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Development of acute withdrawal during periodic administration of amphetamine in rats

Wesley White*,[1], Joram Feldon, Ilsun M. White[1]

Swiss Federal Institute of Technology Zürich (ETHZ), Schwerzenbach, CH-8603, Switzerland

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Abstract

Amphetamine (AMPH) administration appears to produce multiple time-dependent effects during the approximately 24-h period after administration. This study examined the development of these effects. Rats were housed in separate cubicles, had ad-lib access to food and water, and were continuously monitored. After a series of control treatments, different groups received AMPH (1.0 mg/kg ip) at either 33- or 24-h intervals. Light–dark cycles (12–12 h) were "staggered" by 3-h intervals across the rats in each group, so that the effects of AMPH could be readily detected in average activity profiles against the background of light-entrainable activity. Changes in activity indicated that AMPH produced a common sequence of effects on both 33- and 24-h-period schedules: psychomotor stimulation (Hours 1–3 postdrug), withdrawal (activity suppression near Hour 20 postdrug), and recovery (activity increase beginning around Hour 24 postdrug). Withdrawal and recovery effects developed during the first several AMPH administration cycles. These time-dependent changes during the approximately 24-h interval after AMPH administration could reflect changes in motivation and in susceptibility to processes thought to underlie the acquisition of drug abuse (such as the positive and negative reinforcing effects of drug receipt). Short- and long-term responsiveness to drug might then depend on when in the postdrug sequence administrations occur.

Keywords: Psychomotor stimulant; Amphetamine; Chronic administration; Locomotor activity; Circadian; Withdrawal; Recovery; Development; Rat

1. Introduction

Amphetamine (AMPH) and related drugs produce a variety of time-dependent effects during the approximately 24-h period after administration. AMPH is well known to produce an *immediate* state that can be observed for several or more hours postadministration (reviewed in Kalivas et al., 1993; Pierce and Kalivas, 1997; Robinson and Becker, 1986; Robinson and Berridge, 1993; Segal and Kuczenski, 1994). A 1.0-mg/kg dose of AMPH has been shown to produce discriminative stimulus effects, to potentiate conditioned place preferences (reviewed in Hoffman, 1989), and to dramatically increase locomotion and rearing (reviewed in Kuczenski and Segal, 1994). AMPH acts as an indirect dopamine agonist: It releases dopamine from presynaptic endings, and it blocks dopamine reuptake. Many of AMPH's acute affects have been associated with altered transmission in the dopaminergic systems (reviewed in Feldman et al., 1997).

AMPH also produces a transient *withdrawal* state. A comparatively small number of studies have involved administering AMPH to rats and then performing assessments at two or more time points over the subsequent 24 h (Barrett et al., 1992; Edgar and Seidel, 1997; Eikelboom and Stewart, 1981; Lin et al., 2000; Persico et al., 1995; Schindler et al., 1994; Tonge, 1974), but all of these studies have demonstrated that AMPH produces transient physiological or behavioral changes at one or more time points between 12 and 24 h postadministration. The most systemic recent research on transient AMPH-induced withdrawal effects has probably been done by Barrett, Caul, and colleagues (Barrett et al., 1992; Caul et al., 1996, 1997; Stadler et al., 1999). Using drug discrimination procedures, these researchers have shown that 20–24 h after a variety of AMPH treatment regimes—from single moderate doses to

* Corresponding author. Psychology Department, 601 Ginger Hall, 150 University Boulevard, Morehead State University, Morehead, KY 40351, USA. Tel.: +1-606-783-2980; fax: +1-606-783-5077.
E-mail address: w.white@moreheadstate.edu (W. White).
[1] Current affiliation: Psychology Department, 601 Ginger Hall, 150 University Boulevard, Morehead State University, Morehead, KY 40351, USA.

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chronic high doses—rats experience a cue state resembling that produced by low-dose haloperidol administration. They have also shown that such a cue state develops over days involving administrations of 0.75 mg/kg AMPH.

Methamphetamine (Kosobud et al., 1998) and cocaine (White et al., 2000a) have been shown to produce a *recovery* state that is characterized by enhanced locomotor activity beginning approximately 24 h after administration. An increase in autonomic function occurs at approximately the same time (Tornatzky and Miczek, 1999).

The purpose of this study was (1) to determine whether immediate, withdrawal, and recovery states could be identified in locomotor patterns of rats given a moderate dose of AMPH (1.0 mg/kg) and (2) to infer from changes in locomotor activity how these states developed across administration cycles.

Rats are active in bouts that are approximately a half hour in duration, that are separated from one another by one to several hours, and that are clustered primarily during the dark phase of the light–dark cycle (Richter, 1922). Averaging across hours of multiple light–dark cycles yields an activity function that is elevated during the dark—hence the rat is considered nocturnal—and that has a characteristic form for a particular strain of rat (Buttner and Wollnik, 1984). Activity that can be entrained by the light–dark cycle can obscure activity changes that are due to drug administration. Consequently, we manipulated light–dark cycles and treatment times in a manner that highlighted the effects on activity of AMPH administration. Specifically, we gave control treatments on a schedule that produced trendless (relatively "flat") average activity functions. AMPH was then administered on the same schedule, and any changes in activity relative to control were ascribed to the effects of AMPH (White and Timberlake, 1999; White et al., 1999, 2000a). Furthermore, to identify common effects produced by different schedules of administration, we administered AMPH on two different intermittent schedules: 24- and 33-h-period schedules. The 24-h-period schedule was selected, because it has been used commonly in other research. The 33-h-period schedule was used because it allowed more time for AMPH-induced effects to have expression, and because it randomized administration times with respect to light-transition cues and circadian timers (White and Timberlake, 1999).

We hypothesized that AMPH would produce schedule-independent, time-dependent effects on activity that were consistent with immediate, withdrawal, and recovery states, and that these effects would develop across administration cycles.

2. Materials and methods

2.1. Subjects

The subjects were 16 male Sprague–Dawley rats, bred and reared at the Swiss Federal Institute of Technology Zurich. Animals were housed four to a group in Macrolon cages (48 × 27 × 20 cm high), in a temperature- (21 ± 1 °C) and humidity-(55 ± 5%) controlled colony. The colony was on a 12–12-h light–dark cycle. Animals had free access to food (Nafag 9431, Eberle Nafag, Gossau, Switzerland) and water before the experiment. They weighed 250–350 g at the start of the studies. The experimental protocol was approved by an Institutional Review Committee for the Use of Animal Subjects.

2.2. Apparatus

Eight stations were used. Each station consisted of a test chamber (25 × 25 cm floor, Coulbourn Instruments, Allentown, PA, USA) contained within a sound-attenuating shell. Each chamber had Plexiglas front and back walls and metal side walls. The front wall had a large drop-down door. One side wall contained a water spout and an opening that provided access to a food hopper. The floor was a metal grid beneath which was a removable tray filled with sawdust. The ceiling was sheet metal. Outside each cubicle but within each shell was a 7-W fluorescent light. Each station light was connected to an appliance timer that arranged for a 12–12-h light–dark cycle. A fan attached to each shell provided ventilation and masking noise. Stations were contained in a room that was used only for this study.

Mounted on top of the ceiling of each cubicle was an infrared motion detector (Coulbourn Instruments, model E24-61). The sensor of each detector projected through a small hole in the ceiling, enabling the detector to monitor changes in temperature patterns across the entire surface of the floor. The detector was set in the "pulse" mode. Each movement of the animal generated a count, and counts were proportional to the activity of an animal. Each monitor was connected to an interface (Coulbourn Instruments, model E91-12), and interfaces were connected to a computer. Software collected total counts in 5-min bins and wrote these 5-min totals to disk throughout the day.

2.3. Drug

d-Amphetamine sulfate (Sigma) was dissolved in saline to give a concentration of 1.0 mg/ml free base. Control injections consisted of 1.0 ml/kg saline. AMPH and saline were administered intraperitoneally.

2.4. Procedure

The rats were divided into two groups of eight, Group T33 and Group T24. The two groups were run successively. Each group was exposed to the same basic conditions.

2.4.1. Entrainment and habituation

Before being placed in an experimental station, each rat in a group was entrained to one of eight different 12–12-

h light–dark cycles that were staggered by 3 h: One rat had lights on at 1900 h, one at 2200 h, etc. Entrainment occurred in ventilated light-tight wooden cabinets. During entrainment, rats were housed on sawdust bedding in standard cages and had ad-lib access to food and water. Animals were allowed to entrain to a light–dark cycle for 2 weeks. Fresh food and water were provided every several days at irregular local times of day. During the last 3 days, each animal was handled and weighed, again at irregular daytimes.

2.4.2. Acclimation

At 1900 h local time, a rat was placed in each of the eight stations. Staggered 12–12-h light–dark cycles were maintained to the end of the study. Animals were allowed to acclimate to the stations for approximately 39 h. In this and all subsequent conditions, rats had ad-lib access to food and water.

2.4.3. Control treatment

At the end of acclimation, at local time 1000 h, animals received the first of a series of treatments. The two groups of rats received treatments at different intervals. Group T33 received treatments every 33 h, and Group T24 received treatments every 24 h. When a control treatment was scheduled, an experimenter entered the room, removed each rat in succession from its experimental station, weighed it, and either stroked its stomach vigorously (handling cycles) or gave it an intraperitoneal injection of saline (saline cycles), and returned the rat to the apparatus. Four handling and four saline treatments were given. The condition accustomed rats to the injection procedure. Each treatment took less than 2 min, and treatments were performed under "safe light" conditions. The experimental room was entered only at the time of a handling treatment or an injection. During this time, the food bin was refilled, bedding was changed, and, if necessary, the water bottle was replaced. For both schedules, averaging, within a given cycle, across the activity patterns of the eight rats would be expected to balance light-entrained activity across the average activity functions. Systematic deviations from a trendless function could then be ascribed to the effects of treatment.

2.4.4. Amphetamine treatment

Each rat received a series of 10 AMPH injections (1.0 mg/kg ip) at the same period with which it had received the control treatments. The first injection occurred at 1000 h local time. Rats in the T33 group, then, remained in staggered light–dark cycles and received AMPH at 33-h intervals. During a given cycle, all rats were injected at the same local time, but across the eight subjects, the injections occurred at eight different circadian times. Across eight consecutive injection cycles, rats were rotated, in different counterbalanced sequences, through the same eight administration times in the light–dark cycle. For example, Rat 1 was injected, over successive 33-h drug administration cycles at circadian times 0, 9, 18, 3, 12, 21, 6, 15, 0, and 9 (where CT 0 is lights on), Rat 2 was injected at times 3, 12, 21, 6, 15, 0, 9, 18, 3, and 12, and so on for Rats 3 to 8. Rats in the T24 group remained in staggered light–dark cycles and were given AMPH at 24-h intervals. During a given cycle, all rats were injected at the same local time, but across the eight subjects injections occurred at eight different circadian times (0, 3, 6, 9, 12, 15, 18, 21). Across cycles, each rat received treatment at a fixed circadian time. For both schedules, the extended time-dependent effects of AMPH could be assessed for a cycle by averaging across the activity patterns of the eight rats.

2.5. Data analysis

Activity counts were combined into 1-h bins. For each rat hourly values during control and AMPH conditions were then expressed as a percentage of the mean hourly value observed for an animal during the eight control cycles ("% of CON"). We will refer to these values as "standardized" values. Expressing values as a percentage of an individual's mean control value reduced variability due to individual differences in level of activity. Data were analysed using within-subjects ANOVAs and paired *t* tests. Post hoc comparisons were made with Fischer's PLSD test.

3. Results

3.1. Entrainment

During the acclimation phase, activity of each rat was almost entirely confined to the dark period of its light–dark cycle, indicating that each animal was stably entrained to its respective light–dark cycle.

3.2. Immediate effects: Hours 1–4 posttreatment

The upper panel of Fig. 1 shows the immediate effects of control and AMPH treatments on activity of rats treated at 33- and 24-hour intervals. When rats were treated at 33-h intervals, they were more active following AMPH administration than following control treatments, $F(1,7) = 1849.1$, $P < .0001$, activity diminished across posttreatment hours, $F(3,21) = 429.6$, $P < .0001$, and the decrease in activity across hours depended on the nature of the treatment, $F(3,21) = 379.8$, $P < .0001$. The same pattern of results was obtained after 24-h period treatments, $F(1,7) = 2459.7$, $P < .0001$, $F(3,21) = 436.3$, $P < .0001$, and $F(3,21) = 369.9$, $P < .0001$.

The lower panel of Fig. 1 shows immediate activity at different times in the study for animals treated at 33- and 24-h intervals. Average standardized activity during Hours

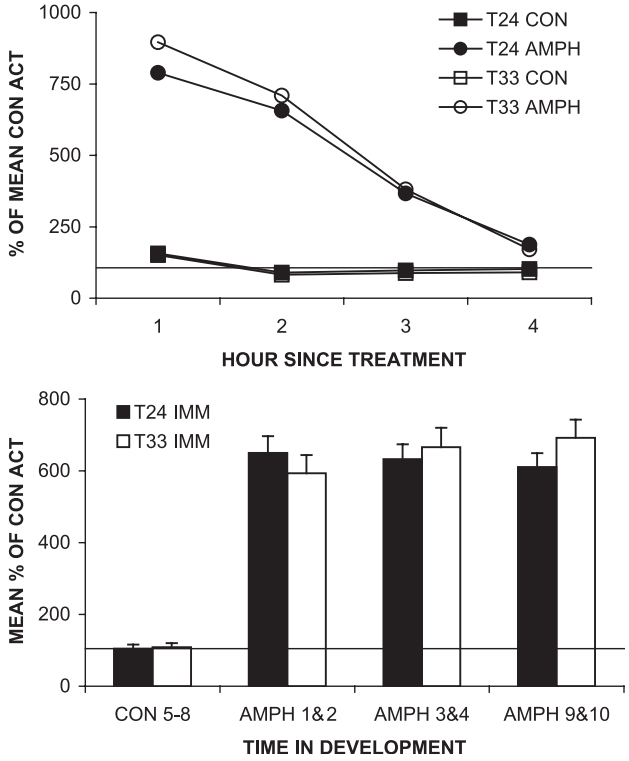

Fig. 1. Upper panel. Mean activity during each of the first 4 h after control (CON) or amphetamine (AMPH) treatments at 33- (T33) or 24-h intervals (T24). For each rat, hourly activity was expressed as a percentage of the mean hourly value observed during the control condition. These standardized values were then averaged across subjects and treatment cycles. Standard errors were smaller than symbols used to plot means. Lower panel. Mean standardized activity during Hours 1–3 postdrug (IMM) of control Cycles 5–8 (CON 5–8), and AMPH Cycles 1 and 2 (AMPH 1 and 2), 3 and 4 (AMPH 3 and 4), and 9 and 10 (AMPH 9 and 10) for rats treated at 33- (T33) or 24-h intervals (T24). For each rat, average standardized activity was found for Hours 1–3 postdrug. These values were then averaged across subjects and cycles. Bars represent standard errors.

1–3 post treatment is shown for the last four control cycles (CON 5–8), for AMPH Cycles 1 and 2 (AMPH 1 and 2), for AMPH Cycles 3 and 4 (AMPH 3 and 4), and for AMPH Cycles 9 and 10 (AMPH 9 and 10). Hours 1–3 were averaged because AMPH-induced effects were largest during these hours. For rats treated at 33-h intervals, a significant increase in activity occurred, $F(3,21) = 50.4$, $P < .0001$. AMPH 1 and 2, 3 and 4, and 9 and 10 were all different from CON 5–8, critical difference (crit diff) = 113.5, $P < .0001$. The mean increase in activity from AMPH 1 and 2 to AMPH 9 and 10 approached significance, $P = .08$. For rats treated at 24-h intervals, activity increased significantly, $F(33,21) = 40.0$, $P < .0001$. AMPH 1 and 2, 3 and 4, and 9 and 10 were different from CON 5–8, crit diff = 113.5, $P < .0001$.

Overall, Fig. 1 indicates that 33- and 24-h period treatment regimes produced comparable effects on immediate activity, and that a large psychomotor stimulant effect was obtained from the first AMPH administration.

3.3. Postimmediate effects: Hours 5–33 or 5–24 posttreatment

Fig. 2 shows the overall postimmediate effects on activity of control (CON, upper left) and AMPH (AMPH, lower left) treatments administered with a period of 33 h (T33). Activity during Hours 1–4 postdrug is not shown. Standardized hourly activity was averaged across rats and across the last eight cycles of each condition. As will be shown shortly, AMPH-induced effects did not change during the last eight AMPH cycles, and so the functions resulting from averaging across these cycles were not complicated by developmental effects. Maintaining rats in a 12–12 h light–dark cycle and averaging across eight consecutive 33-h cycles balances out the effects of the light–dark cycle on activity and makes any consistent time-dependent effects of the 33-h treatment evident. A one-way ANOVA, performed on the control data, failed to reveal a significant effect of control treatment on pattern of activity, $F(28, 196) = 0.8$, $P = .716$. No series of hours were consistently high or low. A separate ANOVA indicated a significant effect of AMPH on the pattern of activity, $F(7,28) = 4.5$, $P < .0001$. Values in the vicinity of Hours 20–22 postdrug were lower than values for most other hours, and values in the vicinity of Hour 27 postdrug were higher, crit diff = 22.8, $P < .05$. The suppression in activity during Hours 20–22 postdrug will be referred to as a "withdrawal" (WITH) effect, and the increase in activity from Hours 25–30 postdrug will be referred to as a "recovery" (REC) effect.

Analogous data for rats treated at 24-h intervals (T24) are also shown in Fig. 2. Hourly activity differed following both control and AMPH treatments, Fs(19,133) = 3.3 and 6.0, respectively, Ps $< .0001$. Control treatments enhanced activity in the vicinity of Hour 11 and reduced activity during Hour 20, crit diff = 19.9, $P < .05$. AMPH treatment reduced activity during Hours 19–21, crit diff = 18.3, $P < .05$. The suppression of activity during Hours 19–21 postdrug will also be referred to as a withdrawal effect.

In each condition eight rats were housed in eight light–dark cycles that were staggered by 3-h intervals: Consequently, averaging across the subjects within a cycle would be expected to balance out the effects of the light–dark cycle on activity and to reveal the effects of treatment. We used this feature of the methodology to quantify the development of the AMPH-induced withdrawal and recovery effects. The upper panel of Fig. 3 shows results for animals treated at 33-h intervals. Mean standardized activity during Hours 20–22 postdrug (WITH) was averaged across the last four control cycles (CON 5–8) and across selected pairs of AMPH administration cycles. A decrease in activity developed during the withdrawal interval, $F(3,21) = 3.2$, $P < .05$. Activity during AMPH administration Cycles 3 and 4 and 9 and 10 was lower than during control Cycles 5–8, crit diff = 27.1, $P < .05$. Inspection of functions from individual cycles (not shown) suggested that the suppression was first evident during AMPH Cycle 2. Average activity during

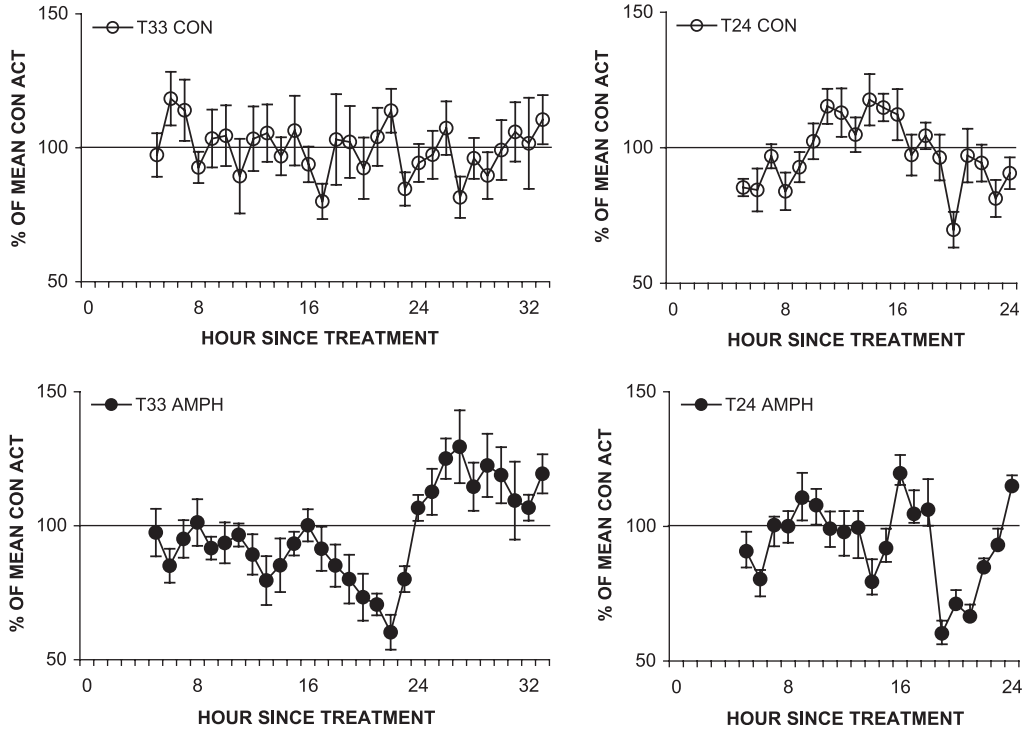

Fig. 2. Mean activity during Hours 5 to 33 or 5 to 24 posttreatment for rats treated at 33- (T33) or 24-h intervals (T24). Effects of the eight CON cycles (upper panels) and the last eight AMPH cycles (lower panels) are shown. For each rat, hourly values were expressed as a percentage of the 1-h mean observed during the control condition. Hourly values were then averaged across subjects within each cycle and then across cycles. Bars are standard errors. The horizontal line in each of the panels indicates the mean control value. Activity during Hours 1–4 is not shown.

Hours 25–30 post treatment (REC) was found for the same times in development. An increase in activity developed during this recovery interval, $F(3,21)=3.2$, $P<.05$. Activity during AMPH 3 and 4 and AMPH 9 and 10 was greater than during CON 5–8 and AMPH 1 and 2, crit dif = 20.4, $P<.05$. Increased recovery activity was first evident during AMPH Cycle 3.

Fig. 3 lower panel shows the development of the AMPH-induced withdrawal effect for rats treated at 24-h intervals. Activity during the withdrawal interval (Hours 19–21 postdrug) decreased during development, $F(3,21)=7.7$, $P<.01$. Activity during AMPH 3 and 4 and AMPH 9 and 10 was significantly lower relative to both CON 5–8 and AMPH 1 and 2, crit diff = 22.8, $P<.05$. In functions plotting individual cycles (not shown) the suppression near Hour 20 seemed to first be evident in AMPH Cycle 3.

Fig. 3 indicates that 24- and 33-h-period schedules of AMPH administration produced withdrawal effects that were comparable in terms of timing and magnitude and that withdrawal and recovery underwent rapid development during intermittent AMPH administration.

4. Discussion

The purpose of this research was (1) to see if changes in locomotor activity, consistent with immediate, withdrawal, and recovery states, occurred between intermittent administrations of AMPH and (2) to infer from changes in activity how these states developed across repeated administration cycles. Evidence for these states and for the development of some of these states across AMPH administration cycles was obtained.

4.1. Amphetamine-induced immediate state

In the present study, 33- and 24-h-period schedules of AMPH administration produced extremely elevated levels of activity indicative of an immediate psychomotor stimulant state. Sensitization, increased immediate effect due to repeated administration (reviewed in Kalivas et al., 1993; Pierce and Kalivas, 1997; Robinson and Becker, 1986; Robinson and Berridge, 1993; Segal and Kuczenski, 1994), was not observed on either schedule. Sensitization is reliably obtained when the withdrawal interval is relatively long (at least several days) and when immediate effects are paired with a unique context. Sensitization may not have been obtained in the present study because the withdrawal interval was comparatively short and because animals received drug in the housing context.

4.2. Amphetamine-induced withdrawal state

An AMPH-induced suppression of activity in the vicinity of Hour 20 postadministration was also evident on both schedules (Fig. 2, lower panels). This suppression may have

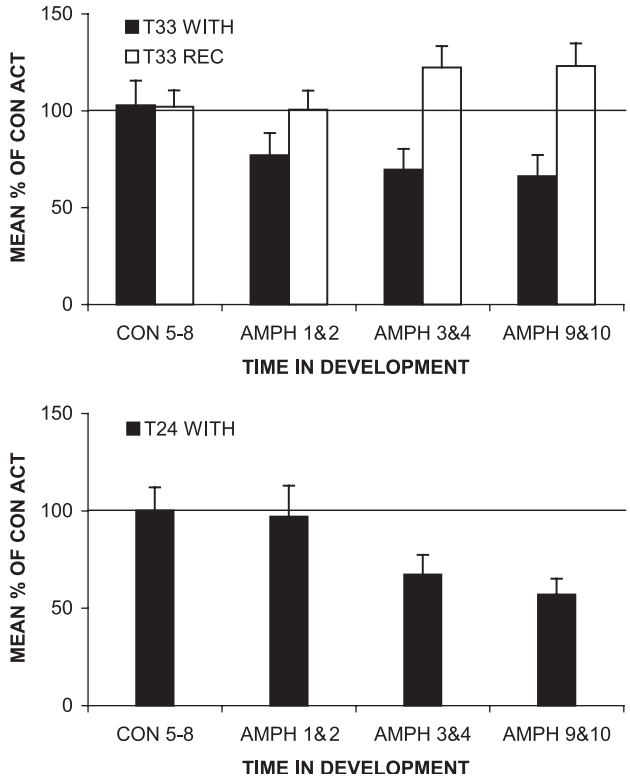

Fig. 3. Upper panel. For rats treated at 33-h intervals (T33), mean withdrawal and recovery activity during the last four control cycles (CON 5–8), and AMPH Cycles 1 and 2 (AMPH 1 and 2), 3 and 4 (AMPH 3 and 4), and 9 and 10 (AMPH 9 and 10). WITH (withdrawal), and REC (recovery) bars are averages of Hours 20–22 and 25–30, respectively. For each rat, hourly values were expressed as a percentage of the 1-h mean observed during the control condition. Hourly values were then matched by circadian time of injection and averaged across withdrawal or recovery hours and cycles. Variability bars are standard errors. Lower panel. For rats treated at 24-h intervals (T24), mean withdrawal activity (WITH) during the same time points in development. Bars are averages of Hours 19–21. Means were calculated as in the upper panel, and variability bars are standard errors.

been an aspect of a transitory withdrawal state that occurred between intermittent AMPH administrations. In humans, termination of AMPH administration produces an abstinence syndrome that includes increased sleep and REM sleep rebound, extreme fatigue, difficulty in concentrating, anhedonia, depression, and anxiety (Grinspoon and Hedblom, 1975). Several studies with rats (Barr and Phillips, 1999; Barrett et al., 1992; Edgar and Seidel, 1997; Eikelboom and Stewart, 1981) have indicated that phenomena analogous to human withdrawal symptoms have maximum expression in the vicinity of Hour 20 post-AMPH. Barrett et al. (1992), using a drug discrimination task, showed that the interoceptive cues present 20 and 24 h after a single high dose of AMPH (10 mg/kg) resembled the interoceptive cues that were present after low-dose haloperidol treatment, that is, after reduction of dopaminergic transmission. This was not the case at the other times tested (4, 6, 8, 12, 16, and 30 h posttreatment). A variety of AMPH administration regimes produce this rebound or withdrawal effect (Caul et al., 1996, 1997; Stadler et al., 1999). Barr and Phillips (1999) found that 20 h after the last AMPH administration of an escalating dose schedule, the break point was reduced on a progressive ratio schedule of responding for 4% sucrose solution. In other words, AMPH-treated rats showed a decreased motivation to obtain natural reward 20 h after AMPH receipt. Edgar and Seidel (1997) gave rats a moderate dose of methamphetamine 5 h after the onset of light and then continuously monitored sleep EEG over the subsequent circadian interval. Nineteen hours after treatment, a REM sleep rebound occurred, and this lasted for 2 to 3 h. Eikelboom and Stewart (1981) gave different groups of rats daily injections of 1, 2, or 5 mg/kg AMPH for several weeks. By 23.25 h postinjection, the rats manifested a context-independent hypothermia that was not observed at other times of day.

The withdrawal state has been identified with a reduction in dopamine receptor sensitivity and the development of tolerance. The ability to discriminate an AMPH cue state (Barrett et al., 1992), AMPH-facilitated self-stimulation of the medial forebrain bundle (Leith and Barrett, 1976), and repetitive movements in response to a novel environment (Persico et al., 1995) all decrease between 12 and 22 h post-AMPH treatment, whereas lateral hypothalamic electrical self-stimulation thresholds increase (Lin et al., 2000). All of these measures normalize subsequently. These results indicate transient blunted reactivity in dopamine function. Persico et al. (1995) and Tonge (1974) identified specific molecular and neurochemical events that are correlated with the occurrence of transient withdrawal. For example, catecholamine levels in the prefrontal cortex and striatum were reduced after 12 h of withdrawal, but they were normal after 24 h.

Our data showed that on both 24- and 33-h schedules, the suppression of activity in the vicinity of Hour 20 post-AMPH was not evident following the first administration, but was detectable after two or three administrations. The suppression was not elicited in "one trial" when a dose of 1.0 mg/kg was used. Instead, the effect depended on some cumulative process. Caul et al. (1997) have similarly shown that a cue state indicative of AMPH withdrawal developed only after repeated administrations of 0.75 mg/kg AMPH.

Twenty-four and thirty-three-hour schedules produced similar effects even though the schedules involved different rates of drug administration.

4.3. Amphetamine-induced recovery state

Thirty-three-hour treatment with AMPH resulted in enhanced activity that began approximately 24 h after AMPH administration (Fig. 2, lower left). This activity did not appear to be a normalization of activity, but instead it appeared to be an "overshoot" relative to control conditions. At the same time, the level of activity appeared to remain in the normal range and did not involve a reinstatement of the excessive levels of activity of the immediate

state. Similar activity has previously been observed in rats following intermittent administration of (1) 2.0 mg/kg methamphetamine at either 24- (Kosobud et al., 1998) or 31-h intervals (Pecoraro et al., 2000) and (2) 20 mg/kg cocaine at 33-h intervals (White et al., 2000a). For the 24-h period group in this study, only a portion of the ascending limb of the recovery could be observed.

The recovery effect is probably accompanied by changes in autonomic function, because the opportunity to self-administer cocaine for 1 h shifts the acrophase of heart rate and body temperature the next day to the time of administration (Tornatzky and Miczek, 1999). Otherwise, little is known about the mechanistic determinants or correlates of the effect. Hypotheses regarding mechanisms have been based on the effects of other treatments, including (1) the effects of giving methamphetamine via drinking water to suprachiasmatic nucleus (SCN)-lesioned rats (Honma and Honma, 1995; Honma et al., 1987, 1988, 1989) and (2) the effects of providing SCN-lesioned rats brief intermittent access to food (Mistlberger, 1994; Stephan et al., 1979a,b). On the basis of the results of these treatments it has been suggested that intermittent psychomotor-stimulant administration may entrain a dopamine-sensitive, SCN-independent mechanism that produces enhanced recovery activity, elevated corticosterone, and increased gastrointestinal function. The AMPH-induced recovery effect has been interpreted as a food-anticipatory state and as a drug-anticipatory state (Kosobud et al., 1998; Tornatzky and Miczek, 1999; White et al., 2000a).

On the 33-h period AMPH-treatment schedule, recovery activity seemed to first appear during Cycle 3. The rate of development of the recovery effect could not readily be evaluated on the 24-h-period schedule.

4.4. Interdependence of AMPH-induced effects

Withdrawal and recovery effects could have been due to independent processes produced by AMPH. For example, the process responsible for the withdrawal effect could have involved a pharmacodynamic process, whereas the process responsible for the recovery effect could have involved an entrainment process. Another possibility is that the recovery effect reflected a compensatory response following upon withdrawal near Hour 20. According to this interpretation, the withdrawal and recovery effects are aspects of a cascade, rather than the result of two independent processes. One form of evidence from the 33-h treatment condition was consistent with the latter interpretation: The rate of development of the two effects appeared to be similar, with the appearance of the withdrawal effect preceding the appearance of the recovery effect by one cycle. The issue of the interdependence of withdrawal and recovery effects is potentially of therapeutic importance, because it would indicate the number of interventions that might be necessary to modify the withdrawal and recovery "side effects" of AMPH administration.

Changes in activity during withdrawal and recovery intervals appeared small compared to changes in activity immediately following drug, but compared to the modulation of activity across the light–dark cycle, changes in activity during withdrawal and recovery intervals were sizeable.

4.5. Psychomotor stimulant-induced states

Using a methodology similar to that used here, White et al. (2000a) showed that 20 mg/kg cocaine produced withdrawal and recovery effects that were very similar to those produced in this study by AMPH. In particular, averaging across eight 33-h period cocaine administration cycles produced a nadir in activity in the vicinity of Hour 20 postdrug and an overshoot in activity during Hours 25–30. Given the shorter duration of action of cocaine and its shorter half-life relative to AMPH, and given the overlap of the drugs in terms of mechanism of action, the potential for a withdrawal effect may be established relatively soon after administration of these psychomotor stimulants via a common—perhaps dopaminergic—mechanism. The similarity in their time-dependent effects provides further criteria for classifying these drugs together.

4.6. Qualifications

Some of our suggestions require qualification. First, the claim that AMPH produces acute withdrawal near Hour 20 is tentative. The evidence for this claim is based on few studies, and the evidence is open to alternative interpretations. To illustrate the latter point, although Edgar and Seidel (1997) observed REM sleep rebound near Hour 20 postmethamphetamine treatment, the rebound commenced just after lights on and coincided with the early inactive period. Endogenous circadian factors may favor the expression of REM sleep rebound at this time regardless of when methamphetamine is administered. Consequently, the occurrence of REM sleep rebound may not follow a fixed 20-h time course as we have implied.

Furthermore, we are not suggesting that a model based solely on changes in locomotor activity can provide a thorough understanding of acute withdrawal. Locomotor activity is a useful dependent measure because it is easily quantified and can be continuously monitored. However, acute withdrawal is defined in terms of multiple symptoms. These symptoms may have different thresholds of elicitation, and they may have different time courses. Therefore, a thorough understanding of acute withdrawal will involve the study of multiple dependent measures that are indicators of these symptoms.

Prior studies that have provided evidence for acute withdrawal have used high and/or escalating doses of drug. In pilot research, we have given a range of AMPH doses at 33-h intervals. Even the highest dose administered (4.0 mg/kg) was followed by hypoactivity near Hour 20 posttreatment and by a normalization of activity around Hour 25

posttreatment (White et al., 2000b). The results suggest a continuity of mechanism across a wide range of doses for this measure. However, the result does not guarantee that the 1.0-mg/ kg dose used in this study is capable of producing other indices of acute withdrawal.

Finally, we have suggested that the mechanisms involved in acute withdrawal not only might be dopaminergic in nature, but might reflect a within-system adaptation: That is, some of the same dopaminergic mechanisms that AMPH affects in the short term might be involved in the proximate expression of acute withdrawal (Koob et al., 1997). These suggestions are intended to be illustrative, and other mechanisms could possibly be involved. For example, because AMPH is an indirect agonist of catecholamines, short-term stimulation of adrenergic mechanisms might be necessary for acute withdrawal. Furthermore, the mechanisms that might have to be stimulated in the short term to produce acute withdrawal might be different from those involved in its proximate expression. We have tried to account for our results in terms of changes in dopaminergic mechanisms in part because these mechanisms have received the most research attention.

4.7. Implications

Placing rats in staggered light–dark cycles, administering AMPH intermittently, and continuously monitoring locomotor activity provided a relatively easy and rapid method for studying the time-dependent effects of AMPH. The a priori identification of consistent time-dependent effects can focus experimental efforts on specific key time points (Hour 20 post-AMPH) and transitions (the first several days of AMPH administration).

If the qualitative changes in activity observed during the 33 h following AMPH administration reflect the capacity of AMPH to induce an extended sequence of states, then different phases of this drug-induced pattern may differentially modulate responsiveness to external events, in a manner analogous to the modulation of responsiveness by different phases of the light-entrained rest–activity cycle (Lemmer, 1995; Reinberg, 1992). With particular respect to drug use and abuse, the AMPH-induced pattern may reflect time-dependent changes in the susceptibility to processes thought to underlie the acquisition of drug abuse, such as the positive and negative reinforcing effects of drug receipt. Consequently, the results of classic behavioral assessments of drug efficacy, such as the tendency to manifest sensitization, the learning of a conditioned place preference, or the self-administration of further drug, may depend on when the assessment is performed relative to a prior drug administration.

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