

## **Food restriction induces functional resilience to sleep restriction in rats**

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## **Abstract**

**Study Objectives.** Sleep restriction leads to performance decrements across cognitive domains but underlying mechanisms remain largely unknown. The impact of sleep restriction on performance in rodents is often assessed using tasks in which food is the reward. Investigating how the drives of hunger and sleep interact to modulate performance may provide insights into mechanisms underlying sleep loss related performance decrements.

**Methods.** Three experiments were conducted in male adult Wistar rats to assess: 1) Effects of food restriction on performance in the simple response latency task (SRLT) across the diurnal cycle (n=30); 2) Interaction of food restriction and sleep restriction (11-h) on SRLT performance, sleep EEG and event-related potentials (ERP) (n=10-13); 3) Effects of food restriction and sleep restriction on progressive ratio (PR) task performance to probe the reward value of food reinforcement (n=19).

**Results.** Food restriction increased premature responding on the SRLT at the end of the light period of the diurnal cycle. Sleep restriction led to marked impairments in SRLT performance in the *ad libitum*-fed group, which were absent in the food-restricted group. After sleep restriction, food restricted rats displayed a higher amplitude of cue-evoked ERP components during the SRLT compared to the *ad libitum* group. Sleep restriction did not affect PR performance while food restriction improved performance.

**Conclusions.** Hunger may induce a functional resilience to negative effects of sleep loss during subsequent task performance, possibly by maintaining attention to food-related cues.

**Key words:** sleep deprivation, attention, motivation, cognition, event-related potentials, circadian rhythm, vigilance, hunger, effort

### **Statement of Significance**

Sleep loss leads to impairments in performance in humans and rodents but the cognitive processes underlying these deficits remain largely unknown. Here these mechanisms were investigated by varying motivational drives for eating and sleep and measuring performance on tasks in which food serves as reward. Results show that hunger and the drive for sleep interact during performance of a simple reaction time task such that hunger greatly reduces sleep loss induced impairments. This 'protective' effect of hunger was accompanied by changes in electrophysiological indices of attentional processes. Identifying the mechanisms underlying protective effects of independent motivations may lead to new countermeasures for the effects of sleep restriction on performance.

## Introduction

Acute and chronic sleep loss and displacement of wakefulness to the habitual rest phase can lead to increases in the propensity to fall asleep, reduction in vigilant attention and performance decrements across a range of other cognitive domains<sup>1-3</sup>. The mechanistic underpinning of these sleep loss-induced decrements in waking function are largely unknown and continue to be discussed (e.g.<sup>4,5</sup>). Progress in characterizing these mechanisms will lead to greater understanding of the contribution of sleep to brain function and may lead to the development of new countermeasures for sleep loss/circadian misalignment associated decrements in waking brain function. Such new countermeasures are needed because sleep loss, sleep disorders, and shiftwork are highly prevalent in industrialized societies<sup>6</sup>.

Rodents are often the preclinical model of choice for the development of new pharmacological and non-pharmacological countermeasures. Development of new countermeasures for sleep loss related performance deficits critically depends upon the translational validity of animal models employed. Studies of the effects of sleep restriction on the sleep electroencephalogram (EEG) and sleep structure have revealed many similarities between humans and rodents<sup>7-9</sup>. However, much less is known about the effects of sleep restriction on performance capacity in rodents and the appropriate means by which to measure decrements in performance in a translationally meaningful manner.

In human and rodent research, the nature of the stimuli used to motivate task performance is different. Human subjects are motivated based on adherence to verbal instructions and/or performance feedback, and occasionally with secondary reinforcers such as monetary rewards<sup>10</sup>. In the majority of rodent studies, food or water is used as a primary reinforcer to reward task performance in food or water-restricted subjects<sup>11-13</sup>. Alternatively, highly palatable fluids have been used as a positive reinforcer in *ad libitum*-fed animals<sup>14</sup>. The impact this may have on the translational validity of the task results has not been established. Results from rodent studies of sleep loss mainly focus on interpretation of data from

the perspective of impairment in cognitive processes or vigilance, whilst rarely addressing the potential interaction of the drives for sleep and other motivational factors such as hunger or thirst.

The human psychomotor vigilance test (hPVT, <sup>15</sup>) is a widely used and extensively validated task sensitive to both circadian and homeostatic sleep manipulations <sup>1 16,17</sup>. The hPVT is an easily implemented practice-free simple reaction time test that provides an index of behavioral effects related to decrements in vigilant attention <sup>1,18</sup>. The hPVT is consistently found to be among the most sensitive tests of performance decrement following sleep loss (e.g., <sup>1,19</sup>). Following experimentally-induced or naturally occurring sleep restriction in humans, canonical deficits in hPVT performance include slowing of response latency and increase in trial omissions, which typically exhibit progressive “time-on-task” effects <sup>20,21</sup>. A rodent-analog of the PVT has been developed <sup>22</sup> and been shown to detect functional deficits following sleep deprivation that bear resemblance to findings in humans <sup>22-24</sup>. However, the precise factors contributing to performance decrement in each species is likely to be complex, including primary cognitive factors <sup>25</sup> but potentially also other motivational factors (e.g., effort and delay discounting, hunger, thirst). In most behavioral studies on the effects of sleep restriction on performance, animals are either food or water restricted yet interactions between the motivation for food/water and sleep are rarely considered.

The aim of the present study was to assess the effects of feeding condition (i.e., *ad libitum*-fed vs. food restriction) at baseline and on sleep restriction induced changes in performance of a simple response latency task (SRLT) in rats <sup>26</sup>. In addition, the effects of sleep restriction and food restriction on performance of a progressive ratio test were measured. In this frequently used instrumental task, rats have to press a lever for a progressively increasing fixed ratio to continue to obtain a food reward <sup>27</sup>. The point at which the increase in lever press requirement becomes too much and the animal stops responding is called the breakpoint. Breakpoint is considered to be an index of present state of motivation of the animal to obtain the reward. The EEG was recorded to quantify sleep and event-related potentials

during the SRLT to gain further insight into cognitive processes, and how they are affected by sleep loss and food restriction<sup>26,28</sup>.

## **Methods**

All experimental procedures were approved by the local Animal Welfare Ethical Review Body and carried out in accordance with the UK Animals (Scientific Procedures) Act 1986.

**Subjects and Housing.** All experiments were performed in an AAALAC [Association for Assessment and Accreditation of Laboratory Animal Care International]-accredited facility. Male adult Wistar rats (Charles River Laboratories, Margate, UK) were group-housed (n=4/cage) in standard home cages (Techniplast SpA, Buguggiate, Italy; Dimensions: 610 x 435 x 215 cm), except for Experiment 2 where animals were housed individually during the period they spent in the sleep restriction chambers. Jolly balls, nestlets and wooden chew blocks were added as environmental enrichment. Experimental rooms were maintained on a 12:12 h light-dark cycle (~35-40 lux at mid-level inside the cage), with controlled ambient temperature ( $23 \pm 1$  °C) and humidity (~50 %). For groups with *ad libitum* access to food, chow was freely available (Teklad Diet, Envigo, Teklad Diets, Madison, WI). Food restricted groups were maintained at no less than 85% of their free-feeding weight by providing 20 g chow per rat/per day after performing the behavioral task. Normal growth curves were maintained during the food-restriction duration. During training for all behavioral tasks, rats were food restricted to facilitate acquisition of operant responding. Water was available *ad libitum*.

**Experiments.** Three experiments were conducted. The performance test of primary interest in this study is the SRLT. In previous studies on the effects of drugs on this task we have used ‘omissions’ as the primary outcome measure (Loomis et al 2015). In our historical data, the Standard Deviation for this measure was

found to be 12.5. In the experiments presented here we are interested in both within and between animal comparisons. For a between groups comparison a sample size of 11 per group would provide 80% power to detect a difference of 15 omissions at a level of  $\alpha=0.05$  (two sided).

### ***Experiment 1: Effect of food restriction and time of testing (diurnal phase) on vigilant attention***

*Simple Response Latency Task (SRLT).* Rats were trained in the SRLT, a sustained attention task<sup>29</sup>. Before the SRLT training started, rats were food restricted for at least a week and maintained on food restriction during the initial training programme. The SRLT was carried out in operant chambers housed in sound and light attenuated chambers (Med Associates, Inc. Fairfax, VT, USA). Training occurred on successive days during daily 30-min sessions for approximately 21 days. Stimuli consisted of the house-light and the magazine light, serving as preparatory and imperative cues respectively (Figure 1a). In the first stage of training, the magazine light/imperative cue was illuminated for 10 s (with a 30-s inter-trial interval) during which a nose poke would earn a palatable sucrose food reward pellet (45-mg TestDiet, Labtabs, Richmond, IN, USA) delivered from an automatic pellet dispenser. The reward would be supplied at the end of this cue even if no nose poke was made. The house-light/preparatory cue remained illuminated throughout these sessions, except for a 5-s timeout period which occurred each time the animal collected a reward. Animals were required to make at least 10 head entries to advance to the second stage, where all rewards had to be obtained by head entry. If the animal did not make a head entry during the presentation of the imperative cue, an omission was recorded that resulted in a 5-s timeout period with no light stimulus. After performing at >75% efficiency over 2 consecutive sessions, animals progressed to the third stage to learn to inhibit premature responding during the preparatory cue. A trial would be initiated by illumination of the house-light and responding had to be inhibited until illumination of the magazine light. During this stage, the interval between preparatory and imperative cue was fixed at 5 s. Premature responses during the preparatory cue resulted in a timeout period. The criterion for progression to the

next stage of training was >75% efficiency over 2 consecutive sessions. Where necessary, animals finding it difficult to meet this criterion would be subjected to remedial training using shorter fixed intervals between cues. After completion of initial training, rats were randomly assigned to parallel food restricted (n=16; weight: 412 ± 4.1 g) or *ad libitum*-fed groups (n=16; weight: 410 ± 4.2 g) for a final training programme to adapt to feeding condition and reach stable baseline performance. In the final stage of training and during the study phase, the preparatory cue consisted of initial presentation of the houselight, followed by a variable time interval (range: 4-6 s), after which the imperative cue (i.e., magazine light) was illuminated (Figure 1a). A period of 10-s following the illumination of the magazine light allowed the rat to perform a nose poke to receive a food reward. An interval of 20-s was imposed between trials. SRLT performance was tested during a 30-min session. Criterion performance for successful training was set at >75% of trial completion efficiency across five consecutive days<sup>29</sup> and was typically reached within 4 weeks from start of training. At completion of the final training programme and beginning of the study phase, body weights were 493.4 ± 7 g for the *ad Libitum*-fed rats and 426.1 ± 7 g for the food restricted group (mean ± SEM). Performance was indexed by the number of correct trials, response errors (i.e., number of premature responses and omissions) and reaction times (i.e., response latency). Data were recorded by in-house programs using MedPC IV software (Med Associates, Inc, Fairfax, VT, USA). During the study phase, rats were subjected to the 30-min SRLT at 6 time points across the 24-h light-dark cycle (i.e., Zeitgeber (ZT) ZT0, ZT3, ZT8, ZT11, ZT16, ZT20) on different days. Each animal was tested 3 times at each time point over a two-week period. Rats were assigned feeding times in a randomised block design across days in order to ensure an equal balance of feeding condition groups across days. The same palatable food reward pellets were used during training and the study phase.

### ***Experiment 2: Effect of sleep restriction and food restriction on vigilant attention***

***Surgery.*** Rats (n=16; ~270-300 g at time of surgery) were anesthetized (2% isoflurane in 100% oxygen) and surgically prepared with a cranial implant for long-term electroencephalogram/electromyogram



(EEG/EMG) recordings, as previously described<sup>29</sup>. The implant consisted of bilateral stainless steel screws (2 frontal [+3.5 mm anteroposterior (AP) to bregma, 2.0 mm mediolateral (ML)] and 2 occipital [-6.4 mm AP, 5.5 mm ML]) for EEG recordings. EEG and ERP signals were recorded as the differential between the left frontal and right occipital electrodes. For the EMG, two Teflon-coated stainless steel wires were positioned under the nuchal trapezoid muscles. The implant was fixed to the skull using cyanoacrylate (Geristore syringeable, DenMat, Santa Maria, CA, USA), applied between the hermetically sealed implant connector and skull, and dental acrylic (Meadway Rapid Repair, Mr. Dental Suppliers Ltd, UK). Locomotor activity was recorded by a miniature transmitter (Minimitter PDT4000G, Philips Respironics, Bend, OR, USA) placed in the abdomen during the same surgical procedure. An analgesic (buprenorphine 0.05 mg/kg) was administered subcutaneously pre-operatively, at the end of the surgery day, and the morning of the first post-operative day. In addition, a non-steroidal anti-inflammatory drug (meloxicam 0.15 mg/kg) was administered orally twice daily for 6 days post-surgery. Prophylactic antibiotic treatment (cefalexin 20 mg/kg) consisted of an oral dose 24-h prior to and immediately before surgery, and twice daily for 7 days after surgery<sup>29</sup>. At least 2 weeks were allowed for recovery from surgery.

*SRLT and sleep restriction.* After recovery, rats were trained for the SRLT as described in Experiment #1. Following SRLT training, rats were housed individually in custom-designed sleep restriction chambers in a sound-attenuated recording room. Each chamber consisted of a rotatable plexiglass rods cylinder (39.7 cm diameter by 32.1 cm depth) inside a Plexiglas frame (637.2 cm<sup>2</sup> floor space). The cranial implants were connected to ultra-low-torque slip-ring commutators (Hypnion, Inc., Lexington, MA, USA) by metal coil reinforced flexible cables, allowing unrestrained movement. Infra-red light and digital video camera allowed continuous remote monitoring. Automated sleep restriction was induced by activating chamber turns based on a Weibull distribution fitted to the survival likelihood of continuous bouts of wakefulness over the course of an 11-h sleep restriction in a historical dataset, as previously described<sup>26</sup>. The purpose of the sleep restriction protocol was to limit the amount of continuous sleep (defined as sleep episodes

that are longer than 20s) that an animal can obtain, which is required for the restorative benefit of sleep. The protocol does allow short (10 second) sleep events prior to the activation of the chamber. Once activated, the motor rolled the cylindrical chamber around its axis for 8 seconds (265° of rotation at 11.5 cm/s), initiating the righting reflex and waking the rat. The chamber turned in a pseudo-random direction to prevent habituation. Seven days prior to the actual sleep restriction experiment, all subjects were sleep deprived for 5 hours to habituate rats to the procedure. A previous study demonstrated that during a 11 h Weibull automated sleep restriction protocol rats obtain only  $197 \pm 13$  min (mean  $\pm$  SEM) of sleep compared to  $369 \pm 12$  min in non-sleep deprived rats <sup>26</sup>.

A crossover study design was then conducted over a 2-week period. On week #1, half of the rats were randomly assigned to the *ad libitum* regime while the other half underwent food restriction. On week #2, food regimes were inverted. At time of testing, rats in the *ad libitum* and food restriction regimes weighed  $516.4 \pm 8.8$  g (mean  $\pm$  SEM) and  $478.1 \pm 6.2$  g respectively. During both weeks, sleep-wake variables were recorded throughout a 24-h baseline, the 11-h sleep restriction period (ZT0-ZT11), and subsequent 37-h period (Figure 1b). Rats were subjected to 40-min SRLT test sessions during baseline, at the end of the sleep restriction and during the recovery period (Figure 1b).

*EEG-EMG analysis.* EEG and EMG signals were amplified (x 10,000) and digitized (400 Hz) with bandpass filters (EEG: 1-300 Hz; EMG: 10-100 Hz, RMS integration; Grass Instrument Co, Quincy, MA, USA). Vigilance states, i.e., waking, NREM sleep and REM sleep, were assessed using SCORE2004™, an automated real-time sleep-wake monitoring system previously validated <sup>26,30</sup>. SCORE2004™ scoring was based on a combination of salient EEG/EMG features on a 10-second epoch basis (i.e., EEG amplitude and zero-crossings, EEG harmonic amplitude and frequency, integrated EMG tone; locomotor activity, drinking and feeding activity). Parameters were matched to individual scoring templates for each animal to determine vigilance states. Visual signal inspection was performed to ensure data quality and effective sleep state determination. EEG power spectra were computed for each 10-s epoch using a fast Fourier transform.

The spectrogram was then sub-divided into the following bands: delta 0.5-3.9 Hz, theta 5.0-8.9 Hz, alpha 9.0-11.9 Hz, beta 12.0-20.0 Hz. The state-specific time series of EEG power in each band was calculated for all EEG-defined epochs devoid of artefacts. Time spent in wakefulness, NREM sleep, REM sleep, and total sleep time were computed (mean  $\pm$  SEM). EEG/EMG recordings were analysed for a 24-h baseline, the 11-h sleep restriction period and 13-h recovery following the SRLT.

*Event-related potentials (ERPs).* Throughout the SRLT, the EEG recordings were time-locked to the imperative cue (i.e., magazine light) using Transistor-Transistor Logic (TTL) signals from the MedPC hardware (Med Associates, Inc., Fairfax, VT, USA) and transferred into the EEG data acquisition hardware (DNR-12 RACKtangle. United Electronic Industries, Inc., Walpole, MA, USA). Grand average ERPs were computed offline using Python 3.6. Data from 50-ms immediately prior to the imperative cue to 1000-ms after the imperative cue were analyzed. ERPs were grouped according to vigilance state staging and only ERPs where the animal was confirmed to be awake by EEG scoring were included in the analysis. ERPs were computed based on recordings obtained during trials with correct responses across each SRLT test. ERP data were analyzed during the SRLT testing at baseline and after sleep restriction.

Data attrition occurred for the EEG/ERP data due to technical recording issues precluding scoring. Thus, the final analysis (SRLT, EEG, ERP) was performed on  $n=10$  and  $13$  in the *ad libitum* and food restricted condition respectively.

### ***Experiment 3: Effect of sleep restriction and food restriction on Progressive Ratio performance***

Rats were trained on the Progressive Ratio (PR) task, prior to assignment to the *ad libitum* ( $n=12$ ) or food restriction group ( $n=12$ ), in a parallel group design. At time of testing, *ad libitum*-fed and food restricted rats weighed  $446.3 \pm 6.8$  g (mean  $\pm$  SEM) and  $388.2 \pm 8.4$  g respectively.

*Progressive Ratio (PR).* PR testing was conducted in standard operant chambers housed in sound and light attenuation chambers (Med Associates, Inc. Fairfax, VT, USA). Two retractable levers were located either side of a recessed magazine where food reward pellets (45-mg TestDiet, Labtabs, Richmond, IN, USA)

were delivered from an automatic pellet dispenser. Data were recorded by in-house programs using the MedPC IV software (Med Associates, Inc., Fairfax, VT, USA). Each daily test session was 30-min long<sup>27</sup>. PR scheduling consisted of presentation of a series of 20 fixed ratio response requirement components, where rats were lever pressing for delivery of a food pellet reward (i.e., the reinforcer). Upon successful completion of a component, response requirements increased according to an exponential function ( $5e^{0.2n}$ )-5, where n is corresponding to the position in the sequence ratio (Figure 1c). A 15-s timeout period was provided between components to allow for consummatory behavior. PR test sessions ended after completion of 20 components or after 30-min, whichever came first. The primary measure of PR performance is the breakpoint, defined as the last ratio i.e., the number of lever pressings completed by the rat to receive the food reward). After the break point the rat stops responding as the requirement to press the lever becomes greater (Fig. 1c). The breakpoint provides an index of the incentive value of the food reward and motivation of the rat to get a reinforcer<sup>31</sup>. Criterion performance was defined as a stable breakpoint for a minimum of three consecutive days, typically achieved within a 2-week training period. After 3-week training, rats were assigned to the *ad libitum*-fed or food restriction group. PR performance was assessed during baseline and after sleep restriction. On the day of sleep restriction, rats were transferred to individual sleep restriction chambers for the 11-h sleep restriction as described in Experiment #2. Data attrition occurred for the PR task due to data acquisition failure (missing data for n=1 and n=4 in the *ad libitum* and food restricted conditions, respectively).

### **Statistical analyses**

Statistical analyses were performed using the SAS software package (version 9.4, SAS Institute, Inc., Cary, NC), except for the PR data which were analyzed using Statistica (version 13.2. Statsoft Ltd, Bedford, UK). All data are presented as mean  $\pm$  SEM.

*Experiment 1.* SRLT parameters were analysed using a mixed model with repeated measures. Feeding condition (i.e., *ad libitum* versus food restriction group) and ‘time of day’ were fixed effects and “subject” was a random effect. A *post-hoc* analysis was applied using a Bonferroni adjustment method. Significance was based on Bonferroni corrected p values.

*Experiment 2.* Outcome variables for SRLT, sleep-wake variables and ERPs were analysed using the mixed effect procedure whereby a repeated measures analysis of covariance was performed. Feeding condition (i.e., *ad libitum* versus food restriction group) and trial day (i.e., baseline, after sleep restriction, recovery) were fixed effects, with minutes of sleep during sleep restriction as covariate. As the study was a crossover design and feeding conditions were administered at different dates, date was also included in the model. A compound symmetry covariance structure was used in the model. Planned comparisons were conducted between feeding condition groups separately on baseline, sleep restriction and recovery days with adjustments made for multiple comparisons. A multivariate mixed effect model was applied to calculate correlation coefficients between the percentage of time spent awake during the SRLT and number of correct trials, premature responses, omissions and median response latency in the presence of repeated measurements within the study design<sup>32</sup>. The experimental group was a covariate, so that the correlations reflected the partial correlation. The model that was successfully fit to the data assumed unstructured variance-covariance structures for both the between-subject and the within-subject matrices. The within-subject correlations between the repeated measures has a Compound Symmetry structure, which assumes that all within-subject correlations between the repeated measures are same. To obtain model to convergence only complete data could be used (n=10). Effect sizes on SRLT parameters were calculated using Hedge’s *g*<sup>33</sup>.

*Experiment 3.* PR performance was analysed using a repeated measures ANOVA factors ‘feeding condition’ (i.e., *ad libitum* versus food restricted), ‘sleep restriction’, and interaction ‘feeding’ x ‘sleep restriction’. Effect sizes on PR performance were calculated using Hedge’s *g*.

## Results

We first assessed the effect of food restriction on several performance parameters in SRLT at six time points across the diurnal cycle (Fig. 2). Number of correct trials did not vary across the diurnal cycle ('Time'  $p=0.249$ ) and was not affected by Food Restriction ('Feeding Condition'  $p=0.199$ ) (Fig. 2a). While the interaction 'Feeding Condition' x 'Time' was significant ( $p=0.010$ ), the *post-hoc* analysis showed no differences in the number of correct trials between the groups at any time of day. Food restriction increased the number of premature responses ('Feeding condition'  $p = 0.018$ ) and these effects varied across the diurnal cycle (interaction 'feeding condition' x 'time':  $p=0.016$ ) with a significant difference between feeding conditions only at ZT11 ( $p = 0.007$ ) (Fig 2c). There was also some indication that food restriction decreased the number of omissions ('Feeding Condition'  $p=0.062$ ; Fig. 2b) and median response latencies made ('Feeding condition':  $p=0.070$ ; Fig. 2d). An effect of time of day was observed for the median response latency ('Time'  $p=0.006$ ), and the number of premature responses ('Time'  $p=0.027$ ) (Fig. 2b-d).

We then assessed the interaction between food restriction and sleep restriction on vigilant attention using the SRLT. The performance of the *ad libitum*-fed group worsened significantly after sleep restriction, with reductions in the number of correct trials and increases in the number of omissions (Fig. 3a-b). Analysis of time on task effects following sleep restriction in *ad libitum*-fed rats showed that the number of correct trials significantly decreased from 20 minutes into the 40-minute session (Fig. 3e). By contrast, the SRLT performance of food restricted rats was not significantly affected by sleep restriction (Fig. 3). After sleep loss, the food restricted group showed significant differences compared to the *ad libitum* group for all parameters. The food restricted group displayed a significantly greater number of correct trials, fewer omissions, more premature responses and faster responses compared to the *ad libitum* group (Table 1; Fig. 3 a-d). The empirical distribution of response times during the SRLT revealed that regardless of sleep

restriction, a higher percentage of faster reaction times was observed in food-restricted rats compared to the *ad libitum*-fed group (Fig. 3f).

We computed effect sizes of food and sleep restriction for SRLT parameters. During baseline (Fig. 4a), a large effect of feeding condition was observed for the premature response parameter, while medium effects were observed for omissions and response latencies. The number of correct trials was less affected by the feeding condition. However, when sleep restriction was applied, the effect sizes of feeding condition were large for all SRLT parameters (Fig. 4b). In food restricted rats, the effects of 11-h sleep restriction were overall small on parameters of trial completion, omissions, and median response latency (Fig. 4c). By contrast, in the *ad libitum*-fed group, trial completion, number of omissions and the median response latency were most responsive to sleep restriction with large effect sizes, while premature responding only displayed a small effect size (Fig. 4d).

To investigate whether there were differences in sleep-wake states and the EEG and whether these differences associated with the differences in SRLT performance described above, we analyzed the sleep/wake recordings. The sleep restriction protocol impacted both groups in a similar manner, with no differences observed in the amount of sleep obtained during the sleep restriction period (Fig. 5a; Table 2). However, during the 40-min SRLT task performed after sleep restriction, food restricted rats maintained significantly more wakefulness than *ad libitum*-fed rats (Fig. 5a-b). In addition, food restricted rats spent more time awake in the period from 30 – 90 min following SRLT testing (Fig. 5b). The spectral composition of the waking EEG was not different between the groups during the SRLT task either at baseline or after sleep restriction (Fig. 5c-d).

We examined the relationship between SRLT parameters and time spent awake during the 40-min SRLT task by computing partial correlations in which the effect of “group” (i.e., Food restricted *versus ad libitum*-fed) was controlled for. The number of correct trials and premature responses showed a linear increase as a function of time spent awake (Fig. 6a,c). The number of omissions and median response

latencies both displayed a significant linear decrease with time spent awake. Thus reduced sleepiness, i.e., overall higher vigilance, is associated with better performance across the two feeding conditions (Fig. 6b,d).

To further explore the effect of food restriction and sleep restriction on attentional processes in the SRLT, we analyzed cue-evoked ERPs only for correct trials. At baseline, the waveforms exhibited two positive components (P1 and P2) and a single negative component (N1). Differences were observed in the ERP amplitude between the two groups, with significantly higher amplitudes in the food restricted rats at 270-292.5 ms and 302.5-310 ms after the onset of the imperative cue (Fig. 7a). Sleep restriction significantly reduced the P2 component of the cue-evoked ERP response in *ad libitum*-fed and food restricted rats compared to baseline (Fig. 7c-d). However, during the SRLT that followed sleep restriction, food restricted rats displayed a significantly larger amplitude in the N1 and P2 components (at 155-175 ms; 215-217.5 ms and 310-355ms respectively) compared to the *ad libitum*-fed group (Fig. 7b).

We then evaluated the effect of food restriction and sleep restriction on the reward value of food using the PR task. The breakpoint, an index of motivation, was significantly greater in food restricted rats compared to the *ad libitum* group both at baseline and after 11-h of sleep restriction (Fig. 8a). Breakpoint was not significantly altered by sleep restriction in either group, and no significant interaction between sleep and food restriction was observed. Effect size estimations showed that the breakpoint was most responsive to the feeding condition, with large effect sizes both at baseline and after sleep restriction (Figure 8b). The size of the effect of sleep restriction was medium in the *ad libitum*-fed group, while it was small in the food restricted group (Fig. 8b).

## Discussion

The present study found that sleep pressure and hunger drive significantly interact to influence behavioral performance in rats and that these effects differ between a simple response latency task and a progressive



ratio task, two tasks which both use food as a reward. Food restricted rats display resilience to the performance decrements following sleep restriction relative to *ad libitum*-fed rats on the simple response latency task, which is often considered a rodent analogue of the human psychomotor vigilance task. These findings have implications for our understanding of the nature of sleep loss induced cognitive deficits and the interpretation of translational research comparing humans and rodents.

***Interaction of feeding status, time of day, sleep debt and task on performance.*** Considering feeding status alone, *ad libitum*-fed animals displayed increased response latencies and decreased premature responding during simple response latency task testing, and decreased breakpoints during progressive ratio test performance. Clearly, less hungry rats are less motivated to perform food-rewarded behavioural tasks. In addition, our analysis of the effects of time of day on performance of the SRLT highlights the need to consider the diurnal phase of assessment of performance, as this may have a significant impact on reaction time and response errors. In many rodent studies evaluating the effects of sleep restriction on performance, testing occurs in the light phase, i.e., the major rest phase<sup>12,23,24,34</sup>. From the perspective of translational validity, it should be borne in mind that this may produce results that are different from those obtained during the active (i.e., dark) phase.

While food restriction improved overall performance in the SRLT and PR tasks, the effects of sleep restriction and its interaction with feeding condition differed between tasks. Following sleep restriction that resulted in a similar magnitude of sleep loss in *ad libitum* and food restricted animals, performance of the SRLT but not the progressive ratio task was impacted. In the SRLT, compared to their pre-sleep restriction baseline, sleep restricted *ad libitum* fed rats exhibited increased numbers of response omissions and a greater “time-on-task” effect where the number of correct trials decreased as a function of session time. By contrast, food-restricted rats were not significantly impaired relative to their pre-sleep restriction performance baseline. This suggests that hunger and the motivation to eat may induce a

functional resilience to the sleep restriction protocol in this test of vigilant attention. It is well accepted that performance of most operant tasks is impaired following sleep-restriction in rats<sup>26,35</sup>. However, the range of effects using either food or water restriction and several different sleep restriction protocols is broad. Our findings are similar to two studies with water-restricted *ad libitum* fed rats exposed to a 24-h acute<sup>22</sup> and chronic intermittent sleep restriction paradigms<sup>23</sup>. Another study using water restriction found significant but less pronounced effects on time-on-task PVT-like measures than would be expected using food reward<sup>24</sup>. Not all studies find that food restricted animals are more resilient to sleep restriction<sup>12,36</sup>, which may critically depend on the performance demands of the task employed. All goal-directed behavioral tasks engage motivational and attentional processes, and while SRLT tasks are most often interpreted in the context of attentional function, performance deficits may also be due to decreases in motivation to respond.

In the PR task, probing the incentive value of food reinforcement, sleep restriction did not affect performance, irrespective of the feeding condition. These results are in accordance with a study employing water restriction throughout a 24-h sleep restriction period, where PR breakpoints also remained unchanged<sup>11</sup>.

### ***Vigilance and attention as mediators of the effects of food restriction.***

While hunger and the motivation to eat may explain in part the functional resilience conferred by food restriction, we further investigated potential mechanisms underlying the protective effect observed in the SRLT after sleep restriction. EEG analyses showed that *ad libitum* fed rats displayed a relatively higher propensity for-sleep in the period subsequent to the task and spent less time awake during the task, compared to food restricted rats. While these effects could imply an effect of food-restriction on build up of homeostatic sleep pressure, as differences were limited to an initial period after the end of the task they more likely represent the relative dominance of the motivation to be awake to eat compared to the

motivation to sleep. Previous EEG analyses of rodent studies have perhaps unsurprisingly shown that rats exhibit more sleepiness during recovery from sleep restriction, compared to baseline condition <sup>22</sup>. Strong physiological links between sleep, wakefulness, hunger and feeding are well described <sup>37,38</sup> and such biology may depend critically upon hypothalamic neuropeptides such as the hypocretins/orexins <sup>39</sup>. The link between arousal and performance capacity was confirmed in the present study by using correlations controlling for the non-specific effect of feeding condition which showed that time awake during the SRLT correlated with the number of correct trials and omissions made. Importantly, performance of an initiated correct trial varied depending on the overall time awake, where increased wakefulness was associated with decreased median response latencies and decreased number of omissions, and more efficient performance. Thus, effects of vigilance state can clearly be observed at the level of individual trial performance within a test session.

Other putative electrophysiological signs of “hunger arousal” were observed in the ERP waveforms synchronized to imperative cues of correct trials. As these trials were completed correctly, the animals were awake and successfully attended to stimuli. It was clear that food restriction and sleep restriction had qualitatively different effects on ERP waveforms. Overall, sleep restriction led to changes in the ERP which can be characterized by a reduced amplitude in the P2 component. Food restriction in non-sleep restricted animals resulted in longer decay phases of the P2 waveform compared to *ad libitum* fed animals. Following sleep restriction, food restricted rats showed larger amplitude of both N1 and P2 components, compared to *ad libitum*-fed animals. As food-restricted animals performed the SRLT test at unimpaired levels following sleep restriction, it is interesting to speculate that the larger P2 amplitude, and enhancement of the N1 component observed in these animals may index the mechanism by which resilience may occur. Increase in the N1 amplitude was previously reported in the context of conditioned learning in a rat psychomotor vigilance task <sup>14</sup>, and has been speculated to be linked to engagement of attentional processes. Our data are also reminiscent of the observed increase of the P2 peak following

the detection of the target tone before a go response in an equiprobable auditory go/no-go task<sup>40</sup>. These and our observations are also consistent with significant increase in P2 amplitude in response to stimulus in the active oddball task compared to the passive paradigm, or in response to target rather than distractor tones in active auditory oddball task<sup>40-43</sup>. All these data imply an association between the P2 component and target detection and active engagement in these tasks<sup>41-43</sup>. The amplitude of P2 was also increased with discrimination in reaction times in oddball task in humans<sup>44</sup>.

Overall these findings suggest that hunger affects attentional processes in the SRLT. This interpretation is strengthened by the observation that PR performance was not affected by sleep restriction in either *ad libitum* or food restricted rats. Thus, it seems reasonable to suggest that the changes in P2 and N1 peak amplitude observed in food restricted animals are likely to be minimally influenced by motivational factors and perhaps result from an increase in attention to the signal. These findings are consistent with the concept of hunger arousal, and resonates with human findings showing heightened attention to food cues in hungry subjects<sup>45</sup>.

**Comparison of human and rodent studies.** Acute total sleep restriction has been reported to increase the reward value of food in humans<sup>46</sup> but whether this is also observed in rats is not well described, and was not supported by the results of the present study. One REM sleep deprivation study in rats has reported a reduction of the reward value of food<sup>35</sup>. Often, no additional incentives are required for humans to perform tasks such as the PVT. However, studies in sleep deprived subjects suggest that secondary reinforcers such as verbal feedback can enhance performance in a choice reaction task<sup>47</sup>. Monetary incentives are also reported to overcome deficits caused by sleep deprivation in human studies<sup>10,48,49</sup>. While nature and availability of the reinforcer may impact task performance, sleep restriction may also differently impact the salience of rewarding stimuli and decision-making processes related to their receipt. For example, following one night of acute sleep deprivation, individuals are willing to make riskier

decisions to optimize gains on gambling tasks <sup>50</sup>. Neuroimaging studies implicate changes in prefrontal cortical and ventral striatal activity and connectivity in sleep restriction induced changes in decision making and emotion regulation <sup>49,51,52</sup>. Further imaging and electrophysiological studies of this nature could determine whether there is a neuropsychological equivalence between food-rewarded studies in rodents and money-rewarded studies in humans.

**Limitations.** There are several limitations to the present study. The first lies in the generalizability of this work. The study only focused on two simple behavioral tasks, the SRLT and progressive ratio task. While both tasks engage motivational and attentional processes, they present different behavioral economics for the animal. Reward value and the amount of effort it takes to receive it are quite different between these two tasks. Therefore, rather than a cognitive account explaining the functional resilience induced by hunger, it may have simply been differences in test economy that were important. Also, other sleep restriction protocols could lead to different effects. A greater range of tasks with differing economies testing a wider spectrum of cognitive domains would help to understand how broadly applicable the present findings are.

**Implications.** It is well documented that sleepiness and cognitive functioning are affected by circadian rhythms and homeostatic pressure in humans <sup>53,54</sup>. It is less certain how sleep loss-induced changes in performance may then be modulated by other homeostatic drives engaging behavior, and whether and how the behavior is rewarded. We have shown in rodents that hunger can induce a functional resilience to sleep restriction, and that a correlate of this resilience can be detected in the ERP signal. Hopefully this work can be used to facilitate greater understanding of the relationships between sleep, sleep loss and cognitive performance in the context of differing motivational demands.

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**Disclosure of statement**

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**Author contribution**

SL performed data collection, was involved in study design, data analysis and interpretation. AMcC, DJD, GG and RWS were involved in study design, data analysis and interpretation. All authors contributed to writing the manuscript.

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## Figure legends

**Figure 1: a) Simple Response Latency Task.** Each testing session lasted 40-min with a sequence of trials. Trials are interspaced with a fixed interval (20-s). The houselight is illuminated to serve as a preparatory cue to trial commencement. After a 5-s variable interval (range 4 – 6-s), the magazine light is illuminated to serve as an imperative cue (i.e., stimulus). A head entry made during the 10-s period when the magazine light is on will result in the delivery of a food pellet (i.e., reward). Head entries made before magazine light onset are recorded as premature responses. A failure to respond to the magazine light within 10-s is recorded as an omission. Both premature responses and omissions are punished with a 5-s time-out period (houselight is off and test chamber in inactivated). **b) Sleep Restriction Experimental Protocol.** Rats were housed under a 12-h:12-h Light (L) – Dark (D) cycle (with lights ON at 08:00). On the baseline day (pre-sleep restriction day), rats underwent either a Simple Response Latency Task (SRLT) or Progressive Ratio (PR) task between Zeitgeber Time (ZT) 3-5. Following the 24-h baseline, sleep restriction commenced at the beginning of the light phase and lasted for 11 hours (ZT0-ZT11). At ZT11, animals were placed immediately into operant boxes for either a 40-min SRLT or a 30-min PR session (“Sleep Restriction day”). Rats were then re-tested in the SRLT during the recovery period (post-sleep restriction day) between ZT 3-5. **c) Progressive Ratio Task.** Following a 20-s lights off period the houselight is illuminated, the lever is presented and activated and the fixed ratio component started (the fixed ratio component is an exponential rate of lever presses required to achieve a food pellet reward). On completion of each component a food pellet is delivered and the house light goes off. This is followed by a 15-s time-out period prior to presentation of the next component.

**Figure 2. Effect of time of day on SRLT performance in food restricted and *ad libitum* fed rats.** a) Number of correct trials, b) number of omissions, c) Number of premature responses, d) median response latency assessed a 6 time points across the 24-h light-dark cycle (data are double plotted). The x-axis depicts

Zeitgeber time (ZT) at which SRLT was performed, rats were tested 3 times for each time point (*ad libitum* fed rats – blue circles (n=16); food restricted rats – red circles (n=16)). Data (mean  $\pm$  SEM) are shown as an overall average of the 3 test times. Asterisks refer to comparison between feeding conditions where \*p < 0.05; \*\*p < 0.01; \*\*\*p < 0.001. Dark bars along the x-axis indicate the 12-h dark (“lights off”) period, light bars along the x-axis indicate the 12-h light (“lights on”) period.

**Figure 3: Effects of feeding status and sleep restriction on Simple Response Latency Task performance.**

a) Number of correct trials; b) number of omissions; c) number of premature responses, (d) median response latencies, on baseline day, following 11-h sleep restriction and recovery after sleep restriction. Red and blue lines refer respectively to food restricted (n = 13) and *ad libitum* fed rats (n = 10). Asterisks refer to planned comparisons of feeding regimen conditions within a test session, where \*p < 0.05; \*\*p < 0.01; \*\*\*p < 0.001. Carots (^) refer to planned comparisons between baseline and after sleep restriction within test sessions, where ^ p < 0.05; ^^ p < 0.01; ^^^ p < 0.001; Hashes (#) refer to planned comparisons between task after sleep restriction and recovery within test sessions, where # p < 0.05; ## p < 0.01; ### p < 0.001. e) Time on Task for number of trials across the 40-min SRLT time course (after sleep restriction: solid symbols, baseline: open symbols). Asterisks refer to planned comparisons of feeding regimen conditions after sleep restriction by 10-min time intervals, where \*p < 0.05; \*\*p < 0.01; \*\*\*p < 0.001. f) Distribution of response latencies during the SRLT following 11-h of sleep-restriction and at baseline. Data are represented as a percentage of total response latencies per 250-ms time bin, ranging from 250-ms to 10,000-ms during 40-min SRLT for *ad libitum* food (ALF) rats (blue circles, n = 10) and restricted food (RF) rats (red triangles, n = 13). Data during 40-min SRLT on baseline day are shown as dotted lines for *ad libitum* (ALF) fed rats (blue open circles, n = 10) and restricted food (RF) rats (red open triangles, n = 13).

**Figure 4: Hedge's g effect sizes of SRLT parameters.** Hedge's g effect sizes were computed for absolute numbers of correct trials, number of omissions, number of premature responses and response latencies. a) Effect of food regimen without sleep restriction (i.e., during baseline). Effect sizes are presented as the absolute value of *ad libitum* minus restricted food groups; b) Food regimen effect in combination with sleep restriction. Effect sizes are presented as the absolute value of *ad libitum* minus restricted food groups; c). Sleep restriction effect in food restricted rats (n=13). Effect sizes are presented as the absolute value of sleep restriction minus baseline groups; d). Sleep restriction effect in *ad libitum* fed rats (n = 10). Effect sizes are presented as the absolute value of sleep restriction minus baseline groups. Green color indicates better performance in the SRLT parameter, while red indicates poorer performance. Dashed lines show small ( $\geq 0.2$ ), medium ( $\geq 0.5$ ) or large ( $\geq 0.8$ ) effect sizes<sup>33</sup>. ALF: *ad libitum*-fed; RF: restricted food; SR: sleep restriction.

**Figure 5: a) Time-course of percentage of time spent awake before, during and after the 11-h sleep restriction.** The dark grey box above the first light period represents SRLT testing on baseline days, the white and light grey box on the top of the second light period illustrates the 11-sleep restriction period followed by the 40-min Simple Response Latency Task (SRLT). *Ad libitum* fed group (blue circles, n = 10) and restricted food group (red circles, n = 13). **b) Time course of wakefulness following the 11-h sleep restriction.** 5-h time course, encompassing the 40-min Serial Reaction Latency Task, immediately following the 11-h sleep restriction for the *ad libitum* fed (blue circles, n = 10) and food restricted (red circles, n = 13) groups. Asterisks refer to comparison between feeding conditions, where  $*p < 0.05$ . **c) EEG spectral power during Waking in food restricted and *ad libitum*-fed male Wistar rats during a Simple Response Latency Task on baseline day.** EEG spectral power was normalized relative to total power; mean  $\pm$  SEM. Frequency bands are labelled and separated by vertical dotted lines. Blue: *ad libitum* fed rats (n = 10), food restricted rats in red (n=13). **d) Wake EEG spectral power in Food Restricted and *Ad Libitum***

**fed male Wistar rats during a Simple Response Latency Task after sleep restriction.** Spectral power data following 11-h sleep restriction were normalized relative to total power, showing the mean and standard error of each group. Frequency bands are labelled and separated by vertical dotted lines. The EEG signals were recorded as the differential between the left frontal and right occipital electrodes. *Ad libitum* fed rats in blue (n = 10), food restricted rats in red (n=13).

**Figure 6: Correlation coefficients between the time spent awake and performance parameters during the Simple Response Latency Task (SRLT).** After controlling for the effects of the experimental groups (i.e., feeding conditions), residuals were plotted for a) Number of correct trials, b) Number of omissions, c) Number of premature responses, and d) Median response latencies, against the residuals for time spent awake during the SRLT. *Ad libitum*-fed rats (blue circles, n=10) and food restricted rats (red circles, n = 10). The reported correlation is the correlation between endpoints on the same subject in the same week.

**Figure 7. Effect of food restricted and *ad libitum* feeding on event-related potentials to the magazine light (imperative cue) during a Simple Response Latency Task.** a) Effect of feeding status on event related potentials in response to the magazine light on baseline day. b) ERP amplitude during SRLT in the *ad libitum*-fed rats at baseline and following 11-h sleep restriction. c) ERP amplitude during SRLT in the food restricted group at baseline and following 11-h sleep restriction. (d) Effect of sleep restriction on the amplitude of ERPs. The EEG signals were recorded as the differential between the left frontal and right occipital electrodes. *Ad libitum* fed rats in blue (n = 10), food restricted rats in red (n=13). Asterisks refer to comparison between feeding conditions (Fig. 7a-b) and between sleep restriction and baseline (Fig. 7c-d), where \*p < 0.05.

**Figure 8. Effect of feeding regimen and sleep restriction on performance in the Progressive Ratio task.**

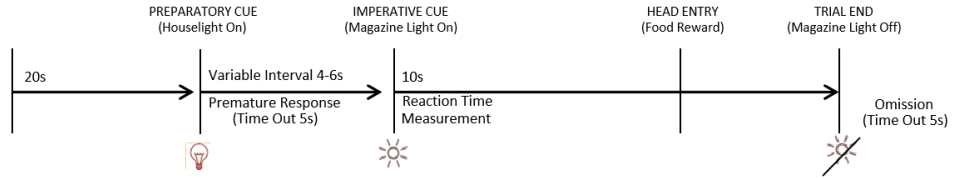
a) Breakpoint (i.e. the maximum fixed ratio of responding the animal will express to gain a single food reward) reached by restricted food rats (red) and *ad libitum* food rats (blue) under baseline condition (BL) or following an 11-h sleep restriction (SR). b) Hedge's *g* effect size of feeding condition and sleep restriction computed for the breakpoint during the PR task; ALF: *ad libitum*-fed; FR: restricted food, BL: Baseline; SR: 11-h sleep restriction (n = 7-11). Green color indicates better performance in the SRLT parameter, while red indicates poorer performance. Effect sizes are highlighted as small (0.2), medium (0.5) or large (0.8).

**Table 1: Effects of feeding condition and 11-h sleep restriction on SRLT parameters in adult male Wistar rats.** DF, F and p-values are shown for each parameter for each condition, food restriction, sleep restriction and their interaction.

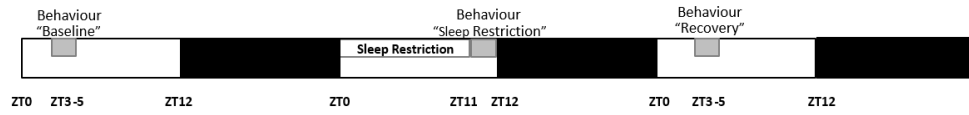
**Table 2: Sleep and locomotor activity during the 11-h sleep restriction in adult male Wistar rats.** Data are shown as mean  $\pm$  SEM. Food restricted rats (n = 13), *Ad Libitum* rats (n=10).

**Figure 1**

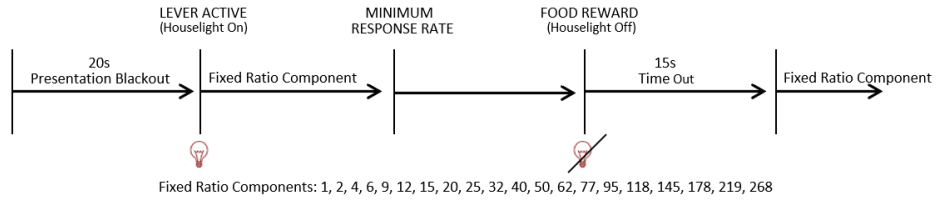
**a Simple Response Latency Task (SRLT)**



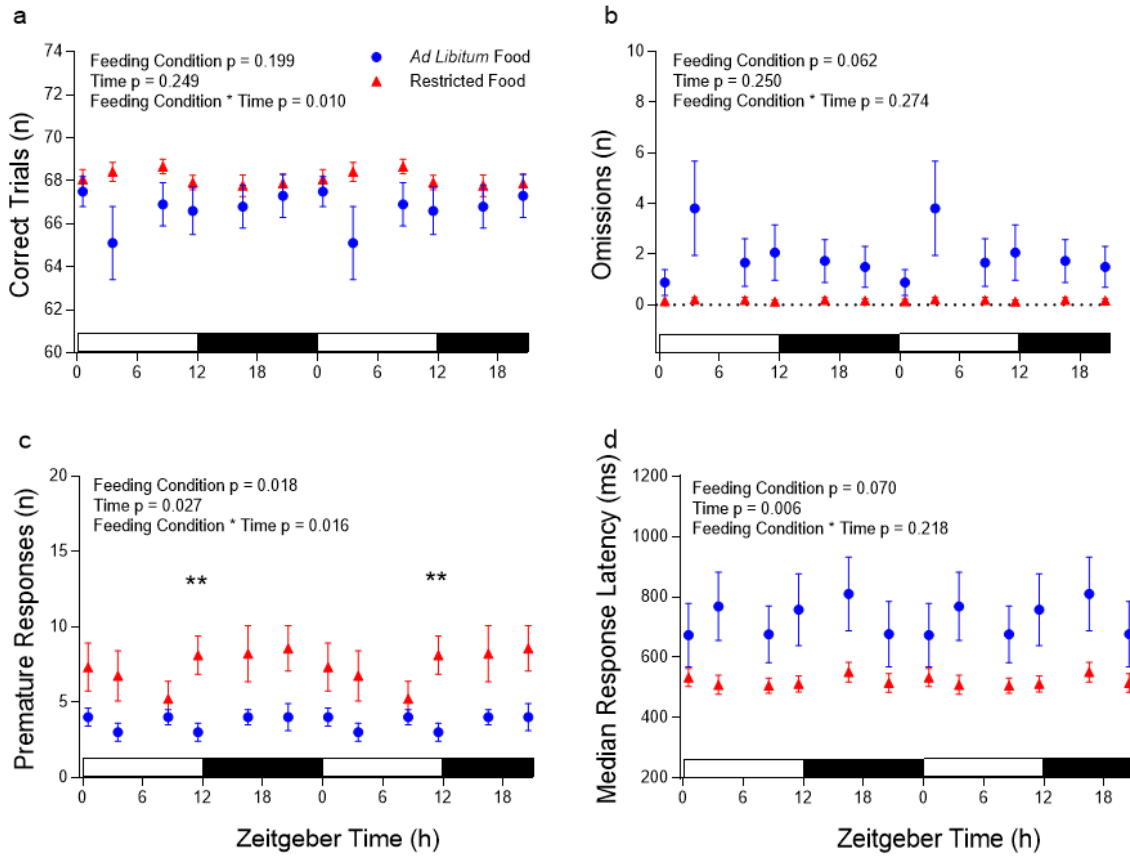
**b Experimental design**



**c Progressive Ratio task**

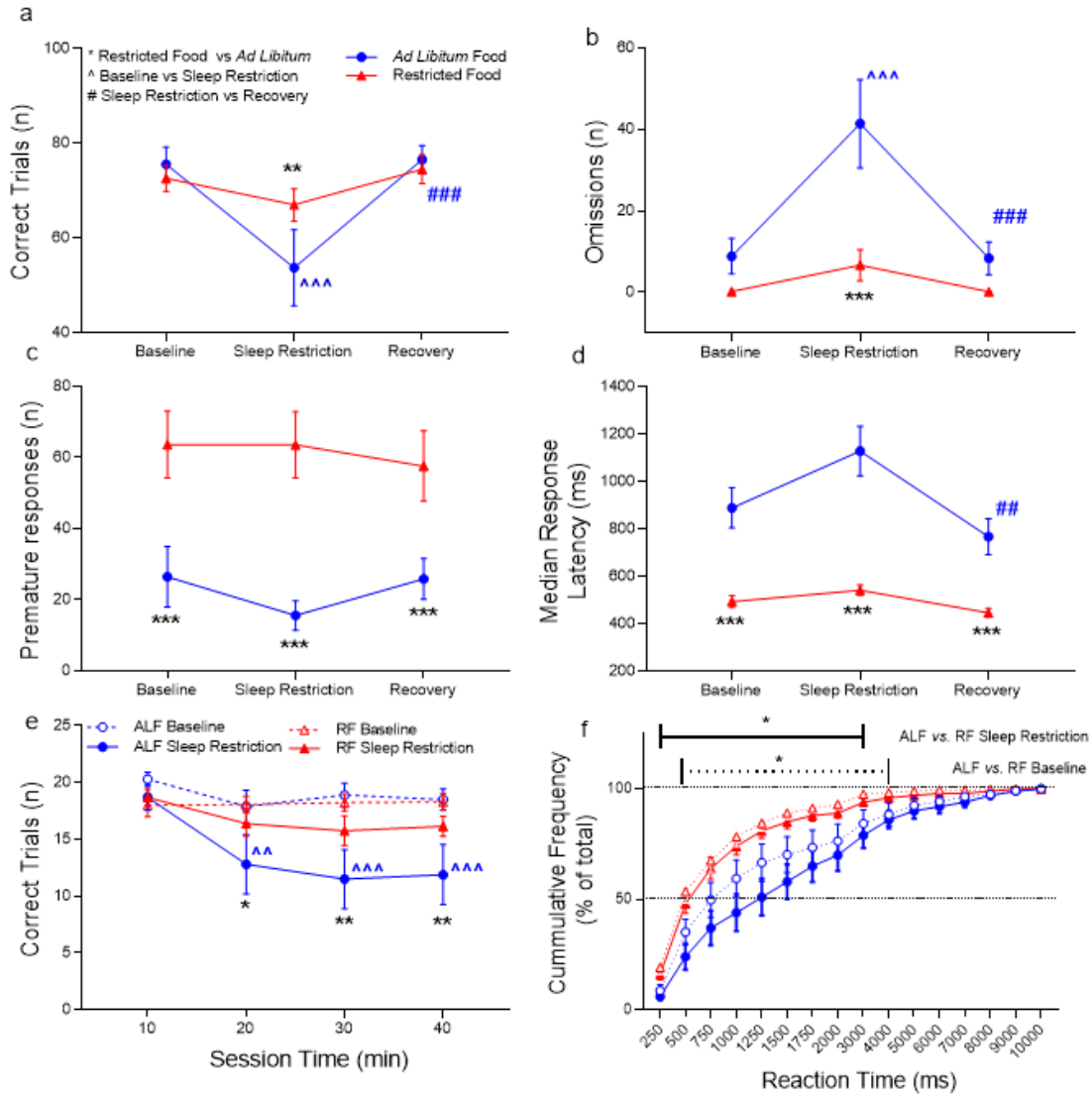


**Figure 2**





**Figure 3**



**Figure 4**

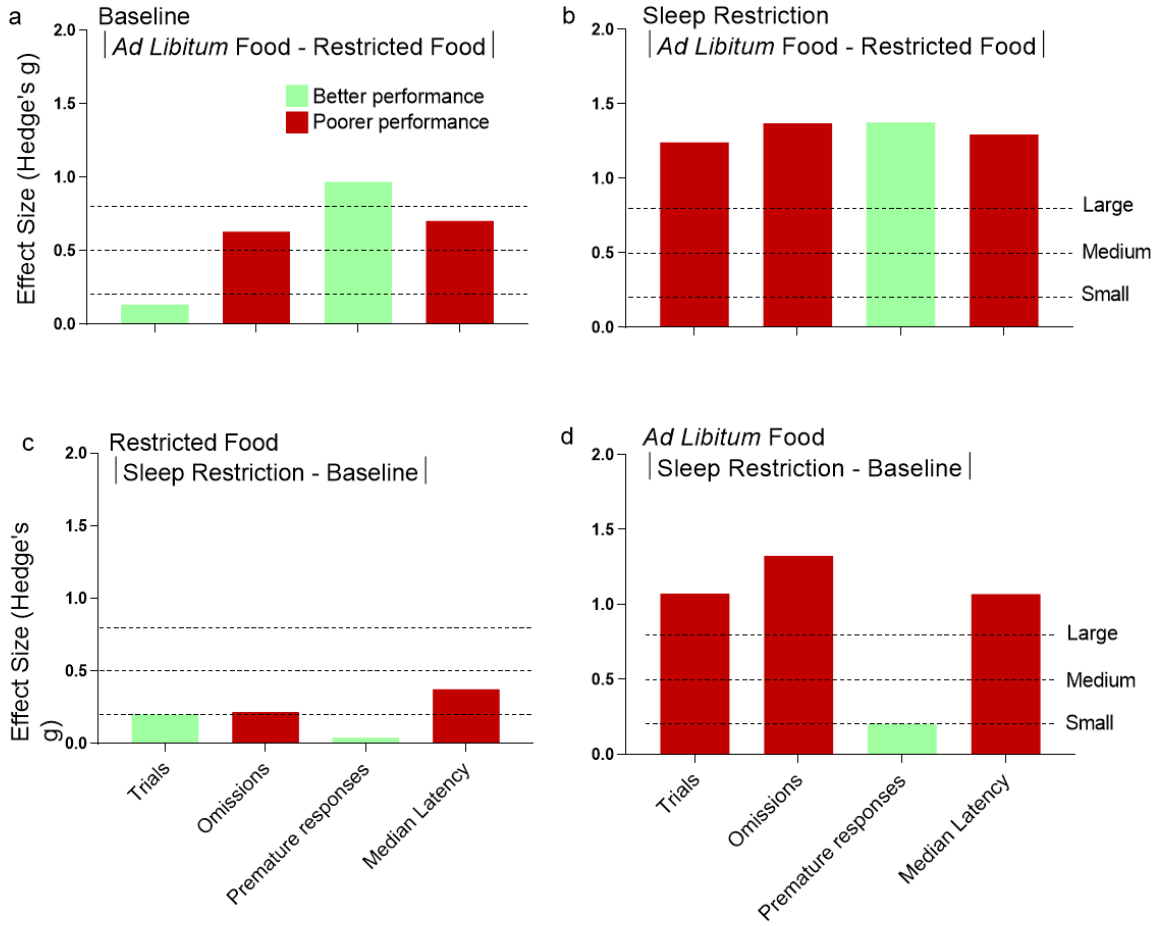


Figure 5

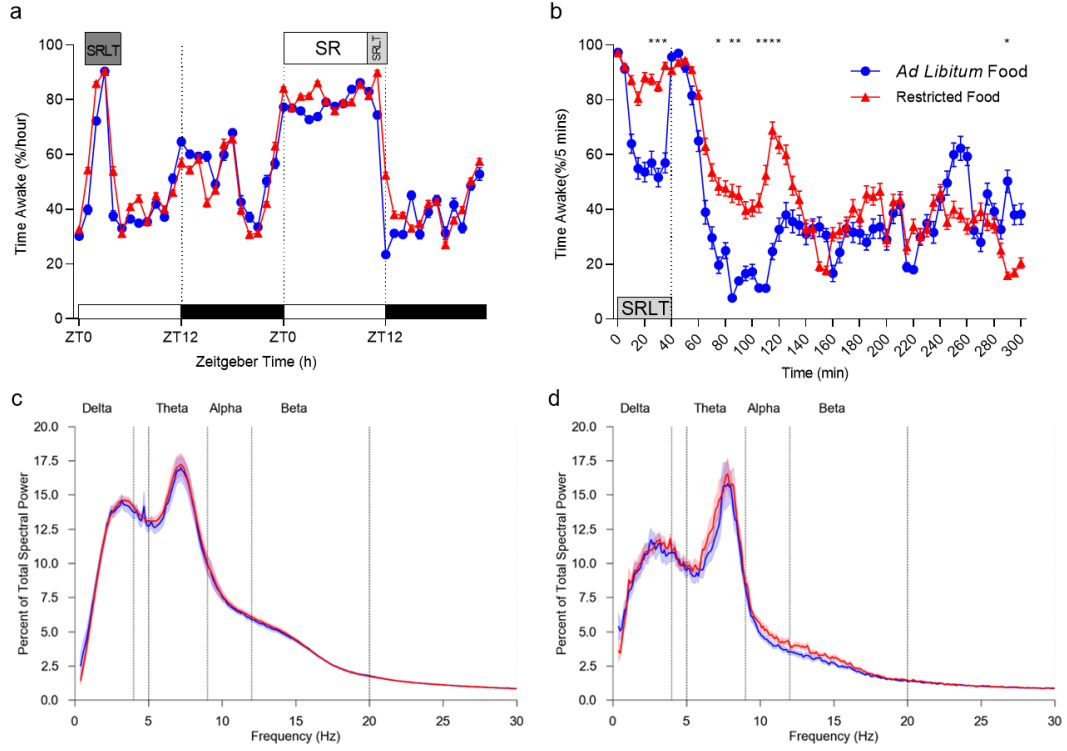


Figure 6

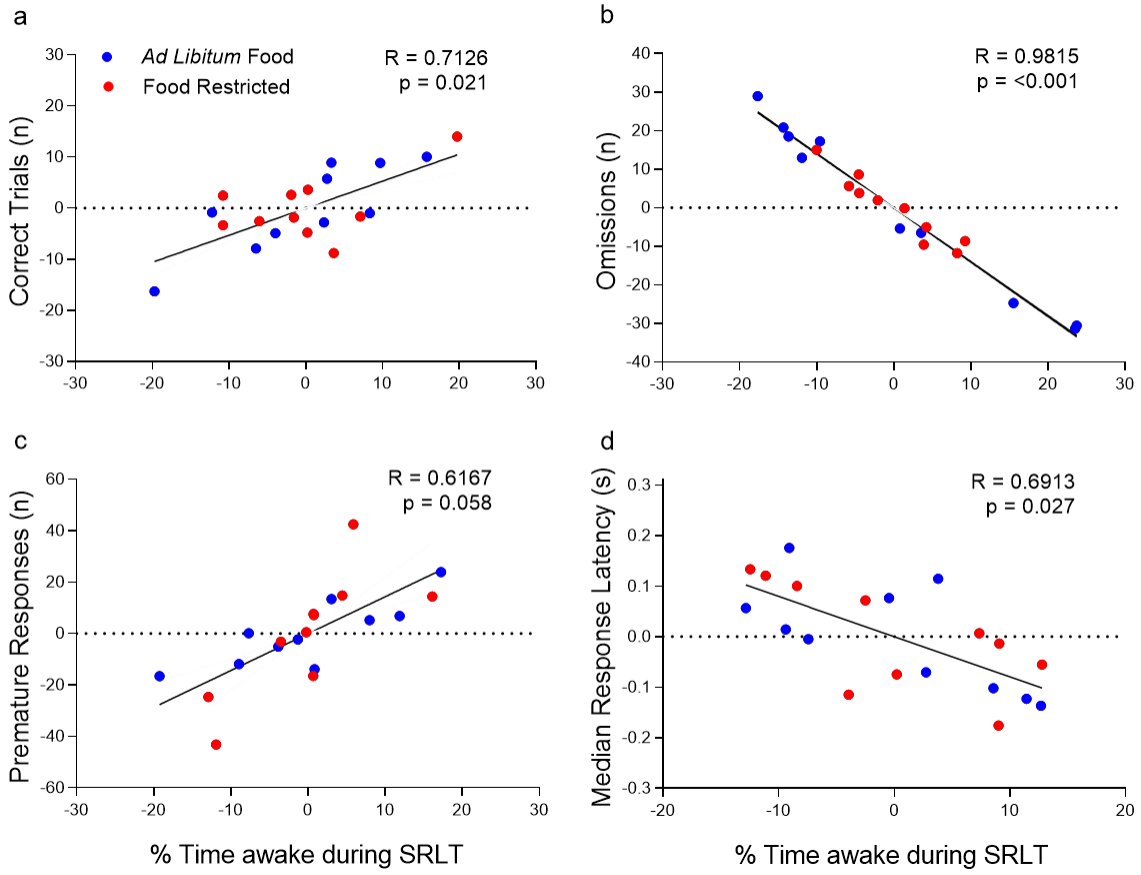


Figure 7

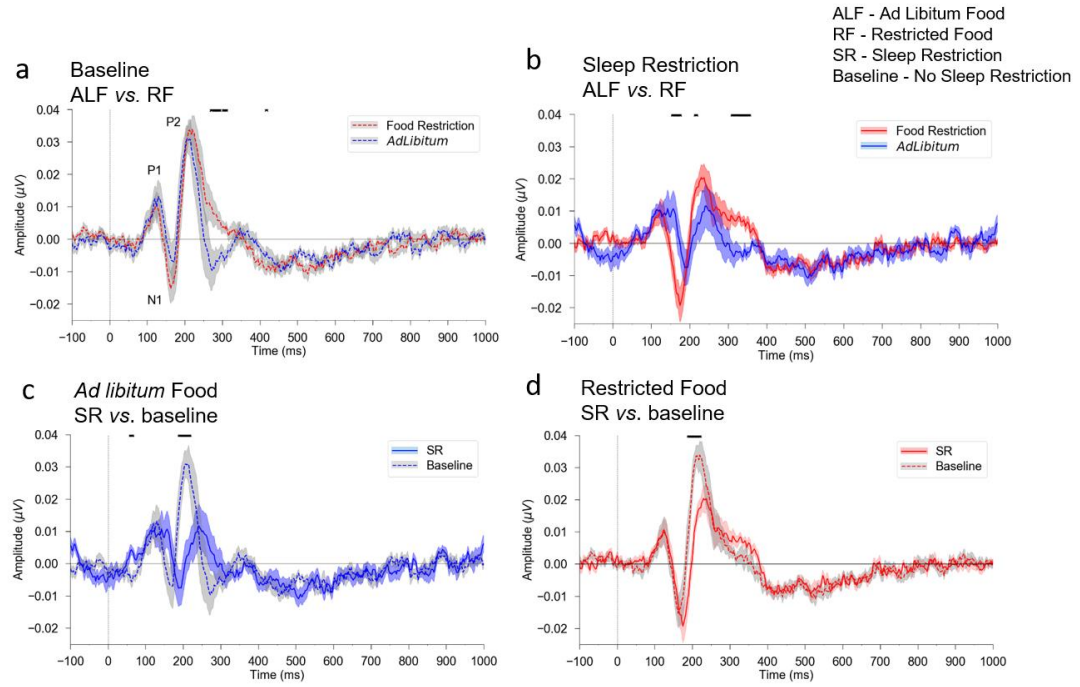
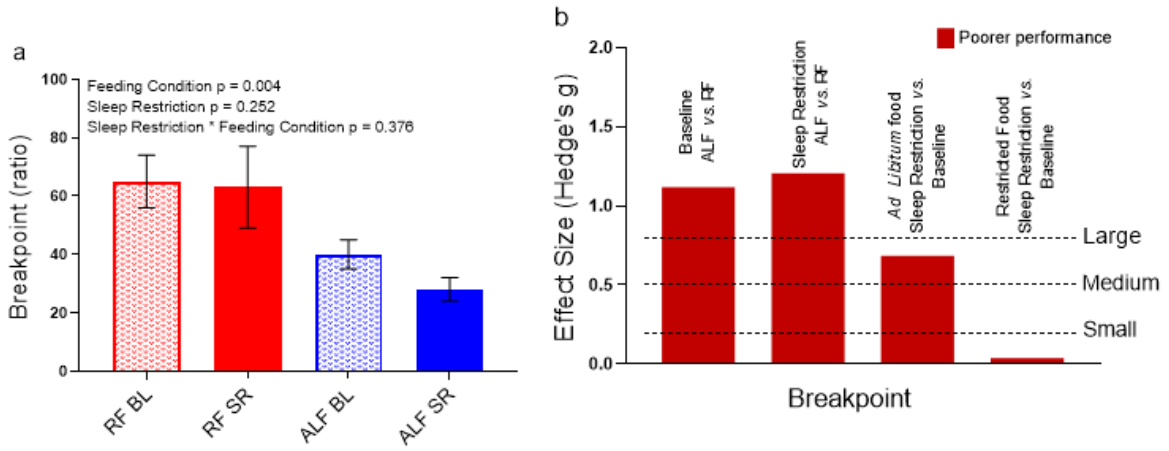


Figure 8



**Table 1**

|                            | <b>Food Restriction</b>     | <b>Sleep Restriction</b>    | <b>Food x Sleep Restriction</b> |
|----------------------------|-----------------------------|-----------------------------|---------------------------------|
| <b>Trials</b>              | 53.6, 1.55, $p = 0.218$     | 50.6, 15.05, $p \leq 0.001$ | 50.6, 4.46, $p = 0.016$         |
| <b>Omissions</b>           | 55.8, 26.86, $p \leq 0.001$ | 51.1, 15.60, $p \leq 0.001$ | 51.1, 6.98, $p = 0.002$         |
| <b>Premature Responses</b> | 52.0, 78.73, $p \leq 0.001$ | 50.2, 0.58, $p = 0.562$     | 50.2, 1.31, $p = 0.279$         |
| <b>Mag Latency</b>         | 52.1, 60.43, $p \leq 0.001$ | 50.5, 5.08, $p = 0.010$     | 50.5, 0.61, $p = 0.548$         |

**Table 2**

| <b>Parameter</b>                   | <b>Food Restricted (n = 13)</b> | <b><i>Ad Libitum</i> (n = 10)</b> | <b>Significance</b> |
|------------------------------------|---------------------------------|-----------------------------------|---------------------|
| <b>Total Sleep (min)</b>           | 125 ± 9                         | 141 ± 17                          | 0.949               |
| <b>NREM sleep (min)</b>            | 124 ± 10                        | 140 ± 15                          | 0.823               |
| <b>REM Sleep (min)</b>             | 0.7 ± 0.3                       | 0.3 ± 0.1                         | 0.219               |
| <b>Locomotor Activity (counts)</b> | 971 ± 8.5                       | 963 ± 11.1                        | 0.920               |