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SHORT REPORT

Sleep Disruption Impairs Sustained Attention in Food-Restricted Rats Using a Food-Reinforced Rodent Psychomotor Vigilance Test

Catherine M Davis[1–3], Victoria Elizabeth Elliott[1,2], Joan Smith[2]

[1]Department of Pharmacology and Molecular Therapeutics, Uniformed Services University of the Health Sciences, Bethesda, MD, USA; [2]Armed Forces Radiobiology Research Institute, Bethesda, MD, USA; [3]Johns Hopkins University School of Medicine, Baltimore, MD, USA

Correspondence: Catherine M Davis, Department of Pharmacology and Molecular Therapeutics, Uniformed Services University of the Health Sciences, 4301 Jones Bridge Road, Bethesda, MD, 20814, USA, Email catherine.davis-takacs@usuhs.edu

Purpose: Sleep disruption (SD) impairs sustained attention, and impairment is quantified with the psychomotor vigilance test (PVT) in humans. In rats, food restriction attenuates SD's effects on sustained attention, limiting translation of rodent vigilance tests. The goal of the current study was to determine if a rodent PVT (rPVT) requiring high baseline performance using food restriction and reinforcement is sensitive to the effects of SD.

Methods: Male Long-Evans rats (n=4) were trained on the rPVT using food reinforcement. Once baseline acquisition criteria were achieved, rats experienced acute SD using an automated sweep bar that moved across the home cage. Rats were tested in the rPVT the day following SD to assess performance-impairing effects.

Results: SD significantly increased lapses, and this effect was specific to shorter response-stimulus intervals. Decreased percent correct responses and increased slow reaction times were also found. These data suggest that many of the performance-impairing effects of SD are not attenuated by food restriction in this procedure.

Conclusion: The rPVT is sensitive to the performance impairing effects of SD in food restricted rats, a common methodology used to train and maintain performance on operant behavioral tests. Thus, food restriction does not appear to attenuate the effects of SD in all attention-related behavioral procedures.

Keywords: food restriction, vigilance, operant behavior

Introduction

Sleep loss reduces sustained attention, degrades cognition, and leads to functional impairment.[1] The human psychomotor vigilance test (PVT) is the "gold standard" objective measure for assessing sleep loss-induced changes in sustained attention, psychomotor speed, impulsivity, and state stability.[2] Rodent versions of the PVT (rPVT) have been developed.[3–12] These tests use water restriction and reinforcement or food reinforcement in ad libitum fed rats to assess SD's performance-impairing effects, given that food restriction can attenuate the effects of SD.[12] More specifically, using the simple response latency task (SRLT), food restriction, but not ad libitum feeding, attenuated the behavioral and physiological effects of SD in rats. Importantly, differences in baseline SRLT performance between feeding conditions were apparent, and could affect this test's sensitivity to SD. We have designed a version of the rPVT using food restriction and reinforcement that requires high baseline levels of performance,[6,7,11] and previously reported robust response-stimulus interval (RSI) and time-on-task effects where healthy, non-SD rats displayed predictable changes in performance.[11] The goal of the current study was to determine if the rPVT is sensitive to the performance-impairing effects of SD, given that food restriction and reinforcement are common methodologies for operant behavior tests in rodents.

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Materials and Methods

Subjects and Apparatus

Animal care was conducted according to Public Health Service (PHS) Policy and the Institutional Animal Care and Use Committee of the Johns Hopkins University and Uniformed University of the Health Sciences approved all procedures. Both programs are accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care. Male Long-Evans rats (n=4, Envigo, East Millstone, NJ) were used as subjects because they are a common strain used in operant behavioral testing and readily learn reinforcement contingencies. Rats were received at approximately 10–12 weeks of age, singly housed in plastic cages with enrichment toys, maintained on a 12:12 h light-dark schedule (lights on at 0600, Zeitgeber time [ZT] ZT0), and at an ambient temperature of 23°C. Rats were maintained at 90% of their free-feeding weights by being fed measured amounts of chow each day after the rPVT session. Water was freely available in the home cage. Rats were run in 30-min sessions at the same time each day (1100; ZT5). Operant chambers contained one nose-poke key, cue and house lights, a food cup for delivery of pellets, and were enclosed in sound-attenuating cubicles equipped with exhaust fans (Med Associates, Burlington, VT). MedPC® IV programs controlled experimental contingencies; all data was recorded on a trial-by-trial basis.

Rodent Psychomotor Vigilance Test (rPVT)

Sessions began with the onset of the house light. After a variable delay of 3–10 sec, the nose-poke key was illuminated. Correct responses were defined as responses on the nose poke key within 2 sec after light onset (ie, 2-sec limited hold, LH) and were reinforced with a pellet. Responses prior to light onset (premature responses) were not reinforced and punished with an 8 sec time out; responses after the 2-sec interval had elapsed or no response (omission) had no programmed consequences. The variable delay for the next trial began after a 1-sec inter-trial interval, timed after the response or the end of the 2-sec LH, whichever occurred first. The number of correct responses, premature responses, omissions, and lapses in responding (omissions plus correct responses greater than twice each rat's mean response latency for that session) were collected. Summary measures were expressed as total numbers and percentages (for details, see Supplementary Methods). Response latencies were recorded in milliseconds and used to calculate reaction times. Each performance measure was also calculated based on the response stimulus interval or time-on-task (ie, performances binned into 6-minute intervals across the 30-min session time). Criteria for inclusion in this study were $\geq$75% percent correct and $\leq$ 25% premature responses for four out of the five daily test sessions during one to two weeks prior to any manipulation.

Acute Sleep Disruption

Rats were moved from their home cages to sleep fragmentation chambers (Model #80391, LaFayette Instrument, LaFayette, Indiana); standard home cages containing an automated sweep bar that moves across the chamber. Sweep duration was set to 'continuous', where the bar traverses the chamber once every 7.5 seconds. Rats were housed in these chambers for two days with the sweep bar stationary as baseline days to ensure that housing alone in this chamber did not impair rPVT performance. Sweep bars were turned on starting at lights off (1800; ZT12) and were run continuously throughout this period and the beginning of the lights on period of the next day until each subject was moved to their rPVT chamber for testing (ZT5). Rats were then returned to standard home cage housing. Two bouts of SD were completed, separated by approximately one month, and the two bouts were averaged together for data analysis. Rats have a polyphasic/fragmented sleep pattern that results in 12–15 hrs of sleep per day, occurring in sleep bouts averaging 6–14 min,[13] and the sweep duration described above likely resulted in few, if any, undisturbed sleep bouts.

Data Analysis

Paired permutation tests were used to assess the effects of SD on the number of lapses, percent correct responses, median reaction time, mean reaction time, mean speed, Q90 reaction times, hits, long RT hits, and omissions, in addition to changes in these parameters based on response-stimulus interval or time-on-task. The paired permutation test has no assumptions about normality, homoscedasticity, or the shape of the distribution. Statistical analyses were completed using

Microsoft Excel. Alpha was set to 0.0625 for significant effects (one-tailed) because this value represented the percentage cut-off where the data from this experiment were the most extreme values in the distribution.

Results

Acute SD significantly increased the number of lapses compared to baseline (Figure 1A; see Figure 1B for mean of differences, a measure of effect size), in addition to the percentage of lapses emitted at shorter response-stimulus intervals (3–8 seconds in duration; Figure 1C). For time on task, SD increased the percentage of lapses starting at 10-min into the rPVT session, and lapses remained elevated for the remainder of the session (Figure 1D). SD significantly decreased percent correct responses (Figure 2A and B depicts mean of differences), and significantly increased Q90 reaction times (Figure 2C and D depicts mean of differences). Animals emitted a high number of correct responses ("hits") after SD, even though it was significantly decreased from baseline (Figure 3A and B depicts mean of differences). SD significantly increased omissions (Figure 3E and F depicts mean of differences) and trended to increase correct trials with long response times (Figure 3C and D depicts mean of differences), but this change did not reach statistical significance (p=0.125; these data were the most extreme permutation in this distribution, however). SD decreased percent correct responses at specific RSIs with fewer correct trials at the shortest RSIs (3–7; Figure S1A; no effects at other RSIs, S1B–E), and decreased percent correct responses as the session progressed starting at 10-min into the session (Figure S2A, but not Q90 RT, Figure S2B). Thus, animals continued to perform at high levels even after

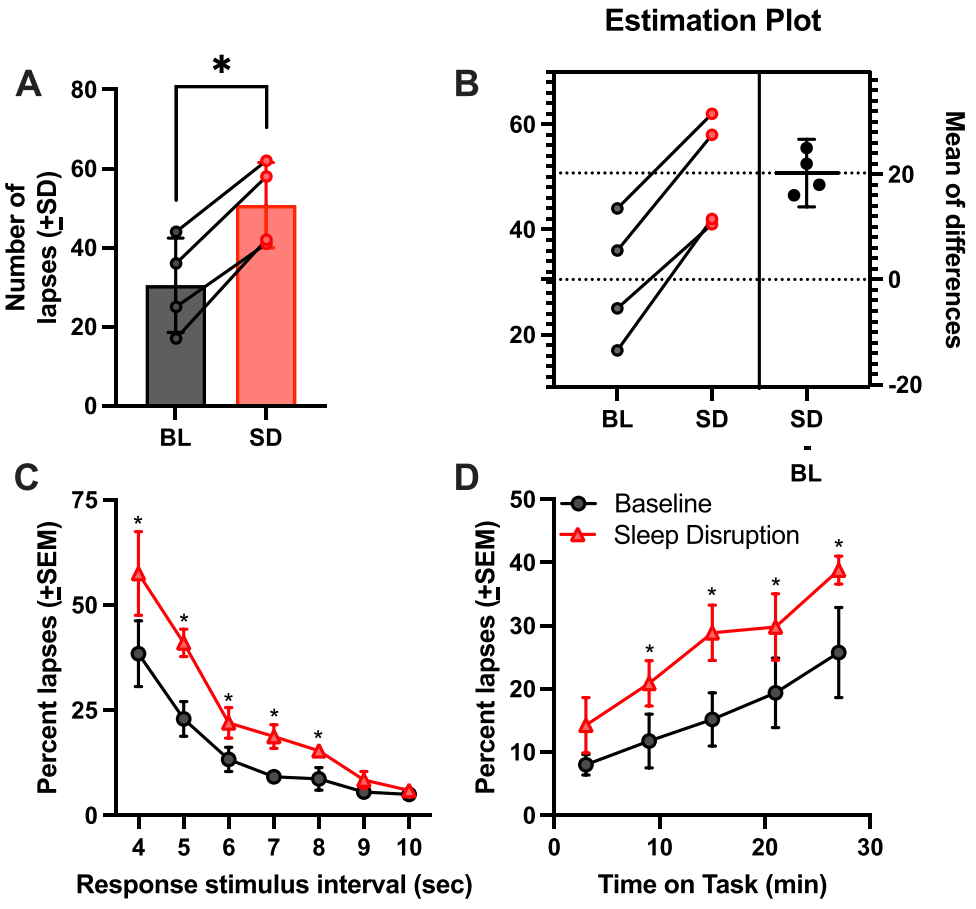

Figure 1 Sleep disruption increases the number of lapses emitted on the rPVT in food-restricted rats when responding is reinforced with food pellets. (**A**) Mean number of lapses increased following acute sleep disruption (SD) compared to baseline (BL). (**B**) Estimation plot showing mean of differences (effect size). Error bars indicates 95% CI of the mean difference. (**C**) SD significantly increased lapses at response stimulus intervals between 3–8 seconds. RSI 4 = RSIs from 3–4 sec, RSI 5 = RSIs from 4.1–5 sec, etc. (**D**) The percentage of lapses emitted increases across time on task starting at 10-min into the 30-min session. *p<0.0625 via paired permutation tests. Symbols represent individual animals and lines connect their performances at BL to those following SD.

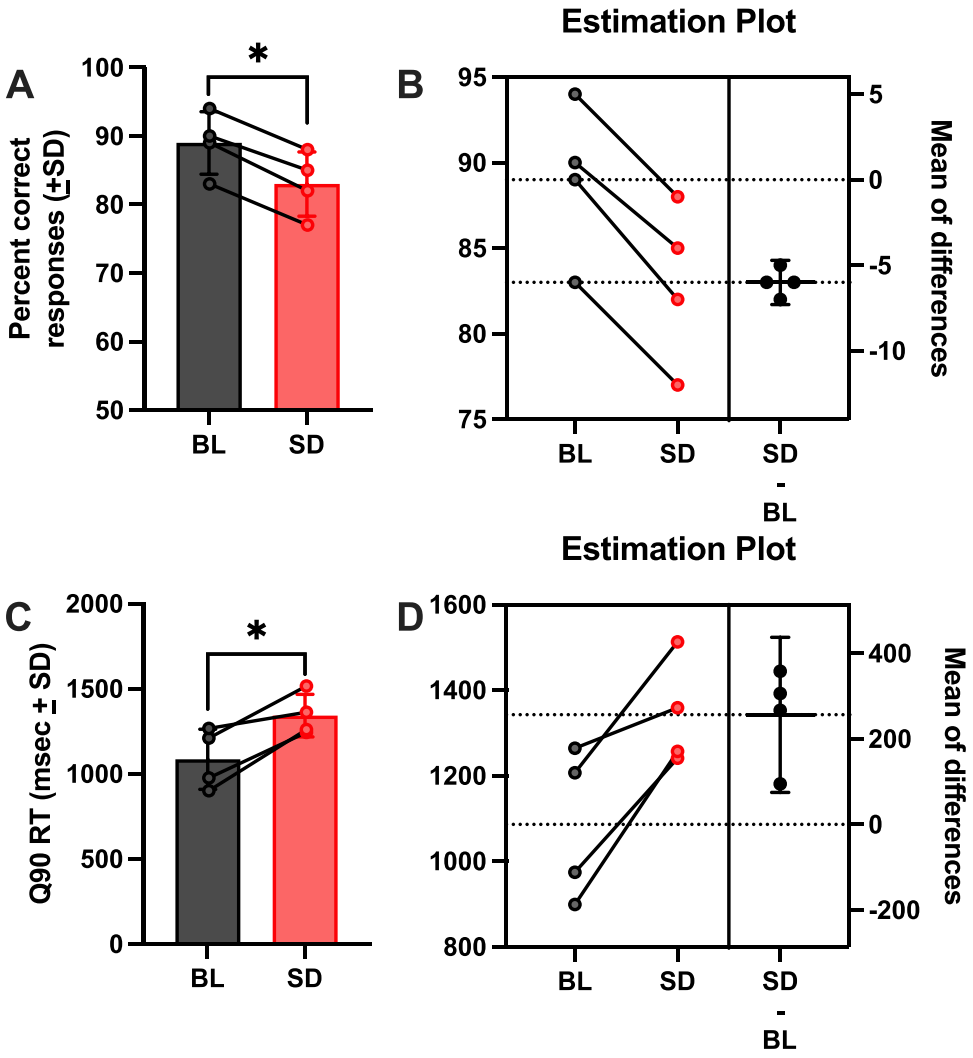

Figure 2 Sleep disruption decreases percent correct responding and increases the slowest 10th percentile reaction times (Q90). (**A**) Percent correct responses were significantly decreased following sleep disruption (SD) when compared to baseline (BL), however, rats still maintained ≥ 75% correct responding following sleep disruption. (**B**) Estimation plot showing mean of differences (effect size) and 95% CI for percent correct responses. (**C**) Sleep disruption significantly increased Q90 reaction times, with an average increase of ~300 msec. (**D**) Estimation plot showing mean of differences (effect size) and 95% CI for Q90 RTs. *p<0.0625 via paired permutation tests. Symbols represent individual animals and lines connect their performances at BL to those following SD.

SD, which suggests rats did not fall asleep during the rPVT session. SD did not affect mean or median reaction times, mean speed, Q10 reactions times, or premature responses (data not shown).

Discussion

We found that SD impaired sustained attention in food-restricted rats performing a food-reinforced rPVT. Specifically, SD significantly increased the number of lapses emitted, and these effects were more robust at shorter RSIs, with no change in emitted lapses at long RSIs between baseline and SD. The most lapses were emitted at the two shortest RSIs, which was accompanied by a significant decrease in percent correct responses, suggesting that lapses following SD during these short RSIs were primarily omissions. SD also slowed the slowest reaction times (Q90 RTs). Thus, following SD, rats had more difficulty responding to quickly occurring stimuli. Further, these results support the translational nature of the rPVT, with the number of lapses increasing following SD in a manner similar to humans experiencing SD[2]. Thus, these data demonstrate that the food-reinforced rPVT in food-restricted rats is sensitive to SD's performance-impairing effects.

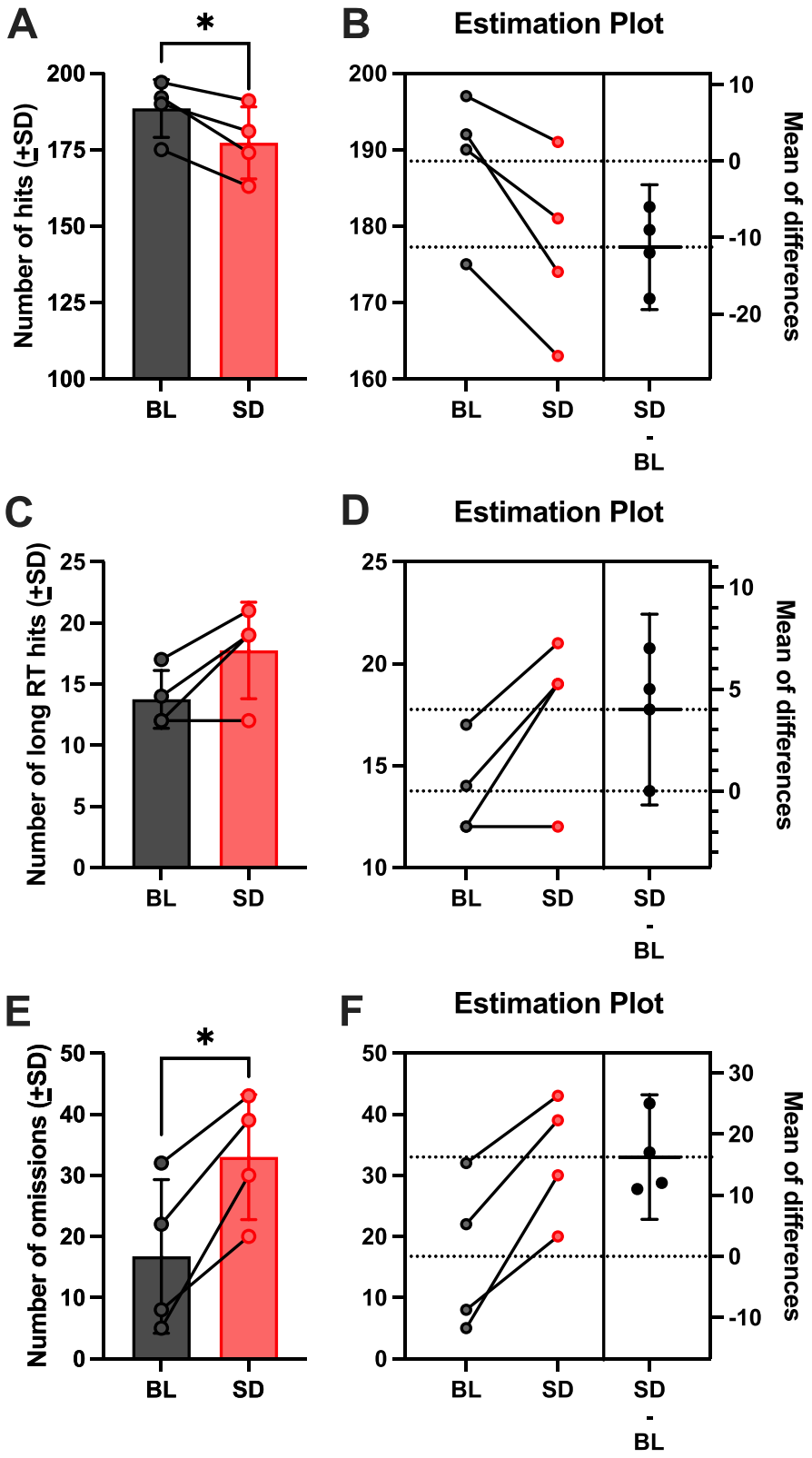

Figure 3 Sleep disruption increases slow response trials and omissions on the rPVT, which leads to a decreased number of hits. (**A**) Mean total hits (correct trials) on the rPVT significantly decreased following sleep disruption (SD) compared to baseline (BL), even though the total number of hits was still high. (**B**) Estimation plot showing mean of differences (effect size) and 95% CI for number of hits. (**C**) Sleep disruption trended to increase 'long hit trials', where animals took twice their mean reaction time to respond correctly (one trial type included in lapses). (**D**) Estimation plot showing mean of differences and 95% CI for number of long hits. (**E**) SD significantly increased the number of omissions (the second trial type included in lapses). (**F**) Estimation plot showing mean of differences (effect size) and 95% CI for number of omissions. *p<0.0625 via paired permutation tests. Symbols represent individual animals and lines connect their performances at BL to those following SD.

Loomis et al[12] recently reported that food restriction results in functional resilience to SD when compared to ad libitum feeding using a simple response latency task (SRLT), where rats respond to a randomly occurring light stimulus. The rPVT and SRLT are similar, but there are important methodological considerations that likely increased sensitivity of the rPVT to SD in food-restricted rats. First, baseline performance on the rPVT was high, with rats displaying an average of 89% correct responding (Figure 2A). In contrast, food-restricted rats performing the SRLT emitted significantly greater premature responses than subjects under ad libitum conditions, even though both groups emitted similar numbers of correct trials and omissions. Thus, regardless of the test or food restriction status, rats with a higher number of correct trials and fewer premature responses are more sensitive to the effects of SD. These data suggest that baseline performance is an important factor for whether the effects of SD are detectable in rodent sustained attention tests. While the rPVT measures lapses, a combination of correct trials with slow response times (2x the mean response time) and omissions, the SRLT measures omissions only. Despite these differences, SD significantly increased omissions on the SRLT under ad libitum food conditions and in the rPVT using food restriction (Figure 3C). SD also increased lapses emitted as the session time increased (time on task; Figure 1D). These effects are similar to those found under ad libitum feeding by Loomis and colleagues,[12] in addition to previous rPVT reports employing water restriction and reinforcement.[3,4,8,9] Finally, while SD did increase Q90 reaction times on the rPVT, it did not affect mean, median, or Q10 reaction times, similar to food-restricted rats performing the SRLT.

There are several limitations to the current study. One is the lack of electroencephalogram and electromyogram (EEG/EMG) recordings, which would have shown the effects of SD on physiological sleep measures, in addition to how food restriction affects these processes. A small group of subjects was used in this study, and while the baseline effects were similar to those reported previously,[11] a larger group size is needed to replicate and extend these findings. Future studies should examine both sexes. Finally, food restriction could eliminate SD effects on mean and median reaction times, but more work is needed to assess this possibility.

Conclusion

From our survey of the literature, this is the first version of the rPVT using food reinforcement in food-restricted rats to show sensitivity to SD, with significantly increased lapses in attention, increased Q90 reaction times, and decreased percent correct responses, and supports RSI and time on task effects of SD. These data demonstrate that the attention-impairing effects of SD can be measured under food restriction, commonly used to train and maintain performance on operant behavioral tests.

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Disclosure

The authors report no conflicts of interest in this work.

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