Shown are the activity counts per 2 min periods. Estimates of passive activity during the sleep deprivation procedure are shown in white. The grey boxes indicate the periods with white light off and dim red light on.
Figure 27  Average total movement during the 12 hour of the sleep deprivation protocol and the matching movement control, and during 12 hour of subsequent recovery (n=6 sleep-deprived and n=8 control rats)

A.) Number of activity counts (± S.E.M.) per hour. B.) Number of activity counts (± S.E.M.) within the 12 hour periods. The grey box indicates the period with white light off and dim red light on. A significant main effect was present for experimental group (F(1, 12) = 7.4; p = 0.019), as well as for the interaction between period and experimental group(F(1.0,12.0) = 248.9; p = 0.000). The time effect was significant for both groups; (t(7) = 13.8; p = 0.000 for SD, t(7) = -9.9; p = 0.000 for control), and for both periods (t(12) = -13.2; p = 0.000 during light phase, t(8.1) = 5.5; p = 0.001 during dark phase).
5.3.2.2. Learning prior to sleep deprivation experiments

To exclude potential differences in discrimination learning before the onset of reversal learning, the numbers of correct responses during the five discrimination sessions (D1-D5) were analyzed with an ANOVA. A main effect of session ($F(2.6,57.7) = 131.8$, $p = 0.000$) and planned simple contrasts showed that rats increased their number of correct responses over the first 3 sessions (D1-D3; $p \leq 0.001$). No difference between the 2 test groups was found ($p = 0.38$ for the group effect and $p = 0.82$ for the interaction).

5.3.3. Experiment 2, Three hours of nap prevention

Nap prevention was also accomplished with gradually increasing mild forced locomotion, using the movement protocol corresponding to the first three hours of the sleep deprivation protocol described before (Leenaars et al., 2011, Chapter 2). Nap prevention (n=8) was always started on Thursday morning, after exposure to the first spatial reversal session. The second session of the first reversal followed immediately after the 3h of nap prevention. Control rats (n=8) were housed in the deprivation devices during the same periods, but never exposed to mild forced locomotion.

5.3.3.1. Activity during and after the nap prevention protocol

To confirm effectiveness of the nap prevention protocol, activity levels were measured as described for experiment 1. A representative actigram for a nap-deprived and a control rat are shown in Figure 28. Averaged half-hour totals for the deprivation period and the subsequent recovery are shown in Figure 29a. Totals for the deprivation period and for the 2,5 hours of recovery after Skinner box testing are shown in Figure 29b. During the sleep disturbance, no-nap rats are more active than control rats. Behavioural activity during the recovery period was not different between the groups. Statistical details are provided with Figure 29.
Figure 28  Representative actigrams of one rat on the nap prevention protocol and of one on the matching movement control

Shown are the activity counts per 2 min periods. Estimates of passive activity during the nap prevention procedure are shown in white. The grey boxes indicate the periods with white light off and dim red light on.
Figure 29  Average total movement during the 3h of the nap prevention protocol and the matching movement control, and during 2.5 h of subsequent recovery (n=8 sleep-deprived and n=8 control rats).

A.) Number of activity counts (± S.E.M.) per half hour. B.) Number of activity counts (± S.E.M.) within the 2.5h periods. Data from the 10:00 – 10:30 interval are deleted, as rats were then being transferred back from the skinner boxes to the sleep deprivation devices. The grey box indicates that in this period white light was off and dim red light on.

Significant main effects were present for both period (F(1.0,14.0) = 39.6; p = 0.000) and experimental group (F(1, 14) = 12.6; p = 0.003), as well as for the interaction between these two factors (F(1.0,14.0) = 84.4; p = 0.000). The time effect was significant for both groups (t(7) = -2.5; p = 0.041 for control and t(7) = 9.4; p = 0.000 for no nap). A clear difference in activity is present during deprivation (t(14) = -6.5; p = 0.000), but behavioural activity during the recovery period was not different between the groups (p = 0.7).
5.3.3.2. Learning prior to nap prevention experiments

To exclude potential differences in discrimination learning before the onset of reversal learning, the numbers of correct responses during the five discrimination sessions (D1-D5) were analyzed with an ANOVA. A main effect of session ($F(3.0, 42.2) = 78.4, p = 0.000$) and planned simple contrasts showed that rats increased their number of correct responses over all sessions (D1-D4 vs. D5; $p \leq 0.000$). No difference between the two test groups was found ($p = 0.15$ for the group effect and $p = 0.32$ for the interaction).

5.3.4. Statistical analysis

All data are presented as average values ± standard error of the mean (SEM). Statistical analyses were performed with SPSS (Chicago, US). Differences were considered significant at $p \leq 0.05$.

The number of sessions needed to reversal learning (e.g. reach 90% correct in the second half of the session) was compared between sleep-deprived and control rats with a Mann-Whitney test. Reversal learning was further analyzed for both the first and the second reversal separately. The numbers of correct responses in 8 blocks of 8 trials during the two sessions of each reversal were analyzed with an ANOVA, with session (R1.1 and R1.2 for the first reversal; R2.1 and R2.2 for the second,) and block (8 blocks of 8 trials within each session) as within-subjects factors, and condition (sleep deprivation or control) as between-subjects factor.

Statistical power was calculated post-hoc for the ANOVAs of the first reversals (R1.1 and R1.2 for experiment 1 and 2), using G*power 3.1 (Faul et al., 2007). The following input was used: a medium effect size of 0.25 (Cohen, 1992), $\alpha = 0.05$ and actual values for other variables.

Activity data during sleep deprivation protocols were collected in 2-minute bins.

For experiment 1, hourly totals were calculated and corrected for passive movement. These values are shown for the deprivation period and for the subsequent 12 hours of recovery. The corresponding periods are shown for non-deprived animals. Totals for these periods were calculated and compared with an ANOVA with period (sleep deprivation or recovery) as within-subject factor, and experimental condition (no nap or control) as between-subject factor. For experiment 2, half-hourly totals were calculated and corrected for passive movement. These values are shown for the deprivation period and for the 2.5h of recovery after Skinner box testing. The corresponding periods are shown for non-deprived animals. Totals for these periods were calculated and compared with an ANOVA with period (sleep deprivation or recovery) as within-subject factor, and experimental condition (no nap or control) as between-subject factor.
The numbers of correct responses during the five discrimination sessions before the onset of reversal learning was analyzed with an ANOVA, with session (D1-D5) as the within-subjects factor and condition (future sleep deprivation or control) as between-subjects factor.

A Huyn-Feldt correction was applied when the assumption of sphericity was violated (Field, 2005).

5.4. Results

5.4.1. Experiment 1: The effect of 12 hours of sleep deprivation on reversal learning

5.4.1.1. The first spatial reversal

Rats needed two or three sessions to reach the criterion of 90% correct responses in the second half of the R1.2 session. There was no significant effect of 12 hours of total sleep deprivation during the light phase on the number of sessions needed to reach criterion (p = 0.7). The effect of 12 hours of sleep deprivation during the light phase on learning a spatial reversal (Figure 30) was further analyzed with an ANOVA, with session (R1.1 vs. R1.2) and block (8 blocks of 8 trials nested within each session) as within-subjects factors, and condition (sleep deprivation or control) as between-subjects factor. Significant main effects were present for both session (F(1.0,21.0) = 424.9; p ≤ 0.001) and block within session (F(7, 147) = 49.4; p ≤ 0.001), as well as for the interaction between these two factors (F(4.6, 97.6) = 4.5, p = 0.001), indicating learning within and over sessions. No significance was found for the effect of interest, i.e. the effect of sleep condition (p = 0.46). No significant interaction between sleep deprivation condition and session (p = 0.21) or block (p = 0.24) or session and block (p = 0.66) was present either, indicating that 12 hour of sleep deprivation does not affect learning of a spatial reversal in this paradigm. The statistical power for the analyses to detect a medium effect size, representing an effect likely to be visible to the naked eye of a careful observer (Cohen, 1992), was 0.90.
5.4.1.2. Potential delayed effects of 12 hours of sleep deprivation on a second reversal

The day after sleep deprivation, rats were exposed to a second reversal; pressing the originally rewarded lever became once again rewarded (data not shown). Potential delayed effects of the 12 hours of sleep deprivation were analyzed with an ANOVA, with session (R2.1 vs. R2.2) and block (8 blocks of 8 trials within each session) as within-subjects factors, and condition (12 hours of sleep deprivation on the preceding day or control) as between-subjects factor. Significant main effects were present for both session (F(1.0,22.0) = 72.1; p ≤ 0.001) and block within session (F(4.7,103.4) = 28.3; p ≤ 0.001), as well as for the interaction between these two factors (F(4.9,108.3) = 10.0, p ≤ 0.001), indicating learning within and over sessions. No significant effect of condition could be observed (p = 0.63). No significant interaction between sleep deprivation condition and session (p = 0.74) or block (p = 0.40) or session and block (p = 0.71) was present either, indicating that 12 hours of sleep deprivation does not have a long-term delayed effect on learning of a spatial reversal in this paradigm either.

5.4.2. Experiment 2: The effect of 3h of nap prevention on the consolidation of reversal learning

5.4.2.1. Consolidation of the first spatial reversal

Rats needed two or three sessions to reach the criterion of 90% correct responses in the second half of the R1.2 session. There was no significant effect of 3 hours of total sleep deprivation during the dark phase on the
number of sessions needed to reach criterion \( p = 0.7 \). The effect of nap prevention (Figure 31) was further analyzed with an ANOVA, with session \( (R1.1 \text{ vs. } R1.2) \) and block (8 blocks of 8 trials within each session) as within-subjects factors, and condition (sleep deprivation or control) as between-subjects factor. Significant main effects were present for both session \( (F(1.0, 14.0) = 84.9; p \leq 0.001) \) and block within session \( (F(6.0, 84.2) = 29.4; p \leq 0.001) \), as well as for the interaction between these two factors \( (F(5.3, 74.1) = 6.1, p \leq 0.001) \). This indicates learning within and over sessions. No significant effect of sleep condition \( (p = 0.31) \) could be observed. No significant interaction between sleep deprivation condition and session \( (p = 0.47) \) or block \( (p = 0.75) \) or sleep deprivation and block \( (p = 0.30) \) was present either, indicating that 3h of nap prevention immediately after the first session of reversal learning does not affect consolidation of learning a spatial reversal in this paradigm. The statistical power for these analyses to detect a medium effect size was 0.81.

Figure 31 Consolidation of the first reversal after 3h of nap prevention

Shown are the correct responses (± S.E.M.) per block of 8 trials for the first (R1.1) and second (R1.2) sessions of the first reversal. Three hours of total sleep deprivation was applied between R1.1 and R1.2 (n=8 sleep-deprived and n=8 control rats).

5.4.2.2. Potential delayed effects of 3h of nap prevention on a second reversal

The day after nap prevention, rats were exposed to a second reversal; pressing the originally rewarded lever became once again rewarded. Potential delayed effects of the 3-hour nap prevention were analyzed with an ANOVA, with session \( (R2.1 \text{ vs. } R2.2) \) and block (8 blocks of 8 trials within each session) as within-subjects factors, and condition (12 hours of sleep deprivation on the preceding day or control) as between-subjects factor (data not shown). Significant main effects were present for both session \( (F(1.0, 14.0) = 87.6; p = 0.000) \) and block within session \( (F(4.9, 68.8) = 16.4; \)
p ≤ 0.001), as well as for the interaction between these two factors (F(6.3, 88.2) = 7.9, p ≤ 0.001), indicating learning within and over sessions. No significant effect of sleep condition could be observed (p = 0.15). No significant interaction between sleep deprivation condition and session (p = 0.07) or block (p = 0.83) or session and block (p = 0.18) was present either, indicating that 3h of nap prevention immediately after the first session of reversal learning does not have a delayed effect on learning of the subsequent reversal either.

5.5. DISCUSSION

We hypothesized that 12 hours of inactive phase sleep deprivation would impair spatial reversal learning, and that 3 hours of active phase sleep deprivation would disturb the consolidation of reversal learning. However, the results of our experiments do not support these hypotheses.

Our first hypothesis was based on the assumption that performance of a behavioural task that is dependent on the functional integrity of the PFC would be particularly sensitive to prior sleep deprivation. Reversal learning in general has indeed been shown to depend on prefrontal functioning (Brown & Bowman, 2002) and the same holds for the paradigm that we used in the present study (Boulougouris et al., 2007; De Bruin et al., 2000). The insensitivity of spatial reversal learning to 12h of total sleep deprivation might be explained in several ways.

First of all, 12h of total sleep deprivation may be too little to cause impairments. However, other animal studies did find evidence for impaired cognitive flexibility after several types of comparably mild sleep disturbance: 24 hours of sleep fragmentation impaired extra-dimensional set-shifting (McCoy et al., 2007) and 12 hours of inactive phase sleep deprivation attenuated task-switching accuracy (Leenaars et al., 2012; Chapter 3). In this respect it is important to note that we used a highly effective method for sleep deprivation; the protocol has been shown to limit slow wave sleep to 0.8% of the 12h period and to completely prevent rapid eye movement sleep (Leenaars et al., 2011; Chapter 2).

A second possible explanation could be that the rat PFC does not respond to sleep deprivation as human PFC does. Sleep deprivation has been shown to alter PFC activity in both humans and rats, but one-to-one comparisons are difficult because of diverging assessment methodologies. Sleep deprivation changes gene expression in the cerebral cortex in a quite similar way across rodent species (Cirelli et al., 2006; Terao et al., 2006). Sleep deprivation in rats initially increases extracellular glutamate in the cerebral cortex, followed by a gradual decline (Dash et al., 2009). These methodologies are not feasible in humans. The only potential example of matching methodologies is that sleep deprivation attenuates PFC glucose uptake in humans (Thomas et al., 2000), while it increases hexokinase, the rate limiting enzyme in glucose metabolism, in the somatosensory cortex in rats (Ramanathan et al., 2010). Future studies should provide comprehensive
evidence to the comparability of the PFC’s response to sleep deprivation in rodents and humans.

A third possible explanation is that sleep deprivation did induce impairments in PFC functioning that are relevant to reversal learning, but that these were compensated for by the recruitment of other brain regions less sensitive to sleep loss. This theory has been elegantly described previously by Hagewoud et al. (2010b).

A fourth possible explanation is that young individuals are relatively insensitive to the effects of sleep deprivation. At the time of the reversal experiments, our rats were approximately 11 weeks old, which may be compared to human young adulthood. A recent meta-analysis indicates that detrimental effects of sleep deprivation in several cognitive domains cannot robustly be observed in school aged children (Astill et al., 2012). Also healthy volunteers in their 20’s do not show any differences in flexibility following SD compared to undisturbed sleep (e.g. Binks et al., 1999). It remains to be investigated whether sleep deprivation affects reversal learning in older rats.

Finally, it could be that reversal learning is just not sensitive at all to acute SD. Previously, McCoy et al. (2007) reported that 24 hours of sleep interruption did impair attentional set-shifting in rats, but not reversal learning of familiar odour and texture discriminations. Walsh et al. (2011) reported that spatial reversal learning of a hidden platform in a Morris water maze is not sensitive to 6h of REM sleep deprivation. Our hypothesis was that the more severe sleep disturbance caused by total sleep deprivation would affect reversal learning. However, the combined evidence of our present results and those of McCoy et al. (2007) and Walsh et al. (2011) shows that reversal learning is relatively robust to the effects of both 24 h partial and 12 h total sleep deprivation. In contrast, repeated sleep deprivation throughout discrimination training resulted in subsequent impaired reversal learning (Hagewoud et al., 2010b), suggesting that a more prolonged partial sleep restriction during discrimination learning is crucial to disrupt subsequent reversal learning.

Besides the robustness of the acquisition phase of spatial reversal learning to twelve hours of SD, we show that three hours of nap prevention after exposure to spatial reversal learning does not affect the consolidation of the task. Several explanations for the insensitivity might be offered.

First, three hours of total sleep deprivation may be too little to cause impairments, even though our method was equally effective in keeping rats awake as it was in the first experiment (Chapter 4). Rats are active throughout the nap prevention procedure as shown by the activity data in the methods section. However, 3h of sleep deprivation in the active phase are sufficient to disturb instrumental learning, the association of an action (lever pressing) with reward (food; Chapter 4).

Secondly, the precise timing of sleep deprivation respective to the learning may be relevant. In concordance with previous experiments, rats were
exposed to the second session of the reversal after a break of 3 hours. Memory consolidation may occur over more prolonged (or delayed) time-windows (Smith, 196; Vertes, 2004), and we cannot exclude the possibility of sleep deprivation effects during other time intervals than the 3-hour interval between the first two reversal sessions we applied. It would be of interest to elongate the interval between the sessions and apply sleep deprivation of a longer duration to examine this possibility. However, at the onset of the second session, rats usually show memory of what happened in the previous session; they start at a higher level of correct responding compared to the first session, indicating that at least some consolidation did occur in the 3-hour interval, even without sleep.

A third explanation could be that the consolidation of reversal learning is truly insensitive to SD. Hagewoud et al. (2010b) recently presented data that support this possibility: five hours of sleep deprivation between daily reversal sessions did not affect learning of a Y-maze reversal. In humans, sleep does not benefit memory consolidation under all circumstances and in all paradigms either (Maquet, 2001; Marshall & Born, 2007). We here show that spontaneous napping is not necessary for the consolidation of reversal learning in rats.

We conclude that sleep deprivation prior to reversal learning does not affect the acquisition phase of spatial reversal learning, and that sleep subsequent to reversal learning is not required to consolidate it. Functioning of those parts of the PFC responsible for flexible responding to familiar cues is apparently spared after a relatively mild sleep deprivation.

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