6. UNALTERED INSTRUMENTAL LEARNING AND ATTENUATED BODY-WEIGHT GAIN IN RATS DURING NON-ROTATING SIMULATED SHIFTWORK

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6.1. Abstract

Exposure to shiftwork has been associated with multiple health disorders and cognitive impairments in humans. We tested if we could replicate metabolic and cognitive consequences of shiftwork as reported in humans, in a rat model comparable to 5 weeks of non-rotating night shifts. The following hypotheses were addressed: 1.) shiftwork enhances body-weight gain, which would indicate metabolic effects and 2.) shiftwork negatively affects learning of a simple goal-directed behaviour, i.e., the association of lever pressing with food reward (instrumental learning), which would indicate cognitive effects.

We used a novel method of forced locomotion to model work during the animals' normal resting period. We first show that Wistar rats are indeed active throughout a shiftwork protocol. In contrast with previous findings, the shiftwork protocol attenuated the normal weight gain to $76 \pm 8$ g in 5 wks as compared to $123 \pm 15$ g in the control group. The discrepancy with previous work may be explained by the concurrent observation that on our shiftwork protocol, rats did not adjust their between-work circadian activity pattern. They maintained a normal level of activity during the “off-work” periods.

In the control experiment, rats were kept active during the dark period, normally dominated by activity. This demonstrated that forced activity, per se, did not affect body weight gain ($85 \pm 11$ g over 5 weeks as compared to $84 \pm 11$ g in the control group).

Rats were trained on an instrumental learning paradigm during the fifth week of the protocol. All groups showed equivalent increases in lever pressing from the first ($3.8 \pm 0.7$) to the sixth ($21.3 \pm 2.4$) session, and needed a similar amount of sessions ($5.1 \pm 0.3$) to reach a learning criterion ($\geq 27$ out of 30 lever presses).

These results suggest that while on prolonged non-rotating shiftwork, not fully reversing the circadian rhythm might actually be beneficial to prevent body-weight gain and cognitive impairments.
6.2. Introduction

Exposure to shiftwork has been associated with health problems, including cardiovascular, gastrointestinal, psychiatric, and neurological disorders (Driesen et al., 2010; Wong et al., 2010). Desynchronization of the circadian system by shiftwork and sleep deprivation may contribute to the development of obesity (Chen, et al., 2010; Ekmekcioglu & Touitou, 2011; Spiegel et al., 2009; Tanaka et al., 2010). Multiple studies report an elevated body mass index (BMI, a measure for body weight relative to height) in shiftworkers as compared to daytime workers (Croce et al., 2007; Kroenke et al., 2007; Morikawa et al., 2007; Parkes, 2002). In a sample of Italian railroad workers, shiftworkers had a higher BMI compared to daytime workers, even though the quality of their diet was better (Croce et al., 2007). Furthermore, shiftwork has recently been associated with abnormal eating behaviour, including emotional eating, decreased dietary restraint, and decreased quality of food choice (Wong et al., 2010). Shiftwork and poor sleep can alter glucose regulation (Padiha et al., 2010; Spiegel et al., 2009), and hypoglycemia has been observed in shiftworking nurses (Inoue et al., 2004). A shiftwork protocol in rats, comparable to 4 weeks of non-rotating night shifts in humans, induced alterations in the rhythmicity of food intake, plasma glucose concentrations, and body-weight gain (Salgado-Delgado et al., 2008).

Besides affecting body weight, shiftwork is generally thought to induce cognitive impairments in humans; shiftwork may attenuate attention span and performance on multitask complex procedures (Van Dongen, 2006a; Van Dongen et al., 2006b; Van Dongen & Belenky, 2009). Shiftwork-induced hypoglycemia may be a possible factor involved, because it can impair cognitive performance (Inoue et al., 2004). As exposing rodents to an altered light-dark rhythm can disturb multiple types of non-appetitively motivated learning (e.g., Craig & McDonald, 2008; Fekete et al., 1985; Gibson et al., 2010; Loh et al., 2010; Tapp & Holloway, 1981), we wondered whether impaired learning of a food-rewarded instrumental response could be demonstrated in a rodent shiftwork model.

Up to now, little is known about the causal mechanisms underlying shiftwork-related health problems, and a valid animal model is needed for research. A repetitive shift of the light-dark (LD) cycle has been proposed as an animal model for shiftwork (Bartol-Munier et al., 2006; Tsai et al., 2005). However, this procedure shows more resemblance to repetitive jet-lag caused by travelling to different time zones than to shiftwork where the LD cycle stays continuous and activity is altered respective to the LD phase (Salgado-Delgado et al., 2010). Another proposed model limits the availability of food and access to an activity wheel to the light (inactive) phase (Murphy et al., 2003). In this model, rats, indeed, voluntarily shift their activity to the light phase, but activity will concentrate around the LD transitions only, which is not quite similar to the activity pattern of humans working ~8 h continuously on a shift.
Forced locomotion in activity wheels during the light phase for 8 h/d over a 4-5-week period has recently been suggested as a more ethologically valid model for shiftwork, comparable to 4 weeks of night shifts with weekends off in humans (Salgado-Delgado et al., 2008, 2010). However, these activity wheels are not very compatible with tethering rats for on-line measurements of physiological and neurochemical parameters needed for the elucidation of underlying neurobiological mechanisms. We recently introduced and validated a novel method for sleep deprivation in rats using a variably rotating upright drum (Figure 1). This method does not induce increases in corticosterone concentrations or locomotor activity above peak levels normally encountered during the circadian cycle, thus limiting potential confounding by stress and locomotor activity (Leenaars et al., 2011; Chapter 2). We used the variable forced locomotion protocol in this device to mimic non-rotating night shifts over a period of 5 weeks. As spontaneous activity during forced locomotion (shift)work protocols has not previously been reported, we measured activity both during and between (shift)work hours. We measured body weight throughout the 5 weeks to obtain an indication of metabolic changes. During the fifth week of the protocol, we tested if this prolonged exposure to work- and/or shiftwork-altered learning of a simple goal-directed behaviour, i.e., the association of lever pressing with food reward (instrumental learning). This task is highly sensitive to sleep disturbance; the data from Chapter 4 show that preventing spontaneous napping during the dark (active) phase for only 3 h after the first learning session impairs performance on subsequent sessions.

6.3. Methods

6.3.1. General procedures

Experiments were performed on 32 male Wistar rats ( \( \approx \) 10 weeks old when starting the experiments; Harlan, Horst, the Netherlands), housed in pairs in type-IV macrolon cages (60 x 38 x 20cm), in two rooms with controlled temperature (20 ± 2°C) and controlled relative humidity (60 ± 20%). All rats were left undisturbed for 1 week after arrival for acclimatization. From the second week onwards, rats were handled and weighed daily. Baseline weight measurements were started after at least 1 week of habituation to daily handling, and continued throughout the experiment. Except for the last experimental week, food was available ad libitum. Water was always unrestricted for all rats.

Rats were divided into four groups for the different experimental conditions: (I) shiftworking rats that would work during the normally inactive light phase from Zeitgeber Time (ZT, ZT0 = light onset) 2 to ZT10 (SHIFTWORK, n = 8); (II) their respective non-treatment controls (SHIFTCON, n = 8); (III) working rats that would work during the active dark phase from ZT14 to ZT22 (WORK, n = 8); and (IV) their respective controls (WORKCON, n = 8). Working and shiftworking rats were exposed to 5 weeks of (shift)work. Instrumental learning was scheduled during working hours of the 5th week; (shift)work was interrupted for the learning sessions.
SHIFTWORK and SHIFTCON rats were housed on a normal LD cycle: lights-on at 07:00 h; lights-off (dim red light) at 19:00 h. Until the last week of the experiment, SHIFTCON rats did not experience any experimental interventions, besides daily handling and weighing. WORK and WORKCON rats were placed in another room from arrival onwards, to be exposed to a reversed LD rhythm; lights-on at 19:00 h; lights-off (dim red light) at 07:00 h. This procedure was necessary to allow the experimenters to work with these groups during office hours. After such an LD reversal, the daily activity pattern (activity measurements described in the next section) shifts over the first few days to adjust to the new light regime (see Section 7.2.3).

All rats were habituated to the reversed LD cycle for at least 12 d before the start of baseline measurements. At the onset of the experiments, the activity pattern of WORK and WORKCON rats had fully adapted to the LD reversal; the activity profile on the Saturdays preceding the onset of the (shift)work protocol did not differ between LD reversed and non-reversed groups (ANOVA, light versus dark period as within-subjects factor (p < 0.001), light-dark reversal as between-subjects factor (p = 0.37), interaction (p = 0.31).

All 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 international ethical standards (Portaluppi et al., 2010).

6.3.2. Home-cage activity measurements

Home-cage activity levels were measured with metal plates connected to piezo-electric elements responding to the amount of vibration, as previously described by Cailotto et al. (2005). Eight plates were connected to a PC to measure activity continuously, and aggregated activity was stored in 6-min bins. Combined measurements were made for two rats housed together in a home cage. Rats always shared a home cage with another rat from the same experimental group. The system was restarted every week for back-up purposes.

6.3.3. Work and shiftwork

Work (during dark, ZT14-22, comparable to a human daytime job) and shiftwork (during light, ZT2-10, comparable to a human night-time job) were modelled by variable forced locomotion using previously described sleep-deprivation devices that we refer to as ‘work-boxes’ (Figure 1, Section 2.3.3 Leenaars et al., 2011; Chapter 2). These boxes consist of a rotating drum (Ø 39 cm, height 37 cm), divided into two semicircular compartments by a stationary central wall. The bottom rotates bidirectionally at varying speed.

The daily (shift)work protocol was similar to the first 8 h of the sequence used previously for total sleep deprivation (Table 1, Section 2.3.3). The rotation speed and the number of directional alternations were gradually increased over time to compensate for increases in sleep pressure. This
total sleep-deprivation protocol is very efficient at keeping rats awake and continuously active, but does not increase the activity levels over normal circadian peak levels (Leenaars et al., 2011; Chapter 2).

On working days (Monday-Friday), rats were placed in the workboxes before ZT2/ZT14, when work started. After daily (Monday-Friday) work finished at ZT10/ZT22, rats were placed back in their home cages. Control rats stayed in their home cages during this period. Light in the work boxes was similar to light in home-cage conditions, with red light for working rats and white for shiftworking rats.

Although no EEG-studies were performed during the current 5-week protocol, we are convinced that sleep deprivation occurs during the 8-h work periods, as was shown for the 12-h protocol (see above, and refer to results Section 6.4.1 for activity during the protocol). Rats could, however, sleep freely in the 16 h between shifts. During work, behavioural activity was measured by detection of infrared displacement, as described previously (Leenaars et al., 2011; Chapter 2). Activity data were collected in 2-min bins. We present these activity data from the first and the fourth week of (shift)work. As this method of activity measurement was different from the method used in the home cages, the data do not permit calculation of energy expenditure during (shift)work protocols compared to baseline.

We briefly observed our rats at least four times during each work period. Both shiftworking and working rats were seen to display the following behaviours: leaning against the non-moving central wall while regularly lifting their paws over the moving floor, walking around in the box, rearing to the side or central wall of the box, eating and drinking (during the first 4 weeks of (shift)work, water and food was available ad libitum from the non-moving central wall), and lying down on the floor for brief periods.

We calculated that when rats would lean against the wall for the full 8 h; on their typical position halfway in the box, they would walk approximately 0.68 km during an 8-h (shift)work episode. This is not much when compared to voluntary wheel running, where male Wistar rats choose to walk >4 km/d (Matiello et al., 2010). Rats do, however, voluntarily move more than minimally required, as will be shown in the results section.

Minimal necessary activity was estimated by measuring activity from an artificial rat, that would “lean against the wall” (comparable to the most typical position of our rats), except when the box would change direction, when it would stay put on the rotating floor until reaching the other side of the wall (Leenaars et al., 2011; Chapter 2). Our observations have shown that rats periodically behave in this manner, although they are generally more active when the box changes direction.

6.3.4. Instrumental learning

On the Monday before the first behavioural training (that started on Wednesday), food was restricted to 16 g/rat/d to increase the motivation
for food rewards. Learning experiments were conducted in 16 Skinner boxes (Med-associates, St. Albans, Vermont, 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, a food tray with tray light between the levers on one wall, while the opposite wall contained a white noise generator and a house light. Every rat was appointed one Skinner box in which they were tested twice a day for 3 days (Wednesday - Friday). Testing consisted of 30 trials in which one of the two levers was presented in a random order for maximally 60 s, single sessions lasted maximally 30 min. Every lever press resulted in a reward (Bio-Serve dustless precision pellets, 45 mg, BioServ, Frenchtown, New Jersey, US). Between testing, rats were transferred back to the workboxes (working groups) or home cages (control groups) for ≈3 h. Outcome measures were the number of lever-presses within the six sessions for within-session behaviour, and the number of sessions required to reach a criterion of ≥27 lever-presses for between-session learning (Cheng & Feenstra, 2006).

6.3.5. Data analysis

Workbox activity levels are only presented for the first and the fourth week of (shift)work. The 8-h work-day totals were calculated from 2-min bins for each work episode. These 8-h totals were analyzed by ANOVA with week (1st vs. fourth) and workday (Monday-Friday) as within subject factors, and condition (WORK vs. SHIFTWORK) as between-subject factor. Home-cage activity data are only presented for the first and fourth week of (shift)work. Hourly totals were calculated from the 6-min bins, and 12-h totals were calculated for each light and dark phase on the weekends following (shift)work. Home-cage behavioural activity was analyzed for the Saturdays subsequent to the first and the fourth work week. Only Saturdays were analyzed, as data from part of a Sunday were missing due to technical malfunction. Separate ANOVAs were performed for both WORK vs. WORKCON and SHIFTWORK vs. SHIFTCON. Circadian phase (light vs. dark) and week (1 vs. 4) were included as within-subject factors, group ((shift)work vs. respective control) as between-subject factor.

To minimize the effects of day-to-day fluctuations in body weight, the average body weight was calculated for each experimental week. Average weights for the last baseline week and the five working weeks were analyzed with an ANOVA with week (1-6) as the within-subjects factor, and both work (daily (shift) work vs. home-cage control) and time of weighing (light versus dark phase, always before the start of work) as between-subject factors. Post-hoc ANOVAs were performed for both light regimes separately.

To evaluate instrumental learning, the number of lever-presses was analyzed by ANOVA with session (1-6) as the within-subjects factor, and both work (daily work vs. home-cage control) and time of testing (light versus dark phase) as between-subject factors. The number of sessions required to reach the learning criterion of ≥27 lever-presses within a session was compared between the four groups (WORKCON, WORK, SHIFTCON, and
SHIFTWORK) with a Kruskal-Wallis test for non-parametric data. For rats that did not reach the learning criterion within the six sessions, a value of 7 was substituted to enable inclusion of non-learners in this analysis.

For all ANOVAs, when the assumption of sphericity was violated, a Greenhouse-Geisser correction was applied. Statistical analyses were performed with SPSS (Chicago, Illinois, US). Differences were considered significant at p = 0.05. All data are presented as average values ± standard error of the mean (SEM).

6.4. Results

6.4.1. Activity during work and shiftwork

Figure 32 Activity (measured by infrared displacement detection) during work

Data from the 09:00 – 17:00-h work intervals are shown. WORK rats were more active during work than SHIFTWORK rats during shiftwork (p = .02). All rats decreased their behavioural activity upon repeated exposure to work (week-effect: p = .003, day-effect: p < .001). Statistical details are provided in the main text.

Rats decreased their behavioural activity with repetitive exposure to work, both from day-to-day and over subsequent weeks (week-effect: $F_{1,14} = 13.2; p = 0.003$; day-effect: $F_{2,1,29.4} = 41.2; p < 0.001$; day-week interaction: $F_{2.8,39.1} = 9.7; p < 0.001$, Figure 32). The effect of condition was also significant ($F_{1,14} = 6.8; p = 0.02$, Figure 32); WORK-rats had a higher behavioural activity than SHIFTWORK-rats during work. None of the interactions with condition was significant, indicating that the decrease in activity with repeated exposure was similar for both groups.
Although activity during work decreased with repetitive exposure, rats did remain active throughout the work periods, as can be seen in two respective actigrams from the five work days in the fourth week of work (Figure 33). Activity levels during the last working day of the 4th week were at the lowest level observed, but still higher than previously observed normal spontaneous activity levels. For SHIFTWORK rats, activity (mean ± SEM) during work equalled 5460 ± 743 counts/8 h (compared to a light-phase baseline level of 1360 ± 304 counts/12 h in our previous study (Leenaars et al., 2011; Chapter 2) where rats were food-restricted), and for WORK rats activity during work equalled 8630 ± 1282 counts/8 h (compared to a food-restricted dark-phase baseline level of 7448 ± 946 counts/12 h in our previous study (Leenaars et al., 2011). Activity levels were also still higher than estimated passive or minimal activity, as plotted in light gray in Figure 33.

Figure 33  Activity (measured by infrared displacement detection) during the fourth week of work

Only data from the work intervals (ZT2-ZT10 for SHIFTWORK and ZT14-22 for WORK) are shown. Periods between work intervals are deleted because rats were then back in their home cages. An estimate of passive or minimal activity is plotted in light gray. Minimal activity was estimated by measuring activity with an artificial rat, that would “lean against the wall” throughout, except when the box would change direction, when it would stay put on the rotating floor until reaching the other side of the wall. (Leenaars et al., 2011; Chapter 2).
6.4.2. Home-cage activity between work and shiftwork exposure

Activity was also measured in the home cages between working sessions. Home-cage behavioural activity from the first and the fourth workweek are presented in Figure 34.

**Figure 34** Home-cage activity (Mean ± SEM as measured by piezo-electric current detection) during the first and fourth work week

From Monday to Friday SHIFTWORK and WORK rats were absent from their home cages to be exposed to 8 h of (shift)work. A.) Home-cage activity of work and control rats in the first week of work; B.) Home-cage activity of work and control rats in the fourth week of work; C.) Home-cage activity of shiftwork and control rats in the first week of work, and D.) Home-cage activity of shiftwork and control rats in the fourth week of work.

For WORK versus WORKCON rats, no effect of work was found on activity during the Saturdays after the first and fourth weeks (p = 0.47); neither were the effect of week nor any of the interactions significant, indicating that work did not affect home-cage activity on free Saturdays. The effect of LD phase was significant (F_{1,0,6,0} = 165; p < 0.001); as expected, rats were more active during the dark than during the light phase. For SHIFTWORK versus SHIFTCON rats, a significant effect of week was observed on activity during the Saturdays after the first and fourth weeks, (F_{1,0,6,0} = 12.3; p = 0.01), and the week*circadian interaction was significant (F_{1,0,6,0} = 10.1; p = 0.02, Figure 34). After the fourth workweek, Saturday-dark-phase activity was lower than after the first work week, for both SHIFTWORK and SHIFTCON rats. None of the other interactions was significant, neither was
the effect of shiftwork (p = 0.9), indicating that after shiftwork, weekend activity levels were unaltered. The effect of circadian phase was significant, (F1.0,6.0 = 320.2; p < 0.001, Figure 35); rats were more active during dark than during light.

Figure 35  Home-cage activity (measured by piezo-electric current detection) during the Saturdays following the first and the fourth workweek

Activity was higher during the dark than during the light phase (p < .001). Home-cage activity was not different between WORK and WORKCON rats (p = .5), or between SHIFTWORK and SHIFTCON rats (p = .9). Under normal light conditions, activity decreased from the first to the fourth week of the experiment (p = .01). Statistical details are provided in the main text

6.4.3. The effect of work and shiftwork on body-weight gain

On average (± SEM), the 32 rats grew from 304.4 ± 3.1 g to 390.3 ± 5.9 g over the course of the experiment, which resulted in a significant effect of week (F1.3,36.2 = 298.3; p < 0.001, Figure 36).

The factor work significantly altered average body weight (F1,28 = 5.6; p = 0.025), and the week*work interaction was also significant (F1.3,36.2 = 5.8; p = 0.02). Time of weighing did not significantly affect body weight (main effect: p = 0.6; interaction with week: p = 0.2; 3-way interaction: p = 0.5). The post-hoc ANOVA for the SHIFTWORK vs. SHIFTCON rats showed that body-weight gain over time was again significant (F1.4,19.1 = 228.6; p < 0.001). Besides, both the interaction of week and shiftwork (F1.4,19.1 = 6.3; p = 0.01) and the effect of shiftwork (F1,14 = 7.9; p = 0.01) were significant, indicating that SHIFTWORK negatively affected weight gain during the course of the experiment. The post-hoc ANOVA for the WORK vs. WORKCON rats showed that body-weight gain over time was again significant (F1.2,16.5 = 103.9; p < 0.001). However, neither the week*work interaction (p = 0.3), nor the main effect of work (p = 0.5) was significant, indicating that WORK
did not affect weight gain during the course of the experiment when compared to WORKCON.

**Figure 36**  Average body weight over the course of the experiment

Body weight increased over the course of the experiment ($p < .001$). Only SHIFTWORK rats grew significantly slower than SHIFTCON rats ($p = .007$). Statistical details are provided in the main text. BL = Baseline, IL = Instrumental Learning.

Food was restricted in the week of instrumental learning. Body weight during instrumental learning decreased in all groups. Values for the pre-diet value on Monday and the last weight measurement on the Friday before learning are provided in Table 11.
Table 11  Weight loss during the final week of the experiment, when food was restricted to increase motivation for instrumental learning

<table>
<thead>
<tr>
<th>Group</th>
<th>Weight (g) on Monday (mean ± SEM)</th>
<th>Weight (g) on Friday (mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHIFTWORK</td>
<td>380.3 ± 7.6</td>
<td>374.4 ± 7.4</td>
</tr>
<tr>
<td>WORK</td>
<td>382.5 ± 12.5</td>
<td>379.0 ± 12.3</td>
</tr>
<tr>
<td>SHIFTCON</td>
<td>420.0 ± 11.3</td>
<td>408.6 ± 11.0</td>
</tr>
<tr>
<td>WORKCON</td>
<td>397.8 ± 12.0</td>
<td>386.5 ± 14.3</td>
</tr>
</tbody>
</table>

6.4.4. The effect of work and shiftwork on instrumental learning

Over the six sessions of instrumental learning, rats improved their performance from 3.8 ± 0.7 lever-presses in the first session to 21.3 ± 2.4 lever-presses in the sixth session (Figure 37a). A significant effect of session reflects this improvement ($F_{2.7,53.3} = 14.8; p < 0.001$). Neither time of testing (light versus dark phase, $p = 0.9$), nor work (shiftwork and work versus control, $p = 0.2$), nor any of the interactions affected lever-pressing over the sessions. On average, groups needed 5.1 ± 0.3 sessions to reach the criterion of ≥27 lever-presses within a session. No difference between the four groups (WORKCON, WORK, SHIFTCON and SHIFTWORK) was found ($p = 0.4$; Figure 37b) on the number of sessions to criterion.
Figure 37 Instrumental learning during the fifth week on a shiftwork protocol

Performance on the subsequent sessions for all four groups. Performance improved over the subsequent sessions ($p < 0.001$). No significant differences were observed between any of the groups. B.) the number of sessions to learning for all four groups. No significant differences were observed between any of the groups. Statistical details are provided in the main text.

6.5. Discussion

In this paper we applied a new shiftwork paradigm. Activity measurements during (shift)work indicated that rats were, indeed, continuously active during WORK and SHIFTWORK protocols. Activity measurements between
work hours showed that rats did not change their home-cage activity as compared to undisturbed control rats. Contrary to previous findings, shiftwork attenuated body-weight gain. Instrumental learning was not affected.

The shiftwork-protocol described in this paper is very effective at keeping the rats active, as indicated by the activity throughout the (shift)work periods (Figure 33), although we did not confirm absence of periods of micro-sleep with EEG-registrations. The activity level observed during (shift)work is comparable to spontaneous activity levels observed in our previous sleep-deprivation studies (Leenaars et al., 2011; Chapter 2), and substantially higher than the estimated minimal activity required during the protocols. Thereby, this procedure is applicable for various shiftwork studies that aim to investigate the effect of activity during the rest phase. The current protocol does not disturb other factors that are relevant to human shiftwork, such as exposure to light during the dark phase.

In contrast with the previous studies by Salgado-Delgado et al. (2008, 2010), we did not observe an increased weight gain (or growth) in our shiftworking rats compared to the other groups. Of note, our control rats showed normal weight gain over time compared to standard data (e.g., Poole, 1987), and age was equal for rats in all groups. We observed that our shiftworking rats did not decrease their nocturnal home-cage activity and/or increase their home-cage activity during the light period on the weekends as compared to undisturbed conditions.

When not working, SHIFTWORK rats and WORK rats were placed back in their respective room, where they were housed together with their controls. Combined housing and other room-related factors may have affected the activity pattern of the control groups. Indications for this effect of social housing can be observed in Figure 34, where mainly the bimodal pattern during the dark phase appears to differ between both control groups, mainly in the 4th week of the protocol. This effect could not be analyzed in detail, as part of the data is missing. Future studies should address the potential masking effects of activity changes in control groups by comparing activity during work protocols to preceding baseline values.

Spontaneous activity is an important factor in body-weight gain; obesity in humans is associated with low levels of spontaneous activity, and elevated levels of spontaneous activity protect against obesity, also in rats (Teske et al., 2008). As both our SHIFTWORK- and our WORK-rats had normal spontaneous activity in their home cage between working sessions, and increased activity levels during work-sessions, it is not surprising that they gained less weight (or grew slower) than the home-cage control groups. Although the activity during work was lower in the SHIFTWORK than in the WORK group (Figure 32), the total increase in activity is likely to be highest in the SHIFTWORK group, as the rats of this group had to work while they would normally be preferably asleep, i.e., they were forced to be active during the period of time when they would normally show the lowest levels.
of spontaneous activity. Accordingly, they expended more energy and gained weight (or grew) slower than rats in the corresponding control group.

Not all human shiftwork studies have found increases in body weight of workers. In a sample of industrial workers, shiftworkers were found to have a lower BMI compared to daytime workers (McNamee et al., 1996). Furthermore, BMI was also not related to shiftwork duration in hospital nurses or factory workers (Ha & Park, 2005). Moreover, BMI was not different between three different shifts of garbage collectors (de Assis et al., 2003). Interestingly, in the latter sample, non-rotating night-shift workers had significantly less sleep compared to workers on daytime shifts. If missed sleep during “night” shifts is not completely compensated for between shifts, subjects will experience a certain degree of sleep deprivation while performing shiftwork. Although BMI in non-growing humans is not very comparable to weight gain in growing rats, it is tempting to speculate that mild sleep deprivation during non-rotating night shifts may prevent body-weight gain. Because “epidemic” short sleep in humans is temporally associated with increasing obesity, concerns, however, exist that short sleep duration may substantially contribute to obesity. Recent review articles, nevertheless, question this hypothesis (Horne, 2008; Marshall et al., 2008).

In contrast to humans, rodents usually decrease their body weight in response to sleep deprivation, in spite of increased food intake. In rats, specific deprivation of rapid eye movement sleep can induce loss of body weight, augment energy expenditure, and decrease metabolic efficiency (Hipolide et al., 2006). As sleep deprivation induces an increase in stereotyped gnawing behaviour, the observed decrease in body weight after sleep deprivation has been attributed to an increase in food spilling (Martins et al., 2007). When corrected for spilling, during short-term sleep deprivation rats decrease their food intake while their energy expenditure increases, and, therefore, they lose weight (Martins et al., 2006). In experiments using liquid food to prevent gnawing-related spillage, hyperphagia was observed after 6 d of sleep deprivation, but without the concurrent increase in body weight (Koban et al., 2008). Rats also lose weight over the course of 8 d of chronic total sleep deprivation (10 or 20 h/d; Barf et al. 2010). Overall, sleep deprivation can, therefore, be concluded to cause weight loss, at least in rodents.

A single exposure to the protocol used in the current study virtually eliminates sleep (Leenaars et al., 2011; Chapter 2). We did not measure sleep in the current repetitive procedure, but did observe continuous activity during the protocols (Figure 33). We therefore assume that rats are kept awake both during work and shiftwork, while they would otherwise have been sleeping at least part of this period. Activity levels in-between work do not suggest any compensation of sleep. Therefore, cumulative mild sleep restriction can be assumed for both the WORK and SHIFTWORK group. Even though our rats did not lose weight, the SHIFTWORK rats, experiencing the most sleep restriction, grew at the slowest rate.
Although we did not measure food intake in this study (this was technically challenging, due to social housing and the type of feeders used), we did observe that while in the workboxes, working rats ate more during their WORK period in the dark phase than shiftworking rats ate during their SHIFTWORK period in the light phase (we had to refill the WORK-rats’ feeding tubes in the workboxes more often). Thereby, we assume that our SHIFTWORK-rats did not shift most of their feeding to their shiftwork period as described by Salgado-Delgado et al. (2008). When these authors prevented the shift in feeding by restricting shiftworkers’ food availability to the dark phase, (when they are back in their home cages,) they also no longer observed an increased growth rate in shiftworking rats (Salgado-Delgado et al. 2010). Alternatively, our shiftwork rats could also have eaten less than the other groups.

As demonstrated in our previous study, the 12-h total sleep-deprivation protocol does not increase the corticosterone levels over normal circadian peak levels (Leenaars et al., 2011; Chapter 2). Increased stress levels would be relevant as they can increase metabolism in rodents, and thus energy expenditure, e.g., by affecting brown adipose tissue (Koban & Swinson, 2005). This could contribute to decreased weight gain in the shiftwork group. Also, it could explain potential differences between the effect of shiftwork on humans and rodents, as adult humans hardly have any brown adipose tissue (e.g., Mattson, 2010). We cannot yet exclude increased stress upon repeated exposure to work in our method, but Salgado-Delgado et al. (2008) have shown that comparable activity protocols do not have a main effect on corticosterone levels.

Further research is required to investigate the mechanisms involved in the attenuated weight gain, that could involve, among others, energy expenditure, food intake, water intake, stress, etc.

While shiftworking rats in the Salgado-Delgado studies (2008, 2010) transferred their activity to the light phase, our SHIFTWORK rats did not. As the light regimes, food availability, and work were comparable in both studies, voluntary home-cage circadian activity patterns must be affected by a different factor. The main difference between these studies is the housing condition; during our experiments, rats were socially housed (in pairs from the same group). During (shift)work, each pair of rats was placed in a workbox, where social interaction could be continued through 13 holes (Ø 1 cm) in the Perspex middle wall. After work, the pairs of rats were placed back together in the home cage. Future studies should investigate if home-cage social interaction between work sessions prevent the compensatory decrease in between-work activity as observed in the studies by Salgado-Delgado et al. (2008, 2010).

Although our unpublished data (in press) show that performance on our instrumental learning paradigm is highly sensitive to mild sleep disturbance, we did not observe any effects of (shift)work on learning. The improvement between sessions on the first day (Cheng & Feenstra, 2006) that was sensitive to sleep deprivation (Chapter 4) was
absent in all groups in the current study. Instead, the improvement in performance was observed from day-to-day (from $3.8 \pm 0.7$ times in the first session to $21.3 \pm 2.4$ in the sixth session). Therefore, this paradigm should still be sensitive to both impairments and improvements in learning.

As we are mainly interested in the effects of sleep disturbance on cognition, we chose to combine this shiftwork protocol with a relatively simple cognitive task, i.e., the learning of a new stimulus-response-reward association. The performance of more complex cognitive tasks (e.g., Christie & Hersch, 2004; McCoy et al., 2007; Leenaars et al., 2012a; Chapter 3) may be more sensitive to disturbance by prolonged (shift)work. When mainly interested in the effect of motivation on task performance, a different type of task could be preferable, for example, a task implementing ratio lever pressing or imposing delays. Our previous study (Leenaars et al., 2011; Chapter 2) shows that the motivation to perform a fixed-interval, 40-s fixed-ratio three lever presses task after 12 h of sleep deprivation can be maintained by adjusting the diet. It would be of interest to determine if this is also the case after prolonged (shift)work.

Some degree of cognitive impairment during shiftwork may generally be experienced by shiftworkers (Van Dongen & Belenky, 2009; Van Dongen et al., 2006). The previously mentioned shiftwork models using light-cycle shifts do disturb multiple types of learning (e.g., Craig & McDonald, 2008; Fekete et al., 1985; Gibson et al., 2010; Loh et al., 2010; Tapp & Holloway, 1981). Unfortunately, to the best of our knowledge no systematic studies have been performed investigating learning performance during shifts outside “normal office hours”, in either humans or rodents. We show that shiftwork by means of forced locomotion does not necessarily lead to alternations in spontaneous circadian activity patterns. Rats may have habituated to the prolonged sleep restriction and overcome the negative effects of sleep deprivation on learning, in line with the study of Loh et al. (2010) showing decreased effect of repeated exposure to phase-shifting on fear conditioning. Surprisingly, circadian phase did not affect learning either; control rats performed equally well on the instrumental learning task during the dark and the light part of the circadian cycle.

Although circadian differences in locomotor activity patterns are commonly reported (Drijfhout et al., 1995; Gritton et al., 2009; Kiwaki et al., 2004; Morrison, 1968; Tang et al., 2007), other types of rodent behaviour are not necessarily different when tested in either the dark or the light circadian phase. In line with the current findings, social memory in rats is independent of circadian time and not affected by phase-shifts of 6 or 12 h (Fekete et al., 1985), and passive avoidance learning is not affected by circadian phase as long as rats are given ≥3 d to adjust to the new cycle (Fekete et al., 1985; Tapp & Holloway, 1981). Moreover, behaviour on an elevated plus-maze is similar during light and dark (Jones & King, 2001). For planning when to perform cognitive experiments, it may be important to note that behavioural testing, itself, can modify circadian locomotor activity patterns (Gritton et al., 2009).
In conclusion, a shiftwork protocol can attenuate weight gain compared to undisturbed control rats when rats do not shift their circadian spontaneous activity levels to the shiftwork period. Furthermore, after 4.5 weeks of habituation to this protocol, shiftwork does not alter instrumental learning. Given the previous and current findings, it is tempting to speculate that while on prolonged shiftwork, not fully reversing the circadian rhythm might actually be beneficial regarding body-weight gain and learning. In the current society, where 24-h work schedules have become more and more common, future studies to find the optimal procedures for managing shiftwork are of high importance to limit the associated health problems.

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