Published in final edited form as:

Brain Res Bull. 2010 October 30; 83(5): 245–254. doi:10.1016/j.brainresbull.2010.06.012.

REM sleep deprivation produces a motivational deficit for food reward that is reversed by intra-accumbens amphetamine in rats

Erin C. Hanlon, Ph.D.¹, Ruth M. Benca, M.D., Ph.D.^{1,2,*}, Brian A. Baldo, Ph.D.², and Ann E. Kelley, Ph.D.^{1,2,*}

¹Neuroscience Training Program, University of Wisconsin-Madison, 6001 Research Park Blvd, Madison, WI, 53719

²Department of Psychiatry, University of Wisconsin-Madison, 6001 Research Park Blvd, Madison, WI, 53719

Abstract

Prolonged sleep deprivation in rats produces a characteristic syndrome of increase in food intake accompanied by, paradoxically, decrease in weight, suggesting a potential alteration in motivation for food reward. Using the multiple platform method to produce REM sleep deprivation (REMSD), we investigated the effect of REMSD on motivation for food reinforcement with a progressive ratio operant task, which yields a measure of the motor effort that a hungry animal is willing to expend to obtain food (the point at which the animal quits responding is termed the “break-point”). We found that REMSD rats decreased the break-point for sucrose pellet reinforcement in comparison to controls, as revealed by a within-session decline in responding. This behavioral deficit is similar to that observed in rats with diminished dopamine transmission within the nucleus accumbens (Acb), and, considering that stimulants are frequently used in the clinical setting to reverse the effects of sleepiness, we examined the effect of systemic or intra-Acb amphetamine on break-point in REMSD rats. Animals were given either systemic or intra-Acb amphetamine injections on days 3 and 5 of REMSD. Systemic amphetamine (0.1, 0.5, or 2.5 mg/kg) did not increase break point in REMSD rats. In contrast, intra-Acb infusions of amphetamine (1, 10, or 30 µg/ 0.5 µl bilaterally) reversed the REMSD-induced suppression of progressive ratio responding. Specifically, the two higher doses of intra-Acb amphetamine were able to prolong responding within the session (resulting in an increased break-point) on day 3 of REMSD while only the highest dose was sufficient following 5 days of REMSD. These data suggest that decreased motivation for food reward caused by REMSD may result from a suppression of dopamine function in the Acb.

Keywords

Multiple platform; operant; food motivation; nucleus accumbens; stimulants

© 2010 Elsevier Inc. All rights reserved.

*Deceased, manuscript submitted posthumously

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Conflict of Interest

The authors declare that they have no competing financial interests. The following is a disclosure of duality of interest for Ruth M Benca: serves in capacities of consulting/advisory/speaking for Glaxo Smith Kline, Neurocrine/Pfizer, Sanofi-Aventis, Sepracor, Takeda and Wyeth

Introduction

Both total sleep deprivation and rapid eye movement (REM) sleep deprivation produce a well-established syndrome consisting of an increase in feeding accompanied by a decrease in weight [8,17,36,39,46]. Although 24-hour food intake can roughly double during the period of sleep deprivation, rats lose weight [17,39]. Increases in food intake are related to increases in energy expenditure and suggest enhanced motivation for food. Although metabolic demands seem to be driving feeding during sleep deprivation, food intake is not high enough to compensate for the increased energy expenditure, suggesting that motivation for food may not be adequate to compensate for observed weight loss. When learning (or maintenance) of an operant task during sleep deprivation was previously investigated, it was found that despite increases in 24 hr food intake, REM sleep-deprived rats show decreased responding for food reward in a 15 min operant task [30]; rats were tested during the period of sleep deprivation for their ability to learn to lever press for food reward or maintain basic responding after they had acquired the task. The overall decrease in responding observed in the sleep-deprived rats was due to a rapid decline in response rate within a session, whereas all groups of animals responded at similar rates at the beginning of the session. These results suggest that the within-session decline in responding was not due to a learning or memory deficit but rather a decreased motivation for food reward.

To further investigate the effects REM sleep deprivation (REMSD) on behavior, we tested rats in another operant task, the progressive ratio task, which is a well-accepted measure of motivation that examines exerted work effort [31,32,60]. In this schedule of reinforcement, the ratio requirement (i.e., the number of lever presses required to earn reinforcement) systematically increases following dispensation of each reinforcer within the operant session. Thus, the animal has to increase responding progressively to obtain the same reward. The last ratio completed by the animal before the session ends is termed the “break-point” and allows for a measure of exerted work effort to obtain reward. Moreover, much is understood about the underlying neurobiological mechanisms that contribute to this behavior.

The progressive ratio task is known to be sensitive to alterations in dopamine transmission within the nucleus accumbens (Acb). The Acb is thought to mediate the incentive reward processes as well as reward-motor integration [33,53]. In contrast to other manipulations of the Acb that increase feeding (e.g. alterations of the opioid or GABAergic systems), dopamine alterations within the Acb do not effect feeding *per se*. Instead, Acb dopamine is thought to be involved in motivation by affecting the work effort an animal will exert to obtain reward. Decreasing dopamine transmission within the Acb alters distribution of behavior in tasks that offer choices between responses with different work effort requirements. Rats that receive infusions of dopamine receptor antagonists or 6-hydroxydopamine (6-OHDA) within the Acb shift behavior from highly active responses (i.e. high rates of lever pressing or barrier climbing) to behaviors that require less work effort, although these responses produce lesser reward or reinforcement value [53]. These findings are reminiscent of a previous report that shows that the overall decrements in responding for food reward in REMSD rats were due to within-session declines in responding [30]. Moreover, infusions of amphetamine into the nucleus accumbens increase break-point [67], whereas intra-Acb infusions of the neurotoxic agent 6-OHDA decrease break point [29]. These data suggest that increasing or decreasing dopamine transmission within the Acb alters break-point. We therefore sought to examine whether intra-Acb amphetamine could reverse the effects of REMSD on break-point.

We also assessed the effect of systemic amphetamine on progressive ratio responding in REMSD and control rats. The data on the effect of systemic amphetamine on break-point in

a progressive ratio task are equivocal, although systemic administration of dopamine antagonists reliably decreases break-point [2,15,47]. Systemic stimulants are frequently used to combat sleepiness in sleep-deprived individuals. We therefore sought to determine the combined effect of systemic amphetamine and sleep deprivation on motivated behaviors and to assess whether systemic amphetamine would reverse REMSD-induced deficits in operant responding.

Materials and Methods

Animals

A total of 111 male Sprague-Dawley rats (Harlan, Madison, WI) approximately 3 months of age and weighing between 300–370g were used in these experiments. Rats were maintained on a 12hr:12hr light-dark cycle and were given food (Harlan Teklad Rat Diet) and water *ad libitum* unless specified otherwise. All animal procedures and facilities were reviewed and approved by the IACUC of the University of Wisconsin-Madison, and were inspected and accredited by AAALAC.

Behavioral testing: Operant Tasks

All rats were put on a restricted diet that maintained body weight at 90% of free-feeding weight while they were trained in the operant task. Baseline weight for each animal was measured prior to training and 90% of free-feeding weight was calculated. Each day, prior to training on the operant task, animals were weighed and subsequently fed rat chow, following training, to achieve 90% of their free-feeding weight. This restricted diet continued until the rats were fully trained, at which time they were returned to an *ad libitum* diet. Water was freely available at all times in the home cage.

Behavioral testing was performed using standard operant chambers (Coulbourn Instruments, Allentown, PA; W: 9.5 in., L: 17 in., H: 8 in.). The chambers contained a lever, a food trough with photosensors, a pellet delivery system, a house light, and a red signal light. Prior to introduction to operant chambers, the animals were habituated to sugar pellets in their home cages in order to familiarize them with this novel food. A time limit was implemented for responding. Therefore, the schedules of reinforcement can also be termed time-constrained progressive ratio schedules. Sessions were 30 min in length for Experiments 1 and 2 and 75 min in length for Experiment 3. We chose to lengthen the session time for the intra-AcbSh amphetamine experiment (Exp. 3) based on previous work in our laboratory (data not shown) and in the literature; Zhang et al. [67] reported an increase in breakpoint following intra-AcbSh amphetamine during a longer testing session. Based on examination of the Zhang et al. [67] data, we extended the testing session to 75 min to observe potential alterations in breakpoint following intra-Acb infusions of amphetamine. During all habituation days the lever was retracted. On the first two habituation days, the schedule of reinforcement was such that a reinforcer was delivered on average every 30 seconds (random time 30 sec [RT30]), followed by two days of a tandem reinforcement schedule such that one press resulted in the delivery of one reinforcer (fixed ratio 1 [FR1]) along with a sucrose pellet randomly delivered on average every 30 seconds (RT30). For the next schedule of reinforcement, one lever press resulted in the delivery of one reinforcer (fixed ratio 1 [FR1]). Once the rats pressed consistently on an FR1 schedule, they were subsequently switched to FR3, and then FR5 schedules. Following achievement of a stable response on the FR5 schedule, rats were switched to a progressive ratio 2 (PR2) schedule for each experimental session, during which the first press by the animal was reinforced, and then the rat was required to increase its responses by two lever presses for each subsequent sugar pellet delivery. For example, an animal was rewarded after 1 press, then after 3 presses, 5 presses, 7 presses, and so on. Thus, rats were required to expend progressively

more effort for each subsequent sugar pellet reinforcer. The number of responses required in the last completed ratio before the session ended was defined as the break-point. Thus, if an animal last completed 35 presses and obtained a sugar pellet before the timed session ended, the break-point was recorded as 35.

In all experiments, responding on the lever resulted in the following sequence of events: the trough light switched on and the food pellet was immediately delivered to the food trough. Lever presses and nose pokes into the food trough were recorded. Animals were observed continuously by an experimenter while in the operant chamber to verify that they were not sleeping.

Surgery

Following behavioral training, as stated above, rats were anesthetized with a ketamine-xylazine mixture (100 mg/kg ketamine and 10 mg/kg xylazine) administered intraperitoneally (i.p.). Bilateral 10-mm stainless steel guide cannulae (23 gauge) were implanted using standard stereotaxic procedures and were secured to the skull with stainless-steel screws and dental cement. Based on the atlas of Paxinos and Watson [49], from bregma and with nosebar 5 mm above interaural zero, guide cannulae were aimed at the nucleus accumbens shell (AcbSh) with coordinates, anteroposterior (AP) +2.9; mediolateral (ML) -1.0; and dorsoventral (DV) -5.3 from skull surface. After surgery, stainless steel wire stylets were placed in the guide cannulae to prevent occlusion. Each rat received an intramuscular injection (0.1 ml/100 g) of sterile penicillin immediately following surgery.

Sleep Deprivation

Following surgery, rats were selectively deprived of REM sleep for 5 days (minimum of 120 hours) using the multiple platform technique [44,46], which results in near-total loss of REM sleep and significant loss of NREM sleep [27,45,46]. In experiment 1, three groups of animals were studied: REM sleep deprived rats (D), apparatus controls (C), and home cage controls (H). In experiments 2 and 3, groups of D and H rats were studied. For all experiments home cage animals were singly housed in standard acrylic cages (24 cm × 45 cm × 21 cm). D and C rats were housed in a large tank (1.73 m × 83 cm) partitioned with clear acrylic into 6 sections (54 cm × 37 cm × 4 cm), such that rats were in visual contact with one another. For experiment 1, D and C rats were alternated in the compartments so that D rats were next to C rats. Each animal had 3 small platforms (diameter 6.5 cm, height 14 cm) for D rats, or 2 large platforms (diameter 13 cm, height 14 cm) for C rats, to minimize confinement stress. Platforms protruded approximately 3 cm from a water bath at room temperature. Animals had overhead access to food and water from standard housing cage tops. The premise of this paradigm is that when D animals enter REM sleep and lose muscle tone, a natural characteristic of REM sleep, they are unable to stay on the small platforms and touch or enter the water below, thus ending the sleep bout. C animals have larger platforms and are more likely to maintain their posture during sleep.

Previous studies have reported that D rats lose approximately 70–100% of baseline REM sleep along with 10–40% of baseline NREM sleep [27,40,43,45,46,51,61]. Furthermore, it has been reported that C rats may experience up to 40% loss of baseline REM sleep and 20% of baseline NREM sleep [27,40,43,51,61]. Thus, based on the literature, the experimental groups in experiment 1 represent varying degrees of sleep deprivation; C rats experienced moderate sleep loss, and D rats, extreme sleep deprivation. Furthermore, in experiments 2 and 3, amphetamine effects were assessed on severely sleep deprived animals (D) and home cage controls (H).

Experimental Design

Three experiments were performed to assess the effects of sleep deprivation on break point in a progressive ratio task and the effect of systemic or intra-AcbSh amphetamine on this behavior. Baseline (SD0) for each of these experiments was the average of the three prior days to the start of REMSD.

Experiment 1—*Break point in a progressive ratio task without food restriction* ($n = 20$; H = 8, C = 6, D = 6). To analyze the effects of REMSD on break point in a progressive ratio task, rats were tested on responding for sucrose pellet reward during each of the five days of REMSD while fed *ad libitum*. Rats had been trained to lever press for food reward in a progressive ratio task while being maintained at 90% of free feeding weight. When asymptotic responding occurred animals were returned to an *ad libitum* diet for four days before being randomly assigned to D, C, and H groups and tested on the progressive ratio task on each of the five days of REMSD.

Experiment 2—*Break point in a progressive ratio task with systemic amphetamine* ($n = 44$; one H group per dose = 20 total, one D group per dose = 24 total). To determine the effect of systemic amphetamine on break point in REMSD rats, animals were tested on a progressive ratio schedule while fed *ad libitum* and were given i.p. injections of one of three doses of amphetamine (0.1, 0.5, or 2.5 mg/kg) or vehicle control (0.9% sterile saline) on days 3 and 5 of REM sleep deprivation. Rats had been habituated to i.p. injections of sterile saline on days -3, -1, and 1 of REMSD. The protocol was otherwise identical to Exp. 1.

Experiment 3—*Break point in a progressive ratio task with intra-AcbSh amphetamine* ($n = 47$; one H group per dose = 23 total, one D group per dose = 24 total). To determine the effect of amphetamine administered directly into the AcbSh on break point in REMSD rats, animals were tested on a progressive ratio schedule while fed *ad libitum* and were given one of three doses of amphetamine or vehicle control intracerebrally on days 3 and 5 of REM sleep deprivation. The protocol was identical to Exp. 1; however on days 3 and 5 of REMSD animals received an intra-AcbSh infusion of either 1, 10, or 30 μg in a volume of 0.5 μl per side of amphetamine or sterile saline. Rats had been habituated to intra-AcbSh infusions of sterile saline on days -3, -1, and 1 of REMSD.

Drugs and Microinfusions

For Experiment 2, amphetamine (from the National Institute on Drug Abuse) was dissolved in sterile 0.9% saline and administered via an i.p. injection in total doses of either 0.1, 0.5 or 2.5 mg/kg in a total volume of approximately 0.3 ml. The vehicle control was sterile 0.9% saline. Rats were taken into the testing room and given i.p. injections of either amphetamine or vehicle immediately prior to being placed in the operant chamber.

For intra-AcbSh microinfusions (Experiment 3), amphetamine (from the National Institute on Drug Abuse) was dissolved in sterile 0.9% saline. The total unilateral dose of amphetamine used in this study was either 1, 10, or 30 μg ; previous experiments demonstrated that the two highest doses increased break point in normal rats in a progressive ratio task [67]. The vehicle control was sterile 0.9% saline. Rats were taken into the testing room, stylets removed, and intracerebral injections of amphetamine or vehicle (total volume of 0.5 μl per side infused over 1 min 33 sec) were administered through a 12.5 mm injector cannulae (30 gauge) with a microinfusion pump (Harvard Apparatus, South Natick, MA). Injector cannulae were left in place for one additional minute post-infusion to allow for diffusion of injectate into the tissue. Injectors were then removed and stylets replaced and rats were immediately placed into operant chambers for testing.

Data Analysis

For Experiment 1, effects of sleep deprivation on lever pressing were analyzed with two-factor ANOVA (sleep condition $\times$ day), with repeated measures for the within-subjects variable, day. Post hoc comparisons were done with the Newman-Keuls test and analyses of simple main effects were used for interactions. Individual days were analyzed with a one-factor ANOVA and post hoc comparisons were done with Fisher's PLSD test.

For experiments 2 and 3, effects of sleep deprivation and dose of amphetamine on lever pressing were analyzed with three-factor ANOVA (sleep condition $\times$ dose $\times$ day), with repeated measures for the within-subjects variable, day. Simple main effects were used to further analyze interactions.

Histology

At the end of behavioral testing on, all animals were deeply anesthetized with an overdose of sodium pentobarbital and perfused transcardially with isotonic saline immediately followed by 10% formalin in phosphate buffer (formalin/PB). Brains were removed and stored in formalin/PB. Brains were cut in 60 μ m sections, mounted and stained with cresyl violet. Placement of injector tips was determined using light microscopy. (This laboratory has regularly been performing intra-Acb infusions for over a decade; all cannulae were in the site of interest and no animals were eliminated due to missed placements). Chartings of representative placements are presented in Figure 1.

Results

Experiment 1

To determine the effects of REMSD on motivation for food reward, we measured the break point in animals performing a progressive ratio task during each of the five days of REMSD. After two days of REMSD, the D and C rats responded less for food reward (had a lower break point) compared to the H rats, although all groups had similar break points during pre-REMSD baseline (Fig. 2A). Analysis of variance for the five days of REM sleep deprivation indicated that break point differed significantly among the D, C and H groups, (sleep condition $\times$ day interaction: $F(10,85) = 4.584$; $p < 0.0001$). Not only did the D rats show the lowest break point, but also over the 5-day period of REMSD these rats diverged progressively from the other two groups (Fig. 2A). Analysis of simple main effects revealed significant sleep condition $\times$ day interactions for D vs. H interaction ($F(5,85) = 6.976$, $p = 0.0001$) and the C vs. H interaction ($F(5,85) = 4.777$, $p = 0.0007$), showing that the break points of the D and C groups were significantly lower than the H group over the period of REMSD. This analysis revealed no significant differences in break point between the C and D groups over the 5 days of REMSD, although the difference between the two groups was consistent with the observation that D rats exhibited the lowest mean break points of the three groups on days 2–5 of REMSD ($F(5,85) = 1.569$, $p = 0.1776$). Further analysis of variance of all groups for the individual days of REMSD revealed significant or near significant differences beginning on day 2 of REMSD (SD2: $F(2,17) = 5.151$, $p = 0.0178$; SD3: $F(2,17) = 4.845$, $p = 0.0216$; SD4: $F(2,17) = 4.714$, $p = 0.0235$; SD5: $F(2,17) = 3.265$, $p = 0.0631$). Post hoc analysis with Fisher's PLSD revealed that these differences were due to both the D and C groups displaying significantly lower break points compared to the H group on SD2 (C vs. H: $p = 0.0103$; D vs. H: $p = 0.0225$) and SD3 (C vs. H: $p = 0.0289$; D vs. H: $p = 0.0116$). In further confirmation of the differences between the D and C groups, only the D group was significantly different than the H group on days SD4 ($p = 0.0072$) and SD5 ($p = 0.0225$).

We further analyzed responses across each 30 min session collapsed across days 2 through 5 of REMSD (Figure 2B). Day 1 of REMSD was omitted from analysis since effects on this day are most likely due to stress of the paradigm [46]. To allow for analysis of within-session responding, the mean of total responses within 3 minute bins on days 2–5 of REMSD were examined. Analysis of variance of all three groups revealed that over days 2–5 of REMSD there were significant main effects of sleep condition ($F(2,237) = 36.944, p < 0.0001$), bin ($F(9,2133) = 33.367, p < 0.0001$), and a sleep condition $\times$ bin interaction ($F(18,2133) = 11.718, p < 0.0001$) showing that across the session, the D and C groups decreased responding more than the H group. Analysis of simple main effects for days 2–5 of REMSD revealed that all groups were significantly different from each other in main effect of sleep condition (D vs. H: $p < 0.0001$; C vs. H: $p < 0.0001$; D vs. C: $p = 0.0229$). The interaction of sleep condition $\times$ bin was significant for D vs. H ($p < 0.0001$) and C vs. H ($p < 0.0001$) comparisons. Thus, the overall response deficit observed in the D rats following two days of REMSD was due to a within-session decline in lever pressing. Taken together with the session to session data, the within-session time course data reveal that behavior of the C group is intermediate to the D and H groups, most likely due to the intermediate amount of sleep restriction observed in this group.

Experiment 2

To assess the interaction between amphetamine and REMSD on break point, we tested rats fed *ad libitum* on a progressive ratio task and administered various doses of amphetamine (0.1, 0.5, and 2.5 mg/kg) or vehicle control (0.9% sterile saline) on days 3 and 5 of REMSD (Figures 3A–B). The amphetamine doses were each tested in separate groups of animals. Similar to what we observed in Experiment 1, REM-sleep-deprived rats exhibited a lower break point than home cage controls during the period of REMSD despite the fact that all groups showed similar levels of responding during baseline. Analysis of variance of break point for all groups over the five days of REMSD demonstrated a main effect of sleep condition ($F(1,36) = 29.489, p < 0.0001$), a main effect of day ($F(5,180) = 59.187, p < 0.0001$), and a sleep condition $\times$ day interaction ($F(5,180) = 11.193, p < 0.0001$). Furthermore, there was an interaction between testing day and the amphetamine dose group to which animals were assigned ($F(15,180) = 4.121, p < 0.0001$). On days SD3 and SD5, when amphetamine was administered, analysis of variance revealed a main effect of sleep condition ($F(1,36) = 12.017, p = 0.0014$), a main effect of dose ($F(3,36) = 11.505, p < 0.0001$), and a day $\times$ dose interaction ($F(3,36) = 2.924, p = 0.047$), reflecting the fact that systemic amphetamine decreased break point in both groups in a dose dependent manner and to a greater extent following the second administration (SD5). However, for both the D and H groups on days SD 2, and 4, when amphetamine was not given, there were no statistically significant differences with regard to amphetamine dose group to which animals were assigned, while the overall breakpoint of rats in the REMSD group remained lower (main effect of sleep condition: $F(1,39) = 24.930, p < 0.0001$). This demonstrates that the sleep deprivation effects on break point were present, and that there were no residual effects of systemic amphetamine on days that it was not administered.

Further analyses revealed that break-point was lower on SD5, particularly in the 2.5 mg/kg amphetamine group, although this effect did not achieve statistical significance ($F(3,36) = 2.089, p = 0.1187$). There was, however, a significant main effect of dose for the H group on days SD3 and SD5 ($F(3,36) = 11.166, p < 0.0001$) demonstrating that break point decreased in a dose-dependent manner. Pre-planned contrasts among means indicated that the two highest doses decreased breakpoint in comparison to the saline control (SD3: H0.5, $p = 0.0626$; H2.5, $p = 0.0029$; SD5: H0.5, $p = 0.0045$; H2.5, $p = 0.0001$).

Analyses of within-session responding for all eight groups over days 2–5 of REMSD (Figure 4A–D) showed that systemic amphetamine decreased responding in both D and H rats. For

data collapsed across days 2–5 of REMSD (see Fig 4) there were significant main effects of sleep condition ($F(1,496) = 189.822, p < 0.0001$), day ($F(3,496) = 10.689, p < 0.0001$), and bin ($F(9,4464) = 89.096, p < 0.0001$). Furthermore, there was a significant bin $\times$ sleep condition $\times$ dose interaction ($F(27,4464) = 2.65, p < 0.0001$) and a bin $\times$ day $\times$ dose interaction ($F(81,4464) = 1.723, p < 0.0001$) showing that both sleep condition and dose produced decreases in responding within a session on days 2–5 of REMSD. Post hoc analysis revealed only a significant main effect of time bin in the D group ($F(9,4464) = 21.727, p < 0.0001$) demonstrating that the D group decreased responding within a session independent of day or dose of amphetamine. Similarly, the H group demonstrated a significant main effect of bin ($F(9,4464) = 82.445, p < 0.0001$) and also main effects of day ($F(3,496) = 16.767, p < 0.0001$), dose ($F(3,496) = 31.209, p < 0.0001$), and a bin $\times$ day $\times$ dose interaction ($F(81,4464) = 1.987, p < 0.0001$) demonstrating that unlike the D group, decreased responding within the session in the H group was related to the dose of amphetamine (i.e. the H groups that received the two highest doses started at a lower rate and showed greater decrements in responding across the session on days 3 and 5 when amphetamine was administered). In summary, the response deficit produced by REMSD was not reversed by systemic amphetamine; in contrast, the higher doses of systemic amphetamine reduced responding in both the H and D groups.

Experiment 3

To assess whether intra-AcbSh administration of amphetamine would restore within-session responding and increase break point in REMSD rats, we tested *ad libitum* fed rats on a progressive ratio task and administered various doses of amphetamine (1, 10, and 30 μ g) or vehicle control (0.9 % sterile saline) bilaterally into the AcbSh on days 3 and 5 of REMSD (Figures 5A–B). These doses were chosen based on previous experiments that have shown an increased break point following amphetamine infusion into the AcbSh [67]. The intra-AcbSh amphetamine doses were each tested in separate groups of rats. As seen in the previous experiments, REM-sleep deprived rats exhibited a lower break point than home cage controls following 2 days of REMSD despite the fact that all groups exhibited similar break points during baseline. Analysis of variance of all groups over the period of REMSD revealed main effects of sleep condition ($F(1,39) = 14.187, p = 0.0005$), day ($F(5,195) = 13.603, p < 0.001$), and a sleep condition $\times$ day interaction ($F(5,195) = 9.698, p < 0.0001$). On days SD3 and SD5, when amphetamine was infused into the AcbSh, analysis of variance revealed a main effect of sleep condition ($F(1,39) = 19.768, p < 0.0001$) and a tendency towards a significant interaction between testing day and the intra-AcbSh amphetamine dose ($F(3,39) = 2.615, p = 0.0647$). This demonstrates that intra-AcbSh amphetamine increased break point. However, for both the D and H, groups on days SD 2 and 4, when intra-AcbSh amphetamine was not given, there were no statistically significant differences with regard to intra-AcbSh amphetamine dose group to which animals were assigned while the main effect of sleep condition ($F(1,36) = 51.392, p < 0.0001$) and main effect of day ($F(1,36) = 4.546, p = 0.0399$) remained. This demonstrates that the sleep deprivation effects on break point persisted, but that there were no residual effects of intra-AcbSh amphetamine on days that it was not infused.

Because of the presence of a strong main effect of sleep condition and a tendency towards a day $\times$ dose interaction, as well as a strong a priori hypothesis that intra-AcbSh amphetamine would alter breakpoint [67] we proceeded with further analyses and post-hoc means comparisons for all groups on days SD3 and SD5 (infusion days). Further analyses revealed that there were no statistically significant differences between days SD3 and SD5 for the H groups. In contrast, analyses of the D groups on days SD3 and SD5 revealed a main effect of dose ($F(3,39) = 2.751, p = 0.05$) and a tendency toward a day $\times$ dose interaction ($F(3,39) = 2.431, p = 0.0796$) demonstrating that amphetamine increased break point in REM sleep-

deprived rats. The 10 and 30 μ g infusions of intra-AcbSh amphetamine preferentially increased break point in comparison to saline in REM sleep-deprived rats on SD3 (10 μ g, $p = 0.0580$; 30 μ g, $p = 0.0204$). On SD5, only the 30 μ g dose of amphetamine increased break point in REM sleep-deprived rats ($p = 0.0348$), showing that following five days of REMSD only the highest dose of intra-AcbSh amphetamine was able to recover some of the sleep deprivation effects on break point. Analyses of within-session responding for all eight groups over days 2–5 of REMSD (Figure 6A–D) revealed significant main effects of sleep condition ($F(1,908) = 186.824$, $p < 0.0001$), dose ($F(3,908) = 6.843$, $p = 0.0001$), and time bin ($F(14,12712) = 112.776$, $p < 0.0001$). Moreover, there was a statistically significant interaction of time bin, sleep condition, day, and dose ($F(126,12712) = 1.441$, $p = 0.0009$). We further collapsed the data across days 2 and 4 (no infusions) or days 3 and 5 (intra-AcbSh amphetamine infusions). On days 2 and 4, analyses revealed a main effect of sleep condition ($F(1,454) = 97.734$, $p < 0.0001$), bins ($F(14,6356) = 84.302$, $p < 0.0001$), and an interaction between time bin, sleep condition, and day ($F(14,6356) = 1.912$, $p = .02$). This demonstrates the fact that sleep deprivation effects on within-session declines in responding were present, and that there were no residual effects of intra-AcbSh amphetamine on days that it was not administered.

When intra-AcbSh amphetamine was infused, on days 3 and 5, analysis of variance revealed main effects of sleep condition ($F(1,454) = 89.217$, $p < 0.0001$), dose ($F(3,454) = 7.225$, $p < 0.0001$), and bin ($F(14,6356) = 35.915$, $p < 0.0001$). There was also an interaction between bins, sleep condition, day, and dose ($F(42,6356) = 1.384$, $p = 0.05$). Further analyses revealed that both D and H groups exhibited main effects of dose (D: $F(3,464) = 13.064$, $p = 0.0001$; H: $F(3,444) = 4.66$, $p = 0.0032$). However, analyses revealed that only the H group exhibited an interaction between bin and dose ($F(42,6216) = 3.542$, $p = 0.001$) and the D group an interaction between bin, day, and dose ($F(42,6496) = 1.711$, $p = 0.0029$). This is consistent with the idea that for the H group, intra-AcbSh amphetamine, in a dose dependent manner, maintained lever pressing throughout the 75 minute session in comparison to the saline control, although this effect was not sufficient to increase overall lever pressing or break point for the session in H rats. Similarly for the D group, amphetamine facilitated lever pressing within a session, in that the rats continued to lever press within a session instead of showing the decline that normally accompanies REMSD. In contrast to the H group, amphetamine in the D group maintained lever pressing enough to increase overall lever pressing and breakpoint for an individual session. Thus, the intra-AcbSh amphetamine was able to reverse the response deficit observed in D rats by maintaining lever presses within a session.

Discussion

These results demonstrate that REM sleep deprivation (REMSD) significantly reduced within-session responding in rats in comparison to home cage and apparatus control rats in a progressive ratio schedule of operant reinforcement for food reward. REMSD also significantly reduced break-point relative to home cage rats and produced a non-significant trend toward reduced break-point in comparison to apparatus controls, where break-point is thought to reflect the degree of motivation to work for a given reinforcer [32,60]. The present findings confirm the previous observation that sleep-deprived rats show diminished levels of responding in food-reinforced operant tasks, including acquisition or maintenance of an operant task, whether or not they were concurrently food deprived [30], and that this decrement in responding is primarily due to a more rapid decline in responding within the testing session. Kirby and Kennedy [34,35] have also reported decreased rates of responding on various schedules of operant reinforcement in REMSD rats. The present study extends these previous findings in that we primarily investigated motivation by determining work

effort rats would exert to obtain food reward, whereas previous work explored REMSD effects on acquisition, maintenance, or reinforcement rates.

One potential confound of the current study is that effects may have been partly due to stress effects produced by the multiple platform method, given that apparatus control (C) animals also displayed decrements in break point, as shown previously in Hanlon et al. [30]. To minimize stress related to the REMSD paradigm, animals were provided with multiple platforms to decrease confinement stress and were housed in a large, portioned tank in view of each other to decrease isolation stress. Nevertheless, both D and C rats may have experienced more stress than H rats. The literature concerned with sleep-deprivation-induced changes in the stress hormone corticosterone is, at this point, equivocal. Some investigators report an increase in plasma corticosterone following sleep deprivation [37,38,62] and others report no change [6,18]. Therefore, it is unclear to what degree stress responses, particularly those mediated by the hypothalamic-pituitary-adrenal axis, contributed to the results in this study.

In terms of sleep deprivation, C rats were almost certainly intermediate between H and D rats and thus represented a partially sleep-deprived group as well. Electroencephalogram (EEG) studies have reported reductions of approximately 70–100% of baseline REM sleep amounts in sleep-deprived D rats using the multiple platform method [27,40,43,45,46,51,61], as well as approximately 10–40% of non-REM (NREM) sleep [27,40,43,45,51,61]. In most of these studies, C rats also showed variable decreases in REM and NREM sleep; with decrements as much as 40% and 20% from baseline, respectively [27,40,43,51,61]. It is therefore likely that in the present study C rats experienced some sleep loss, although probably not to the same extent as D rats. Indeed, we observed that the D rats were always most extreme in behavioral outcomes, suggesting that the impairments observed in the D group are due at least in part to REMSD and not solely to the stress of the paradigm. Nevertheless, even if the observed effects of sleep deprivation using the platform technique are due to a combination of sleep loss and stress, the findings in this study suggest that the decrements in operant responding may be due to alterations in dopamine activity in the nucleus accumbens.

Progressive Ratio Task

In the current experiment, the overall reduction in break point in D rats was due to a significant within-session decline in responding. All groups responded similarly during the first minutes of the operant session, but the D rats showed reduced responding later in the session relative to the H and C controls. The within-session decline in responding is similar to that seen with systemic DA antagonist treatment on fixed ratio responding for food reward [65,66]. Moreover, rats subjected to 6-OHDA-induced dopamine depletion in the Acb shift behavior from highly active responses (i.e. high rates of lever pressing or barrier climbing) to behaviors that require less work effort, although these responses obtain fewer reinforcers [54]. Acb dopamine may not be necessary in free feeding *per se*, because dopamine receptor antagonists do not alter total food intake or latency to feed; rather, Acb dopamine transmission is thought to modulate motivational arousal and the work effort an animal will exert to obtain reward [5,55]. Because REMSD decreases breakpoint, while increasing free feeding, we suggest that Acb dopamine is involved in REMSD-induced decrements in food motivation, resulting in a decline in exerted work effort. Moreover, we suggest that the observed response deficit may be due to decreased dopamine transmission within the Acb.

This hypothesis is supported by the extensive data indicating that responding in a progressive ratio task involves dopaminergic transmission within the Acb [2,29,67]. In the present study, we targeted the AcbSh because of the finding that intra-AcbSh amphetamine

infusions increase breakpoint slightly more than intra-AcbC infusions [67]. We found that discrete infusions of amphetamine directly into the AcbSh rescued the behavioral deficit produced by REMSD; specifically, intra-AcbSh infusions of amphetamine dose-dependently increased break point in D rats. On day 5 of REMSD only the highest dose of amphetamine (30 μ g) was able to recover the REMSD-elicited response deficit. The overall increase in break point observed in the D rats following amphetamine infusion into the AcbSh was due to sustained responding within the session; the two higher doses of amphetamine (10 and 30 μ g) infused directly into the AcbSh prevented the within-session decline in responding observed in D rats. This preservation of responding within a session led to an overall increase in break point in the D rats, which may have been due to amphetamine-induced elevations in AcbSh dopamine levels. Although amphetamine affects transmission of other monoamines, its behavioral effect in the Acb is thought to be due primarily to its dopamine releasing effects [23,58,64]. This suggests that decreased dopamine transmission within the AcbSh may have contributed to the decline in responding for food reward observed in Experiment 1 and in our previous study [30].

The observation that REMSD rats have decreased motivation for food may seem contradictory to the data showing that REMSD produces an increase in 24-hour food intake. However, consuming freely available food and working for food reward likely involve somewhat separate motivational systems and mechanisms [33]. Furthermore, the effects of REMSD on food motivation may prevent feeding behavior (and underlying Acb dopamine mechanisms) from effectively compensating for the increased metabolic demand. It is likely that increases in 24-hour food intake are driven by incompletely met metabolic demands, whereas deficits in operant responding for food reward may directly reflect deficiencies in dopamine transmission within the Acb.

Sleep Deprivation and Dopamine

Several studies have examined potential changes in dopamine systems induced by sleep deprivation. It has recently been reported that dopaminergic cells within the ventral periaqueductal gray matter are most active during waking, diminish firing during non-REM sleep, and cease firing during REM sleep [42,56]. Thus, persistent firing of dopaminergic neurons would be expected during times of extended wakefulness. The effects of sleep deprivation on dopamine systems (as indexed by extracellular dopamine levels, metabolites, turnover, and dopamine receptors levels) are equivocal, however. Some studies have reported an increase in striatal dopamine levels [26] and increased levels of the dopamine metabolite 3,4-dihydroxyphenylacetic (DOPAC) in the striatum [21,22] and the medial preoptic area [52] following different lengths of sleep deprivation. Similar findings were reported in other species; for example, dopamine and DOPAC were increased in the hypothalamus of hamsters deprived of total sleep for 4 hrs [4] and DOPAC levels were increased in the striatum and Acb of mice deprived of sleep for 48 hrs [3]. Nevertheless, many researchers have reported unchanged dopamine levels [1,7,9,14,22], decreased dopamine levels [9,52], or unchanged DOPAC levels [16,19] in several regions of the rat brain following sleep deprivation. Furthermore, human studies investigating the dopamine metabolite homovanillic acid (HVA) in cerebrospinal fluid did not reveal differences between sleep-deprived and control groups [25,41]. Despite these ambiguous data on dopamine, DOPAC, and HVA levels following sleep deprivation, there have been consistent reports of an increase in dopamine D1 receptors in the limbic system [16,20] and both D1 and D2 receptors in the striatum [28] and several other brain regions [48] following varying lengths of sleep deprivation. Although these findings do not lend much insight to the state of dopaminergic transmission following sleep deprivation, the consistent finding of increased dopamine receptor sensitivity following sleep deprivation may reflect compensation for diminished presynaptic dopamine levels.

REMSD and systemic amphetamine

Stimulants such as amphetamine are frequently used in the clinical setting to reverse the effects of sleep deprivation. Thus, it was important to investigate the potential interaction between REMSD and systemically administered amphetamine on behavior. In the current experiment, systemic amphetamine did not reverse the effects of REMSD. In fact, the second administration (on SD5) of the highest dose of systemic amphetamine (2.5 mg/kg) resulted in further decrements in break point in the D rats. Also, the higher doses of amphetamine (0.5 and 2.5 mg/kg) decreased break point in the H group on both days of administration (SD3 and SD5).

Our findings are in agreement with studies reporting that systemic amphetamine decreased break point in non-sleep-deprived rats in a progressive ratio task [15,24,47,57,63]. Caul and Brindle [15] reported that higher doses of amphetamine (0.5, 0.75, and 1 mg/kg) administered 20 min prior to the 18 min test session decreased break point, whereas lower doses (0.0625, 0.125, and 0.25 mg/kg) did not affect behavioral responding. However, there were also reported increases in break point following amphetamine administration [13,50,59]. Our findings are similar to those reported by Caul and Brindle [15]; we also observed a decrease in break point following the higher doses (0.5 and 2.5 mg/kg) and no change in responding after i.p. administration of a low dose of amphetamine (0.1 mg/kg). The cause of the discrepancies in the literature is unclear, but it could be due to multiple factors including dose of amphetamine investigated, route of administration, latency to test following administration, length of operant sessions, and ratio requirements within the progressive ratio task. Some have argued that the findings can be explained by the “rate-dependency” theory of amphetamine action [58]; thus, it is hypothesized that the effect of systemic amphetamine on operant responding is dependent on the pre-drug baseline rate of responding. Simply stated, low baseline responding rates are increased whereas high baseline responding rates are decreased by systemic amphetamine. In a progressive ratio operant task the initial response requirements are relatively low; however, depending on the rate of ratio increase, the response requirements quickly increase. Therefore, it is possible that the high response requirement towards the end of the sessions in the current experiment were negatively affected by the higher doses of systemic amphetamine administration, as the “rate-dependency” theory posits. Additionally, high doses of systemic amphetamine are known to be anorexic agents [10,11]; in this way amphetamine may be producing decrements in responding for food reward. In the present study, the effects of direct intra-Acb amphetamine on operant behavior are opposite to those seen with systemic administration of the drug.

Conclusions

Based on the literature and the findings in the current study, we hypothesize that sleep deprivation functionally decreases dopaminergic transmission within the Acb, either by decreasing dopamine levels in the synapse or causing subsensitivity of postsynaptic dopamine receptors (or both), resulting in a decreased motivation for food reward. This hypothesis is supported by the fact that decrements in responding observed in REMSD animals are similar to those seen in animals that have received infusions of dopamine receptor antagonists or 6-OHDA, and that amphetamine primarily exerts its action via dopaminergic neurons in the Acb. Therefore, we propose the following model: throughout the period of sleep deprivation dopaminergic neurons are continually active; at some point, dopamine turnover is not able to maintain normal levels for release due to the persistent activation. Consequently, the releasable pool of dopamine declines following sleep deprivation. Systemic injections of amphetamine were apparently not able to counteract this, either because high enough doses were not utilized to effectively increase dopamine transmission within the Acb and/or because behaviorally effective doses exert effects on

extra-Acb brain regions and monoamine systems that might oppose amphetamine's actions in the Acb. Discrete infusions of amphetamine into the Acb, however, reversed REMSD-elicited deficits in transmission by releasing available dopamine from the terminal and blocking re-uptake and catabolism of dopamine in the synapse, thus effectively increasing dopamine transmission and restoring motivation for food reward in REMSD animals.

In the present study, systemic amphetamine administration did not improve REMSD-elicited deficits in operant responding, nor did it improve measures of coping responses and anxiety as previously reported [44]. In contrast, many human studies have reported an increase in arousal and improvement on various cognitive tasks following stimulant administration during the period of sleep deprivation (for review see: [12]). It is not clear, however, whether stimulants are simply reversing 'sleepiness', which can impact performance, or are also reversing primary deficits in brain function produced by sleep deprivation. We propose that general arousal levels, and specific motivational processes, may be mediated by distinct substrates (for example, ascending noradrenaline versus dopamine systems) during a period of sleep deprivation. Therefore, a better understanding of the interaction between stimulants such as amphetamine and sleep deprivation on behavior is needed.

Acknowledgments

We express our sincere appreciation Matt E. Andrzejewski, Ph.D. for his expertise while preparing this manuscript. This work was supported by National Research Service Award 5 F31 MH070342-02 to E.C.H., NIMH grant MH52226 to R.M.B., and NHLBI grant RO1 HL086465-01 to R.M.B and A.E.K.

References

1. Aalto J, Kiianmaa K. Role of brain monoaminergic systems in the increased ethanol drinking caused by REM-sleep deprivation. *Alcohol Alcohol*. 1987 Suppl 1:313–317.
2. Aberman JE, Ward SJ, Salamone JD. Effects of dopamine antagonists and accumbens dopamine depletions on time-constrained progressive-ratio performance. *Pharmacol Biochem Behav*. 1998; 61:341–348. [PubMed: 9802826]
3. Asakura W, Matsumoto K, Ohta H, Watanabe H. REM sleep deprivation decreases apomorphine-induced stimulation of locomotor activity but not stereotyped behavior in mice. *Gen Pharmacol*. 1992; 23:337–341. [PubMed: 1511844]
4. Asikainen M, Deboer T, Porkka Heiskanen T, Stenberg D, Tobler I. Sleep deprivation increases brain serotonin turnover in the Djungarian hamster. *Neurosci Lett*. 1995; 198:21–24. [PubMed: 8570087]
5. Baldo BA, Sadeghian K, Basso AM, Kelley AE. Effects of selective dopamine D1 or D2 receptor blockade within nucleus accumbens subregions on ingestive behavior and associated motor activity. *Behav Brain Res*. 2002; 137:165–177. [PubMed: 12445722]
6. Bergmann BM, Everson CA, Kushida CA, Fang VS, Leitch CA, Schoeller DA, Refetoff S, Rechtschaffen A. Sleep deprivation in the rat: V. energy use and mediation. *Sleep*. 1989; 12:31–41. [PubMed: 2538910]
7. Bergmann BM, Seiden LS, Landis CA, Gilliland MA, Rechtschaffen A. Sleep deprivation in the rat: XVIII. Regional brain levels of monoamines and their metabolites. *Sleep*. 1994; 17:583–589. [PubMed: 7531362]
8. Bhanot JL, Chhina GS, Singh B, Sachdeva U, Kumar VM. REM sleep deprivation and food intake. *Indian J Physiol Pharmacol*. 1989; 33:139–145. [PubMed: 2592037]
9. Bliss EL. Sleep in schizophrenia and depression--studies of sleep loss in man and animals. *Res Publ Assoc Res Nerv Ment Dis*. 1967; 45:195–210. [PubMed: 6083190]
10. Blundell JE, Latham CJ. Characterisation of adjustments to the structure of feeding behaviour following pharmacological treatment: Effects of amphetamine and fenfluramine and the antagonism produced by pimozide and methergoline. *Pharm. Biochem. Beh*. 1980; 12:717–722.

11. Blundell JE, Rogers PJ. Effects of anorexic drugs on food intake, food selection and preferences and hunger motivation and subjective experiences. *Appetite*. 1980; 1:151–165.
12. Bonnet MH, Balkin TJ, Dinges DF, Roehrs T, Rogers NL, Wesensten NJ. The use of stimulants to modify performance during sleep loss: a review by the sleep deprivation and Stimulant Task Force of the American Academy of Sleep Medicine. *Sleep*. 2005; 28:1163–1187. [PubMed: 16268386]
13. Brennan K, Roberts DC, Anisman H, Merali Z. Individual differences in sucrose consumption in the rat: motivational and neurochemical correlates of hedonia. *Psychopharmacology(Berl)*. 2001; 157:269–276. [PubMed: 11605082]
14. Carlini EA. REM sleep deprivation and dopamine in the CNS. *Rev Pure Appl Pharmacol Sci*. 1983; 4:1–25. [PubMed: 6142502]
15. Caul WF, Brindle NA. Schedule-dependent effects of haloperidol and amphetamine: multiple-schedule task shows within-subject effects. *Pharmacol Biochem Behav*. 2001; 68:53–63. [PubMed: 11274708]
16. Demontis MG, Fadda P, Devoto P, Martellotta MC, Fratta W. Sleep deprivation increases dopamine D1 receptor antagonist [3H]SCH 23390 binding and dopamine-stimulated adenylate cyclase in the rat limbic system. *Neurosci Lett*. 1990; 117:224–227. [PubMed: 2149748]
17. Everson CA, Bergmann BM, Rechtschaffen A. Sleep deprivation in the rat: III. total sleep deprivation. *Sleep*. 1989; 12:13–21. [PubMed: 2928622]
18. Everson CA, Crowley WR. Reductions in circulating anabolic hormones induced by sustained sleep deprivation in rats. *Am J Physiol Endocrinol Metab*. 2004; 286:E1060–E1070. [PubMed: 14871886]
19. Fadda P, Martellotta MC, De Montis MG, Gessa GL, Fratta W. Dopamine D1 and opioid receptor binding changes in the limbic system of sleep deprived rats. *Neurochemistry International*. 1992; 20 Suppl:153S–156S. [PubMed: 1365415]
20. Fadda P, Martellotta MC, Gessa GL, Fratta W. Dopamine and opioids interactions in sleep deprivation. *Progress in Neuropsychopharmacology and Biological Psychiatry*. 1993; 17:269–278.
21. Farber J, Miller JD, Crawford KA, McMillen BA. Dopamine metabolism and receptor sensitivity in rat brain after REM sleep deprivation. *Pharmacol Biochem Behav*. 1983; 18:509–513. [PubMed: 6135227]
22. Farooqui SM, Brock JW, Zhou J. Changes in monoamines and their metabolite concentrations in REM sleep- deprived rat forebrain nuclei. *Pharmacol Biochem Behav*. 1996; 54:385–391. [PubMed: 8743599]
23. Fibiger HC. Drugs and reinforcement: a critical review of the catecholamine theory. *Annu. Rev. Pharmacol. Toxicol*. 1978; 18:37–56. [PubMed: 348064]
24. Foltin RW, Evans SM. The effects of D-amphetamine on responding for candy and fruit drink using a fixed ratio and a progressive ratio schedule of reinforcer delivery. *Pharmacol Biochem Behav*. 2001; 69:125–131. [PubMed: 11420077]
25. Gerner RH, Post RM, Gillin JC, Bunney WE Jr. Biological and behavioral effects of one night's sleep deprivation in depressed patients and normals. *Journal of Psychiatric Research*. 1979; 15:21–40. [PubMed: 219193]
26. Ghosh PK, Hrdina PD, Ling GM. Effects of REMS deprivation on striatal dopamine and acetylcholine in rats. *Pharmacol Biochem Behav*. 1976; 4:401–405. [PubMed: 180546]
27. Grahnstedt S, Ursin R. Platform sleep deprivation affects deep slow wave sleep in addition to REM sleep. *Behav Brain Res*. 1985; 18:233–239. [PubMed: 4091961]
28. Hamdi A, Brock J, Ross K, Prasad C. Effects of rapid eye movement sleep deprivation on the properties of striatal dopaminergic system. *Pharmacol Biochem Behav*. 1993; 46:863–866. [PubMed: 7906037]
29. Hamill S, Trevitt JT, Nowend KL, Carlson BB, Salamone JD. Nucleus accumbens dopamine depletions and time-constrained progressive ratio performance: effects of different ratio requirements. *Pharmacol Biochem Behav*. 1999; 64:21–27. [PubMed: 10494993]
30. Hanlon EC, Andrzejewski ME, Harder BK, Kelley AE, Benca RM. The effect of REM sleep deprivation on motivation for food reward. *Behav Brain Res*. 2005; 163:58–69. [PubMed: 15967514]

31. Hodos W. Progressive ratio as a measure of reward strength. *Science*. 1961; 134:943–944. [PubMed: 13714876]
32. Hodos W, Kalman G. Effects of increment size and reinforcer volume on progressive ratio performance. *J. Exp. Anal. Behav.* 1963; 6:387–392. [PubMed: 13963807]
33. Kelley AE, Baldo BA, Pratt WE, Will MJ. Corticostriatal-hypothalamic circuitry and food motivation: Integration of energy, action and reward. *Physiol Behav.* 2005; 86:773–795. [PubMed: 16289609]
34. Kennedy CH. Effects of REM sleep deprivation on a multiple schedule of appetitive reinforcement. *Behav Brain Res.* 2002; 128:205–214. [PubMed: 11796165]
35. Kirby M, Kennedy CH. Variable-interval reinforcement schedule value influences responding following REM sleep deprivation. *J Exp Anal Behav.* 2003; 80:253–260. [PubMed: 14674732]
36. Koban M, Le WW, Hoffman GE. Changes in Hypothalamic CRH, NPY, and POMC Gene Expression During Chronic REM Sleep Deprivation of Rats. *Endocrinology.* 2005; 147:421–431. [PubMed: 16210372]
37. Kollar EJ, Pasnau RO, Rubin RT, Naitoh P, Slater GG, Kales A. Psychological, psychophysiological, and biochemical correlates of prolonged sleep deprivation. *Am J Psychiatry.* 1969; 126:488–497. [PubMed: 4308894]
38. Kollar EJ, Slater GR, Palmer JO, Docter RF, Mandell AJ. Stress in Subjects Undergoing Sleep Deprivation. *Psychosomatic Medicine.* 1966; 28:101–110. [PubMed: 5930959]
39. Kushida CA, Bergmann BM, Rechtschaffen A. Sleep deprivation in the rat: IV. paradoxical sleep deprivation. *Sleep.* 1989; 12:22–30. [PubMed: 2928623]
40. Landis C. Altered sleep patterns with the platform method of REM sleep deprivation in rats. *Sleep Research.* 1996; 25:469.
41. Livrea P, Puca FM, Barnaba A, Di Reda L. Abnormal central monoamine metabolism in humans with "true hypersomnia" and "sub-wakefulness". *European Neurology.* 1977; 15:71–76. [PubMed: 323018]
42. Lu J, Zhou TC, Saper CB. Identification of wake-active dopaminergic neurons in the ventral periaqueductal gray matter. *Journal of Neuroscience.* 2006; 26:193–202. [PubMed: 16399687]
43. Machado RB, Hipolide DC, Benedito-Silva AA, Tufik S. Sleep deprivation induced by the modified multiple platform technique: quantification of sleep loss and recovery. *Brain Res.* 2004; 1004:45–51. [PubMed: 15033418]
44. Martinez-Gonzalez D, Obermeyer W, Fahy JL, Riboh M, Kalin NH, Benca RM. REM sleep deprivation induces changes in coping responses that are not reversed by amphetamine. *Sleep.* 2004; 27:609–617. [PubMed: 15282995]
45. McDermott CM, LaHoste GJ, Chen C, Musto A, Bazan NG, Magee JC. Sleep deprivation causes behavioral, synaptic, and membrane excitability alterations in hippocampal neurons. *J Neurosci.* 2003; 23:9687–9695. [PubMed: 14573548]
46. Mendelson WB, Guthrie RD, Frederick G, Wyatt RJ. The flower pot technique of rapid eye movement (REM) sleep deprivation. *Pharmacol Biochem Behav.* 1974; 2:553–556. [PubMed: 4371007]
47. Mobini S, Chiang TJ, Ho MY, Bradshaw CM, Szabadi E. Comparison of the effects of clozapine, haloperidol, chlorpromazine and d-amphetamine on performance on a time-constrained progressive ratio schedule and on locomotor behaviour in the rat. *Psychopharmacology (Berl).* 2000; 152:47–54. [PubMed: 11041315]
48. Nunes GP Junior, Tufik S, Nobrega JN. Autoradiographic analysis of D1 and D2 dopaminergic receptors in rat brain after paradoxical sleep deprivation. *Brain Res Bull.* 1994; 34:453–456. [PubMed: 8082038]
49. Paxinos, G.; Watson, C. The rat brain in stereotaxic coordinates. Fourth Edition, 2 edn.. San Diego: Academic Press Inc.; 1998.
50. Poncelet M, Chermat R, Soubrie P, Simon P. The progressive ratio schedule as a model for studying the psychomotor stimulant activity of drugs in the rat. *Psychopharmacology (Berl).* 1983; 80:184–189. [PubMed: 6136063]

51. Porkka-Heiskanen T, Smith SE, Taira T, Urban JH, Levine JE, Turek FW, Stenberg D. Noradrenergic activity in rat brain during rapid eye movement sleep deprivation and rebound sleep. *Am J Physiol.* 1995; 268(Pt 2):R1456–R1463. [PubMed: 7611522]
52. Ramesh V, Lakshmana MK, Rao S, Raju TR, Kumar VM. Alterations in monoamine neurotransmitters and dendritic spine densities at the medial preoptic area after sleep deprivation. *Sleep Res Online.* 1999; 2:49–55. [PubMed: 11382882]
53. Salamone JD, Correa M. Motivational views of reinforcement: implications for understanding the behavioral functions of nucleus accumbens dopamine. *Behav Brain Res.* 2002; 137:3–25. [PubMed: 12445713]
54. Salamone JD, Correa M, Mingote S, Weber SM. Nucleus accumbens dopamine and the regulation of effort in food-seeking behavior: implications for studies of natural motivation, psychiatry, and drug abuse. *Journal of Pharmacology & Experimental Therapeutics.* 2003; 305:1–8. [PubMed: 12649346]
55. Salamone JD, Wisniecki A, Carlson BB, Correa M. Nucleus accumbens dopamine depletions make animals highly sensitive to high fixed ratio requirements but do not impair primary food reinforcement. *Neuroscience.* 2001; 105:863–870. [PubMed: 11530224]
56. Saper CB, Cano G, Scammell TE. Homeostatic, circadian, and emotional regulation of sleep. *Journal of Comparative Neurology.* 2005; 493:92–98. [PubMed: 16254994]
57. Schulze GE, Paule MG. Acute effects of d-amphetamine in a monkey operant behavioral test battery. *Pharmacol Biochem Behav.* 1990; 35:759–765. [PubMed: 2345755]
58. Seiden LS, Sabol KE, Ricaurte GA. Amphetamine: effects on catecholamine systems and behavior. *Annu Rev Pharmacol Toxicol.* 1993; 33:639–677. [PubMed: 8494354]
59. Smith JK, Neill JC, Constall B. Post-weaning housing conditions influence the behavioural effects of cocaine and d-amphetamine. *Psychopharmacology (Berl).* 1997; 131:23–33. [PubMed: 9181632]
60. Stewart WJ. Progressive reinforcement schedules: a review and evaluation. *Australian Journal of Psychology.* 1975; 27:9–22.
61. van Luijckelaar EL, Coenen AM. Electrophysiological evaluation of three paradoxical sleep deprivation techniques in rats. *Physiol Behav.* 1986; 36:603–609. [PubMed: 3714830]
62. von Treuer K, Norman TR, Armstrong SM. Overnight human plasma melatonin, cortisol, prolactin, TSH, under conditions of normal sleep, sleep deprivation, and sleep recovery. *J Pineal Res.* 1996; 20:7–14. [PubMed: 8648563]
63. Wiley JL, Compton AD. Progressive ratio performance following challenge with antipsychotics, amphetamine, or NMDA antagonists in adult rats treated perinatally with phencyclidine. *Psychopharmacology (Berl).* 2004; 177:170–177. [PubMed: 15243736]
64. Wise RA. Catecholamine theories of reward: a critical review. *Brain Res.* 1978; 152:215–247. [PubMed: 354753]
65. Wise RA, Spindler J, deWit H, Gerberg GJ. Neuroleptic-induced "anhedonia" in rats: pimozone blocks reward quality of food. *Science.* 1978; 201:262–264. [PubMed: 566469]
66. Wise RA, Spindler J, Legault L. Major attenuation of food reward with performance-sparing doses of pimozone in the rat. *Can J Psychol.* 1978; 32:77–85. [PubMed: 737579]
67. Zhang M, Balmadrid C, Kelley AE. Nucleus accumbens opioid, GABAergic, and dopaminergic modulation of palatable food motivation: contrasting effects revealed by a progressive ratio study in the rat. *Behav Neurosci.* 2003; 117:202–211. [PubMed: 12708516]

Figure 1. Histological reconstructions of representative cannulae placements. Nucleus accumbens shell placements are denoted by circles. Numbers represent distance from bregma in millimeters. Adapted from the Atlas of Paxinos and Watson [49].

Figure 2.

The effect of REMSD on break point in a progressive ratio 2 operant task. Error bars represent one SEM. Note that the y-axis scales differ in A and B. Furthermore, baseline is an average of the three previous days preceding the period of REMSD. **(A)** Mean break points in 30-min sessions are shown for sleep-deprived (D), apparatus control (C), and home cage (H) groups fed *ad libitum*. * $P < 0.05$ for the C vs. H comparison. # $P < 0.05$ for D vs. H comparison. **(B)** Within-session responding for sessions on days 2–5 of REMSD in PR2 fed *ad libitum*. For the sake of clarity, the mean numbers of responses for each group are shown in three-minute bins across the 30-minute session.

Figure 3.

The effect of systemic amphetamine on break point in REMSD. Baseline is an average of the three previous days preceding the period of REMSD. Various doses of systemic amphetamine were administered on days 3 and 5 of REMSD to the (A) home cage and (B) REM sleep-deprived animals. Administration of amphetamine is denoted with an arrow. Error bars represent one SEM.

Systemic Amphetamine Within Session Bins

Figure 4.

Within-session responding for the all groups on days 2–5 of REMSD. Error bars represent one SEM. Note that scale on y-axis differs between home cage and REMSD groups. Testing days were separated according to systemic amphetamine treatment, for the sake of clarity. Responding for the home cage animals are shown in **A** and **B** in which the animals received no treatment on days 2 and 4 (**A**), and were administered systemic amphetamine on days 3 and 5 (**B**). Responding rates for the REM sleep-deprived rats are shown in **C** and **D** in which the animals did not receive amphetamine treatment on days 2 and 4 (**C**), and were administered systemic amphetamine on days 3 and 5 (**D**).

PR2 with IntraAcbSh Amphetamine

Figure 5.

The effect of intra-nucleus accumbens amphetamine on break point in REMSD. Baseline is an average of the three previous days preceding the period of REMSD. Various doses of intra-Acb amphetamine were administered on days 3 and 5 of REMSD to the (A) home cage and (B) REM sleep-deprived animals. Intra-Acb administration of amphetamine is denoted with an arrow. Error bars represent one SEM.

IntraAcbSh Amphetamine Within Session Bins

Figure 6.

Within-session responding for the all groups on days 2–5 of REMSD. Error bars represent one SEM. Note that scale on y-axis differs between home cage and REMSD groups. Testing days were separated according to systemic amphetamine treatment, for the sake of clarity. Within-session lever presses for the home cage animals are shown in **A** and **B**; the animals received no treatment on days 2 and 4 (**A**), and were given intra-Acb amphetamine on days 3 and 5 (**B**). Responding for the REM sleep-deprived rats is shown in **C** and **D**; the animals did not receive amphetamine treatment on days 2 and 4 (**C**), and were given intra-Acb amphetamine on days 3 and 5 (**D**).