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The effects of caffeine and rapid eye movement (REM) sleep deprivation on free operant responding under a VI 30-S schedule of reinforcement

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Abstract

Past research has shown that the effects of 96 hr of Rapid Eye Movement Sleep Deprivation (REMSD) on positively reinforced behavior is dependent upon the schedule of reinforcement maintaining the behavior. One one hand, lean schedules of reinforcement after REMSD maintained low rates of behavior. On the other hand, rich schedules of reinforcement after REMSD maintained behavior at baseline levels. Other research has shown that the use of stimulants reversed the effects of REMSD on operant tasks. The current study investigated the effects of caffeine on rats' lever pressing after 96-hr REMSD. During baseline, doses of vehicle were administered 15 min prior to sessions in which the delivery of reinforcers occurred according to a variable-interval 30-s schedule. After reaching stability rats were exposed to 96-hr REMSD or an aquatic tank control (TC). Following this 96-hr period, a dose of 10 mg/kg of caffeine or vehicle was administered 15 min prior to the session. Ninety-six hours of REMSD did not result in a decrease in responding when using a variable-interval 30-s schedule of reinforcement. Pre-session injections of caffeine resulted in no change in lever pressing regardless of sleep condition. I discuss possible reasons for an inability to replicate previous findings including weight of animals and size of elevated platforms in regard to animal weight. I also discuss the inability to alter rats' lever pressing using caffeine in the context of potency and environment contingencies. Finally, I discuss future directions for research of REMSD and schedules of positive reinforcement.

Introduction

Sleep

Sleep is a vital behavior that is necessary for most organisms to survive. The absence of sleep can be fatal for organisms if deprived of sleep for about 3-4 weeks (Rechtschaffen, Gilliland, Bergmann, & Winter, 1983). Sleep comprises of two parts: Rapid Eye Movement (REM) sleep and non-REM (NREM) sleep. Non-REM sleep is comprised of stages N1, stage N2, and slow wave sleep, or stage N3. Previously, stage N3 was labeled as stages 3 and 4 until 2007 (Iber, Ancoli-Israel, Chesson, & Quan, 2007). REM sleep occurs between cycles of NREM sleep and its onset is typically identified by rapid eye movement as well as loss of muscle tonality.

Sleep is an unconditioned motivating operation (UMO) in most organisms (Laraway, Snycerski, Michael, & Poling, 2003). The only time in which sleep will not function as an effective reinforcer is when an organism is either actually asleep or is sleep satiated (Laraway, Snycerski, Michael, & Poling, 2003). Many researchers study the importance of sleep through methods of sleep deprivation (Pilcher & Huffcutt, 1996). Researchers observe the various behavioral effects that are produced as a function as sleep deprivation such as increases in problem behavior (Kennedy & Meyer, 1996), difficulty discriminating stimuli (Magill et al., 2003), increased food intake (Kushida, Bergmann, & Rechtschaffen, 1989; Mendelson, Guthrie, Frederick, & Wyatt, 1974), and decreased response rate for food acquisition behaviors such as lever pressing under various schedules of reinforcement (Hanlon, Andrzejewski, Harder, Kelley, & Benca, 2005; Hanlon, Benca, Baldo, & Kelley, 2010; Kirby & Kennedy, 2003).

Sleep Deprivation

Research with humans has shown that full sleep deprivation alters qualitative measures, such as alertness and mood (Penetar et al., 1993), while also altering quantitative measures including reaction time and response latency (Magill et al., 2003). Specifically, thirty hrs of sleep deprivation, when compared to 6 hrs of sleep deprivation, produced significant increases in response times across running memory tasks, logical reasoning tasks, math processing tasks, and visual vigilance tasks in males age 18-35 (Magill et al., 2003). Participants took longer to discriminate letters they had previously seen (running memory), label AB logical statements as true or false (logical reasoning), solve simple addition or subtraction problems (math processing), and detect random appearances of a visual stimulus over a 40-min time period (visual vigilance). Also, after 30 hrs of sleep deprivation participants had more errors when tracking a stimulus on a computer monitor with a mouse and detected stimuli less often in the visual vigilance task (Magill et al., 2003). Researchers also suggest that sleep deprivation is at least one of the observed factors that affect marksmanship for Navy SEALs during "Hell Week" training (Tharion, Shutkitt-Hale, & Lieberman, 2003). After 73 hrs of full sleep deprivation, accuracy of rifle marksmanship during a simulation task degraded (Tharion, Shutkitt-Hale, & Lieberman, 2003).

Although these representative studies use full sleep deprivation, a method in which participants are not allowed to obtain any sleep, sleep is often studied through REM sleep deprivation (REMSD), a technique in which participants are awoken when they begin to fall into REM sleep. REM sleep deprivation (REMSD), a type of partial sleep deprivation, elicits similar results to full sleep deprivation in animal research (Kushida, Bergmann, & Rechtschaffen, 1989). The method typically used to deprive rats

of REM sleep is the inverse flower pot technique (Mendelson et al., 1974). Rats are placed in an aquatic setting where a platform is elevated slightly above water. The platform is big enough for the rat to stand on, but not big enough for the rat to acquire REM sleep. When a rat goes into REM sleep it falls in the water. This is the result of a loss of muscle tonality that occurs when an organism enters REM sleep. When rats fall into the water they awaken and climb back onto the pedestal. This method selectively eliminates almost all REM sleep (Maloney, Mainville, & Jones, 1999). The inverse flower pot technique has been used in various animal studies to show that the effects of sleep deprivation are dependent upon the amount of sleep deprivation, as well as the environmental contingencies of avoidance and positive reinforcement.

Operant Behavior and Sleep. Before 2000 little research was published on the effects of sleep deprivation and negative reinforcement (Kennedy et al., 2000). Most research found an relation between sleep deprivation and negatively reinforced behavior in applied settings (Kennedy & Itkonen, 1993; O'Reilly, 1995). Kennedy and Itkonen (1993) observed a negative correlation between the amount of sleep obtained by three teenage children diagnosed with retardation and the amount of escape-from-instruction behavior. These studies suggested that sleep deprivation affected negatively reinforced behavior. In a rodent model, Kennedy and colleagues (2000) sought to observe the interaction between REMSD and avoidance responding. They found that avoidance responding in a free-operant procedure increased after 48-hr REMSD when compared to a baseline of ad libitum sleep. The change in response rate occurred as a result of shortened interresponse times (IRTs) and the increases in responding occurred independent from response-shock intervals (Kennedy et al., 2000). The authors suggested

two possible reasons for the change in response rate after REMSD. First, rats are more active, in general, after sleep deprivation (Albert, Cicala, & Siegel, 1970). Second, REMSD alters the organism's sensitivity to stimuli within the environment (Kennedy et al., 2000). For example, studies have shown that sleep deprived rats have a lowered pain threshold (Hicks et al., 1978).

To understand the behavioral mechanisms of which REMSD operates, Kennedy (2002) observed the effects of REMSD under schedules of reinforcement. Although Kennedy had shown that sleep deprivation followed by avoidance conditioning led to an increase in avoidance responding, this was not the case under appetitively reinforced behavior. Under a multiple fixed-interval (FI) fixed-ratio (FR) schedule of reinforcement, responding decreased compared to baseline following 96-hr REMSD (Kennedy, 2002). However, this decrease was temporary for three of four rats being tested. After multiple trials under REMSD, these three rats returned to baseline levels of responding. This helped clarify the behavioral mechanisms of which REMSD affected behavior. The results showed that REMSD does not increase all behavior. However, Kennedy was unable to determine the cause of the decrease in responding. The results could have been due to REMSD or an interaction between food deprivation and REMSD.

To separate behaviors motivated by REMSD and food deprivation, Kennedy (2002) made REM sleep unattainable during sessions. When REM sleep was unattainable, responding under the influence of reinforcement was similar in control and REMSD conditions. Therefore, REMSD has different interactions with operant behavior. Under schedules of aversive conditioning, avoidance responding increased after REMSD. On the other hand, REMSD appeared to either increase or have no effect on responding

under schedules of reinforcement (Kennedy, 2002). This phenomenon occurs even though animals will increase food intake after REMSD (Hanlon et al., 2005).

To further investigate the relation between REMSD and appetitive responding, Kirby and Kennedy (2003) studied the effects of different variable-interval (VI) schedules of reinforcement. REMSD appeared to cause a decrease in operant responding under some appetitive schedules. However, this effect was observed across a select range of schedules of reinforcement. Using a VI schedule, Kirby and Kennedy observed the effects of reinforcer density without the interaction of different schedules of reinforcement that evoke distinctly different types of responding. The authors compared response rates of rats after 96-hr REMSD to that of baseline response rates where sleep was readily available.

Kirby and Kennedy (2003) took four rats and shaped lever pressing behavior via shaping through successive approximation. They began using CRF schedules, changed to a VI 5 s, and progressively added 5 s to the schedule of reinforcement across multiple days. The study began once the rats were responding under a VI 30-s schedule. Sessions comprised of four 10-min bins that were separated by 1-min blackout components. The dependent variables were response rates and rate of reinforcement from the last two bins of each session. Baseline sessions continued until stability was reached. Stability occurred once the session's responding rate was ± 10% of the response rate from the previous 15 sessions. Rats were then placed into one of three experimental conditions for the next 96 hours. Rats were either kept in their home cage (HC), placed in an aquatic tank with a large platform (Tank Control, TC), or placed in an aquatic tank with a small platform (REMSD). This cycle continued until rats completed the three experimental

conditions 9 times under a VI 30-s schedule. Rats repeated the same cycle under a VI 15-s schedule of reinforcement, completed the 9 conditions, and then repeated the cycle again under a VI 30-s schedule of reinforcement.

Kirby and Kennedy (2003) found that the schedule of reinforcement that maintained responding was the controlling variable of the effect of REMSD. Under a leaner schedule of reinforcement (VI 30 s) rats' lever pressing greatly decreased after 96-hr REMSD. When the schedule of reinforcement was changed to VI 15 s, the response rate was consistent with baseline levels. In other words, a dose of 96-hr REMSD, found previously effective in appetitive conditioning (Kennedy, 2002), can be attenuated by environmental contingencies (Kirby & Kennedy, 2003). Kirby and Kennedy (2003) discussed that the two establishing operations, REMSD and food deprivation, were two operations competing to control behavior. Under a lean schedule of reinforcement, REMSD is the effective establishing operation for non-food-seeking behavior. Under a rich schedule of reinforcement, food deprivation becomes the dominant operation establishing motivation for food-seeking behavior.

Stimulants. Like environmental contingencies, stimulants can attenuate the effects of sleep deprivation. A study conducted by Hanlon and colleagues (2010) found that certain doses of intra-accumbens amphetamine reversed the effects of sleep deprivation on operant tasks. This finding suggests that the use of stimulants may reduce or reverse the behavioral effects of sleep deprivation.

Caffeine

Caffeine is a central nervous system stimulant that operates on the A_1 and A_{2A} adenosine receptors (Roehr & Roth, 2008). Adenosine is a neuromodulator that decreases

the rate of neural firing and neuron's neurotransmitter release (Prince & Stevens, 1992; Roehr & Roth, 2008). Adenosine builds in the basal forebrain as an organism stays awake, promoting sleep (Julien, Advokat, & Comaty, 2010), and is metabolized when an organism is asleep, thereby providing low levels upon waking. Caffeine blocks adenosine receptors which commonly precedes an increase in overall behavior. It follows that caffeine, an adenosine antagonist, should delay or prevent the onset of sleep. Lab studies have shown that caffeine delays the onset of sleep as well as interfere with the quality of sleep (Roehr & Roth, 2008). Caffeine administration has also been suggested as a method for sleep disruption (Paterson et al, 2009).

Although it does not operate on the same receptors as other stimulants such as amphetamine, caffeine is well established as a psychoactive stimulant. At certain doses caffeine increases lever pressing maintained under a FI schedule of reinforcement (McMillan, 1979; Mechner & Latranyi, 1963). Research has shown that caffeine reduces and sometimes reverses the behavioral effects of sleep deprivation (Bonnet, Gomez, Wirth, & Arant, 1995; Lagarde et al., 2000; Magill et al., 2003; Penetar et al., 1993; Tharion et al., 2003; Wesensten, Belenky, Thorne, Kautz, & Balkin, 2004).

Research Question

Kirby and Kennedy (2003) found that the differing response rates and rates of reinforcement under sleep deprivation may be due to competing motivating operations. When the rate of reinforcement available to the organism increases, the response rate reduction effect of REMSD can be attenuated, in turn maintaining response rates at baseline levels. When rats were given stimulants, such as caffeine, an increase in response rate was observed at a dose of 10 mg/kg (McMillan, 1979; Mechner & Latranyi,

1963). Based on this information, it is possible that caffeine can do pharmacologically what only rich schedules of reinforcement do under similar methods. The current study will investigate this possibility through systematic replication of the Kirby and Kennedy (2003) study with extension by administering caffeine prior to the experimental session under a REMSD and tank control condition.

Expected Findings

Effective REMSD & caffeine. If 96-hr REMSD and caffeine are effective as they have been in previous studies, then REMSD will systematically decrease responding that is being maintained under VI 30-s schedules of reinforcement, and caffeine will increase responding independent of sleep or schedule conditions. This would mean that caffeine would not only work as an establishing operation for food-seeking behavior, but that caffeine alter a response rate the same way enrichment of a reinforcement schedule can alter response rate.

Non-effective REMSD & caffeine. If 96-hr REMSD and caffeine are not effective, responding will not change across conditions. This could have multiple conclusions. An inability to observe a REMSD effect on response rate could be due to a difference in methods or it could mean that 96-hrs of REMSD is not effective at lowering response rates maintained under schedules of reinforcement. If caffeine is not effective in this study a few conclusions could be reached. A lack of change in response rate could mean that 10 mg/kg is not an effective behavior-altering dose of caffeine. Also a lack of behavior change could mean that caffeine is ineffective at altering response rates under VI schedules of reinforcement.

Effective REMSD & non-effective caffeine. If 96-hr REMSD is effective, as it has been in previous studies, then REMSD will systematically decrease responding under VI 30-s schedules of reinforcement. If caffeine has no effect across all sleep conditions then the same conclusions can be reached as discussed in the previous paragraph. However, if caffeine is only ineffective at increasing response rates after REMSD, then we can conclude that REMSD decreases the potency of caffeine when measuring behavior change in a VI-30 s schedule of reinforcement.

Non-effective REMSD & effective Caffeine. If 96-hr REMSD is not effective, then REMSD will have no effect on responding independent of the schedule of reinforcement. If caffeine is effective, as it has been in previous studies, responding should increase independent of the schedule of reinforcement and sleep conditions. Here we can reach conclusions for REMSD and caffeine as expressed in the first and second expected findings, respectively.

Method

Subjects

The subjects were 12 experimentally naive male Sprague-Dawley rats individually housed in plastic cages (23cm x 20.5cm) in a colony room illuminated from 6:00 a.m. to 6:00 p.m. on a 12-hr dark-light cycle at a temperature of 21^{O}C - 27^{O}C and 45% - 55% humidity. Water was given ad libitum. After a stable ad libitum weight was reached the rats were kept at a body weight of 80% or greater. Weight stability was defined as daily weight that is ±10 grams of the average weight of the rat from the prior two weeks. Training included only food pellet deliveries. At least one hr after training sessions were completed, the rats were fed the remainder of their controlled food regimen. The food regimen was 10 - 15 g per day delivered in the form of 45-mg pellets (Bio-Serv, Frenchtown, NJ; TestDiet®, Richmond, IN) earned during experimental sessions and Harlan (Madison, WI) Rodent Diet (8604) delivered about one hr after each session.

Apparatus

The experiment was conducted in Med-Associates (Georgia, VT) rat operant chambers (ENV-008CT) individually housed in a ventilated, sound and light attenuated enclosure. Each operant chamber contained two retractable response levers (ENV-112CM) that were located on the front wall evenly spaced on either side of an opening through which pellet reinforcer delivery occurred. A houselight was located at the top center of the back wall of the operant chamber. Below the light and across from the feeder opening was a third retractable lever that was not used in the current study. Above each lever were three colored LED stimulus lights (red, yellow, and green, left to

right). A 4.0 KHz (80 db) speaker, controlled by a Med Associates Audio Stimulus Generator (ANL-926), was located on the front wall of the chamber above the pellet dispenser. A computer using MED-PC IV software programming controlled the operant chambers.

REM Sleep Deprivation (REMSD). REMSD was accomplished through the pedestal-over-water method described by Morden, Mitchell, and Dement (1967). Aquatic tanks where rats were housed during REMSD were 29 cm high, 25 cm wide, and 50 cm in length. The platform was 16 cm high, 7.5 cm in diameter, and 9 cm from the tank wall. Each platform was 1 cm above water level.

Tank-control. The control condition was also achieved using the pedestal-over-water method. Aquatic tanks where rats were housed for the tank control (TC) condition were 29 cm high, 25 cm wide, and 50 cm in length. The platform was 16 cm high, 15 cm in diameter, and 9 cm from the tank wall. Platforms 15 cm in diameter allowed rats to stay on the platform when entering REM sleep. Each platform was 1 cm above water level.

Procedure

General Procedure.

Pretraining. All pretraining sessions lasted for 43 min or until 250 reinforcers were delivered. During the first session the food receptacle in each chamber was baited with 25 pellets prior to the session, and all levers remained retracted; also, one pellet was delivered according to a random time 30-s schedule throughout the session. A 2000 Hz tone occurred for 500-ms concurrently with each pellet delivery. The next four sessions involved an autotraining procedure in which prior to each pellet delivery, the left lever

extended and the three LEDs above the extended lever illuminated. If the rat did not press the lever within 10 s, the lever retracted, a pellet was delivered, and the three LEDs extinguished. If the rat pressed the lever within 10 s, the lever retracted, the pellet was delivered and the LEDs extinguished. After 10 lever presses, an operant contingency was put in place such that the rat had to press the lever in order for food delivery to occur. After the operant contingency was in place the procedure changed to a free-operant CRF procedure. Each session began when the house light illuminated, the lever extended into the chamber and the three designated LEDs illuminated. The session ended once the rat received 250 pellets or 50 min had passed. At the end of each session, the lever retracted, the three LEDs extinguished, and the houselight extinguished. Once consistent responding occurred across sessions all rats advanced to the baseline condition.

Baseline. All sessions occurred between 9:30-10:30a.m. Sessions consisted of four 10-min components, each separated by a 1-min blackout component. Each component began with the illumination of the houselight and the extension of the left lever. After 10 min the blackout component occurred. During the blackout component the houselight extinguished and the extended lever was retracted. At the end of the session the extended lever was retracted, and the houselight extinguished simultaneously. Baseline sessions occurred until the stability criterion was met. All baseline sessions entailed a VI 30-s schedule across components in a single four-component baseline session. Only one session occurred each day. Stability was achieved once the final two components in a session were within ± 10% of the mean of the previous 10 baseline sessions. Previous baseline session averages were determined by the final two components of those prior sessions. Once the stability criterion was met, 6 assigned rats

were exposed to either the REMSD or REMSD+Caffeine condition, and 6 assigned rats were exposed to the TC or TC+Caffeine.

REMSD, REMSD+Caffeine, TC, and TC+Caffeine Phases.

After the stability criterion was met, a rat was exposed to a REMSD, REMSD+Caffeine, TC, or TC+Caffeine condition for 96 hr. During this time no testing occurred. The REMSD and REMSD+Caffeine required placing a rat in a REMSD tank. REMSD was accomplished through the pedestal-over-water method described by Morden, Mitchell, and Dement (1967). In the REMSD condition rats received .1 mL/g of saline (0.9% sodium chloride) via Intra Peritoneal (IP) injection 15 min prior to running the experimental sessions. In the REMSD+Caffeine condition rats received an IP injection of 10 mg/kg dose of caffeine dissolved in saline 15 min prior to running the experimental session. The TC was identical to the REMSD tank condition, but rats were presumably able to access REM sleep ad libitum. This was achieved using a larger platform. Fifteen min prior to the experimental session, rats received an IP injection of saline. In the TC+Caffeine condition rats received an IP injection of 10 mg/kg dose of caffeine dissolved in saline 15 min prior to running the experimental session. The TC was chosen for two reasons. First, the TC kept the environment as similar as possible to REMSD conditions. This eliminated any possible differential ‘stress’ effects of an aquatic. Secondly, Kirby and Kennedy (2003) showed similar response rates and food pellet rates across TC and home cage conditions. During all conditions the rat had access to water ad libitum and the restricted diet available through a wire top.

All rats received alternate injections of caffeine and saline injections across experimental days. Following the exposure to REMSD, REMSD+Caffeine, TC, or

TC+Caffeine, each rat was exposed to a single session identical to the session used during baseline. Following the experimental session the rat returned to its home cage where restricted diet, ad libitum REM sleep, and ad libitum water were available. The following day, rats returned to baseline sessions. Once stability was reached the rat repeated the previous 96 hr and received the opposite injection from their previous experimental day.

Results

With only 6 rats the responses per minute across the baseline average (lines), baseline standard deviation (broken lines), saline (closed circle), and caffeine conditions (open circles) for all rats are shown for the REMSD and TC groups. The response per minute for baseline is the average of the last two baseline sessions before stability. Each data point for caffeine and saline conditions is the average response rate across all four components of the designated session.

Responses Per Minute.

REMSD. Figure 1 shows the response rate per minute across the baseline average (lines), baseline standard deviation (broken lines), saline (closed circle), and caffeine conditions (open circles) for rats in the REMSD groups. As seen in Figure 1, average baseline response rates varied greatly although all rats were trained using the same autoshaping procedure. Average baseline response rates ranged from 13 to 94 responses per minute across rats in the REMSD group. Ninety-six hours of REMSD did not have any consistent effect on response rate. The rate of responding for S-1-1 maintained baseline levels following 96-hr REMSD. Response rates increased following 96-hr REMSD for rats S-1-5, S-1-6, and S-1-8. Each individual rat's response rate following 96-hr REMSD were within the respective range of baseline responding (10-113 responses/min) with a slight upward trend. Caffeine also had no clear effect on response rates following 96-hr REMSD. Response rates for all rats, with the exception of S-1-5, were within the range of baseline responding. Three rats (S-1-1, S-1-4, S-1-8) had clear upward trends in response rate following 96-hr REMSD and an injection of caffeine.

Again, response rates were above and below baseline averages with the majority of data points being within ±1 population standard deviation of the average baseline responding.

Tank Control. Figure 2 shows the response rate per minute across the baseline average (lines), baseline standard deviation (broken lines), saline (closed circle), and caffeine conditions (open circles) for rats in the TC groups. Similar to the REMSD group, baseline responding varied greatly among six rats as seen with baseline averages and standard deviations. Baseline response rates ranged from 20 to 131 responses per minute. Ninety-six hours of TC had no clear effect on rats' response rate. The rates of responding for S-1-13, S-1-7, S-1-9, and S-1-10 maintained baseline levels following 96-hr TC. Response rate for rat S-1-2 decreased across experiment sessions following 96-hr TC. Caffeine also had no clear effect on response rate following 96-hr TC. As seen in Figure 2 response rates under the caffeine conditions were slightly above or at baseline levels of responding. Three rats in the TC+caffeine condition (open circles) had slight upward trends across the three caffeine days and the remaining three rats had small downward trends.

Rate of Reinforcement

Figure 3 shows the pellet delivery per minute across the baseline average (lines), baseline standard deviation (broken lines), saline (closed circle), and caffeine conditions (open circles) for rats in the REMSD and TC groups, respectively. The rate of pellet delivery is the average of pellets earned per minute of the last two components with the corresponding session. Baseline pellet delivery is the average of the final two components of each session within the last 15 days of baseline.

As seen in Figure 3, the rate of pellet delivery was not different between REMSD and TC groups. Rate of pellet delivery did not change as a function of drug condition. Across all rats the rate of pellet delivery was consistent with little to no difference across rats.

Discussion

The purpose of this research was to confirm the results of Kirby and Kennedy (2003) and to explore the relation of a psychoactive stimulant on lever pressing under similar methods. An effect of REMSD on lever pressing maintained under a VI 30-s schedule of reinforcement and pellets earned was not observed. Unlike Kirby and Kennedy (2003), 96-hr REMSD did not systematically decrease lever pressing compared to baseline response rates. This absence in effect is observed across different rates of responding, different weights, fluctuations in weight, within subjects, and between groups. Also unlike Mechner and Latranyi (1963), caffeine at a dose of 10 mg/kg also appeared to have no systematic influence on response rate or pellets earned. Again this was observed across different rates of responding, different weights, fluctuations in weight, within subjects, and between groups.

Past research had shown that 96-hr REMSD reduced responding under appetitive conditions when sleep could be obtained (Kennedy, 2002). Ninety-six hrs of REMSD was the lowest amount needed to decrease lever pressing maintained by appetitive reinforcement across all subjects. Kennedy showed that 96-hr REMSD reduced responding under a multiple FI FR schedule of reinforcement unless REMSD was unattainable during a session. REMSD did not affect motivation for food, but it reduced food-reinforced behavior. Other studies also show that 96-hr REMSD effectively decreases lever pressing under appetitive reinforcement (Hanlon et al 2005, Hanlon et al 2010).

In a pilot study using the inverted flower-pot method, Youngblood and colleagues (1997) concluded a 10g:1cm^2 weight/platform area ratio was required to achieve a

behavioral change due to partial sleep deprivation. For example, a rat maintained at 350 g would be placed on a platform 35 cm^2 in order to achieve a change in behavior. Kirby and Kennedy (2003) used a similar ratio, maintaining a weight:platform area ratio range between 9.09-.65g:1 cm^2. The areas of the REMSD platforms used in the current study were 44cm^2. The ratio used in the current experiment to achieve REMSD ranged between 6.48-7.95:1. At both ratio extremes there was no clear change in response rate between and within subjects. As for a suitable control condition, Youngblood and colleagues (1997) used a weight/platform area ratio of 1:1. On the other hand, Kirby and Kennedy (2003) used a ratio ranging from 2.27-2.42:1 for the TC condition. Tank Control ratios ranged between 1.6-1.99:1 in this experiment. It appears that the range of TC ratios used in the current experiment was large enough to produce similar responding to baseline response rates. Overall, it seems that the size of platforms were adequate for the TC condition, but the size of platforms used for REMSD may have been too large to evoke a change in behavior caused by REMSD.

While the ratio of weight/platform area may play a role in acquiring a change in behavior through REMSD, another factor that may contribute to our findings could be weight of the subjects. In previous research using REMSD all rats were maintained at a weight range of 400-425 g (Kennedy et al., 2001; Kennedy 2002, Kirby & Kennedy, 2003). This weight, combined with 96-hr REMSD, resulted in behavioral changes when compared to baseline responding. As mentioned earlier, this is found in both aversive and appetitive schedules of reinforcement. The current study maintained all subjects between 285-350 g. It could be that these weights were too low to see any effect of 96-hr REMSD.

To date there is no research investigating the interaction between subject weight and REMSD on behavior changes.

Besides weight, the possibility of short-term weight loss during REMSD could play a factor for such high levels of responding after 96-hr of REMSD. It could be that the rats were so hungry that they maintained high levels of lever pressing even after 96 hr of REMSD. Previous research has found that full sleep deprivation and REMSD results in a decrease in weight even after there is an increase in food intake or maintenance of baseline rates (Everson, Bergmann & Rechtschaffen, 1989; Kushida, Bergmann, & Rechtschaffen, 1989; Mendelson et al., 1974). This suggests that the baseline-level rates of responding after 96-hr REMSD in this project were not caused by weight loss or the motivating operation of decreased food intake.

Caffeine did not appear to have any systematic effect on lever pressing in either the REMSD or TC conditions. There are a few possible reasons why there was no change in lever pressing. First, caffeine may not increase lever pressing when the behavior is maintained by a VI schedule of reinforcement. The effects of drugs on operant behavior can be changed based on the environment such as the schedule of reinforcement maintaining a behavior (Dews, 1955). Previous research only found an increase in lever pressing and licking after caffeine administration under a FI schedule at a dose of 10 mg/kg of caffeine (McMillan, 1979; Mechner & Latranyi, 1963). Second, this study was limited to one dose of caffeine. Therefore, we cannot conclude that caffeine is ineffective at changing lever pressing under a VI schedule of reinforcement. To answer this question future research should compile a dose-response of caffeine using this protocol. Last, we cannot effectively conclude that caffeine does not interact with 96-hr REMSD. As

mentioned earlier, REMSD was ineffective at altering response rates, which differs from previous research (Kennedy et al. 2000; Kennedy, 2002; Kirby & Kennedy, 2003).

Originally I had hoped to not only replicate the findings of Kirby and Kennedy (2003) but I had also hoped to negate the effects of REMSD using caffeine. According to these data, 96-hr REMSD does not appear to lower response rate under a VI-30 s schedule of reinforcement and a 10 mg/kg dose of caffeine does not appear to alter response rate either. However, this study contributes to the literature in a few different ways. These data indicate that 96 hr REMSD may not have a strong effect on response rate under VI schedules of reinforcement. REMSD may still alter behavior maintained under a schedule of reinforcement, but that effect may become more salient only when using a smaller platform or a schedule of reinforcement that is more sensitive to changes in operant behavior. This also applies to the use of caffeine. It is crucial to study a dose range with a low dose that has no effect on behavior and a high dose that disrupts most behaviors. A dose range offers a full picture of a drug's effects in a specific context. As mentioned above, a 10 mg/kg dose of caffeine is not effective to reach a conclusion of the effects of this psychoactive stimulant on lever pressing under a VI-30 s schedule of reinforcement. Instead, this research can only conclude that a 10 mg/kg dose of caffeine is ineffective at changing response rates under a VI-30 s schedule of reinforcement. Future research looking into REMSD and psychoactive stimulants in a similar context will be able to provide conclusive results if they use platforms small enough to evoke changes in response rates, and, if given the time, use a dose-range covering extreme doses of the drug.

Table 1
Order of Conditions

	Schedule of Reinforcement					
	VI 30-s					
Rat ID	Condition 1	Condition 2	Condition 3	Condition 4	Condition 5	Condition 6
S-1-1	REMSD +Caffeine	REMSD	REMSD +Caffeine	REMSD	REMSD +Caffeine	REMSD
S-1-2	TC	TC +Caffeine	TC	TC +Caffeine	TC	TC +Caffeine
S-1-13	TC +Caffeine	TC	TC +Caffeine	TC	TC +Caffeine	TC
S-1-4	REMSD	REMSD +Caffeine	REMSD	REMSD +Caffeine	REMSD	REMSD +Caffeine
S-1-5	REMSD	REMSD +Caffeine	REMSD	REMSD +Caffeine	REMSD	REMSD +Caffeine
S-1-6	REMSD +Caffeine	REMSD	REMSD +Caffeine	REMSD	REMSD +Caffeine	REMSD
S-1-7	TC +Caffeine	TC	TC +Caffeine	TC	TC +Caffeine	TC
S-1-8	REMSD +Caffeine	REMSD	REMSD +Caffeine	REMSD	REMSD +Caffeine	REMSD
S-1-9	TC +Caffeine	TC	TC +Caffeine	TC	TC +Caffeine	TC
S-1-10	TC	TC +Caffeine	TC	TC +Caffeine	TC	TC +Caffeine
S-1-11	REMSD	REMSD +Caffeine	REMSD	REMSD +Caffeine	REMSD	REMSD +Caffeine
S-1-12	TC	TC +Caffeine	TC	TC +Caffeine	TC	TC +Caffeine

Note: REMSD = 96 hr Rapid Eye Movement sleep deprivation. TC= 96 hr Tank Control

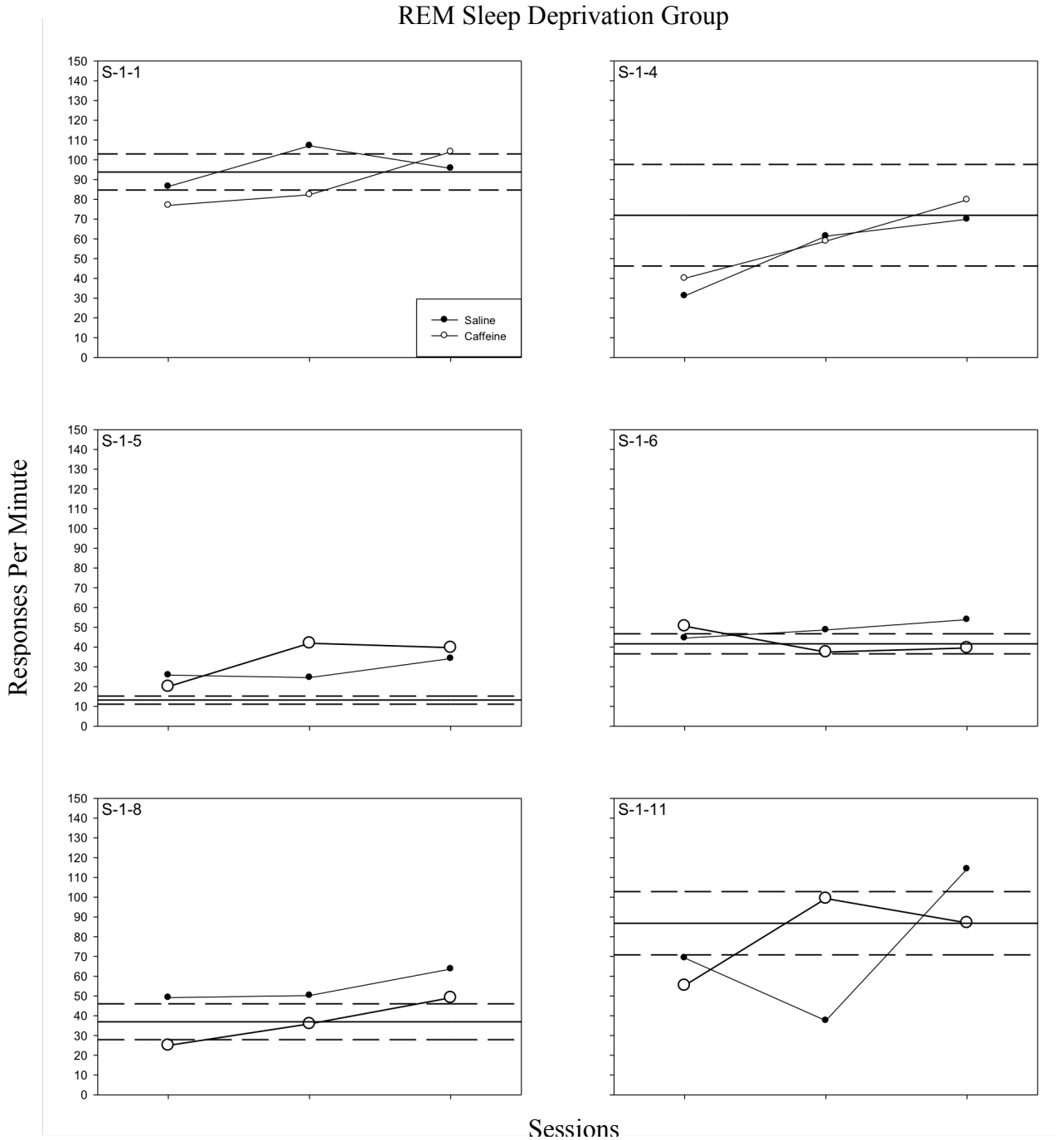

Figure 1. Shows the individual response rate as a function of REMSD and drug condition.

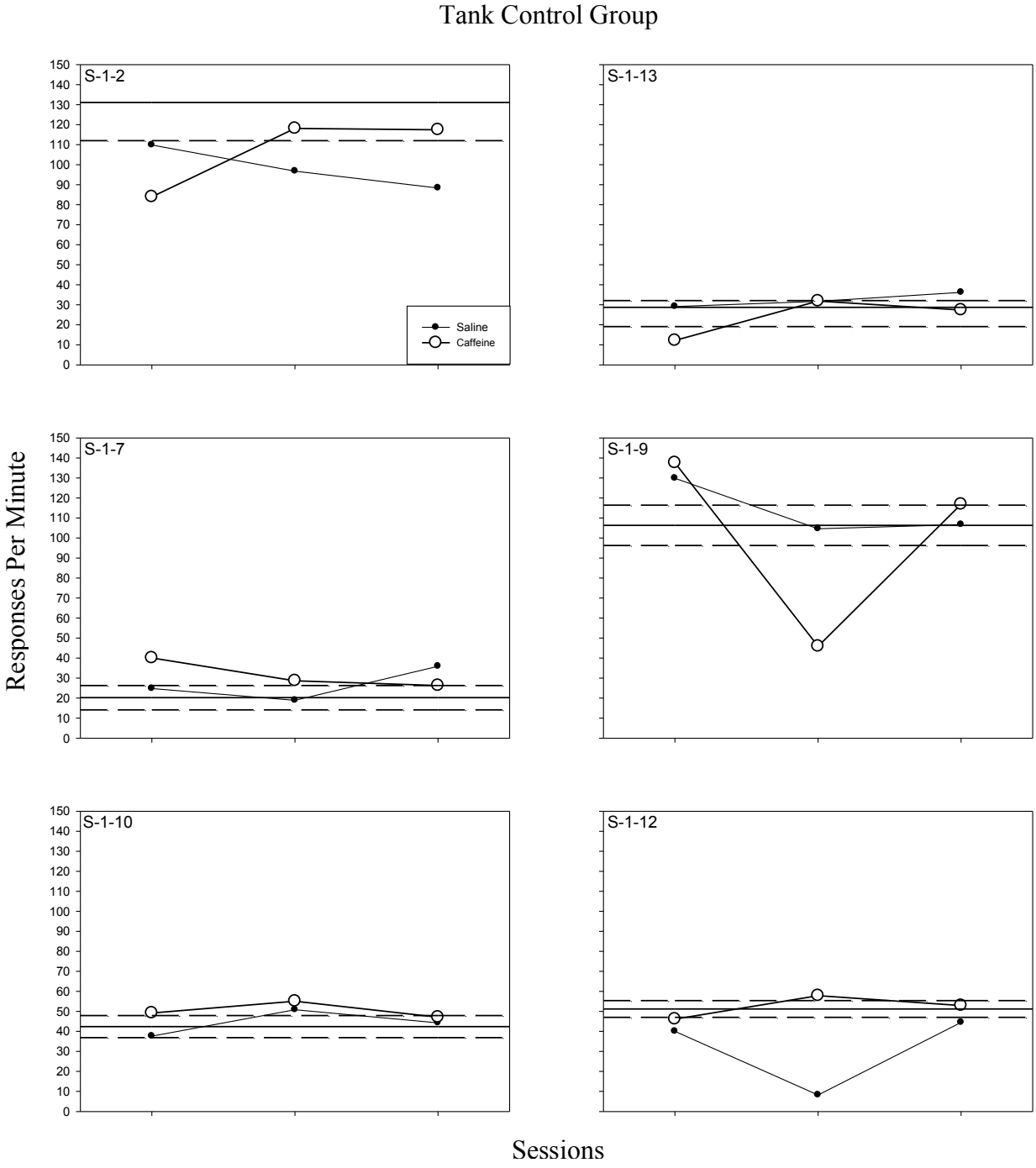

Figure 2. Shows the individual response rate as a function of TC and drug condition.

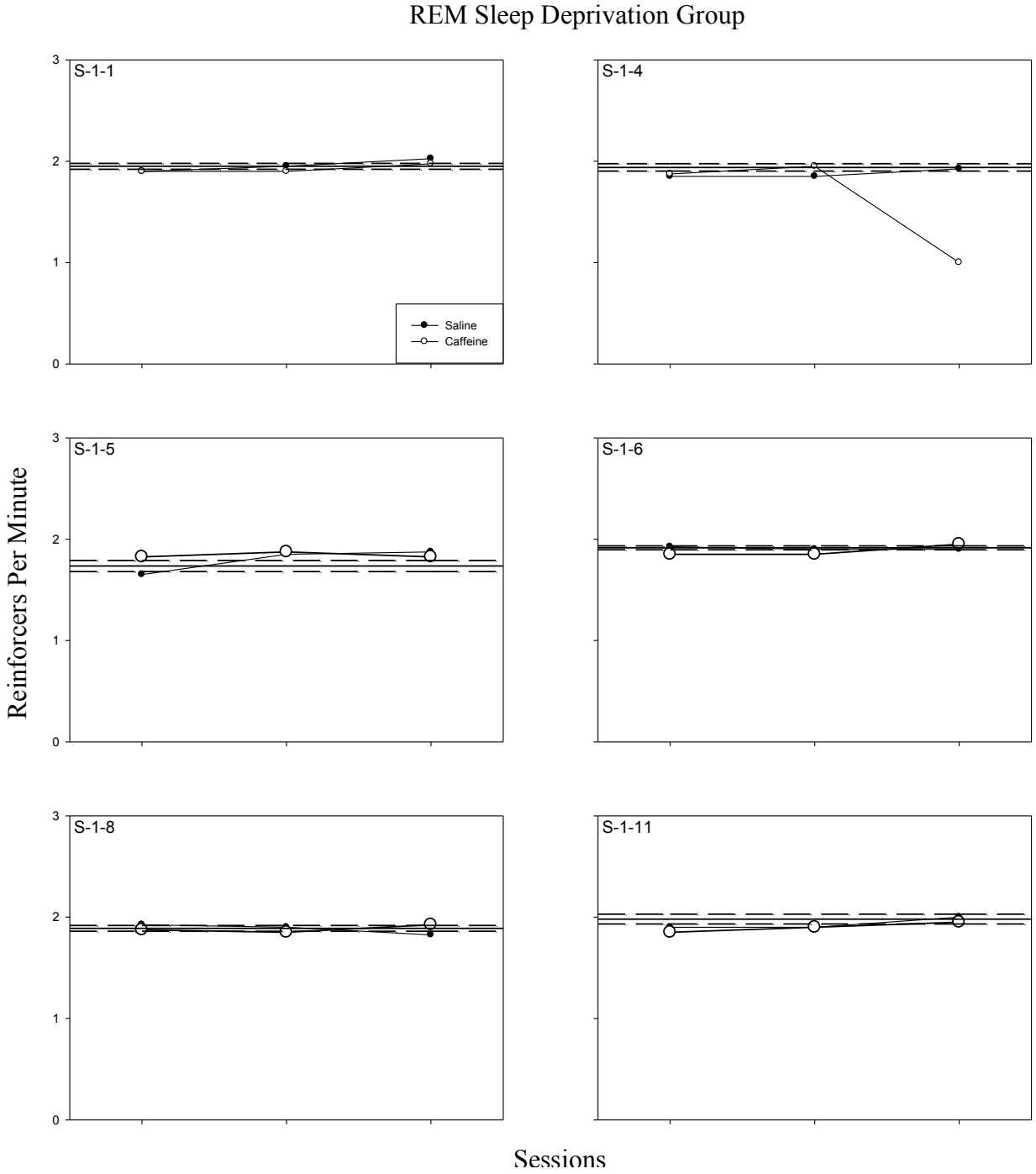

Figure 3. Shows the individual rate of reinforcement as a function of REMSD and drug condition.

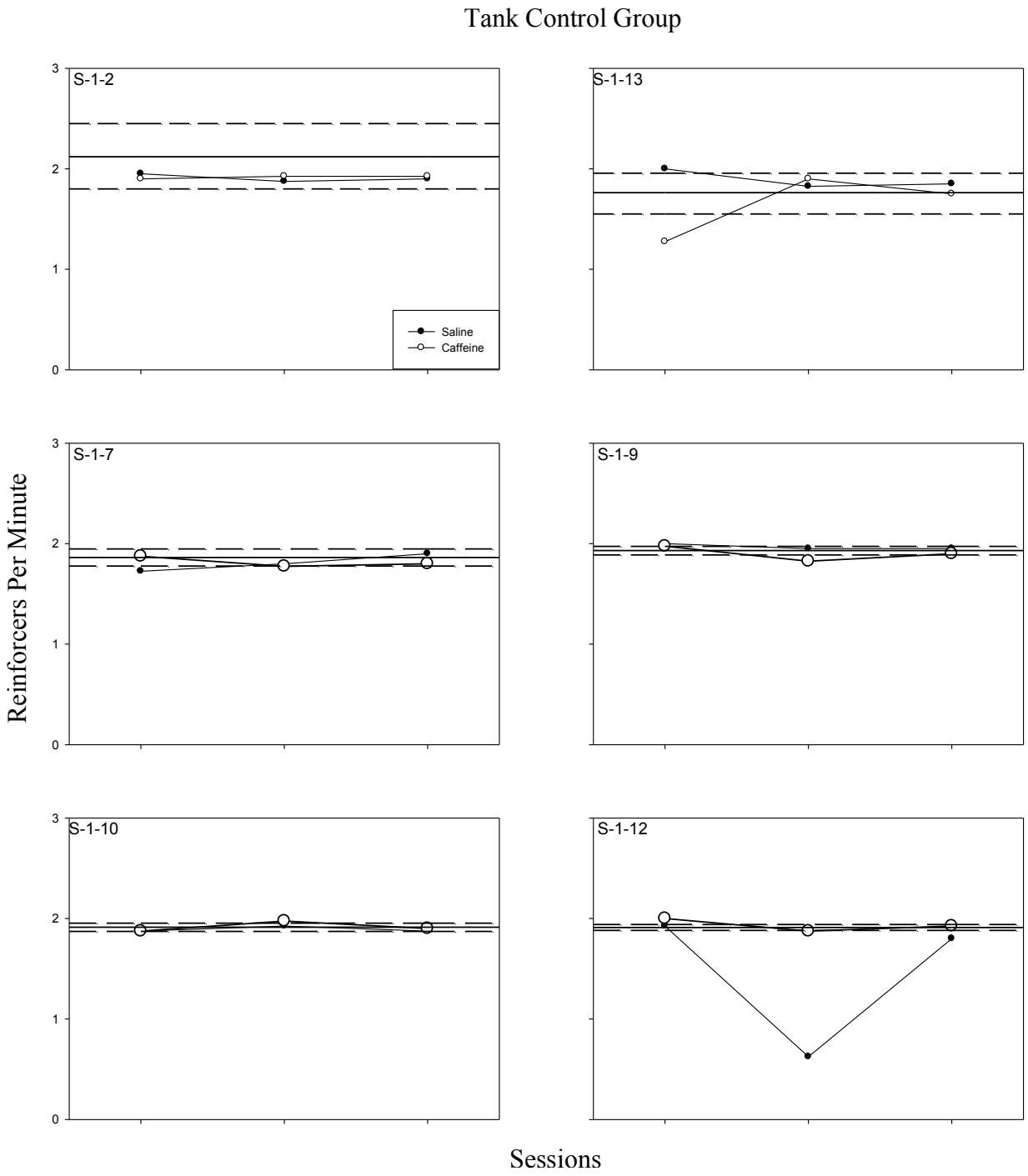

Figure 4. Shows the individual rate of reinforcement as a function of TC and drug condition.

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