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Amphetamine and Morphine May Produce Acute-Withdrawal Related Hypoactivity by Initially Activating a Common Dopamine Pathway

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Abstract

Rats given drugs of abuse such as amphetamine or morphine show longer-term effects, that is, signs of acute withdrawal, including hypoactivity, hypophagia, and blunted affect, sometime between 12 and 24 h after treatment. This research explores the possibility that signs of acute withdrawal produced by different drugs of abuse are instigated by overlapping mechanisms. The specific objectives of the research were to see if amphetamine and morphine produced longer-term hypoactivity, and to see if any longer-term hypoactivity elicited by the drugs could be blocked by SCH23390, a dopamine D1 antagonist. Six groups of rats, with eight rats in each group, were exposed to a series of five-day tests. Near light onset of Test Day 1, each animal was given control administrations, consisting of a saline treatment (1.0 ml/kg) followed 30 m later by a saline posttreatment, and locomotor activity was monitored for the next 24 h. On Test Day 3, each animal was given experimental administrations, and locomotor activity was again monitored for 24 h. Each group received only one combination of experimental administrations across tests. Experimental administrations consisted of saline, amphetamine (2.0 mg/kg), or morphine (5.0 mg/kg), followed by saline or SCH23390 (0.05 mg/kg). All administrations were subcutaneous. Amphetamine and morphine produced longer-term hypoactivity, having similar time courses and magnitudes. SCH23390 blocked the longer-term hypoactivity produced by both drugs. Saline and SCH23390 produced no changes in longer-term activity in their own right. The time course of amphetamine-elicited longer-term hypoactivity resembled that of amphetamine-elicited longer-term hypophagia observed in a prior study. Approximately ¼ of the animals given amphetamine or morphine did not show longer-term hypoactivity (“low withdrawal” rats). Amphetamine and morphine may initiate the cascade of events resulting in signs of acute withdrawal by producing activation in a common pathway that uses dopamine as a neurotransmitter. Different signs of acute withdrawal (hypoactivity and hypophagia) may involve the short-term activation of the same common pathway. Low withdrawal rats may have a different vulnerability to amphetamine and

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may show differences in drug assessment outcomes relative to animals that manifest distinct signs of acute withdrawal.

Keywords

amphetamine; morphine; activity; withdrawal; sign; dopamine antagonist

1. Introduction

When rats are administered amphetamine (2.0 mg/kg), they show longer-term hypoactivity, a reduction in activity 12 to 24 h later (White et al, 2004; White & White, 2006), and long-term hypophagia, a reduction in food intake over a similar time period (White et al, 2015). The longer-term hypoactivity and hypophagia appear to be aspects of an acute withdrawal or “hangover” state, because other measures change during this period in a manner consistent with acute withdrawal, including affect (Barrett et al, 2005). These measures normalize by 24 h after treatment. The similarity in time course of longer-term hypoactivity and of longer-term hypophagia suggest that the two putative indicators of acute withdrawal may share determinants.

The methods used to assess the effects of amphetamine on longer-term activity and longer-term food intake differed in the intermittency with which drugs were administered. In research involving activity as the dependent measure, amphetamine was given repeatedly at periods of 24 or 33 hours (White et al, 2004; White & White, 2006), whereas in research involving feeding, drug treatments, including amphetamine, were given at 5-day intervals (White et al, 2015; White et al, 2010; White et al, 2007).

In the present study, amphetamine was given at 5-day intervals, the schedule used in feeding studies. The purpose was to see whether, when schedules of administration were equated, the time course with which longer-term hypoactivity occurred resembled the time course, formerly seen, of longer-term hypophagia. Similarity of time courses under these circumstances would suggest shared determinants of elicitation. Given that longer-term hypophagia could be prevented by administering a dopamine D1 receptor antagonist (SCH 23390) around the time of amphetamine administration (White et al, 2015), we also examined whether D1 antagonist could prevent longer-term hypoactivity. This outcome would provide another form of evidence that the same mechanism was involved in the elicitation of longer-term hypoactivity and hypophagia. Finally, further goals of this research were to see if morphine, a narcotic, produced similar longer-term hypoactivity, and to see if any longer-term hypoactivity produced by morphine could be prevented by D1 antagonist. These outcomes would suggest that amphetamine and morphine produced some aspects of acute withdrawal via a similar mechanism.

In the present study, “short-term effects” will be defined as drug-induced changes occurring during the first six hours following treatment. Short- and longer-term effects of morphine on activity have been examined in several studies. In the short-term, morphine produced a biphasic pattern, first suppressing activity for approximately 1.5 to 2 hours, and then, after approximately 1 to 1.5 hours, enhancing it for 1 to 2 hours (Magnus-Ellenbroek &

Havemann-Reinecke, 1993; Sisti & Lewis, 2001). In the longer-term, high morphine doses (above 40 mg/kg) received daily reduced activity sometime between hours 13 and 24 following administration (Kim et al, 2005; Kumar et al, 1971; Silva and Heyman, 2001). The purpose of the latter studies was to model abuse. The present research used a lower dose (5 mg/kg) more intermittently administered, and it showed a more detailed time course of effects. Some short-and longer-term effects of amphetamine on activity have been discussed previously (White et al, 2004; White & White, 2006) and will not be reviewed here.

Amphetamine is an indirect dopamine agonist that promotes release of dopamine from vesicles and attenuates dopamine reuptake (Feldman et al, 1997; Waddington, 1993). Morphine is a mu-opioid receptor agonist (Chartoff & Connery, 2014; Mazei-Robison & Nestler, 2012). Dopamine antagonists might plausibly be expected to alter effects produced by morphine because dopamine and opioid neurotransmitter systems interact, most familiarly, via the ventral tegmental area (VTA). By binding to mu-opioid receptors in the VTA, morphine inhibits the inhibitory GABA neurons that synapse on dopaminergic dendrites in the VTA, indirectly exciting dopamine neurons, and leading to an increase in dopamine output to nucleus accumbens (NAc), the prefrontal cortex (PFC), and the amygdala (Chartoff & Connery, 2014; Mazei-Robison & Nestler, 2012; Tanda & Di Chiara, 1998). Through this shared pathway, amphetamine and morphine could initiate the cascade of events leading to longer-term hypoactivity, and a D1 antagonist could prevent the longer-term hypoactivity produced by either drug.

The procedure used in the present study was similar to that used in prior research involving feeding as the dependent measure (White et al, 2015; White et al, 2010; White et al, 2007). Rats received a series of five-day tests. At the start of Day 1 of a test, animals received a control treatment, and at the start of Day 3, animals received an experimental treatment such as amphetamine or morphine. Patterns of activity on Day 1 and Day 3 were compared. Each five-day test began with a one-day re-baseline in the eventuality of baseline shifts due to repeated apparatus exposure, to aging, or to drug-induced shifts in food-intake set point (Koob & Bloom, 1988). Drug was administered at light onset, the start of the inactive period, so that motivational deficits due to amphetamine administration, which tend to be greatest 15 to 24 hours post-treatment, would coincide with the active period and so be easier to detect. The beginning of the inactive period is also the time at which recreational drug use presumably peaks in humans.

Activity was used as the dependent measure because it is sensitive to the effects of drug treatment: An animal accomplishes most functional behaviors (eating, drinking, exploration, etc.) via activity, and the diminution of any functional behavior by drug would probably be reflected in reduced activity. Additionally, activity is potentially informative with respect to antagonist results: A complete blockade of drug-induced longer-term hypoactivity by an antagonist would suggest a blockade of acute withdrawal signs generally, and a normalization of all functional behaviors.

2. Materials and Methods

2.1. Animals

The subjects were 48 adult male outbred Wistar rats (Harlan, Indianapolis, IN). Eight rats were in each of six groups. Each group was in a different treatment condition. The six groups/treatment conditions were run one after the other from May 2014 to July 2015. Several shipments of animals were received prior to and during this period. The eight subjects in each group were randomly selected from a recent shipment. After arriving, subjects were housed in pairs in an animal colony in an animal vivarium. This colony was used to house only those groups of animals that were involved in this study. The colony was on a 12-12 hour light-dark cycle, and the animals had free access to food (5001 Rodent Diet, Lab Diet) and tap water. A week before the start of a treatment condition, each animal in the group was housed in an individual cage. On each of several days prior to the start of a treatment condition, each animal was briefly handled, weighed, and rubbed on the back of the neck.

2.2. Apparatus

Activity was monitored in one of four stations. Each station consisted of an Activity Test Chamber (Med Associates, ENV-515) placed in a Sound Attenuating Cubicle. Each chamber had clear plastic walls and was 43 cm X 43 cm X 30 cm high. Near the floor of each wall was a strip containing 16 infrared sources or detectors (Med Associates, ENV-258). Each source or detector was spaced 2.5 cm center to center, and strips were positioned so that each source or detector was 3.7 cm above the floor. A plastic insert was placed in an activity chamber before an animal was placed in the chamber. Each insert was approximately 42 cm X 42 cm X 7.3 cm high and had finger holes so that it could easily be inserted or removed. Each of the eight rats in an experimental group was assigned its own insert. Water was available from a bottle secured in a metal holder affixed to a chamber wall. The bottle could contain as much as 300 ml of water. Each chamber could be covered with a removable top that contained ventilation holes.

Each sound attenuating cubicle (Med Associates, ENV-017M-027) consisted of compressed wood and was 73 cm wide by 60 cm deep by 59 cm high. The cubicle and chamber could be illuminated by a low-profile 15-cm long light fixture that was mounted near the top of one cubicle wall. The fixture contained a fluorescent bulb (Lampi, F4T5WW). The light-fixture in each station was connected to an appliance timer that controlled a 12-12 hour light-dark cycle within each station. Light-dark cycles in the stations and in the animal colony were synchronized. Each cubicle contained a fan that ventilated the station and masked noises.

Strips containing infrared sources and detectors were connected to a 48 Channel IR Controller (Med Associates, ENV-520). The controller of each station was connected to a computer that ran activity monitoring software (Activity Monitor, Version 6.00, Med Associates). The stations and control equipment were located in a room in the animal vivarium that was adjacent to the animal colony (the "running room"). The room was used only for this study. Temperature and humidity within the room were well regulated.

2.3. Drugs

All drugs were obtained in powdered form from Sigma-Aldrich (St Louis) and were mixed in saline. The following drug concentrations were used: 2.0 mg/ml d-amphetamine base; 5.0 mg/ml morphine base; and 0.05 mg/ml SCH 23390 hydrochloride (D1 antagonist). Saline was used as the control treatment (1.0 ml/kg).

2.4. Procedure

Animals in each group were first habituated to the open field stations, and then they received a series of tests.

2.4.1. Habituation—Prior to being tested, the eight animals in a treatment condition were divided into two subgroups, with four animals in each subgroup. On alternating days, the two subgroups were placed in the open field stations near light onset and were allowed to habituate for the next 24 hours. Each subgroup was given three days of habituation.

Stations were prepared prior to receiving an animal: Approximately 40 g of bedding (Sani-Chips, P.J. Murphy Forest Products) and 40 g of chow (Lab Diet, 5001 Rodent Diet) were placed in an insert, and the insert was placed in an activity chamber. A station's water bottle was refilled as needed. Each animal in a subgroup was always placed in the same station during habituation. Activity was monitored throughout the time an animal was in a station. At the end of a day of activity monitoring, each insert was removed, wiped out, thoroughly rinsed with hot water, and allowed to completely air dry before it was next used with the same subgroup of animals. These practices continued throughout testing.

2.4.2. Testing—Generally, each test was five days in duration, each group was given a series of six tests, and tests occurred one after the other. For exceptions, see below. A typical test is shown in Figure 1. At light onset of test day 1, a subgroup of four animals was brought from the animal colony. Each animal was given a control treatment (saline), and was placed in its assigned activity monitoring station. 30 min later, each animal was removed, was given a control posttreatment (saline again), and was placed back in its activity monitoring station. Activity was monitored from 10 min after light onset, until light onset of the next day (23 h and 50 min). At light onset of day 2, the subgroup of animals was removed from the stations, and it spent day 2 in the animal colony (a “colony day”). On day 3 (experimental day), the same subgroup of four animals was subjected to conditions similar to those of day 1, except that they received a treatment-posttreatment combination that depended on their assigned treatment group (see below). The subgroup spent day 5 of a test in the animal colony.

For a second subgroup of four animals, their day 1 and day 3 occurred on days when the first subgroup was in the animal colony. On these days they were exposed to the same conditions to which the first subgroup had been exposed. In other words, over a five-day period all eight rats in a treatment group were tested once.

Table 1 shows the day 3 treatment-posttreatment combinations to which the six different groups of eight animals were exposed. Treatments were either saline, amphetamine, or morphine, and they were followed 30 minutes later by posttreatments of saline or the

dopamine D1 antagonist SCH 23390. All treatments were given subcutaneously, under the loose skin on the back of the neck. Body weights were recorded at the time of treatment.

2.4.3. Exceptions—One group received fewer than six tests, on a couple of occasions an additional off day occurred, and on several occasions data were lost or were unusable. Here are the specifics.

For the Amphetamine-D1 Antagonist group, subgroup 1 received 5 tests, and subgroup 2 received 4 tests: Unanticipated construction adjacent to the experimental room made it impossible to conduct remaining tests under suitable conditions in a timely manner. For the Saline-Saline group, an extra colony day occurred before the experimental day of test 4 for subgroup 2. For the Amphetamine-Saline group, an extra colony day occurred between the end of test 1 and the start of test 2.

For the Saline-Saline group, due to a power outage, data were lost for hours 1–8 of the control day for test 6 of subgroup 2. For the Saline-D1 Antagonist group, intermittent construction noises occurred in an area adjacent to the running room during the experimental day of test 5 for subgroups 1 and 2, and so the data from that test were not used. For the Morphine-Saline group, due to a power outage, data for the experimental day of test 3 for subgroup 1 was lost. In these cases, results were based on the remaining tests.

2.5. Compliance Statement

The experimental protocol was approved by the Institutional Review Committee for the use of Animal Subjects and was in compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals.

2.6. Dependent Measures

Horizontal distance moved was collected every five minutes.

2.7. Data Analysis

Data were analyzed using repeated measures ANOVAs. Significant effects were analyzed with additional ANOVAs, followed up by Fisher's PLSD post hoc comparisons or t-tests (paired or unpaired).

3. Results

3.1. Habituation

Groups were given three days of habituation to insure that levels and patterns of 24-hour activity were stable prior to drug testing. To show that activity was stable, for each treatment group the mean cumulative percent change in activity from the last habituation day to the saline control day of test 1 was found. The change was found for successive three-hour bins from hours 7 to 24 following light onset. Results for each group are shown in Figure 2.

When a function does not show a large excursion from 0 across bins, no change in level or pattern of activity occurred from the last habituation day to the control day of test 1: In other words, levels and patterns of activity had become reproducible or were stable. In order to

assess whether these functions reflected stability, a 6 (treatment group) by 6 (3-h bin) ANOVA was done on the data used to produce Figure 2. No main effect of treatment group was obtained, $F(5, 42) = 1.428$, $p = .234$, nor a main effect of 3-h bin, $F(5, 210) = .945$, $p = .4526$, nor a treatment-group by 3-h bin interaction, $F(25, 210) = .754$, $p = .7955$. In other words, all groups had stable patterns of activity prior to testing. Because no group showed a large excursion from 0 prior to testing, any large excursions seen subsequently could be ascribed to the effects of treatment, and not to the effects of additional habituation to the stations.

3.2. Short-term Effects

Figure 3 shows the short-term impact of different treatments on distance moved. The figure shows, for each group, the difference in distance moved on control day 1 and experimental day 3 (experimental – control). Results are shown in half hour bins for the first six hours following treatment. To produce a function, the mean for each subject in a treatment group was found across tests, and then a group mean across subjects was found.

To begin to assess the impact of different treatment conditions on short-term activity, a 6 (treatment group) by 12 (30-min bin) ANOVA was done. Activity differed between treatment groups, $F(5, 42) = 70.389$, $p < .0001$, it differed across time, $F(11, 462) = 13.826$, $p < .0001$, and the pattern of activity across time differed for groups, $F(55, 462) = 21.694$, $p < .0001$. For each treatment group, a one way ANOVA was done to see if activity differed across time for that specific group. For the Sal-Sal group, activity from the control day to the experimental day did not differ across 30-min bin, $F(11, 77) = 1.706$, $p = .0878$, whereas for the other groups it did, $F_s(11, 77) = 4.165$ to 70.961 , $p_s < .0001$.

For each 30-min bin, a one-way ANOVA was done across groups to assess differences in distance moved. Groups differed from 30-min bins 1 to 9, $F_s(5, 42) = 3.12$ to 61.05 , $p_s = .0175$ to $< .0001$. Post hoc comparisons using Fischer's PLSD test revealed the following significant difference (all $p_s < .05$).

The Sal-Ant group differed from the Sal-Sal group only during bin 9, that is, D1 antagonist did not produce an effect on distance moved that was substantially different from saline.

The Amp-Sal group had a higher level of activity than the Sal-Sal group from bins 1–9. The Amp-Ant group had a level of activity similar to that of the Amp-Sal group during bin 1, prior to the receipt of D1 antagonist, but the Amp-Ant group had a level of activity similar to that of the Sal-Sal group thereafter: D1 antagonist blocked amphetamine-elicited hyperactivity.

The Mor-Sal group had a higher level of activity than the Sal-Sal group during bins 5–8. Compared to Mor-Sal, Mor-Ant suppressed activity during bin 2 and attenuated activity during bins 5–7. Mor-Ant also reduced activity relative to Mor-Sal during bin 1. The reduction in activity during bin 1 following Mor-Sal relative to Sal-Sal did not reach the level of significance.

3.3. Longer-term Effects

3.3.1. Longer-term Effects Overall—Figure 4 shows how different treatments affected longer-term activity, activity occurring 7 to 24 hours after treatment.

Results are shown as cumulative percent change functions. The starting data for these functions were total distance moved, in three-hour bins, during control and experimental days of each test. Within a test, each three-hour total, for both control and experimental days, was converted to a percentage of activity that occurred from hours 7–24 of the control day. Then, for each test, the difference in distance moved from the control day of a test to the experimental day of a test was found for each three-hour bin (experimental – control). These differences were then cumulated across bins. Finally, an average was found across tests for each subject, and a group average was found across subjects in a treatment condition.

Handling data in this way has a variety of advantages: Converting values to percent of control reduces between-subject variability and provides a more meaningful measure of change than distance moved. Finding the difference between experimental and control removes much of the variation in behavior entrained by the light-dark cycle, better revealing the magnitude of the effects of treatments; and it condenses the data, yielding, for each condition, a single function, thereby simplifying the display and statistical analysis of the results of multiple conditions. Finally, cumulating changes can show the progressive increase in the magnitude of effects obtained.

To assess the effects of treatments on longer-term activity, a 6 (treatment group) by 6 (3-h bin) ANOVA was done on the data used to construct Figure 4. Overall, the change in activity varied by treatment group, $F(5, 42) = 12.824$, $p < .0001$, the change in activity varied across time, $F(5, 210) = 9.326$, $p < .0001$, and the change in activity across time depended on treatment, $F(25, 210) = 9.456$, $p < .0001$.

For each group, a one-way ANOVA was done, where the factor was time (6 3-h bins). For the Sal-Sal group, the change in activity varied across time, $F(5, 35) = 2.943$, $p = .0255$, but it differed from 0 only during bin 13–15, $t(7) = -2.466$, $p = .0431$. For the Sal-Ant group, the change in activity did not vary across time, $F(5, 35) = 2.111$, $p = .0872$, though it differed from 0 during bins 7–9 and 16–19, $t(7) = -2.636$ and -3.053 , $ps = .0336$ and $.0185$. All changes from 0 were transient and marginal, so Sal-Sal and Sal-Ant did not markedly alter the pattern of activity seen after control day Sal-Sal.

Amp-Sal was followed by a progressive cumulative reduction in activity that began during bin 13–15 and continued through bin 22–24, $F(5, 35) = 45.711$, $p < .0001$, Fischer's PLSDs, $ps < .05$. The change in activity was different from 0 from bin 13–15, $t(7) = -3.937$ to -7.577 , $ps = .0056$ to $.0001$. On the other hand, following Amp-Ant, the change in activity did not vary across bins, $F(5, 35) = 1.413$, $p = .2438$, and the change did not differ from 0 during any bin, $t(7) = -1.182$ to -2.271 , $ps = .2757$ to $.0574$. Amphetamine treatment produced a gradual cumulative decline in longer-term activity, and D1 antagonist post-treatment prevented this decline.

Mor-Sal was followed by a progressive cumulative reduction in longer-term activity that began during bin 10–12 and continued through bin 19–21, $F(5, 35) = 23.968$, $p < .0001$, Fischer's PLSDs, $ps < .05$. The change in activity was different from 0 from bin 10–12, $ts(7) = -2.723$ to -10.512 , $ps = .0296$ to $<.0001$. Following Mor-Ant, the change in activity varied across bins, $F(5, 35) = 3.181$, $p = .0180$, but this was because the cumulative change in activity was greater during bins 19–21 and 22–24 than during other bins, Fischer's PLSDs, $ps < .05$. Nevertheless, the change did not differ from 0 during any bin, $ts(7) = .153$ to 1.788 , $ps = .8827$ to $.1170$. As was the case with amphetamine, morphine treatment produced a gradual cumulative decline in longer-term activity, and D1 antagonist post-treatment prevented this decline.

For each bin, a one-way ANOVA was done, where the factor was treatment group (six groups). Groups did not differ during bin 7–9, $F(5, 42) = 1.409$, $p = .2406$. During bin 10–12, the difference among groups was almost significant, $F(5, 42) = 2.201$, $p = .0721$, and at each bin from 13–15 to 22–24 groups differed significantly, $Fs(5, 42) = 6.417$ to 15.481 , $ps = .0002$ to $<.0001$. Mor-Sal differed from all groups from bins 10–12 to 22–24, Fischer's PLSDs, $ps = .0353$ to $<.0001$, except during bins 19–21 and 22–24, when Amp-Sal was similar, Fischer's PLSDs, $ps > .05$. The Mor-Sal group aside, Amp-Sal differed from remaining groups from bins 16–18 to 22–24, Fischer's PLSDs, $ps = .0340$ to $<.0001$. At least in this study, the average reduction in cumulative activity started and ended earlier following Mor-Sal than following Amp-Sal, and both treatments produced a similar minimum.

One other apparently systematic difference occurred: During bins 13–15, 19–21, and 22–24, the cumulative percent change was greater following Mor-Ant than Amp-Ant, Fischer's PLSDs, $ps = .0432$ to $.0020$. The results may reflect a differential impact of D1 antagonist on the capacity to rebound from treatment with amphetamine and morphine.

3.3.2. Longer-term Effects during Individual Tests—Figure 5 shows, for Amp-Sal (upper panel) and Mor-Sal (lower panel) treatment groups, cumulative percent change in activity across subjects for individual tests. For each treatment condition, after test 1, similar results were obtained across tests, and the progressive reduction in cumulative activity did not appear to systematically decrease or increase across tests, that is, effects analogous to tolerance and sensitization were not evident.

3.3.3. Longer-term Effects Shown by Individual Subjects—Figure 6 shows cumulative percent change in activity across tests for individual subjects in Amp-Sal (upper panel) and Mor-Sal (lower panel) treatment groups.

For the Amp-Sal group, the function for each animal was an average of six tests. Animals in the Amp-Sal group appeared to show different magnitudes of cumulative percent reduction in activity. To assess this, for each animal a 6 (test) by 6 (3-h bin) ANOVA was done to see if a cumulative reduction in activity did in fact occur across 3-h bins. Five “high withdrawal” rats (animals 1, 3, 4, 5, and 7) had a significant cumulative percent reduction in activity across bins, $Fs(5, 25) = 6.892$ to 18.174 , $ps = .0004$ to $<.0001$. Three “low withdrawal” rats (animals 2, 6, and 8) did not, $Fs(5, 25) = 1.360$ to 2.102 , $ps = .2728$ to $.0986$. For these low

withdrawal rats, follow-up one-sample t-tests indicated that bin values did not differ from 0, $t(5) = .061$ to -2.199 , $p = .9536$ to $.0792$.

For the Mor-Sal group, the functions for rats 1–4 were based on five tests, and the functions for rats 5–8 were based on six tests. For each animal a 5 or 6 (test) by 6 (3-h bin) ANOVA was done to see if cumulative percent activity decreased across 3-h bins. Six “high withdrawal” rats (animals 2, 3, 4, 5, 6, and 8) had a significant cumulative percent reduction in activity across bins, $F(5, 20 \text{ or } 25) = 2.956$ to 16.388 , $p = .0371$ to $<.0001$, and one “low withdrawal” rat (animal 1) did not, $F(5, 20) = 1.259$, $p = .3199$. For this low withdrawal animal, bin values did not differ from 0, $t(4) = -.057$ to -1.548 , $p = .9574$ to $.1966$. Rat 7 was a borderline case: it did not have a significant percent reduction, $F(5, 25) = 2.579$, $p = .0516$, but four of six bin values differed from 0, $t(5) = -2.641$ to -4.184 , $p = .0459$ to $.0086$.

3.4. Comparison of High and Low Withdrawal Rats

For the Amp-Sal group, additional analyses were done to assess whether selected attributes of the high and low withdrawal rats differed, and so might be predictors of the magnitude of longer-term hypoactivity. High and low withdrawal rats did not differ in any attribute assessed including: average weight at the time of test 1 control; average change in weight from test 1 control to test 6 control; average total activity during the dark phase of control days; average pattern of activity during the dark phase of control days; average level of short-term responsiveness to amphetamine; or the average pattern of short-term responsiveness to amphetamine. Small sample sizes made rigorous comparisons difficult, and in subsequent research such comparisons would need to be done with larger sample sizes.

4. Discussion

4.1. Summary

During the first six hours following administration, amphetamine (2.0 m/kg) and morphine (5.0 mg/kg) produced changes in activity resembling those previously reported: Amphetamine substantially increased activity during the first several hours, and morphine modestly increased activity around the third hour. D1 antagonist, which produced little change in short-term activity in its own right, blocked these increases in activity.

Amphetamine and morphine produced longer-term hypoactivity having similar time courses, and drug-induced hypoactivity was also blocked by D1 antagonist, though antagonist itself did not produce longer-term changes in activity. Individual differences were observed: Three rats treated with amphetamine followed by saline, and one rat treated with morphine followed by saline, were “low withdrawal” animals, that is, they did not show longer-term hypoactivity.

4.2. Amphetamine and Morphine and Longer-term Hypoactivity

The longer-term hypoactivity produced by amphetamine and morphine were similar in time course and magnitude and could be blocked by a D1 antagonist. The results suggest that the

longer-term hypoactivity produced by the drugs may have had common determinants. Longer-term hypoactivity has at least two sets of determinants, initiators and expressers. Initiators are factors that occur shortly after drug administration and that start the sequence of events resulting in longer-term hypoactivity. Expressers are factors that are responsible for the proximate manifestation of longer-term hypoactivity. Co-administration of a D1 antagonist with amphetamine or morphine prevented longer-term hypoactivity, suggesting that an initiator common to the two drugs could have been an increase in dopamine neurotransmission in the mesolimbic dopamine pathway. Amphetamine increases dopamine neurotransmission by promoting release and reducing reuptake of dopamine (Feldman et al, 1997), whereas morphine removes the inhibition of interneurons on ventral tegmental dopamine neurons, thereby increasing dopamine neurotransmission in output regions, such as the prefrontal cortex, the nucleus accumbens, and the amygdala (Chartoff & Connery, 2014; Mazei-Robison & Nestler, 2012). Consequently, the common initiator of longer-term hypoactivity could have been a change in one or more dopaminergic output regions of the ventral tegmental area (VTA). Expressers presumably would be downstream of these regions.

The hypothesis that morphine produces longer-term hypoactivity by first modifying dopamine neurotransmission of the VTA suggests that most drugs of abuse, which modulate VTA function in a way such that dopamine neurotransmission is increased (Wanat et al, 2009), would be expected to produce longer-term hypoactivity that could be blocked by D1 antagonist administration. More generally, the cascade of events by which different drugs of abuse produce at least some withdrawal signs may be initiated through the mesolimbic dopamine system, the same pathway responsible for the short-term reinforcing effects of drugs.

Alternatively, because mu receptors are found, not only in the VTA, but in a variety of other brain regions (Mansour et al, 1994), morphine may modulate dopamine function and produce longer-term hypoactivity by directly acting at regions outside the VTA.

4.3. Schedule of Amphetamine Administration and Longer-term Hypoactivity

In prior research, amphetamine was repeatedly administered at 24- or 33-hour intervals (White et al, 2004; White & White, 2006). In the present study, amphetamine was repeatedly administered at five days intervals. In each of these studies, longer-term hypoactivity was obtained, and the time course and the magnitude were similar.

4.4. Longer-term Hypoactivity and Hypophagia Following Amphetamine

Previously, we reported that rats treated with amphetamine every five days showed longer-term hypophagia (White et al, 2015; White et al, 2010; White et al, 2007). In the present study, rats treated with amphetamine every five days showed longer-term hypoactivity that was similar to the longer-term hypophagia seen in our previous research, in terms of both time course and magnitude. Moreover, like the longer-term hypophagia (White et al, 2015), the longer-term hypoactivity in the present study was blocked by a D1 antagonist. Thus, shared determinants of longer-term hypoactivity and hypophagia are indicated. If longer-

term hypophagia results from increased VTA dopaminergic neurotransmission, then morphine administration would be expected to produce longer-term hypophagia.

The results of the current study suggest a preliminary conceptual model of the organization of some of the signs of acute withdrawal. A symptom cluster consists of two or more symptoms that are related to each other and that occur together (Kim et al, 2005). Longer-term hypoactivity and longer-term hypophagia seem to have the defining characteristics of a “cluster” as it is meant in this sense: They entail a departure from normal function, they share determinants, they are similarly responsive to different manipulations, and they have parallel time courses. Because they are objective measures they are properly considered signs rather than symptoms. Consequently, longer-term hypophagia and longer-term hypoactivity could be considered aspects of a “sign cluster”. Symptom clusters may reveal specific underlying dimensions of symptoms and may have utility for identifying and assessing treatments (Kim et al, 2005). Sign clusters may have similar uses.

4.5. Amphetamine and High versus Low Withdrawal

In the present study, one group of animals (N=8) received six tests of amphetamine followed by saline. Five of the animals in this group had a significant decline in cumulative longer-term activity (high withdrawal rats), and three did not (low withdrawal rats). Most of the critical activities in which an animal engages (eating, drinking, exploration, etc.) involve a locomotor component. Thus, a large reduction in longer-term activity may reflect a substantial reduction in one or more functional activities and so a substantial acute withdrawal state. The absence of a reduction probably reflects a less sizeable acute withdrawal state. Consequently, the difference in the expression of longer-term activity in high and low withdrawal rats appears to be a simple behavioral indicator of differential expression of acute withdrawal.

The extent of longer-term hypoactivity may signify more than just the magnitude of acute withdrawal: It might be, for example, a biomarker of the degree of susceptibility to drug use. One line of reasoning suggests that low withdrawal rats might be less susceptible to drug use. Following exposure to chronic high doses of amphetamine, withdrawal symptoms can be highly aversive. A major reason animals purportedly self-administer amphetamine is to terminate such symptoms (Koob, 2015). Low withdrawal rats would not self-administer for this reason, and so may show a lower susceptibility to drug use. Whether the administration regime used in the present study produced a withdrawal sufficiently aversive to incentive self-administration is uncertain.

Another line of reasoning suggests that low withdrawal rats might be more susceptible to drug use. Following drug administration, low withdrawal rats would experience a short-term hedonically positive primary reaction, but, in contrast to high withdrawal rats, they would not experience a longer-term hedonically negative after reaction (Solomon, 1980). In other words, for low withdrawal rats the ratio of costs to benefits for self-administration would be lower.

Whether an animal was a high or low withdrawal rat could not be predicted from other attributes, such as starting weight or weight gain, baseline level or pattern of activity, or

short-term responsiveness to amphetamine. And so the presence or absence of longer-term hypoactivity following drug may have unique predictive value with respect to drug susceptibility.

The distinction between low and high withdrawal rats suggests some obvious lines of research. One line of research might assess whether low and high withdrawal rats differed in drug self-administration or in correlates of self-administration. Another line might assess whether low and high withdrawal phenotypes have a genetic basis.

4.6. Integration of Short and Longer-term Effects

A final issue raised by the results is the way in which short and longer-term effects of drug are integrated to produce outcomes in drug assessments. Little consideration has been given to the possibility that such integration might occur: Drug outcomes assessments and the variability in them are assumed to be due primarily to individual differences in the immediate reaction to drug. Good grounds do exist for supposing that current responding is little affected by remote consequences: Animals discount events that are even briefly delayed (Volkow & Baler, 2015; Sellitto et al, 2011). Acute withdrawal might be conceived of as a remote event that can have little impact on a current decision to approach a drug paired context or to self-administer a drug. However, the phenomenon of taste aversion demonstrates that an event (illness) occurring many hours after a signal (a taste) can impact responsiveness to that signal (Welzl et al, 2001). The present findings indicate that the longer-term impacts of a drug such as amphetamine can differ between subjects. If animals differ in longer-term effects, and if short and longer-term effects of drug are integrated, animals can vary on drug outcome assessments, even if short term impacts are identical.

A variety of drug outcome assessments could depend on the way in which short and longer-term effects of drug administration are integrated, including conditioned place preference, resistance to punishment during drug-seeking, persistent responding in the absence of drug, sensitivity to develop escalation of drug taking, and heightened relapse and reinstatement. Conditioned place preference with amphetamine can be used to illustrate. The degree of approach during a test of conditioned place preference may be related not only to the state with which a context is paired, but also to the later state that the context predicts. If short-term and longer-term effects are integrated, outcomes during tests of conditioned place preference might depend on whether an animal manifested high or low withdrawal. A low withdrawal rat would be expected to show higher conditioned place preference during a test, because during training the context had been paired with a positive affective state, but it did not predict a negative affective state. A high withdrawal rat would be expected to show lower conditioned place preference during a test, because during training the context had been paired with a positive affective state, but it also predicted a negative affective state. Some of the variability in conditioned place preference results may be due to such differences.

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Highlights

- Longer-term hypoactivity (LH) is a reduction in activity 12–24 h following drug
- LH is a sign of acute withdrawal
- Amphetamine (Amph) and morphine (Morph) given every 5 days elicit similar LH
- The LH elicited by both drugs was blocked by co-administration of a D1 antagonist
- Amph and Morph elicit some signs of withdrawal via a common dopamine pathway

Figure 1.

Diagram of a typical test. Most tests were five days long. Conditions in effect during each day are shown. At light onset of Day 1, each subject received control administrations (CON), consisting of saline treatment followed 30 m later by saline posttreatment. On Day 3 subjects received experimental administrations (EXP), consisting of saline, amphetamine, or morphine followed 30 m later by saline or D1 antagonist, depending on group. The light-dark bar shows when lights were on or off. Grey indicates when animals were in activity monitoring stations. Animals spent Days 2, 4, and 5 in the animal colony.

Figure 2.

Mean cumulative percent change in longer-term activity from the last habituation day to day 1 of test 1 for each treatment group. Longer-term activity is from h 7 to 24 following treatment, and it is shown in 3-h bins. The bar across the top of the figure indicates when lights were on or off. Values did not differ from 0: Levels and patterns of activity had become stable by the time of the first test.

Figure 3.

Short-term effects of treatments. Each function is for a different treatment group and shows the mean change in distance moved (m) from the control days of tests to the experimental days (experimental - control). Data are from h 1–6 following treatment, in 30 m bins. For each subject in a treatment group, data were averaged across tests, and then a group average was found across subjects. The horizontal line shows the time of posttreatment. Error bars are standard errors.

Figure 4.

Longer-term effects of treatments. Each function is for a different treatment group and shows the mean cumulative percent change in longer-term activity from the control days of tests to the experimental days (experimental – control). Longer-term activity is from h 7 to 24 following treatment, and the cumulative change is shown in 3-h bins. The bar across the top of the figure indicates when lights were on or off. For each subject in a treatment group, data were averaged across tests, and then a group average was found across subjects. Details regarding how each function was produced and the advantages of summarizing data in this way are described in the body of the text.

Figure 5.

Mean cumulative percent change in longer-term activity during each test for animals in the amphetamine-saline treatment group (upper panel) and in the morphine-saline treatment group (lower panel). For the morphine-saline group, the function for Test 3 was based on rats 5–8. All other functions were based on all eight rats in a treatment group. The bar across the top of each figure indicates when lights were on or off.

Figure 6.

Mean cumulative percent change in longer-term activity for individual subjects in the amphetamine-saline treatment group (upper panel) and in the morphine-saline treatment group (lower panel). For the morphine-saline group, the functions for rats 5–8 were based on five tests. All other functions were based on six tests. The bar across the top of each figure indicates when lights were on or off.

Table 1

Test Day 3 Treatment and Post-Treatment Combination for Each Group/ Drug Treatment Condition

Group	Treatment	Post-Treatment
Saline - Saline	1.0 ml/kg Saline	1.0 ml/kg Saline
Saline - D1 Antagonist	1.0 ml/kg Saline	0.05 mg/kg SCH 23390
Amphetamine - Saline	2.0 mg/kg Amphetamine	1.0 ml/kg Saline
Amphetamine - D1 Antagonist	2.0 mg/kg Amphetamine	0.05 mg/kg SCH 23390
Morphine - Saline	5.0 mg/kg Morphine	1.0 ml/kg Saline
Morphine - D1 Antagonist	5.0 mg/kg Morphine	0.05 mg/kg SCH 23390

Note. Groups were run between May 2014 and July 2015. Each group was run for a period of approximately six weeks, and the order in which groups were run was: Morphine-Saline, Morphine-D1 Antagonist, Saline-D1 Antagonist, Amphetamine-Saline, Amphetamine-D1 Antagonist, and Saline-Saline.