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Effects of Amphetamine on Schedule-Induced Polydipsia

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Abstract

A series of experiments examined the effects of amphetamine (AMPH) at various doses administration for different length of time on a schedule-induced polydipsia (SIP) and possible associations with behavioral activation. Two stages of a two-week AMPH treatment were introduced with interposed interval of two months. In terms of behavioral activation, AMPH induced a robust depression across stages but with less potency in the second one. As for the SIP performance, the effects manifested qualitative difference in the two stages. For the first stage, there were no differential effects of AMPH on stereotypy intensity during the facultative phase of the inter-rewarding interval. However, AMPH reduced the high frequency of licks in the adjunctive (schedule-induced) phase and increased the low frequency of nose pokes in the terminal (schedule-dependent) phase. In the second stage, AMPH had no effect on the frequency of licking whereas the efficiency of licking and the frequency of nose pokes were reduced. These results were interpreted to support the current viewpoint that the behavior of SIP displaying is relevant to the function of central dopaminergic systems. The results were further discussed in the considerations of behavioral competition, stress coping strategy, and also the impact of AMPH at different time.

Key Words: amphetamine; schedule-induced polydipsia; locomotor activity; stereotyped behavior; behavioral competition; stress coping strategy

Introduction

Amphetamine (AMPH) is one of the major psychotomimetics to be abused, and it may induce a state of behavioral activation, psychological euphoria and/or even hallucinations (45). In animal studies, converging evidence indicates that AMPH-induced behavioral expression can be influenced by certain controlling variables, which can be broadly categorized into dosage, length of treatment and experimental context (2, 34, 43, 49). In terms of dosage, some earlier studies suggest that a low dose of AMPH-enhanced locomotor activity; whereas high-dose conditions lead to a variety of behavioral patterns with more repetitive and/or stereotyped characteristics in nature (43, 49, 51, 53). For the length of treatment, it is evident that effects of acute AMPH administration manifest differently with those of chronic administration [for review, see (52)]. In terms of experimental context, it is worth noticing that in recent years

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interest has been shifted onto the relationship between situational context and AMPH-related behaviors, in which the level of motivation (for example, the degree of hunger) and anxiety (for example, the coping capacity in a long term period of tension anxiety) were shown to be crucial determinants (27).

In this study, we aimed to provide further information regarding AMPH manipulations, especially in the interaction between treatment length and environmental context accompanied with the schedule-induced polydipsia (SIP), focusing on the role of stress-coping ability in AMPH-induced psychopathologies. Among animal models of coping strategies, SIP has earned its reputation in providing useful data in both quantitative and qualitative profiles [for review, see (10)]. As firstly demonstrated by Falk (11, 12, 13) that periodic schedule of food pellet can not only be regarded as a procedure of reinforcement, but also generate a burst of vigorous drinking in rats, namely the SIP. The responses prior to the next pellet need to be shaped *via* a series of scheduled activities, including three striking behavioral phases termed terminal (*i.e.* schedule-dependent), adjunctive (*i.e.* schedule-induced) and facultative behaviors. In rats, these successive phases of behavior correspond equivalently to pressing, licking, and locomotion (38). The properties of such behavior, particularly the adjunctive and facultative ones, have been ascribed to neither the static nor the dynamic attributes of superstitious behavior (12), but a kind of displacement or supplementary behavior which normally occurs when rats are situated in arousal or vigilant conditions (4, 12, 32, 55). One theory appeared to suggest that the cause of SIP is for the purpose of coping and mood buffering of rats to reduce their emotional stress or arousal (5). This hypothesis has been further supported by the findings that rats that engaged in excessive drinking displayed decreased plasma levels of corticosterone during the course of a SIP session (8). Thus SIP phenomenon may reflect, in a broad sense, a coping strategy to the intrinsic levels of anxiety (4, 24), which may be partially related to certain clinical psychiatric disorders with obsession in nature, that is, obsessive-compulsive disorders (20, 36, 44, 54, 58).

The effects of amphetamine on SIP had been reported previously in which the water intake in SIP can be suppressed by amphetamine. This effect of amphetamine may have been due to the reduction of high rates of licking and/or a competition between licking and locomotor or other amphetamine-induced activities (9), although others may interpret this to be a behaviorally nonspecific drug effect in that changes in activity consistently preceded or accompanied reduction in water consumption (31). In terms of drug addiction, recent evidence shows that the mechanism of AMPH addiction is also largely relevant to the processing of dependence-withdrawal schedule (7, 37, 48), in which a boost of AMPH may resume an abuse outbreak despite a long period of abstinence (25, 39). In terms of AMPH exposure in different situational contexts, psychological profiles or behavioral patterns may become interactive complex. Among them stress coping strategies are concerned in the present study as they may be associated to a some extent with the mechanism of drug dependence (58). Thus, research interests centered on the disparity between different episodes of AMPH exposure, such as comparing the stress coping mechanism (6, 28, 40, 42) and behavioral activation (2, 6, 28, 40) between the first time exposure of AMPH and the subsequent ones. This might be useful in helping to interpret the development of AMPH dependence.

Thus, by employing a two-stage design with different lengths of repeated AMPH injections, we used SIP as a behavioral task to explore more details regarding anxiety coping mechanism. The data were also expected to contribute to the understanding of certain clinical issues imbued with obsession or repetition in nature, such as obsessive-compulsive disorder (OCD).

Materials and Methods

Subjects

Twenty-seven 300±50 g adult male Sprague-Dawley rats which successfully established SIP were individually housed in cages with a 12-h light/12-h dark cycle at an ambient temperature of 25°C throughout the study. All subjects had the 85% free feeding weight maintained by food restriction. Water was available at all times. Procedures involving experimentations on animals were done in accordance with the guidelines of the National Defense Medical Center and the National Science Council.

Apparatus

The SIP experiment consisted of a number of subsystems with four identical operant chambers ($25\times28\times30$ cm^3). The sides and ceiling of the chambers were made from 0.2-cm thick clear Plexiglas, and a grid floor was constructed out of 0.6-cm diameter stainless-steel rods spaced 1.5 cm apart. A food magazine was located at the center of the front wall and connected to a pellet dispenser to deliver the Noyes precision food pellets (45 mg/pellet). Water could be easily accessed from the drinking tube connected to a water bottle. Every chamber contained three sets of infrared lights to sense behavioral responses, such as nose pokes, pellet drops and licks.

These sensors were connected to a set of 12 event counters. The software, which operated these sensors, was written in C^{++} language, operated in the Windows 95 environment. During sessions, these systems automatically recorded and accumulated the occurring responses in each event (19). The test of locomotor activity was carried out in four-activity chamber ($45\times45\times30$ cm^3) and was quantified using the automated activity video tracking systems (Chromotrack/ Polytrack, San Diego Instruments Inc. San Diego, CA, USA).

Experimental Procedures

A design of three groups in a two-stage amphetamine treatment. Established SIP rats were injected four times daily with either d-AMPH sulfate (RBI, USA) or saline in their home cage. To prevent from AMPH-induced adverse effects, the dosage has been arranged on a basis of divided administration. The final injection was given approximately 14 hours before each morning's test. Rats were assigned randomly into three groups. Each group (n = 9) receives four times of injection at 1500 h, 1600 h, 1700 h, and 1800 h, respectively. Group 1 (G1) rats received saline as a control. Group 2 (G2) rats received 0.25 mg/kg, and group 3 (G3) rats received 2.5 mg/kg d-AMPH sulfate intraperitoneally (IP) for each time of injection; *i.e.*, G2 rats received a total dose of 1.0 mg/kg while G3 rats received a total dose of 10 mg/kg 14 h before the session started. All rats had a two-week course of daily AMPH-dosing (*i.e.*, stage 1) and experienced a two-month interval drug withdrawal and then a second treatment program (*i.e.*, stage 2, with the identical protocol as stage 1). Mean value of the result from the initial 3 days (day 0 to 3 and 74 to 77) was calculated to reflect the short-term effects, whereas that of the last 3 days (day 11 to 14 and 85 to 88) was calculated to reflect the long-term effects of AMPH treatment.

Behavioral measurement. Before beginning, around 8:00 AM, rats were placed into separate cages made of transparent plastic ($45cm \times 45cm \times 30cm$) for a novel session in the open-field condition. In this session, spontaneously or AMPH-induced locomotive activity (total-walking distance) and stereotyped behaviors were recorded for 30 min. Locomotor activity was indexed by total walking distance and was recorded by using the automated activity video tracking systems (Chromotrack/Polytrack, San Diego Instruments Inc.). Two experienced observers rated the stereotypy score over this 30-min session. Following the novel session, rats were immediately put into the operant chambers to start SIP session for another 30-min period. In this session, operant performances during the two-pellet interval were automatically quantified. Two observers rated the stereotypy score in the facultative phase of this SIP session.

Schedule-induced polydipsia. On the first day of behavioral training the rats were placed into the operant chambers with the food trays containing 30 food pellets. No behavioral data were recorded. On all subsequent sessions a single food pellet was delivered into the food tray every 60 sec (fixed- time [FT] 60-s schedule) with a 30-min session every training day. The following measures were recorded for each rat in each session: 1) the number of nose pokes, 2) the number of pellets earned, 3) the number of licks, 4) the volume of water consumed, 5) lick efficiency (the number of licks/the volume of water consumed). The training sessions (30 min/day) continued for at least 15 days. If a rat consumed more than 12 ml water for at least three consecutive days, we selected this rat as a successfully established SIP rat. After the SIP session, all rats were allowed to drink freely. The water bottles were filled with 100 ml fresh water and installed immediately before each daily experimental session. As for the chaw, food restriction was employed in order to maintain their body weight at 85% of free-feeding weight.

Stereotyped behaviors. The intensity of stereotyped behaviors will be assessed by using the scale established by Ellinwood and Balster, 1974 (10), which is revealed as: Score 1, lying down, eyes closed (*i.e.*, asleep). Score 2, lying down, eyes open (*i.e.*, inactive). Score 3, normal grooming or chewing cage litter (*i.e.*, regional activities). Score 4, moving about the cage, sniffing, rearing (*i.e.*, alert and active). Score 5, running movement (*i.e.*, hyperactive). Score 6, repetitive exploration of the cage at a normal level of activity (*i.e.*, slow patterned behavior). Score 7, repetitive exploration of the cage with hyperactivity or biting attacks (*i.e.*, fast patterned behavior). Score 8, remaining in the same place in the cage with fast repetitive head and/or foreleg movement (*i.e.*, restricted behavior). Score 9, backing up, jumping, seizures, abnormally maintained postures, and dyskinetic movements (*i.e.*, dyskinetic-reactive behavior).

Statistic Analysis

The baseline values obtained from the 3-day means (day -3 to day 0 and day 71 to day 74) were used as 100% to make the comparison among the conditions of different treatment-length. During the basal period, all rats received vehicle (saline) as control treatment. For the operant performance and locomotor activity among groups, Two-way ANOVA was employed in the present experiment with treatment-length as a repeated measurement factor. Whenever appropriate, *post hoc* comparisons were made using Student-Newman-Keuls' test to reveal the dif-

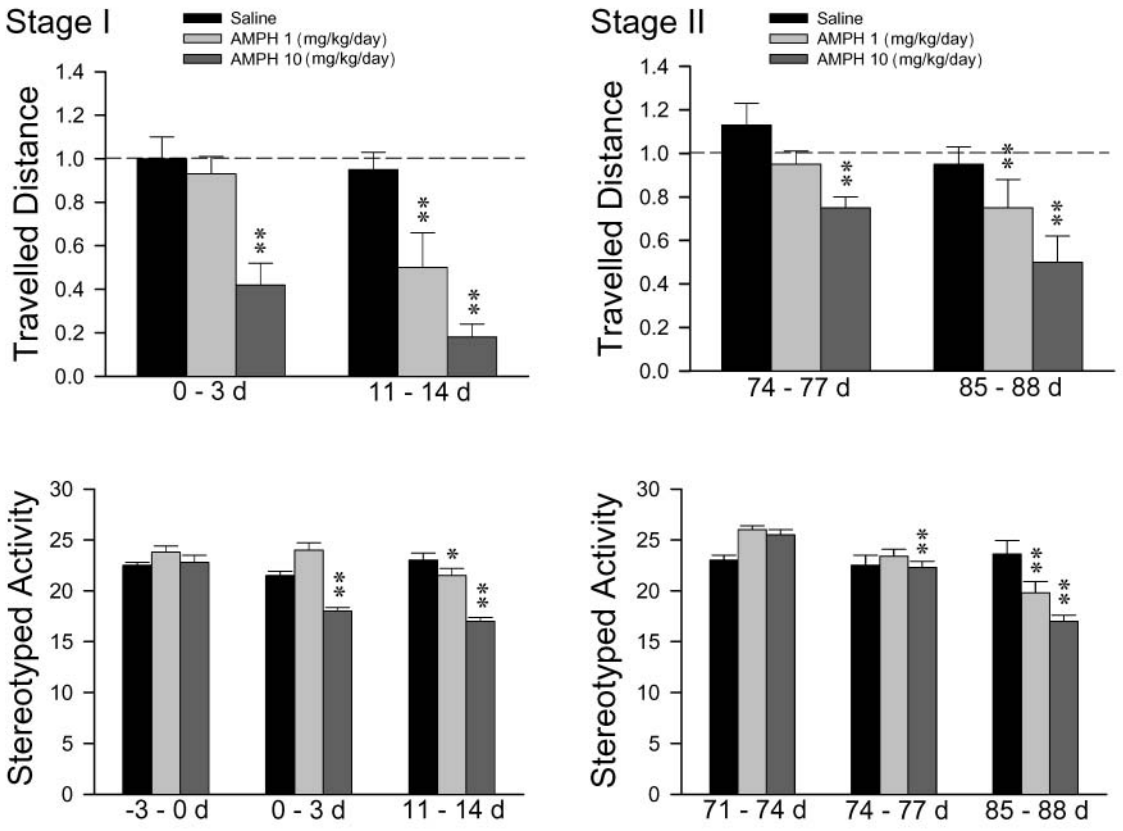

Fig. 1. The effects of the amphetamine (AMPH) on locomotor responses and stereotyped activity in SIP rats. Left panel: AMPH effects on rats in stage I in the short- (day 0 to day 3 (0-3 d)) and long- (day 11 to day 14 (11-14 d))-term courses. Right panel: AMPH effects on rats in stage II in the short-(day 74 to day 77 (74-77 d)) and long-(day 85 to day 88 (85-88 d))-term courses. Through this experiment, the respective baseline value (day -3 to day 0 (-3-0 d) and day 71 to day 74 (71-74 d)) of locomotor responses was taken as 100% to compare the mean values obtained from the short- and long-term courses with the representation by using G1 (saline, n = 9), G2 (AMPH 1 mg/kg/day, n = 9), and G3 (AMPH 10 mg/kg/day, n = 9). The intensity of stereotyped activity (II) was expressed as the amount recorded. Error bars are used to represent SEM and the statistical difference compared from the basal level, when further depicted (*$P < 0.05$; **$P < 0.01$). See main text for a detail description.

ference between group pairs of interest. Stereotyped behaviors were ranked on an ordinal scale and non-parametric methods were used, including Wilcoxon matched-pairs signed-ranks test or Friedman analysis of variance by ranks. All comparisons were based on two-tailed probabilities and results were expressed as mean ± SEM. The criterion for statistical significance was $P < 0.05$.

Results

Locomotor Activity Immediately Following AMPH Administration

Regarding locomotor activity, rats received AMPH became apparently more hyperactive than their saline controls. There was a main effect of AMPH [$F_{(1,16)} = 10.2$, $P < 0.01$].

Locomotor and Stereotyped Activities in the First Stage of AMPH Regime

As shown in the left panels of Fig. 1, there was no differential effect on baseline locomotor activity among the groups of saline control (G1), AMPH 1 mg/kg (G2), and AMPH 10 mg/kg (G3) with the results of 5823±590, 5416±419, and 5557±311 cm/30 min, respectively. The effect of different treatment-length on locomotor activity was statistically significant ($F_{(1,24)} = 30.8$, $P < 0.01$). There were differential effects on dose ($F_{(2,24)} = 12.4$, $P < 0.01$) and the effect of interaction between *dose* and treatment-length ($F_{(2,24)} = 4.1$, $P < 0.01$). As indicated in the left upper panel of Fig. 1, the results of the saline group were similar in both the short-term and long-term treatments, whereas comparisons using the Newman-Keuls' method revealed that values of G2 in the long-

term was much lower than the baseline level (q = 6.7, $P < 0.01$), and values of G3 both in the short-term and long-term AMPH regimes were much lower than the baseline level (q = 7.4, $P < 0.01$ and q = 16.2, $P < 0.01$, respectively). On the other hand, the basal scores of stereotyped behaviors measured by the nine-point rating scale showed no difference among the groups of G1, G2, and G3 with the results of 22.2± 0.6, 23.4±0.5, and 22.2±0.8/30 min, respectively. However, the effect of *treatment-length* (G2: χ^2 = 9.4, df = 2, $P < 0.01$; G3: χ^2 = 12.8, df = 2, $P < 0.01$) was statistically significant. As shown in the left bottom panel of Fig. 1, the intensity of stereotypy activities of G1 rats was similar in both the short-term and long-term AMPH treatments. Post hoc comparisons using the Newman-Keuls' method revealed that G2 rats showed reduced stereotypy activities in the long-term regime when compared with baseline level (q = 4.7, $P < 0.05$), and the stereotypy activities in both the short-term and long-term AMPH treatments of G3 rats were much lower than those of the baseline level (q = 4.96, $P < 0.01$ and q = 5.97, $P < 0.01$, respectively).

Locomotor and Stereotyped Activities in the Second Stage of AMPH Regime

As shown in the right panels of Fig. 1, locomotor activity of the second stage of AMPH-dosing regime is characterized in Fig. 1-(I) (right panel). The baseline level of locomotive activity for G1, G2, and G3 rats were 6071±496, 5552±464, and 5502±261 cm/30 min. There were significant effects for *treatment-length* ($F_{(1,24)}$ = 21.6, $P < 0.001$), dose ($F_{(2,24)}$ = 10.4, $P < 0.01$) and *treatment-length* and *dose* interaction ($F_{(2,24)}$ = 4.6, $P < 0.01$). Further analysis using the Newman-Keuls' method, as indicated in the right upper panel of Fig. 1, revealed that the G3 was comparatively lower than the basal level in the short-term treatment (q = 6.5, $P < 0.01$), and G2 and G3 values were comparatively lower than the basal level in the long-term treatment (q = 5.1, $P < 0.01$; q = 9.7, $P < 0.01$, respectively). On the other hand, the basal scores of stereotyped behaviors measured for G1, G2, and G3 rats were 22.7±0.3, 25.3±0.4, and 24.5±0.5/ 30 min, respectively. A statistically significant effect of *treatment-length* (G2: χ^2 = 12.3, df = 2, $P < 0.01$; G3: χ^2 = 12.0, df = 2, $P < 0.01$) was found. As indicated by the right bottom panel of Fig. 1, treatment-length did not make any difference on the intensity of stereotypy activities of G1 rats, whereas that the intensity of stereotypy activities of the G2 rats in the chronic stage was greatly lower than the basal stage (q = 4.92, $P < 0.01$), and as low as those of the G3 rats in their acute and chronic stages (q = 3.5, $P < 0.01$; q = 4.9, $P < 0.01$).

Behavior in SIP Session during the First Stage of AMPH Regime (Fig. 2 and 3)

Pellet earned. All rats were maintained at 85% of free-feeding weight by food restriction. While repeated injections of AMPH were given for 14 days, all group rats ate the pellets almost immediately after delivery (30 pellets consumed) throughout the session.

Water intake (Fig. 2). The basal stages of water intake for G1, G2, and G3 rats were 14.1±1.3, 13.5± 1.0, and 15.3±1.8 ml/30 min. The effect of *treatment-length* in water intake was statistically significant ($F_{(1,24)}$ = 11.5, $P < 0.01$), while significant effects for *dose* ($F_{(2,24)}$ = 27.7, $P < 0.01$) and were *dose* and *treatment-length* interaction ($F_{(2,24)}$= 8.9, $P < 0.01$) were also found. As shown in the left upper panel of Fig. 2, water intake of G1 and G2 rats were similar in both the short-term and long-term treatments. However, as compared to the basal stage, Newman-Keuls' method revealed that G3 rats in both the short and long-term treatments exhibited significantly lower water intake (q = 4.78, $P < 0.05$; q = 9.76, $P < 0.01$ respectively).

Licks and lick efficiency (Fig. 2). Basal level of licks for G1, G2, and G3 rats were 2338±229, 2114±223, and 2153±223 licks/30 min, respectively. The main effects of *treatment-length* and *dose* in licking were statistically significant ($F_{(1,24)}$ = 13.3, $P < 0.001$ and $F_{(2,24)}$ = 4.7, $P < 0.05$, respectively). As indicated in the middle and bottom left panels of Fig. 2, rats in G1 exhibited similar levels in the short and long-term treatments. Rats of G2 in long-term treatment showed a reduced number of water licking as compared with basal level (q = 4.1, $P < 0.05$) and rats G3 showed reduced number of water licking in both short and long-term treatments than the values in baseline (q = 3.9 $P < 0.05$ and q = 5.8, $P < 0.05$, respectively). The lick efficiency has no statistical difference in all groups of rats during both the short and long-term treatments throughout this stage.

Nose poke (Fig. 3). Nose pokes in general occurred in the terminal phase of the 60-sec interval of SIP session. The number of baseline for G1, G2, and G3 rats were 1059±166, 903±63, and 789±115 pokes/ 30 min, respectively. Significant effects for the treatment-length ($F_{(1,24)}$ = 14.4, $P < 0.01$) as well as *dose* and *treatment-length* interaction ($F_{(2,24)}$ = 3.1, $P < 0.05$), were found. As shown in the left upper panel of Fig. 3, the G1 values of both short and long-term treatments were similar. Newman-Keuls' method was used to make further comparisons and revealed that the long-term drugged rats of G2 appeared to be much higher than the baseline level (q = 4.8, $P < 0.05$). The values of G3 rats in both the short and long-term treatmenty were significantly higher than those of the baseline (q = 3.0, $P < 0.05$ and q = 6.1, P

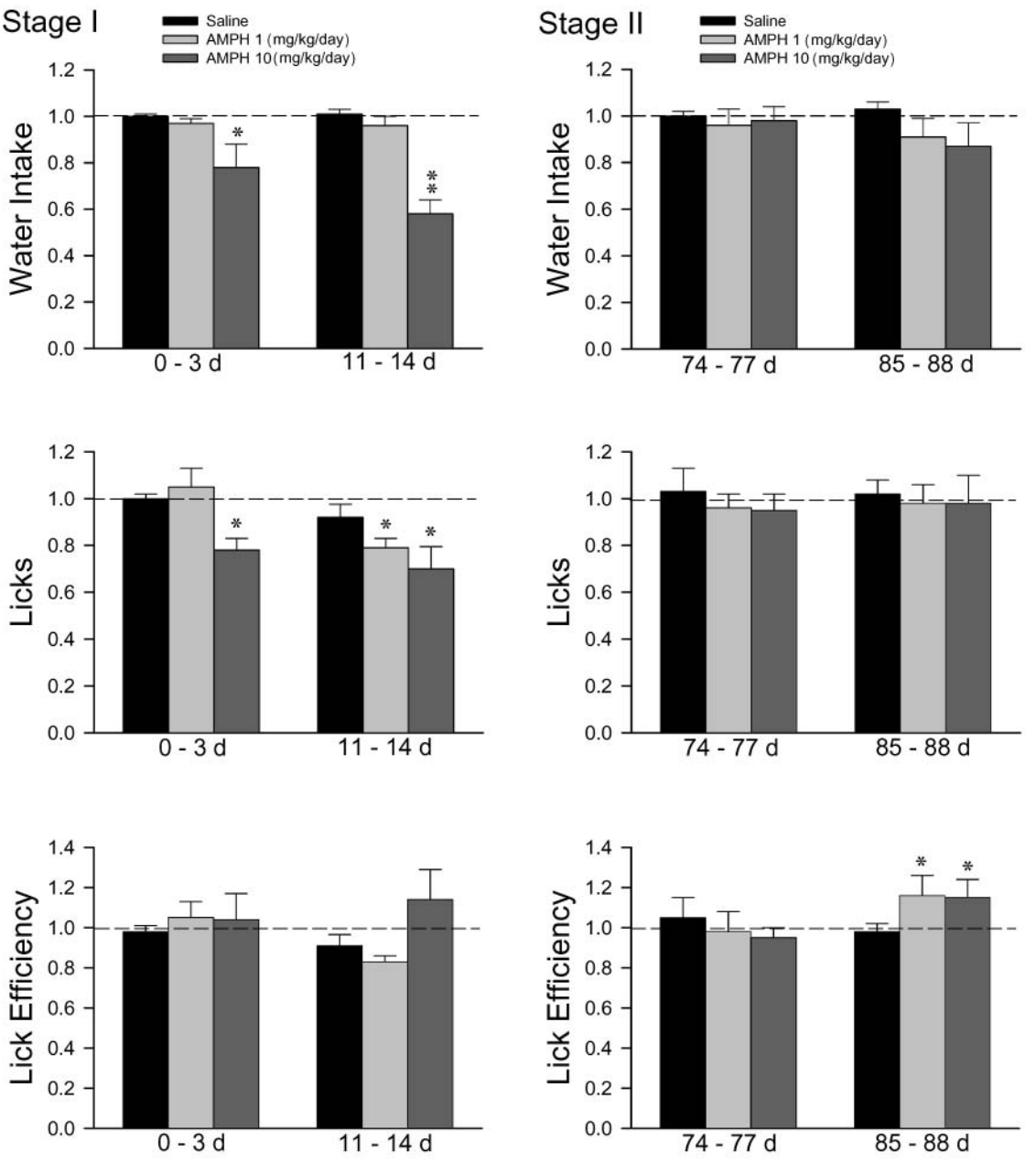

Fig. 2. Effects of AMPH on water intake, and licks, and lick efficiency in SIP rats. Left panel: AMPH effects on rats in stage I in the short- (day 0 to day 3 (0-3 d)) and long- (day 11 to day 14 (11-14 d))-term courses. Right panel: AMPH effects on rats in stage II in the short-(day 74 to day 77 (74-77 d)) and long-(day 85 to day 88 (85-88 d))-term courses. Through the experiment, the respective baseline value (day -3 to day 0 (-3-0 d) and day 71 to day 74 (71-74 d)) of water intakes, licks and lick efficiency was taken as 100% to compare the mean values obtained from the short- and long-term courses with the representation by using G1 (saline, n = 9), G2 (AMPH 1 mg/kg/day, n = 9), and G3 (AMPH 10 mg/kg/day, n = 9). Error bars are used to represent SEM and the statistical difference compared from the basal level, when further depicted (*$P < 0.05$; **$P < 0.01$). See main text for a detail description.

< 0.01, respectively).

Stereotyped behaviors (Fig. 3). The intensity of stereotypy activity occurring in the facultative period over this session had been recorded. The baseline scores of stereotyped behaviors measured for G1, G2, and G3 rats were 23.3±0.4, 22.2±0.7, and 23.4±0.5/30 min, respectively. As shown in the left bottom panel of Fig. 3, all rats in three groups exhibited approximate activities. No differential effects can be found in either treatment-length conditions.

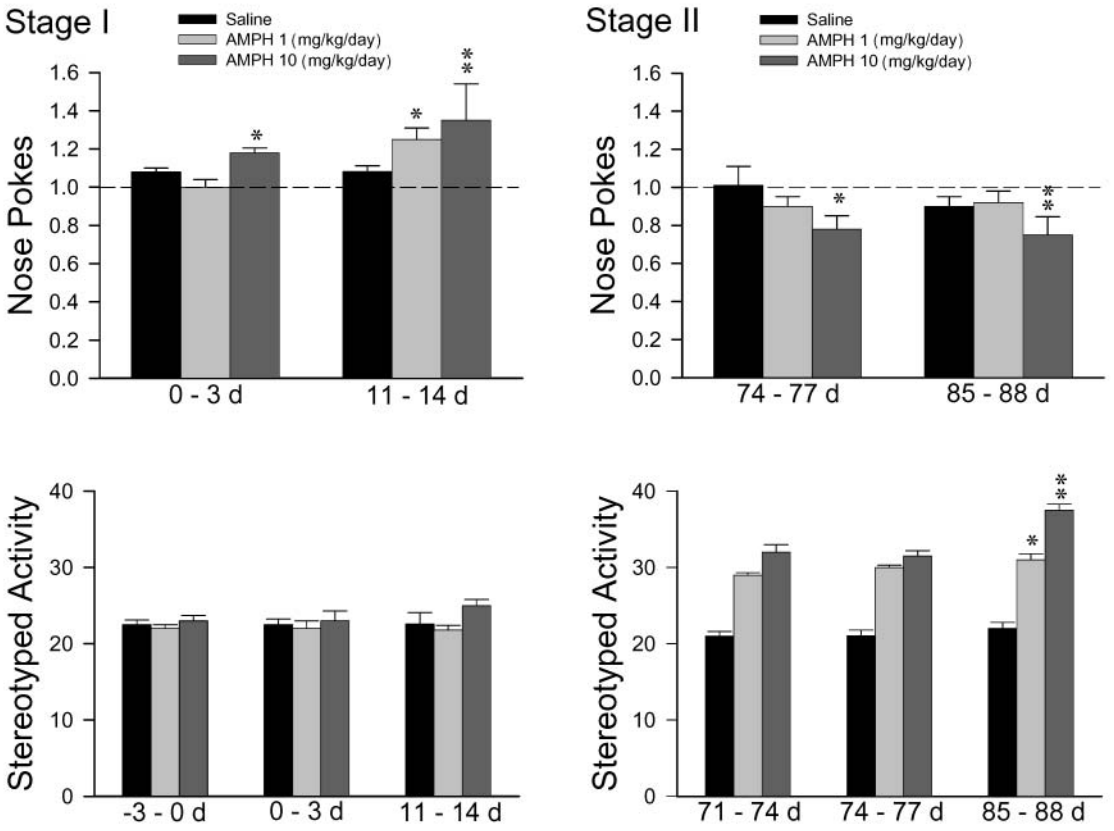

Fig. 3. Effects of AMPH on nose pokes and stereotyped activities in SIP rats. Stereotyped activities were observed and recorded during the SIP test. Left panel: AMPH effects on rats in stage I in the short-(day 0 to day 3 (0-3 d)) and long-(day 11 to day 14 (11-14 d))-term treatment courses. Right panel: AMPH effects on rats in stage II in the short-(day 74 to day 77 (74-77 d)) and long-(day 85 to day 88 (85-88 d))-term treatment courses. Through the experiment, the respective baseline value (day -3 to day 0 (-3-0 d) and nose pokes from day 71 to day 74 (71-74 d)) were taken as 100% to compare the mean values obtained from the short- and long-term courses with the representation by using G1 (saline, n = 9), G2 (AMPH 1 mg/kg/day, n = 9), and G3 (AMPH 10 mg/kg/day, n = 9). The intensity of stereotyped activity was expressed as the amount recorded. Error bars are used to represent SEM and the statistical difference, as compared from the basal level, when further depicted (*$P < 0.05$; **$P < 0.01$). See main text for a detailed description.

Behavior in SIP Session during the Second Stage of AMPH Regime

Pellet earned. All rats were maintained at 85% of free-feeding weights by food restriction before the tests. While repeated injections of AMPH were given for 14 days, all group rats ate the pellets almost immediately after delivery (30 pellets consumed) throughout the session.

Water intake. As shown in Fig. 2, the basal level of water intake for G1, G2, and G3 rats were 11.4± 1.8, 13.4±1.4, and 11.6±0.3 ml/30 min, respectively. In Fig. 2, the right upper panel showed that the amount of water intake in three groups were similar.

Licks and lick efficiency. As shown in Fig. 2, the basal level of water licking for G1, G2, and G3 rats were 1636±174, 1784±264, and 1789±260 licks/30 min, respectively. The licks of all rats in three groups were similar ($F_{(2,28)} = 0.45$, not significant). The baseline level of licking efficiency for G1, G2, and G3 rats were 162±37, 133±11, and 153±20 licks/ml. The effect of *dose* was statistically significant ($F_{(2,28)} = 5.9$, $P < 0.05$) and there was a statistically significant interaction between *dose* and *treatment-length* ($F_{(2,28)} = 3.1$, $P < 0.05$). Further analysis using Newman-Keuls' method revealed a reduction in lick efficiency (increased value) occurred on G2 and G3 rats in the long-term treatment, as compared to the baseline level (G2: q = 3.3, $P < 0.05$; G3: q = 4.5, $P < 0.05$, see Fig. 2, middle and bottom right panels).

Nose poke. As shown in Fig. 3, shows the amount of nose pokes in baseline G1, G2, and G3 rats to be 1170±207, 921±156, and 1046±170 pokes/30 min, resepctively. The effect of *dose* was significant ($F_{(2,28)} = 3.6$, $P < 0.05$), and so was the effect of interaction between *dose* and *treatment-length* ($F_{(2,28)} = 3.1$, $P < 0.05$). As shown in the upper right panel of Fig. 3, the amount of nose poke of the G1 and G2 rats

in both the short and long-term treatments were similar, whereas those of the G3 rats were much lower than the baseline level ($q = 4.3$, $P < 0.05$ and $q = 5.2$, $P < 0.01$, for short and long-term treatments respectively).

Stereotyped behaviors. In Fig. 3 the basal scores of stereotyped behaviors measured for G1, G2, and G3 rats were 21.1±0.3, 29.2±0.2, and 32.4±0.6/30 min, respectively. There were statistically significant differences in G2 and G3 rats (G2: $\chi^2 = 12.3$, df = 2, $P < 0.01$; G3: ($2 = 9.4$, df = 2, $P < 0.01$). As shown in the bottom right panel of Fig. 3, *Post hoc* comparisons using Newman-Keuls' method revealed that the intensity of stereotypy activities in the long-term treatments of both G2 and G3 rats were higher than that of the correspondent baseline level (G2: $q = 4.9$, $P < 0.05$ and G3: $q = 4.1$, $P < 0.01$, respectively).

Discussion

AMPH and Locomotor Activity

AMPH-induced behavioral activation may exhibit in various forms such as heightened vigilance, increased stereotyped behavior, and locomotor activity [for review, see (21)]. Locomotor activity can normally be used to verify the effect of AMPH prior to any further experimental assessment. Similar to previous reports (1, 14, 46), in the present study a robust form of behavioral hyperactivity was observed immediately after low dose AMPH administration. However, after long-term AMPH treatment, rats generally showed motor inertia and therefore revealed behaviorally as decrease in vigorousness of spontaneous locomotive activities and stereotypes. This was most pronounced and presented with a dose- and time course-dependent manner in the novel sessions (upper panel, Fig. 1). The phenomenon can be treated, in a sense, as behavioral depression after withdrawal from AMPH [note in the present experiments, behavioral test was carried out 14 hours after the last dose of AMPH. For more reference about AMPH withdrawal induced behavioral depression, see (3, 22, 33)].

AMPH and SIP

A major purpose of this study was to investigate whether the AMPH-induced behavioral outputs might have effects on SIP in a two-stage design of experiment. Thus, we focused on any behavioral index of SIP which exhibited altered levels in the second stage of AMPH treatment. The cardinal outcomes obtained from this study showed that AMPH led to a reduction of the water intake and licks during the SIP, and this effect decreased in the second stage injection program. During the SIP, nose pokes revealed a mismatched pattern of nose pokes occurred. They increased in the first time but decreased in the second time, with a dose dependent tendency. As to the stereotyped activity, apparently AMPH raised the frequencies only at the second stage. These findings will be discussed in great detail regarding the treatment length and the treatment times (*i.e.*, first stage versus second stage) of AMPH effects on water intake, licks, stereotyped activity and nose pokes. Certain interpretations will be raised based on the concept of behavioral competition and stress coping strategies.

Behavioral Competition

The present study indicated that while there was less of an effect on general locomotive activity, water intake and number of licks on SIP session, stereotyped activity in SIP was found to be raised during the second stage (Fig. 3). This did not occur in the test of spontaneous locomotor activity (Fig. 1). It appears to support the idea that (a) stereotyped behavior should not be considered on the same continuum as locomotor activity, thus these two behaviors may be mediated by different mechanisms (46); and (b) the involvement of dopamine-induced behavioral activation in a given task depends, to a degree, on the imbued characteristic of the context. While spontaneous locomotive activity reflects an energy output to the novel environment, stereotyped behavior in a conditioned context as SIP may reflect behavioral shifting (35) or heightened reactivity in response to a learned anxiety (16). The underlying mechanism needs to be investigated further. However, as revealed from the present experiment, it seems that SIP will intensify the AMPH-induced stereotyped responses, which may be relevant to "behavioral competition" occurring in the intermittent-reinforcement interval. Behavioral competition generally refers to a contest among the factors inherited dissimilar characteristics shared with the same space at the same time (29, 57). This generally helps to explain certain psychological phenomenon in which the frequency of one component increased but the other reduced (15, 18). In the present experiment, behavioral competition might be seen in different patterns in the stage-dependent context. In the first stage of long-term treatment was, AMPH in a large dose was likely to decrease the amount of licks (activity in adjunctive phase) but increase the amount of nose poke (activity in terminal phase). This suggests that competition or response relocation exists between the different phases of SIP procedure is in a manner of dose-dependent and/or time-dependent pattern. Thus, the decreased amount of licks was due to a failure in the response competition between water licking and nose poking. However, in the second stage of long-term treatment, nose pokes decreased in large dose condition in which

the stereotyped activities increased. Thus, behavioral competition occured again but in a different pairing manner. As to the underlying mechanism, contingency or interactive effect of stress-coping strategy and changed motivational value may contribute to the rationale. It is possible that in the first stage of long-term treatment, stress can be balanced or alleviated by the AMPH-induced nose poke, whereas stress evaluating process changed in the second stage of long-term treatment. Therefore, AMPH affected less in increasing nose poking or even exhibited a tendency of decreasing nose poke, which was likely due to the response competition by the raised stereotyped activity in facultative phase.

Repeated Treatment and Re-Established Amphetamine Impact

The effects of AMPH may depend to a great extent on the treatment length. In general, acute administration of AMPH leads to hyperactivity and increased arousal level [for review, see (23)]. As the treatment continued on a repeatedly administration schedule, drug dependence gradually occurred and a reverse tolerance to the ambulation-increasing and/or stereotypy-producing effect might occur (53). This can be seen in both clinical aspects (as AMPH addiction in humans (47)) and animal studies (as stereotypy activity and certain psychotic equivalent behaviors in rodents [for review, see (50)]. In the present experiments, it was observed that in the low dose condition, spontaneous locomotor activities decreased as the drugged schedule was prolonged (Fig. 1). However, in the high dose condition and mainly in the first stage, the AMPH effects on water intake and the amount of licks in SIP were enhanced (Fig. 2). On the other hand, the effect of long-term AMPH treatment on the second stage refers to a re-established AMPH impact, which appeared to be different with the first stage. The disparities can be revealed in three aspects. Firstly, the amount of nose poke apparently went oppositely. While the amount has been increased in the chronic period of first stage, it decreased in the second stage. Secondly, the decreased amount of adjunctive water intake has been compensated to some degree in the second stage, where the rats exhibited a better lick efficiency. Thirdly, the observed stereotyped activity was seemingly to be built up in the second stage long-term treatment (Fig. 3). Possible interpretations may be relevant to stress-coping strategy.

Stress Coping Strategies

The result of the present experiment demonstrated dissociations between the amounts of nose pokes and water intake. As the amount of nose poke represents an inner urge toward the rewarded pallet during the terminal phase of SIP, it appeared that the inner drive reflected an AMPH build-up motivational state, rather than an association with adjunctive water intake. In other words, AMPH might reset the rewarding value of the pallet to a higher level (17, 30), hence the rats revealed a greater behavioral output just before the time of earning the pallet. Accordingly, since the excessive drinking during SIP cannot be explained solely by the motivational state as reflected by the amount of nose poke, the possible rationale regarding how AMPH might influence drinking capacity became justified. First, it was found that in the first stage long-term period of treatment, repeated AMPH administration led to a remarkable decrease of water intake, which is not seen in the second one. One of the explanations could be that the thirst level of drugged rat was changed. However, since the amount of such a pathological drinking in all the experimental contexts was far beyond the need for reducing the level of thirst, this sould hardly be justified. It needs to be interpreted in more comprehensive viewpoints in which stress coping strategy is likely involved. Thus, stress induced from the intermittent rewarding procedure may be coped within SIP. As the amount of water is far beyond the need, some researchers suggested that the excessive water intake did contribute to lessen the stress-related discomfort (27). The reduced water intake seen in the long-term AMPH condition in the present study was very likely because repeated AMPH impaired the dopamine response to stress (56), which then in turn reduce the reactivity of response to the stress induced by SIP (41). Therefore, rats did not exhibit such an “excessive drinking” as usual.

There may be an alternative explanation that the excessive activity for coping stress has been shifted from drinking to nose poking, given that at the same time water-licking decreased, nose-poking evidently facilitated. Since the nose-poking behavior happened just before the drop of pellet and was usually named as terminal behavior or schedule-dependent behavior, the effects on such behavioral output can normally be facilitated by AMPH, as reported elsewhere previously (26). It could be possible that as closer to the end of the waiting period, the anxiety and tension went up. However, we should be cautious in making such an interpretation because (a) the degree of increased nose poking was modest and not always consistent as seen in the second stage; (b) the nose-poking behavior occurred just before the drop of rewarded pallet and was hence imbued with more motivational component toward the reinforcer rather than in a stress coping manner.

In conclusion, the present experiment exhibited that repeated AMPH administration in a discrete and

separate treatment procedure had different effects on the established performance of SIP rats. With an intermission of drug withdrawal period, rats on the second stage of AMPH treatment displayed significant deviation in behavioral performances in both the activity and SIP sessions. While they exhibited fewer nose pokes and more stereotyped activities during the SIP, their water intake and the number of licks remained unchanged. AMPH-induced behavioral competition and altered stress coping strategies may contribute to the interpretation of these results.

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