7. CONCLUDING REMARKS & GENERAL DISCUSSION

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CONCLUDING REMARKS & GENERAL DISCUSSION

The work described in this thesis starts to address the question how a lack of sleep may induce cognitive impairments. This discussion will start with a brief summary of the work presented before. It will continue with a reflection on methodology, including data not presented elsewhere. The last section is dedicated to a brief discussion of the findings and their interpretation in light of related previous literature on sleep and cognition. This section includes suggestions for future research.

7.1. Summary of the preceding chapters

The introduction starts with a resume of the general knowledge and hypotheses on sleep, and its role in cognitive functioning. It includes a description of the behavioural and electroencephalographic characteristics that we can observe during sleep, which are used to define sleep and distinguish it from wake behaviour. This is followed by the general hypotheses on why we sleep, including sleep's contribution to cognitive functioning, and the specific cognitive domains mostly affected by sleep loss. It provides an overview of common human sleep disorders, which can result in cognitive impairment, and finishes with a description of rodent models for both sleep deprivation and cognitive performance.

Chapter 2 introduces a new method for inducing sleep deprivation in rats, based on variable forced locomotion. Contrary to some other methods of forced locomotion (e.g. Roman et al., 2006), this method does not induce significant stress, as indicated by the observation that corticosterone levels did not exceed the levels normally seen during the 24-hour day. Moreover, the method did not have the drawback of potential confounding of experimental results by an increase in locomotor activity, as may be the case in some other methods. When our method was applied for 12h of sleep deprivation during the light phase, activity levels did not exceed those normally seen during undisturbed conditions.

When testing behaviour in a sleep-deprived state, other possible confounders have to be addressed as well. Notably, the effects of sleep deprivation on a specific cognitive domain may depend on nonspecific cognitive effects that affect performance on the task of interest. For example, the motivation to “work” for a reward may be decreased, and fatigue may slow motor functioning. These potential problems were investigated using a task on which rats show vast levels of lever pressing to receive food rewards, which makes this task highly sensitive to decreases in motivation and motor impairment. Potential decreases in motivation were limited by imposing a food restriction to 12g/rat/day.

Chapter 3 describes the modelling of one sleepless night and one night of disturbed sleep in humans, with 12h of inactive-phase sleep deprivation or sleep disruption in rats. It tests the effect of this sleep disruption on cognitive flexibility and introduces a new switch-task. While 12h of total sleep deprivation during the light (inactive) phase decreases accuracy on
switch-task performance, 12h of repetitive sleep disturbance during the inactive phase does not alter task-switching.

Chapter 4 described the impairment in instrumental learning; the simple association between lever pressing and food reward, after 3h of active phase nap-prevention. EEG was measured before and between task performance. Learning is accompanied by an increase in REM sleep. Baseline sleep parameters do not predict subsequent individual differences in learning abilities.

In chapter 5, both 12h of inactive-phase sleep deprivation (as a model for one sleepless night) and 3h of active-phase nap prevention did not disturb performance on a different cognitive task: spatial reversal learning. Total sleep deprivation for 12h during the inactive phase does not impair the acquisition of a spatial reversal, and 3h of nap-prevention during the active phase does not impair the consolidation of reversal learning. This indicates that also in rats, sleep-related cognitive deficits are not generalized but limited to certain cognitive domains.

In chapter 6, rats were exposed to 5 weeks of non-rotating shiftwork. They showed no learning deficits on an instrumental learning task (the same task as used in chapter 4) in their 5th week on this protocol, which shows that rats may somehow habituate to regular sleep deprivation for 8h per day on 5 days per week (both in the active and in the inactive phase). Furthermore, the undisturbed control groups in this study demonstrate that instrumental learning is similar during the active and the inactive phase.

7.2. Reflection on methodology

When investigating the effect of sleep deprivation on cognition in rats, many factors besides sleep deprivation itself can influence the results. The relevant literature has been summarised in chapter 1, while chapter 2 describes the studies which address potential confounding factors with our novel method for sleep deprivation implementing variable forced locomotion. This section will start with a further discussion of the results from chapter 2, and additional results on recovery-sleep after short-term active-phase sleep deprivation, as implemented in chapters 4 and 5.

Combining data from different experiments performed during this project can shed some light on the relative effect of two of the potential confounding factors that can influence the results besides sleep deprivation: inversion of the light-dark cycle and behavioural task structure.

Inversion of the light-dark cycle; keeping rats in the laboratory with the lights on during our night and with the lights off during our day, is often implemented to allow experimenters to work during normal office hours while performing tests during the dark phase of the animals. As the rats are normally raised under a normal light schedule, this procedure involves a phase-shift of 12 hours, which may induce something like a jet-lag, that could affect subsequent experimental results.
Behavioural tasks are designed to answer specific research questions. The tasks themselves will be designed in a certain manner, but not all task parameters are necessarily relevant to answer the research question. Tasks can e.g. consist of a certain number of trials, and trials can be separated by breaks (intertrial intervals) of a certain duration. As tasks implementing different intertrial intervals were used during this project, the effects of the duration of the intertrial interval on performance could be investigated. Additional data are present for the behavioural tests from chapters 3 and 5.

These combined and new data are presented in section 7.2.1 to 7.2.6.

7.2.1. A new automated method for rat sleep-deprivation (chapter 2)

In chapter 2 a novel method for sleep deprivation has been described. Although the upright drum and the type of movement induction are conceptually similar to the disc-over-water-method, our method is more effective in preventing sleep, and does not suffer from the aversive aspects of water. The most likely reason for the increased effectiveness is the variability of the forced locomotion; the movement protocol changes every hour, according to the pre-programmed sequence in Table 1, section 2.3.3, thereby providing novelty and minimal arousal throughout. Both the arousal, novelty, and the increasing amount of locomotion over time are thought to counteract increasing sleep pressure during the sleep deprivation.

7.2.1.1. Variable forced locomotion protocols

Most automated paradigms use standard protocols that are similar throughout deprivation procedures, which may be unnecessary stressful at the onset of sleep deprivation while not being sufficiently arousing at the later stages. Our variable method is sufficiently arousing from the onset to the end, and only parts of the protocol may be minimally stressful as indicated by the temporary mild increase in corticosterone. Because of the high effectiveness and low stressfulness, it can be recommended for sleep deprivation studies to implement protocols with variable forced locomotion; mild movement at the onset of the deprivation procedure and gradually more challenging movement over time.

7.2.1.2. Confounding by stress and activity

At the end of the 12h sleep deprivation protocol, rats were as easy to handle as when not deprived (casual observations), consistent with the absence of a stress response. However, it would be elegant to confirm the absence of the stress response with additional measurements. Ultrasonic vocalisations in the 22kHz range are thought to relate to a negative state in the rat (e.g. Brudzynski, 2007). This is a non-invasive method that could be implemented to register rat “mood” at multiple time points during the deprivation protocol.

Besides the lack of stress, rats maintain spontaneous activity throughout, but they do not have to move faster than they voluntarily would. Although the used infrared-displacement detection is very sensitive, it is difficult to
distinguish active movement from passive movement associated with maintaining a fixed position on a rotating of bottom plate. Ideally, movement should therefore be registered on the rat, and not confounded by a passive change of location.

Unfortunately, the available actimetric equipment that is so often used in humans (e.g. Gohar et al., 2009) is not yet sensitive enough to detect rat movement. An alternative could be EMG in one of the leg muscles, although this is relatively invasive and therefore not ideal. Current telemetric devices for rats registering location (e.g. Drijfhout et al., 1995) will also be activated by the passive movement of the bottom plate of our deprivation device, and will therefore not provide additional information compared to our infrared displacement detection. Theoretically, it should be possible to deduct actual rat movement by combining measurements of the rat’s location (either by infrared or by telemetry) and registration of the movement of the bottom plate. This could well be the most viable movement detection method to aim for.

7.2.1.3. Modelling one sleepless night

In this thesis, twelve hours of inactive (light) phase sleep deprivation was used to model one sleepless night. This model has some construct validity when investigating the effects of staying awake for one night as it induces 12h of continuous wakefulness during the normally inactive phase. However, important differences occur between experimental sleep deprivation in rodents and human sleep deprivation, limiting construct validity. First of all, while humans may choose to stay awake when they choose to work (or participate in an experiment) during the night, rodent sleep deprivation is never voluntary. Also, the cause of sleep deprivation in our model is external, while insomnia can also be caused by internal factors (see introduction).

Sleep has many similarities in rats and humans, warranting the use of rats as model animals in sleep research and enabling careful extrapolation of the results. First of all, in both species sleep follows the behavioural patterns provided in the introduction; sleep is preceded by preparatory behaviours, both humans and rats have a typical sleep posture, sleep occurs regularly within the circadian cycle, arousal threshold is increased during sleep and recovery sleep occurs after sleep deprivation. Second, changes in the EEG show a great degree of overlap. Third, sleep can similarly be affected by pharmacological interventions.

However, rat sleep is not exactly the same as human sleep. Sleep is more fragmented in rats than in humans, and in rat sleep EEG only 2 sleep stages can easily be distinguished, compared to 5 in humans (see introduction). Because of the differences in sleep architecture, sleep deprivation of similar duration may differentially affect both species.

Besides working with different species, laboratory circumstances are not similar to real life. An important phenomenon which may affect laboratory animal studies, is the lack of need for laboratory animals to be awake.
There is no acute danger, and there is no need to hunt for food, which is regularly provided. Zoo animals are known to sleep longer in confinement compared to their wild congeners (Horne, 2006). Assuming that this is also the case for our rats, their surplus sleep may actually make them relatively resistant to the effects of short-term sleep deprivation. Fortunately, our method for sleep deprivation in rats does reproduce cognitive impairments after sleep deprivation (chapter 3). This proves that the method does have decent face validity, and can be used for future mechanistic studies.

7.2.1.4. Motivation after sleep deprivation

The effects of sleep deprivation on a specific cognitive domain may depend on nonspecific cognitive effects such as the motivation to "work" for a reward. Motivation can be investigated with fixed-interval (FI) fixed-ratio (FR) tasks. Chapter 2 describes performance of sleep-deprived rats on an FI40FR3 task. On a 16g/rat/day diet, sleep deprivation decreases the response rate on this task. In the following part of this discussion, it will be shown that motivation is not only affected by internal state (which is altered by amongst others sleep deprivation and food restriction), but that task structure also substantially affects motivation.

The continuous presence of a lever in the Skinner-box stimulates continuous responding, thereby inhibiting the initiation of recovery sleep between trials. It is generally thought that variable ratio and interval schedules lead to the highest rate of responding (Feldman et al., 1997; Mazur, 1983). It would therefore be of interest to see if a VI40VR3 task would be relatively robust to the effects of sleep deprivation compared to the FI40FR3 task used here.

In the current studies, when motivated performance was affected by sleep deprivation, rats usually still performed relatively normally on the first trials of these tasks and only ceased responding later on (e.g. Figure 42; comparable data for the other tasks not shown). This may indicate that motivation after sleep deprivation decreases with prolonged time-on-task.

Not many compounds are available that can truly alleviate all the symptoms of sleep deprivation. However, in humans, the relatively novel stimulant Modafinil can restore multiple executive functions, as well as alertness, after sleep deprivation to normal baseline levels (Killgore et al., 2009; Wesensten, 2006). In a non-sleep deprived state Modafinil can increase motivation in rats (Young & Geyer, 2010). As rats are less motivated to perform certain instrumental tasks after sleep deprivation, it would be of interest to test if the administration of Modafinil can restore normal motivation in a sleep-deprived state. If so, the decreased performance on the FI40FR3 task described in chapter 2 could be used as a model for decreased motivation after sleep deprivation.
7.2.2. Additional results: inactive phase sleep deprivation and recovery sleep

Chapters 4 and 5 describe the cognitive effects of short-term (3h) active-phase sleep deprivation, using the same method as described above. Rats normally nap substantially during their active phase. The amount of sleep that is prevented by sleep deprivation in the active phase, and subsequent recovery sleep, were quantified by EEG measurements during the experiments in chapter 4.

Rats were exposed to 2 daily sessions of instrumental learning. EEG was measured before, between and after these sessions. Sleep deprivation was scheduled during the 3 hours between the first and the second session. EEG could not be measured during learning, but recovery sleep was observed during the 6 30-minute intervals following the second session of instrumental learning. Methods are described in detail in chapter 4.

Sleep deprivation effectively prevented sleep; during the 3 hour sleep deprivation protocol, SWS decreased from 39.4% to 0.8% of the time and REMS from 1.4% to 0.0% compared to the same time on the preceding day.

Recovery sleep, or increases in sleep following sleep deprivation compared to baseline sleep, were tested for the half-hourly intervals subsequent to the second session of instrumental learning using ANOVAs with time (six 30-minute intervals) and day (baseline or recovery) as within-subject factors. Planned simple contrasts were used to locate potential time effects and interactions. Results are shown in Figure 38.

For SWS duration, a generalized effect over the 3h period was observed (F1.0,15.0 = 13.9; p = 0.002), indicating a generalized increase in SWS. For REMS duration, the interaction between time and day was significant (F5.75 = 2.8; p = 0.024), but the simple contrasts for this effect were not significant. The significant day-by-time interaction for REMS duration could not be located with the simple contrasts, therefore individual time points were compared post-hoc using paired Student's t-tests. These post-hoc t-tests indicated that the increase in REMS was only significant during the fourth half hour (t16 = -2.1; p = 0.048). Recovery effects were also observed when sleep deprived rats were compared to undisturbed control rats (e.g. p = 0.001 for SWS and p = 0.008 for REMS; data not shown).

For cortical spindle duration, both the day-effect (F1.0,9.0 = 11.0; p = 0.009) and the day-time interaction (F5,45 = 3.2; p = 0.015) were significant. Spindle duration was decreased during the 1st - 3rd and 5th half hour of recovery (p = 0.038). For cortical spindle number, the day * time interaction was significant (F5,50 = 3.6; p = 0.007). Cortical spindle numbers were increased during the 1st half hour of recovery sleep (p = 0.002). For parietal spindle duration, the day-effect was significant (F1.0,9.0 = 9.2; p = 0.014), spindle duration was decreased throughout recovery sleep. For parietal spindle number, the day * time interaction was significant (F5,50 = 3.6; p = 0.007). The number of spindles was increased during the first half hour of recovery sleep (p = 0.021).
No significant recovery effects were present for spindle amplitude (data not shown).

**Figure 38** Recovery sleep after 3h of sleep deprivation and subsequent task exposure, compared to baseline sleep within the same subjects.

To conclude, even a brief 3h period of sleep deprivation during the active phase results in significant recovery sleep.
7.2.3. Inversion of the light-dark cycle

As the current thesis focuses on rat studies, and rats are preferably nocturnal animals, their light-dark cycle is often inverted to enable experiments during their active, dark phase, without causing substantial sleep deprivation and night shifts for the experimenters. Unfortunately, few studies have been performed to identify the minimal time required for a complete behavioural and physiological adaptation to the inverted light-dark cycle (Bertoglio and Carobrez, 2002).

Inversion of the light-dark cycle is not necessary for all experiments. In the current thesis, if continuous measurements were made for prolonged periods, rats were housed under normal light conditions. In chapter 6 we present an experiment with equal numbers of rats on a normal and an inverted light cycle, for which locomotor activity measurements were performed. Also, baseline electroencephalographic (EEG) registrations, and thereby sleep architecture data, are available for normal light-dark conditions (all EEG-experiments in chapter 2 and the EEG-experiment in chapter 5, in total n = 20), as well as for inverted light conditions (all EEG-experiments in chapter 3, n = 43). Comparisons of spontaneous locomotor activity and time spent sleeping under normal light conditions and after inversion of the light dark cycle are described in section 7.2.3.1 and 7.2.3.2 respectively.

7.2.3.1. Additional results: Locomotor activity after a light-dark reversal

The experiment described in chapter 6 includes both a group on regular light cycle (n = 16) and a group on an inverted light cycle (n = 16). For these rats, activity was measured from arrival from the animal supplier (Harlan, Horst, the Netherlands) onwards, by means of piezo-electric elements responding to vibration, connected to metal plates below the home cages, as previously described (Cailotto et al., 2005). These data allow for a study of the adaptation of the locomotor activity pattern to an inversion of the light-dark cycle.

At our animal supplier (Harlan), rats are housed under a regular 12:12h light-dark cycle. Rats are transported in dark boxes, and housed in the requested light-dark cycle upon arrival. In Figure 39 the activity of these rats is plotted in hourly intervals, for the first 11 days after arrival. Separate lines represent activity of the group maintained on the regular light cycle and the group that were exposed to the inverted light cycle from arrival onwards.

After arrival, a clear circadian pattern can be observed in the locomotor activity, consistent with the normal light cycle at the supplier. The non-inverted group shows high locomotor activity during the dark phase (19:00-7:00) while their activity is low during the light phase (7:00-19:00). The group that underwent a 12h phase-shift upon arrival slowly adapts to the inverted light-dark-cycle. After 11 days, they show high locomotor activity during the dark phase (7:00-19:00) and low activity during the light phase (19:00-7:00).
Figure 39  locomotor activity (measured by piezo-electric current detection for cages housing 2 rats) under normal and inverted light conditions from arrival onwards

Data are presented as mean ± SEM.

Total locomotor activity throughout the light and the dark period on day 12 after inversion (from Sunday 7:00 to Monday 7:00) was compared with an ANOVA with circadian phase (light or dark) as within subject factor and circadian phase inversion as a between-subject factor. Activity at preceding intervals was not tested as data for half of the cages was not available due to a technical failure. A Greenhouse-Geisser correction was applied as data violated the assumption of sphericity.

In these data (Figure 40), a clear effect of circadian period was observed (light versus dark, $F_{1.0,14.0} = 398.0; p = 0.000$), locomotor activity is higher during dark than during light. However, no between group effect of light regime ($p = 0.37$) was present, and the interaction between light regime and light-dark activity was not significant either ($p = 0.31$). From these results we can conclude that 12 days after phase-shifting, the activity of the inverted light group had fully adapted to the inverted light regime. In future experiments, a 12 day adaptation period to inverted light conditions is therefore sufficient as far as spontaneous locomotor activity patterns are concerned.
Reversal of the light-dark cycle is rather common for behavioural experiments, in order to test animals in their active phase during office hours. Circadian differences in locomotor activity patterns are commonly reported (Kiwaki et al., 2004; Morrison, 1968; Tang et al., 2007). More complex rodent behaviour may not depend as much on the light-dark phase of the circadian cycle.

For example, social memory in rats is independent of circadian time and not affected by phase-shifting for 6 or 12h either (Reijmers et al., 2001). Besides, passive avoidance learning is not affected by circadian phase when rats had time to adjust to the new cycle (Fekete et al., 1985). Also not all behaviours on an elevated plus-maze are different between light and dark (Bertoglio and Carobrez, 2002).

Differences in performance of complex cognitive tasks between the light and the dark phase in rodents have to my knowledge not yet been reported. As most of these cognitive tasks are preceded by a training period at the same circadian time, entrainment to being awake during the light phase (by task performance) could potentially prevent impairments related to testing during the inactive phase.

Although the effect of circadian phase on behaviour tested with laboratory paradigms may thus be limited, theoretically, it is logical to teach animals...
something in their active phase, and not to disturb them during their inactive phase. However, the light-dark reversal that is implemented to enable this, may affect the sleep-wake rhythm itself. The data provided above show that effects of phase reversal on activity patterns are no longer detectable after 12 days. The next section evaluates the effect of phase reversal on the sleep pattern.

7.2.3.2. Additional results: Sleep-wake patterns after habituation to a light-dark reversal

For the EEG studies described in this thesis (chapters 2, 4 and 5), baseline parameters were always measured before investigating the effects of experimental interventions. Baseline data are therefore available 4 or more weeks after inversion of the light-dark cycle (chapter 4, n = 43) and can be compared to those obtained under standard light-dark conditions (chapter 2 and 5, n = 20). When these data are synchronized on light-onset; the so called Zeitgeber-time (ZT0) zero, we can compare the relative circadian distribution in sleep time.

Figure 41 Sleep & wake times under normal and inverted light conditions

Data are presented as mean ± SEM. *: p = 0.001. Statistical details are provided in the main text.

Baseline data are available for 17 corresponding time-points (data from the first 10h after connection to the EEG-system are disregarded as preliminary observations indicated that rats were then still habituating to the sleep deprivation devices and to being tethered). The total sleep times per hour
(within-subject factor) were compared between both groups (normal versus inverted light, between-subject factor) with an analysis of variance (ANOVA). A Greenhouse-Geisser correction was implemented as the assumption of sphericity was violated. Wake-duration was not analysed, as it is redundant.

The main effect of Zeitgeber-time was significant ($F_{10.7,566.7} = 42.4; p = 0.000$, Figure 41), confirming the well-known effect of Zeitgeber-time on the amount of sleep. The main between-group effect was not significant ($p = 0.56$). The interaction between Zeitgeber-time and light-dark-conditions was significant ($F_{10.7, 566.7} = 4.7; p = 0.000$, Figure 41). This interaction was due to two time-points; the time-point right after light-onset (ZT1, $F_{1,53} = 9.3; p = 0.004$); rats on an inverted light schedule (19:00-7:00) sleep less during their first hour after light-onset than rats on a normal light schedule (7:00-8:00); and the one-but-last time-point of the light phase, (ZT11, $F_{1,53} = 4.1; p = 0.047$), when rats on an inverted light schedule (5:00-6:00) sleep less than rats on a normal light schedule (17:00-18:00). These effects were confirmed by post-hoc student's t-test for ZT-1 and ZT-11 (ZT1: $t_{62} = 4.9; p = 0.000$ and ZT11: $t_{62} = -2.6; p = 0.010$). No differences occurred between both light schedules during any of the other 15 time-points.

Next, the total sleep time during the 12h light phase was compared between rats housed under normal and inverted light with a student's T-test. While sleep differences occur between normal and inverted light-dark cycles, total light-phase sleep-time does not differ significantly between both light conditions (509.7 ± 6.7 minutes in inverted light and 486.8 ± 11.0 minutes in normal light, $p = 0.07$).

The presented data for the normal and the inverted light group that were used in these analyses were combined from several experiments; implementing different protocols for habituation to the sleep deprivation devices and tethering. However, these data do indicate that even after prolonged exposure to inverted light (at least 4 weeks), sleep patterns may still differ between normal and inverted light conditions.

One cannot conclude if any of the two sleep patterns is preferable. As laboratory sleep will always differ from natural sleep, these minor differences are likely to be irrelevant. Rats under a normal light cycle are meant to sleep while people are working in the laboratories, and their sleep may therefore be mildly disturbed by increased arousal levels. The effect of disturbance by experimenter-presence is minimized by the radio continuously playing in all animal rooms, but rats housed under normal light conditions still sleep more in the first hour after light onset (ZT1; 7:00-8:00) before experimenters arrive in the laboratories, and less when experimental activity is ongoing later during the light phase (ZT11; 17:00-18:00).

7.2.3.3. Concluding remarks on the light-dark reversal

Overall, from the locomotor activity registrations it can be concluded that rats adjust their activity patterns to a 12h phase-shift within 12 days. Under
laboratory conditions, a minor difference in sleep architecture can be present when rats are housed under inverted compared to normal light schedules.

Circadian rhythmicity is not limited to activity- and sleep patterns, but also comprises behaviours like feeding, drinking, exploratory and social behaviour (Bertoglio and Carobrez, 2002). Furthermore, physiological circadian rhythmicity has been observed in body temperature, neurotransmitter release, neurotransmitter-receptor-activation, second-messenger systems and immediate-early gene-expression (Bertoglio and Carobrez, 2002). Circadian patterns of voluntary wheel-running activity have been shown to adjust to a 12h phase-shift within 7 days (Sage et al., 2004). Five days after a complete light-dark reversal, the temperature rhythm has phase-advanced for 10.8h (Jarosz et al., 2001). Different mouse strains also adjust their spontaneous locomotor activity patterns to a inverted day-night cycle within 9 days (Kopp et al., 1998).

Although the studies in this thesis do not include data on how fast any of the physiological rhythms adjusts to an inversion of the light-dark cycle, they can shed some light on adjustments of the behavioural rhythms, comprising spontaneous locomotor activity and the times spent sleeping or awake. For sleep-related experiments, an inverted light-dark cycle, allowing sufficient time (≥12 days) for habituation, will be preferable. The inverted light-dark cycle may minimise sleep disturbance, while experiments can easily be performed during the rats’ active phase.

7.2.4. General task structure

The data in chapter 2 indicate that rats can be kept motivated to perform a task after sleep deprivation by implementing a diet of 12g/rat/day (supplemented with 2.8g of pellets during task performance). However, satiety and sleepiness are not the only factors influencing motivation to perform an operant task.

The motivation to perform certain behaviours arises from a combination of internal factors (hunger, thirst, sleepiness and other drives) and external factors (type, amount and timing of a reward, Minamimoto et al., 2009). In free operant tasks such as the Fixed Interval 40s Fixed Ratio 3s (FI40FR3) task described in chapter 2, the incentive to act is measured by the response rate; in our case the number of lever presses, which is substantially higher than the minimal requirements for being rewarded (1148 ± 254 on the reported baseline day in the 12g/rat/day diet group, while 192 lever presses would have been sufficient to receive all 64 rewards). In choice-tasks such as the switch-task (chapter 3) and spatial discrimination (chapter 5), comprising both a rewarded and a non-rewarded response option, the motivation is more closely reflected by the number of omissions; trials that the rats did not perform (Minamimoto et al., 2009).

Tasks will be designed in a certain manner, and certain task parameters may affect motivation. Dependent on the specific task, trials can be separated by breaks (intertrial intervals) of a certain duration. As tasks implementing
different intertrial intervals were used during this project, the effects of
the duration of the intertrial interval on task completion and the number of
omissions could be investigated.

7.2.4.1. Additional results: short and long intertrial-intervals in the reversal
task

At the start of this project, the first behavioural choice-experiments after
sleep deprivation were performed using the reversal task (chapter 5) as it
had been used previously in our institute. Although we expected our rats to
be sufficiently motivated on a 12g/rat/day diet, many rats did not fully
complete the task, as indicated by a huge number of omissions. When
directly observed, at the end of the testing session many rats were quietly
sitting in the skinnerbox, apparently uninterested in the lever. Sleep
depression apparently still decreases the motivation to perform
instrumental tasks on a 12g/rat/day diet, which is a serious problem when
the effects of sleep deprivation on cognition are to be studied.

The lack of motivation to finish the former version of reversal task can be
explained in several ways. First of all, motivation may decrease because of
a lack of comprehension of the new task. Results in chapter 5 show however
that rats can learn the classic reversal task perfectly after 12h of sleep
depression, indicating that this explanation is not relevant. Second, total
task duration may have been too long. In the FIFR-task described in chapter
2 rats are responding continuously throughout the task, which lasted
approximately 45 minutes. However, rats started to omit trials after 8-16
trials on the reversal-task, after less than 16 minutes, thereby rendering this
explanation unlikely. Satiety is also not expected to be of influence,
because on the new task contingency rats earn fewer rewards because of
making errors. At the time rats start omitting trials (after 16 trials), rats
have received on average 0.6 ± 0.3 rewards, while at the end of the FI40FR3
task, rats are still responding after having received 59.5 ± 1.2 rewards.

An important difference between the FIFR-task from chapter 1 and the early
reversal task can potentially explain the difference in motivation to finish
the task. The levers are continuously present in the FIFR task, enabling
continuous responding, whereas trials in the reversal task are separated by
breaks, called InterTrial Intervals (ITI's), where rats can do nothing but wait
for the start of the next trial. These ITI breaks may be ‘boring’ to the rats,
increasing the chance of them dozing off.

The early version of our reversal task is exactly similar to the reversal task
described in chapter 5, the only difference is the duration of the ITI, which
is 30s in the early task. Because the 30s ITI's were potentially responsible for
the lack of motivation, the task was adjusted by decreasing the ITI to 5s
(thereby also decreasing the total task duration), and the experiment was
repeated (chapter 5).
Figure 42  Omissions within blocks of 8 trials after 12h of sleep deprivation and undisturbed control condition with 2 different task structures for a 2-lever reversal of spatial discrimination.

Here the number of omissions (trials on which the rat made no response at all) is shown for both conditions (sleep deprivation or undisturbed control) in both experiments (the early and the late reversal task, Figure 42, n = 12 in all groups). The number of omissions is plotted for both the first reversal session, right after sleep deprivation or undisturbed control condition, and the second reversal session, after a 3h break (uninterrupted sleep-wake behaviour for all groups). Omissions are plotted for both sessions in 8 blocks of 8 trials each.

These data were analysed with an ANOVA including session (1 and 2) and block within session (1-8) as within-subject factors, and condition (4 groups; sleep deprivation or control for both 5s ITI and 30s ITI) as between subject factor. A Greenhouse-Geisser-correction was applied as the assumption of sphericity was violated. Significant effects occurred for all within subject factors, indicating both session and time-on-task related differences in the number of omissions (p = 0.000 for session; p = 0.000 for the session*group-interaction; p = 0.000 for block; p = 0.000 for the block*group interaction; p = 0.000 for the session*block interaction and p = 0.001 for the session*block*group interaction). The between-group effect was also
significant \((F_{3,44} = 10.7; p = 0.000)\), and post-hoc LSD-analyses indicated that rats in the sleep-deprived-ITI30 condition had more omissions than rats in any of the other conditions \((p = 0.003\) with the 30s ITI control condition, \(p = 0.000\) for both 5s ITI conditions). None of the other between-group effects was significant.

The decreased motivation on the task with 30s compared to 5s ITIs can be seen at trend-level during normal conditions, and becomes very clear after sleep deprivation. From these data we can conclude that either long ITIs or overall long task-duration decreases the motivation to perform an instrumental task, and that motivation to perform an instrumental task is not only dependent on the rats' internal motivational state and reward properties, but also on task structure. In line with these findings, previous work in our group shows that omissions are comparably low in a reversal task with ITIs of 10s (Van der Meulen et al., 2006).

As rats are willing to perform the FIFR-task for 45 minutes continuously (chapter 2), task-duration does not appear to be a relevant factor; ITI-duration is likely to be a very important factor in motivation. For future studies, faster paced tasks with shorter break durations are therefore preferable.

7.2.4.2. Additional results: Short and long intertrial-intervals in the switch-task

Comparable observations as for the earlier reversal task were made in an early version of the switch-task, which was first performed at around the same time. The structure of the switch-task was based on the reversal-task, implementing 30s ITIs in the early switch-experiment. For task-switching, within-subject comparisons were made between a control and a sleep-deprived state.

In the 30s ITI-experiments, rats were left undisturbed during the control condition. In the 5s ITI switch experiments (chapter 3), the control condition consisted of a movement control protocol. In the 30s ITI-experiments, after sleep deprivation rats started to omit trials \((33.3 \pm 10.7\) omissions), while under undisturbed control conditions, none of the rats omitted any trial (Figure 43). In the 5s ITI-experiments, none of the rats \((n = 15)\) omitted any trial under either sleep deprived or movement control conditions (Figure 43).
Figure 43  Omissions after 12h of sleep deprivation and (movement or undisturbed) control condition with 2 different task structures for a conditional discrimination task offered in blocks (a "switch-task")

Intertrial intervals (ITI) were either 30s or 5s. Data are presented as mean ± SEM. The number of omissions was 0 ± 0 for all data points except with the 30s ITI after 12h sleep deprivation, where the number of omissions was 33.3 ± 10.7.

From this comparison we can once more deduce that long ITIs decrease the motivation to perform an instrumental task, which becomes clear after sleep deprivation. Task-duration was again not considered to be relevant as tasks of comparable length were finished without omissions (chapter 2).

7.2.4.3. Concluding remarks on general task structure

Overall, from these data it is clear that motivation to perform an operant task is not only dependent on the rats' internal motivational state and the task's reward properties, but also on the task structure. Faster paced tasks with shorter break durations are preferred by rats, as indicated by a trend to lower numbers of omitted trials already under baseline conditions (Figure 42 and Van der Meulen et al., 2006) but even more clearly so in a sleep-deprived state (Figure 42 & Figure 43).

Although the effect of decreasing the ITI-duration on motivation is very clear from these data, to the best of my knowledge, no published results are available on how task structure and ITI-duration affect rats' motivation to perform an instrumental task. Variable ITI's are often implemented in attentional tasks such as the 5-choice serial reaction time task (e.g. McGaughy et al., 2002, Robbins, 2002, Godoi et al., 2005, Cordova et al., 2006, Navarra et al., 2008). In these paradigms however, rats have to respond to a brief stimulus (500ms) at the end of the ITI, and a decreased performance after longer ITIs is thought to reflect attentional impairments.
In the current studies, the stimulus to which the rat has to respond is present throughout the trial, thereby decreasing the importance of continuous attention and the comparability between paradigms. The effect of variation in the ITIs has been reported for monkeys, where increasing the ITI decreased motivation, as indicated by a higher percentage of incompleted trials and a longer response latency (Minamimoto et al., 2009). Interestingly, increasing the response-reward delay had similar effects, indicating that short breaks decrease the motivation to perform an operant task, regardless of their location within the task.

Concluding, long ITIs appear to decrease the motivation to perform a task. As sleep deprivation has an even more pronounced effect on mood than on cognition in humans (Pilcher & Huffcutt, 1996, Horne, 2006, refer to section 7.8.3), decreased motivation could be responsible for observed impairments in task performance after sleep deprivation, both in humans and in rodents. Although the increased sensitivity may be attractive for certain purposes, it can obscure the cognitive effects. It may therefore be wise to prevent unnecessary breaks and to keep ITIs as short as possible when developing new tasks, at least when interested in cognition instead of motivation.

### 7.2.5. Switch-task parameters

In chapter 3, a novel switch-task for rats was described. This switch-task is based on a simple conditional discrimination; with one stimulus (light or tone) rats have to perform one spatial discrimination (left or right lever), with the other stimulus the other spatial discrimination. For an individual rat, the stimulus-response association was always the same; for half of the rats the left lever was rewarded when the light stimulus was present and the right lever when the sound stimulus was present, for the other half of the rats, the opposite rule was applied. As the order of learning the rules could potentially affect the results, this was also counterbalanced for, creating four experimental conditions (Table 12).

<table>
<thead>
<tr>
<th>SRA’s</th>
<th>First SRA learned</th>
</tr>
</thead>
<tbody>
<tr>
<td>Light $\rightarrow$ left lever; sound $\rightarrow$ right lever</td>
<td>Light $\rightarrow$ left lever</td>
</tr>
<tr>
<td>Light $\rightarrow$ left lever; sound $\rightarrow$ right lever</td>
<td>Sound $\rightarrow$ right lever</td>
</tr>
<tr>
<td>Light $\rightarrow$ right lever; sound $\rightarrow$ left lever</td>
<td>Light $\rightarrow$ right lever</td>
</tr>
<tr>
<td>Light $\rightarrow$ right lever; sound $\rightarrow$ left lever</td>
<td>Sound $\rightarrow$ left lever</td>
</tr>
</tbody>
</table>

SRA = Stimulus Response Association

As only four rats were trained on each condition, the data sets are too small to reliably investigate potential effects of the order of learning the stimulus response associations or of the stimulus response associations themselves. In chapter 3, the data from these four conditions were therefore always pooled; average latencies and percentages correct were calculated for all switch-trials and all 5th repetition trials over whole sessions for each rat. However, additional analyses can be performed to investigate if performance on light and sound trials is different.
7.2.5.1. Additional results: Differences in responding to light and sound stimuli during baseline

Differences may occur in responding to sound and light trials. Therefore, data from a baseline experimental session after the last interventions from chapter 3 were analysed without pooling the data for sound and light trials. Differences between light and sound trials were tested with an ANOVA including trial type (light versus sound) and trial (first versus fifth) as within-subject factors. For latencies (Figure 44), no main effect of stimulus type was observed ($p = 0.2$). Significant latency switch-costs occurred ($F_{1,12} = 15.9; p = 0.002$), and the interaction of trial and stimulus was significant ($F_{1,12} = 12.0; p = 0.005$). Post-hoc paired-samples t-tests for both trial types indicated that the latency-switch-effect was only significant for light blocks ($T_{12} = 0.001$), but not for sound blocks ($p = 0.7$).

![Figure 44 Baseline switch-task performance on light versus sound trials](image)

Significant accuracy switch-costs were also observed when both stimulus types were separately analysed ($F_{1,12} = 5.3; p = 0.040$, Figure 44). Stimulus type and the interaction of stimulus-type with trial were not significant ($p = 0.08$ for both the main effect and the interaction), indicating that the switch-effect on accuracy was similar for both stimuli.

7.2.5.2. Variability in response latencies

The absence of significant latency switch-costs in the sound blocks described above, and in some of the experiments described in chapter 3, may be due to the relatively high variation in latencies on this task. A potential manner to improve the latency data in future studies is by adjusting the task to prevent the occasionally present long response times.
affecting the data. In the current task, rats can respond to the stimulus for up to 30s. Usually, they respond within a second or 2, but latencies for occasional slow trials were included in the analyses. The task could be adjusted to prevent slow trials by decreasing the maximal response time to e.g. 2s.

Alternatively, data analysis might be adjusted to ignore these slow trials. A pilot with the data from the infusion experiment in chapter 3 shows that this procedure reduces the SEM for the latency data with 40-79%. When re-analysing the data this way, the latency switch-costs became significant ($F_{1,10} = 14.4; p = 0.004$). Treatment (saline versus the muscimol-baclofen mixture) did still not affect response latencies ($p = 0.2$ for the treatment and $p = 0.4$ for the treatment-by-trial interaction).

7.2.5.3. Task symmetry

A manner to tackle the difference between responses to sound and light stimuli is by developing a more symmetric task. Two distinct conditional discriminations using compound stimuli could be offered, and the discrimination of choice made dependent on the presence or absence of another stimulus. This type of task could be developed e.g. with nose-poke units with multiple coloured stimulus-lights. For example, when a sound is present rats can be made to respond to a red light, and when the sound is absent, to a green light, ignoring irrelevant other lights. The reason that the current studies used a single conditional discrimination is the relative simplicity. On a more complex switch-task, latencies are expected to increase and accuracy is expected to decrease. The performance may thereby become less comparable with human switch-task performance, which is highly accurate and fast (see chapter 3).

7.2.5.4. Task switching with only one stimulus

In theory, rats can perform the type of conditional discrimination task used in the current studies by observing only one out of the two stimuli, e.g. with a light, press the right lever, without a light, press the left lever, ignoring the presence of a sound stimulus on the no-light trials. To verify that both the light and the sound stimulus were used in task performance, after finishing the chapter 3 experiments, rats were exposed to a new experiment. Versions of the task containing only one stimulus were used, alternating e.g. blocks of normal light trials with blocks without stimulus, which were rewarded when the lever usually rewarded on sound-trials was pressed. Both light-only and sound-only sessions were tested. Performance was compared with the preceding baseline days. Beforehand, we verified that no day-of-week effect was present for the respective days in normal training weeks (chapter 3), using an ANOVA with both day (baseline versus altered task) and trial (first versus fifth) as within-subject factors.

7.2.5.4.1. Additional results: Light-only task-switching

To investigate if the sound-stimuli were essential for switch-task performance, rats were exposed to a task switching between light-trials and
no-stimulus trials, where the no-stimulus trials were rewarded when the sound-matched lever was pressed. When rats had to switch between blocks of light trials and blocks of no-tone trials (rewarded as tone trials), latency (Figure 45) was not altered compared to the preceding baseline day \((p = 0.07)\). The interaction between trial and day was not significant either \((p = 0.4)\). Latency switch-costs were observed as a significant effect of trial \((1st vs. 5th, F_{1,12} = 14.7; p = 0.002)\). To conclude, latencies are unaltered by removing the sound stimulus.

For accuracy (Figure 45), the effect of trial \((1st versus 5th, F_{1,12} = 118.3; p = 0.000)\), a main impairing effect of task alteration \((F_{1,12} = 51.2; p = 0.000)\) and the interaction between these factors \((F_{1,12} = 40.2; p = 0.000)\) were significant. Post-hoc paired-samples \(t\)-tests indicated that switch-costs were significant in both baseline \((t_{12} = -2.3; p = 0.04)\) and light-only \((t_{12} = -13.8; p = 0.000)\) conditions. To conclude, removing the sound stimuli impairs accuracy on the switch-task, mainly on the switch-trials. After the first 2 blocks and the first 2 switches, accuracy and latency stayed constant throughout the rest of the session.

Figure 45 Switch-task performance in sessions were one of the stimuli was removed.

7.2.5.4.2. Additional results: Sound-only task-switching

To investigate if the light-stimuli were essential for switch-task performance, rats were exposed to a task switching between sound-trials and no-stimulus trials, where the no-stimulus trials were rewarded when the
light-paired lever was pressed. For one of the rats in this condition, latency data were deleted from the analysis as too many errors were present. When rats had to switch between blocks of tone trials and blocks of no-light trials (rewarded as light trials), latency (Figure 45) was not altered compared to the preceding baseline day (p = 0.1). The interaction between day and trial was not significant either (p = 0.3). The effect of trial (1st vs. 5th) was significant (F1,11 = 8.8; p = 0.013), indicating latency switch-costs. To conclude, latencies are unaltered by removing the light stimulus.

For accuracy in sound-only sessions (Figure 45), the effect of trial (1st versus 5th, F1,12 = 103.9; p = 0.000), a main impairing effect of task alteration (F1,12 = 221.4; p = 0.000) and the interaction between these factors (F1,12 = 82.1; p = 0.000) were significant. Post-hoc paired-samples t-tests indicated that switch-costs were significant in sound-only (t12 = -12.1; p = 0.000) conditions, but not on the preceding baseline day (p = 0.1). In conclusion, removing the light stimuli impairs accuracy on the switch-task, mainly on the switch-trials. As with light-only switching, after the first 2 blocks and the first 2 switches, accuracy and latency stayed constant throughout the rest of the session. Overall, it can be concluded that both sound and light stimuli are used in performing the switch-task, as deleting either of these stimuli impairs performance.

7.2.5.5. Additional results: High-paced task-switching

To test if rats could also perform a switch-task while switching between the 2 stimuli at a higher pace, rats were exposed to a task where they had to switch between stimulus-response associations after 1 or 2 trials of one type. To compare switch and repetition trials, only the 2nd trials of a block (when present) could be chosen as repetition trials. Performance on the fast version of the switch-task was compared with first and second trials on the preceding baseline day (Figure 46).
Figure 46 Performance of a high-paced version of the switch-task

Switch-costs were observed for both latency ($F_{1,12} = 36.4; p = 0.000$) and for accuracy ($F_{1,12} = 12.0; p = 0.005$); second trials were performed faster and more accurate than first trials. Latencies were increased ($F_{1,12} = 6.7; p = 0.024$) and accuracy was decreased ($F_{1,12} = 7.8; p = 0.02$) on the fast switch task compared to the preceding baseline day. No interaction between task-type and trial was observed ($p = 1.0$ for latencies and $p = 0.2$ for accuracy), indicating that the effects were generalised and not switch- or repetition specific. Concluding, rats can perform a switch-task at a higher pace.

Using a higher pace of switching, a switch-task of a certain length can comprise more switch- and repetition trials. A faster switch-rate could therefore be advantageous, as the average of the switch- and repetition trials is then based on a higher number of observations, and thereby more reliable. However, this alteration is not preferable as latencies are increased and accuracy is decreased on the faster version of the task. The performance will become less comparable with human switch-task performance, which is highly accurate and fast (see chapter 3).

7.2.5.6. Additional results: Reversal of the conditional discrimination

Using the switch-task, a more complex form of cognitive flexibility could be tested by reversing the stimulus-response associations. In a pilot experiment, two rats, after extensive (18 weeks) experience with task-switching, were exposed to reversed stimulus-response associations for a single session. Performance remained below chance levels (15% correct and 33% correct respectively) and did not improve over this session; correct
responses were occasionally made throughout the session. This indicated that after long training, the type of cognitive flexibility needed to reverse the stimulus-response associations is hardly present within a single session. Reversing stimulus-response associations in this switch-task is therefore not likely to be a useful short-term procedure.

7.2.5.7. Concluding remarks on task switching parameters

The switch-task from chapter 3 is a useful paradigm for future studies. The additional results describe that differences occur in responding to light and sound stimuli. For future experiments, these stimuli could be analysed separately, but it is most important to implement strict counterbalancing for the various stimulus response associations (SRAs).

Performance of the switch task remains fairly good when one of the stimuli is no longer presented, and also when the task is offered at a higher-paced switch-rate. Performance is severely impaired in a single-session exposure to a reversal of the conditional discrimination. As also mentioned in the discussion within chapter 3, the switch-task can be altered in many ways to address specific experimental needs.

7.2.6. Spatial reversal learning, additionally measured variables

Chapter 5 describes the number of correct responses after a spatial reversal. Other parameters were registered during these tasks, further confirming the absence of an effect of sleep deprivation on spatial reversal learning.

No clear differences between sleep-deprived and well-rested groups were observed on: The number of omissions (generally none or one), the number of nosepokes made, the latency to press the levers and the activity during the task (as measured by infrared displacement detectors, similar to those used in the sleep deprivation devices). The moment of the first reversed response (on the newly rewarded lever) in the first reversal session was also compared between the sleep-deprived and well-rested groups, as an indication for perseveration. Again, no clear differences were present (data not shown).

7.2.7. Concluding remarks on methodology

The current thesis describes a novel automated method for sleep deprivation, with several advantages over other available methods: It is automated and highly efficacious, while potential confounding by stress and locomotor activity are limited. The advantages are thought to be related to the variability of the forced movement protocol.

Inversion of the light-dark cycle, allowing sufficient time (12 days) for habituation, is preferable when performing sleep-related experiments. The inverted light-dark cycle may minimise sleep disturbance, while experiments can easily be performed during the rats’ active phase.
Concerning task structure, long ITI's appear to decrease the motivation to perform. It would be advisable to prevent unnecessary breaks and to keep ITI's as short as possible when developing new tasks, at least when interested in cognition instead of motivation.

The switch-task from chapter 3 is a useful paradigm for future studies, but as differences occur in responding to light and sound stimuli, it is most important to implement strict counterbalancing for the various SRAs. The switch-task can be altered in many ways to address specific experimental needs.

7.3. Reflection on sleep and cognition

7.3.1. Task switching and sleep disturbance (chapter 3)

From the results in chapter 3 we can conclude that task switching accuracy is impaired after 12h of sleep deprivation. Milder sleep deprivation (occurring in the control conditions and the sleep fragmentation condition) did not have such effects. A dose-response relationship between the amount of sleep deprivation and the resulting cognitive impairments could be postulated. Alternatively, a threshold amount of sleep is needed to be able to perform certain cognitive tasks, and sleep deprivation that does not bring the amount of sleep below this threshold will not affect cognitive performance. To distinguish between these two relationships between sleep and cognition, switch-task performance should be compared after sleep deprivation of various durations.

7.3.1.1. Task switching and PFC-inactivation

The studies in chapter 3 show that the rat mPFC is involved in performing the switch task. However, while sleep deprivation induces switch-specific accuracy impairments, PFC-inactivation has a more generalised effect on both switch and repetition trials. This implies that sleep deprivation probably has a more complex effect than simply inhibiting the mPFC. The finding of impaired task switching accuracy after 12h of sleep deprivation can be used to investigate the mechanisms underlying cognitive impairments after sleep deprivation. It would be of interest to see if stimulant compounds that decrease sleepiness can reverse this effect. By testing several compounds from different pharmacological classes, such as caffeine, amphetamine, modafinil and nicotine, the involved receptor system(s) may be revealed. Next, it would be of interest to perform neurochemical measurements during task-switching in a sleep-deprived and a control state. Using microdialysis, for example mPFC dopamine, noradrenalin and serotonin release could be measured. If neurotransmitter release is not altered by sleep deprivation, post-synaptic alterations should be further investigated.

7.3.2. Nap prevention disturbs instrumental learning (chapter 4)

Chapter 4 shows that three hours of total sleep deprivation during the active phase (nap prevention) following initial task-exposure disturbs subsequent
instrumental learning. Instrumental learning occurs within discrete sessions, and learning may occur both within and between sessions. Lever pressing during the first session of instrumental learning did not appear to be altered by experimental interventions in this study. Sleep deprivation was limited to the 3h after the first session of instrumental learning. This one-time brief period of sleep deprivation may alter memory processing within the period of sleep deprivation, but it may also affect subsequent behaviour. Sleep deprivation either affects processing of previously acquired information, or the learning process within the next session(s), or both.

In line with the hypothesis that sleep-deprivation affects processing of previously acquired information, alterations in sleep can be observed in this period in non-deprived control rats; exposure to an instrumental learning paradigm affects spontaneous subsequent sleep in the 3h after initial task-exposure. The main effect observed was an increase in REM duration within this interval.

The increase in REM-sleep occurs after learning, but not after a subsequent non-learning session. Furthermore, the increase in REM-sleep also occurs after late learning in slow-learning rats.

The results of both sleep deprivation and EEG measurements are in line with the hypotheses that sleep is beneficial for memory consolidation (e.g. Rudoy et al., 2009) and that some form of memory processing takes place within a 3h post-task exposure time window (e.g. Sara et al., 1999). The possibility that previous sleep deprivation also affects the learning process within the next session(s) cannot however be rejected.

7.3.2.1. Mechanisms underlying sleep-dependent consolidation processes

In the current studies, only cortical EEG was measured. Alterations in cell firing as indicated by the surface EEG are present after learning, but potential alterations in deeper brain regions that do not clearly affect the EEG may also occur. For example Eschenko & Sara have shown that during SWS 2h after odour discrimination learning, neuronal firing in the locus coeruleus is transiently increased without affecting the cortical EEG (Eschenko & Sara, 2008). The anatomical location of sleep-related learning was not determined in the current studies. Instrumental learning was previously reported to depend on neural circuits comprising dopaminergic and glutamatergic transmission between the nucleus accumbens, prefrontal cortex and amygdala (Kelley et al., 2003), providing an indication of the mechanistic whereabouts.

Using virtually the same paradigm as in chapter 4, during task performance, dopamine was found to increase in the nucleus accumbens (Cheng & Feenstra, 2006). During the first session, this increase was more pronounced in rats that learned the task than in rats that did not learn the task within two sessions, indicating relevance of this dopamine release for learning. As our 3h nap-prevention protocol between the first and the second session drastically disturbs instrumental learning, one possible mechanism is that it affects dopamine transmission.
After release, dopamine can bind to presynaptic autoreceptors or to post-
synaptic receptors of the D1 (stimulatory) or the D2 (inhibitory) type.
Dopamine receptors can influence cellular function through mechanisms
comprising stimulation or inhibition of adenylate cyclase, thereby increasing
or decreasing the second messenger cyclic adenosine monophosphate
(cAMP) and modulation of inositol phosphate production, but also by direct
effects on potassium and calcium channels (Feldman et al., 1997, Cooper et
al., 2003). These reactions or alterations that occur further downstream,
like the stimulation or inhibition of RNA synthesis or neurogenesis, may
somehow be altered by sleep deprivation. Recent work shows that sleep
depression reduces the normal increase of the transcription factor
phosphorylated cAMP response element binding protein, which may underlie
the simultaneously observed impaired memory formation (Hagewoud et al.,
2010a).

Recently, a number of studies has focussed on the effect of sleep
depression on hippocampal neurogenesis in relation to memory formation,
which might be another possible mechanism in disturbed instrumental
learning after nap prevention. Neurogenesis can be affected by sleep,
circadian time and activity (Meerlo et al., 2008a). Prolonged sleep
depression does reduce hippocampal neurogenesis independent of adrenal
stress hormones (Meerlo et al., 2008a).

Although these findings are interesting for learning over the course of a few
days, it is unlikely that they are very relevant to the findings in chapter 4.
First of all, as neurogenesis itself is unlikely to be severely affected by our
brief period of nap prevention. Neurogenesis is impaired by 4 or 7 days of
sleep fragmentation, but not by a single day (Guzman-Marin et al., 2007).
Differentiation, proliferation and integration of newly formed neurons may
be affected by shorter periods of sleep deprivation (Meerlo et al., 2008a),
but these processes usually take longer than the few hours in which many
rats learn this task. Last, although occasional neurogenesis may occur in
regions throughout the cortex, neurogenesis is generally limited to a few
restricted areas within the brain: mainly the hippocampal dentate gyrus and
the subventricular zone (Cheung et al., 2007, Hagg, 2009), areas that are
generally not thought to be involved in instrumental learning.

7.3.2.2. Concluding remarks on nap-prevention and instrumental learning

Overall, spontaneous napping after a session of instrumental learning is
important for the consolidation of this learning, although it is not essential
to all subjects (chapter 4). Spontaneous napping is also not essential to all
types of memory consolidation; consolidation of a previously learned spatial
discrimination is not altered by a similar post-task period of nap prevention
(chapter 5). The negative effect of post-task exposure sleep disturbance can
furthermore be prevented by substantial habituation to sleep deprivation,
as indicated by the results described in chapter 6. The relationship between
sleep and learning is extremely complex.
7.3.3. Spatial reversal learning and sleep deprivation (chapter 5)

The data in chapter 5 show that twelve hours of total sleep deprivation during the light phase, as a model for one sleepless night, did not alter PFC-dependent reversal learning. Also, three hours of total sleep deprivation during the active phase subsequent to reversal learning, preventing spontaneous napping, did not affect consolidation of reversal learning. These conclusions were based on the number of correct responses throughout the sessions of reversal learning and are strengthened by additionally measured variables described in section 7.2.6.

7.3.3.1. Inconsistent results between studies

The results from chapter 5 clearly indicate that spatial reversal learning is relatively robust to the effects of sleep deprivation. Other authors have shown that 24 hours of sleep interruption did not impair reversal learning of familiar odour and texture discriminations in rats either (McCoy et al., 2007). However, spatial reversal learning on a Y-maze is sensitive to the effects of sleep deprivation in mice, although performance was not different from control conditions either when sleep deprivation only commenced after the first session of Y-maze reversal learning (Hagewoud et al., 2010). The Y-maze task is different from ours; the number of trials per day is lower (6 per day) than in spatial reversal learning in a skinnerbox (2×64 trials per day), and trials last a bit longer, as mice have to walk over the T-maze. This however is unlikely to explain the differences in the results. The trial duration is longer and the number of trials needed to learn a reversal are also lower in the McCoy et al. (2007) study, where rats have to walk to a bowl and dig out their reward (instead of quickly pressing a close by lever).

The sleep-deprivation induced impairment in the Hagewoud (2010) study, in contrast to the other two, can be explained either by a species-effect; mice are perhaps more sensitive to the effect of sleep deprivation on reversal learning than rats, or by a critical effect of the amount of sleep deprivation; in the Y-maze study, mice were exposed to 5h of daily sleep deprivation immediately after training. During the first reversal session, mice had been exposed to 6 days of previous discrimination sessions, and sleep deprivation at that stage therefore totalled 30h (Hagewoud et al., 2010).

It would be of interest to test if longer sleep deprivation (e.g. 23h or longer) does impair spatial reversal learning in our set-up, and if repeated 5h post-training sleep deprivation in rats does impair reversal learning on a comparable Y-maze paradigm. My hypothesis is that more severe sleep disruption would induce stronger cognitive impairments (although also decreased motivation after more severe sleep disruption could affect the results). After long-duration total sleep deprivation, spatial reversal learning could be impaired.
7.3.3.2. Sleep deprivation and reversal learning; alternative strategies

Interestingly, while training on a similar spatial discrimination on T-maze, mice in the sleep-deprived condition were shown to use a different learning strategy, and sleep deprived mice had increases in the transcription factor pCREB, a critical element in memory formation, in different brain regions (Hagewoud et al., 2010). Although reversal learning is robust to the effects of 12h of sleep deprivation, this does not mean that the PFC is performing normally. It would be of interest to test if reversal learning activates the same brain regions in a sleep-deprived and a control state.

7.3.4. Shiftwork and instrumental learning (chapter 6)

The data in chapter 6 show that when rats were trained on an instrumental learning paradigm during the fifth week of shiftwork, instrumental learning was not affected by (shift)work or by circadian phase. Furthermore, shiftwork decreased the normal weight gain. This result contrasts with previous studies. The difference probably arose because our shiftwork rats do not decrease their home-cage activity between the periods of shiftwork, as they did in the previous studies (Salgado-Delgado et al., 2008, 2010). The difference in home-cage activity could be related to experimental factors, and it would be of interest to investigate in our paradigm if single housing would alter the results. Also, it would be of interest to see if the shiftwork procedure itself (the sleep deprivation devices with a variable movement protocol versus the Lafayette activity wheels with a continuous protocol) is of effect.

7.3.4.1. Sleep and body weight

With respect to the effect of sleep deprivation on body weight gain, there are no studies proving that chronic short sleep can cause a substantial weight gain. The main two health issues related to prolonged exposure to shiftwork are peptic ulcers and cardiovascular disease (Horne, 2006). Regular short sleepers are usually not overweight and overweight people are usually not short sleepers (Horne, 2008). When people are (at a risk to become) overweight, it would therefore be advisable for them to take more exercise instead of sleeping more (Horne, 2008).

However, the general notion remains that short sleep may, in a subpopulation of humans, contribute to obesity (Spiegel et al., 2009). A main methodological problem arises when investigating this phenomenon in model animals. Whereas humans truly fast during their sleep, releasing some of its energy stores during sleep to prevent waking up hungry in the middle of the night, this phenomenon hardly occurs in animals. Bigger herbivores and carnivores are digesting their meals during their sleep, thereby supplying their bodies with a continuous nutrient influx (Horne, 2006). Small rodents that are generally used in laboratory studies however do not have consolidated sleep and wake up regularly to nibble on some lab chow (or to look for food in the wild, Horne, 2006). As this truly is a substantial difference in physiology, it may be very difficult to reliably
investigate the relationship between body weight gain and sleep in model animals.

However, important information may still arise from studies in model animals. An important finding from the current study in combination with results described in literature (Salgado-Delgado et al., 2008, 2010), is, that reversing the rhythm may increase body weight gain, while shiftwork without reversing the spontaneous activity pattern prevents this increase. In line with this theory, non-rotating night-shift workers had similar BMIs compared to day-time shifts, even though sleeping less (de Assis et al., 2003). Controlled studies in humans could test if not fully reversing the activity pattern is also preferable during human shiftwork.

7.3.4.2. Instrumental learning

Instrumental learning was not altered by regular work or shiftwork. It would be of interest to see if instrumental learning is altered by shiftwork protocols where between-work home-cage activity is decreased compared to non-working rats. Reversing the circadian activity pattern during shift work appears to be disadvantageous regarding increases in body weight gain. What happens to cognition during shiftwork when the circadian activity pattern is reversed is presently not known.

Although instrumental learning is robust to the effects of (shift)work, in parallel to the discussion on chapter 5, this does not necessarily mean that the brain is functioning completely normally. It would be of interest to test if instrumental learning during undisturbed control conditions and during work and shiftwork activates the same brain regions.

In the current study, rats were trained on an instrumental learning paradigm during the 5th week of (shift)work. Habituation to the daily period of work may prevent negative effects on cognition. It would therefore be of interest to investigate when habituation starts to occur, and to elucidate underlying brain mechanisms.

Instrumental learning should be tested during the first week of (shift)work. As a 3h period of sleep deprivation after first task exposure was sufficient to disturb instrumental learning (chapter 4), impaired instrumental learning would be expected up to some point at least in the 1st week of (shift)work. EEG measurements could show if sleep efficiency during sleep is increased. Increased sleep efficiency during sleep deprivation, thereby maintaining sleep homeostasis, has been described previously (Leemburg et al. 2010). Up to the present, there is no support for the idea that regular exposure to sleep deprivation can lead to tolerance or immunity to the effects (Horne, 2006), so this finding could be of great importance.

7.3.5. Sleep and cognition; other future perspectives

Besides the studies suggested in the preceding paragraphs, other interesting research questions came up during the current work. A few of these will be described here.
First of all, a number of rodent cognitive tasks have been described in the introduction, and some of these could very well be implemented in sleep research. Of particular potential is the 5-CSRTT (see introduction). Attentional performance on this task is negatively affected by sleep deprivation (Cordova et al., 2006), but the task itself has many more possibilities because it uses five differential responses.

7.3.5.1. Motor sequence learning

In humans, motor sequence learning is sensitive to sleep, both on a conscious task consisting of tapping a numerical sequence on the numeric keys on a standard keyboard, and on a subconscious task when a standard sequence is continuously offered on a CSRTT without telling the subjects (Walker et al., 2002, Cajochen et al., 2004, Cohen et al., 2005, Fischer et al., 2006). In mice, the 5-CSRTT has already been used to model sequence learning (Christie and Hersch, 2004), but the effect of sleep on sequence learning in rodents has not yet been described.

7.3.5.2. Observational learning

Sleep has also been shown to benefit observational sequence learning; when sleep followed the viewing of a video of a finger-tapping hand, performance was improved if the viewed sequence was congruent with the tested sequence (Van Der Werf et al., 2009). Observational learning also occurs in rats; observation of instrumental responding enhances instrumental learning in naive rats (Zentall & Levine, 1972). Perhaps observational learning also occurs for sequence learning on the 5-CSRTT, and the effect of sleep on observational learning may then be further investigated in rats.

7.3.5.3. Mood

Besides the cognitive effects, sleep deprivation has an even more pronounced effect on mood (Pilcher & Huffcutt, 1996, Horne, 2006). Sleep deprivation usually impairs mood, but it can actually improve mood in patients suffering from major depressive disorder (Meerlo et al., 2008b). Although mood is not an important topic in this thesis, during the experiments from chapter 3 and in other (not reported) experiments, an interesting observation was made. Rats that were exposed to 3 or 12h of total sleep deprivation were somewhat less active afterwards, but responded normally to handling. However, rats that were exposed to 12h of sleep fragmentation had an altered behavioural response to handling; they were moving more than usual and more escape-prone, consistent with increased ‘grumpiness’. From this observation the theory arises that sleep fragmentation may decrease mood more strongly than total sleep deprivation. If sleep-interrupted rats do show depression-like symptoms in, for example, the Porsolt forced swim test (learned helplessness) or in a sucrose preference test (anhedonia), sleep fragmentation might be used to model a depression-like state in rodents.
7.3.5.4. Neurochemistry

Eventually, this line of work intends to resolve the mechanisms underlying sleep-deprivation induced cognitive impairments. In chapter 3, I first mentioned the hypothesis that after sleep deprivation, neurotransmission is altered in certain brain regions. Therefore, neurochemical studies should be performed before, during and after sleep deprivation, and during task performance in a normal and a sleep-deprived state. Neurotransmission can be altered on either the presynaptic or the post-synaptic side. Presynaptic alterations should be observable on neurotransmitter release, which can be measured in freely-moving animals with for example microdialysis, voltammetry and biosensors. On the post-synaptic side, for example receptor density can be measured with immunohistochemistry or receptor autoradiography (Feldman et al., 1997). Receptor density does not necessarily correlate with the induction of second messengers, but second messengers such as cAMP and IP3 can also be measured with microdialysis (e.g. Cadogan et al., 1994, Gur et al., 1996).

7.4. Concluding remarks

In the introduction, I described that we still do not know exactly what sleep is and why we need it (Franken et al., 2009). A lack of sleep does however have very clear consequences for cognition, as described in the introduction and in chapters 3 and 4, but the mechanisms underlying cognitive impairments after sleep deprivation are currently not known. To elucidate this mechanism, rodent models are needed, and the current thesis describes a number of new and in this respect very useful models. In this discussion, additional data were presented to provide supplementary information on these models.

In humans, simple, dull and monotonous tasks are very sensitive to the effects of sleep deprivation, but cognitively demanding, relatively interesting or exciting tasks are relatively robust to the effects of short-term sleep deprivation (Horne, 2006). Naturally, there are limits to the amount of sleep deprivation up to which people are willing, motivated and still able to perform a task.

Also in rats, some cognitive functions are relatively robust to a lack of sleep (e.g. chapter 5). The lack of an observable effect of sleep deprivation on task performance does however not mean that brain function is unaltered; on relatively interesting tasks, the phenomenon of reactive reinforcement (see chapter 3) may prevent observable deficits. Besides, as indicated by studies in mice, other strategies may be used to perform a task in a sleep-deprived state, and other brain regions may be activated (Hagewoud et al., 2010a). Substantial experimental work is still needed to elucidate how cognition benefits from sleep. Suggestions for future studies were made throughout this discussion.

Although personal health and cognition can clearly be affected by a lack of sleep, the main risk of sleep deprivation consists of sleepiness-related accidents (Dement, 1999). Sleepiness-related crashes induce more
mortality than alcohol-related crashes, irrespective of traffic density (Dement, 1999, Horne, 2006). A problem in this respect is the inability of human beings to accurately monitor their own degree of sleepiness, their tendency to fall asleep and their moments of sleep (Dement, 1999, Horne, 2006). Furthermore, a lack of sleep may impair decision making and increase risk-taking (see introduction), which will make sleepy drivers more inclined to keep driving and less inclined to pull over and take a nap. When sleep-deprived, we should not try to drive or operate dangerous equipment.

On the other side, mild sleep deprivation might even be beneficial in certain circumstances (e.g. shiftwork, chapter 6). Thus, overestimating the importance of sleep for other health factors than sleepiness-related accidents may lead to unfounded concerns about a mild lack of sleep, and may thereby increase "medical consumption", stress and the use of over the counter sleep aids (Horne, 2006).

Although the effects of sleep deprivation on cognition and weight are regularly overrated in both scientific and popular literature, sleep is important, and further research is essential to find methods that can help people suffering from the consequences of bad sleep.

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