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Diurnal differences in rat's motor response to amphetamine

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Abstract

The dose–response characteristics and time-course of amphetamine's effect on motor activity after a single injection given to rats at four different times of the light/dark cycle was investigated using a computerized infrared motor activity recording system. After 7 days of acclimation and 2 days of baseline activity recording, rats received a single subcutaneous injection of vehicle (saline) or 0.6, 1.25 or 10 mg/kg amphetamine at 08.00, 14.00, 20.00 or 02.00. Recording was then resumed for an additional 36 to 48 h. The locomotor indices analyzed were horizontal activity, total distance, vertical activity, stereotypic activity and number of stereotypic movements. All doses (0.6, 1.25 and 10 mg/kg) significantly elevated ($P < 0.01$) locomotor activity compared to baseline at all times of administration. At all injection times, the maximum increase over baseline generally occurred following the 1.25 mg/kg dose of amphetamine ($P < 0.001$). The effect of the lower doses (0.6 and 1.25 mg/kg) on forward locomotion remained the same throughout the light/dark cycle regardless of the large difference in baseline motor activity between the light and dark phases. However, the effects of 10 mg/kg amphetamine on general stereotypic behavior, as well as the ability to cause subsequent depression of nocturnal forward ambulation, were dependent on the time of drug administration. These results showed that the circadian rhythms of locomotor and stereotypic effects of amphetamine are different. © 1998 Elsevier Science B.V.

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1. Introduction

The stimulatory and behavioral effects of the psychomotor stimulant amphetamine were reported as early as 1932 (Downs and Eddy, 1932). The pattern of locomotor activity response to amphetamine in the rat is dose-related. Low doses of the drug elicits an increase in overall locomotor activity, such as forward ambulation, spontaneous movements, rearing and intermittent sniffing (Robinson and Becker, 1986; Kuczenski and Segal, 1988). High doses elicit a different behavioral pattern which is characterized by early and late phases of increased locomotor activity that are interrupted to a greater degree by a period of focused, highly repetitive, stereotyped movements such as head bobbing, licking, repetitive rearing, continual sniffing and gnawing the cage floor as the dosage increases (Ernst and Smelik, 1966; Randrup and Munkvad, 1975; Schiorring, 1971; Kolta et al., 1985).

Most studies of the behavioral effects of acute and/or chronic administration of stimulants in the rat have been conducted during the light phase (i.e. the sleep time of the rat) with little attention given to other times of the day, although motor behavior varies considerably throughout the light/dark cycle (Honma et al., 1986; Paulson and Robinson, 1994; Gaytan et al., 1996a). Many drugs, including stimulants, have also been shown to vary in their pharmacokinetics and their efficacy throughout the day (Scheving et al., 1968, 1994; Smolensky and D'Alonzo, 1993). Scheving et al. (1968) reported that the LD_{50} of amphetamine, which is considered an indirect dopamine receptor agonist, was much greater during the dark phase than during the light phase. Moreover, the neurotransmitters reported to be underlying the regulation of motor activity, as well as in the response to stimulants, have been shown to exhibit circadian rhythms peaking during the middle of the dark cycle, with fluctuations in dopamine levels as well as in dopamine, α and β-adrenoceptor densities (Lemmer and Berger, 1978; Kafka et al., 1981, 1985; Bruinink et al., 1983; Lemmer et al., 1985). These fluctuations in neurotransmitter levels and receptor densities may result in variable motor responses when a stimu-

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lant is given during high and/or low levels of endogenous neurotransmitters throughout the light/dark cycle. Tolerance to the stimulatory effects of amphetamine develops if it is infused continuously during the light phase, but not during the dark phase (Martin-Iverson and Iversen, 1989). There are, however, no studies on diurnal effects of acute or intermittent administration of stimulants.

The present study was initiated to investigate whether differences in the time of drug administration influences the locomotor and stereotypic response to a single administration of three different amphetamine doses. For this purpose, a comprehensive investigation of the dose–response characteristics for locomotor activity immediately and for 24 h after a single amphetamine injection at the beginning and middle of the rat's inactive period (i.e. light phase), and at the beginning and middle of the active period (i.e. dark phase), was performed. The initial studies focused on: (1) investigating relationships between locomotor and stereotypic behavior throughout the light/dark cycle during the normal state and after drug administration; (2) characterizing any variation in the dose–response relationship across the four different times of administration and (3) ascertaining whether there are any persistent alterations in the normal circadian pattern of locomotor activity after a single administration of amphetamine.

2. Materials and methods

Male Sprague–Dawley rats ($n = 140$) weighing 150–170 g were housed in the experiment room in groups of four at an ambient temperature of 21 ± 2°C and relative humidity of 37–42%. Animals were maintained on a 12:12 light/dark schedule (light on at 07.00) for a minimum of 7 days before experimentation in order to internally synchronize their neuroendocrine systems. On the last day of acclimation, rats were weighed and individually housed in the experimental cages and allowed a minimum of 12 h of accommodation to the test cages before recording of locomotor activity began. Food pellets and water were supplied ad libitum throughout the experiment.

2.1. Apparatus

Omnitec Digiscan RXYZM (16) DVA computerized animal activity monitoring system cages were used. The automated system has been described in detail (Dougherty et al., 1990; Gaytan et al., 1996a). In short, the activity chambers consist of clear acrylic open field boxes ($40.5 \times 40.5 \times 31.5$ cm) with 2 levels of infrared motion sensors. The first and second level of sensors were 6 and 12.5 cm, respectively, from the cage floor. The activity monitoring system checked each of the beams at a frequency of 100 Hz to determine whether beams were interrupted. The interruption of any beam was recorded as an activity score. Interruption of two or more consecutive beams separated by at least one second was recorded as a movement score. Cumulative counts were compiled and downloaded every 10 min into OASIS data collection program.

The following indices of motor activity were studied. Total distance and vertical activity, which measure the amount of forward ambulation and rearing, were analyzed, respectively, and were used to assess these two specific locomotor effects elicited by amphetamine. Stereotypic activity measures the repeated interruptions of the same beam(s) from any of the sensor arrays. Number of stereotypic movements measures the number of different episodes of stereotypic activity with at least a one second interval before the beginning of another episode. Stereotypic activity and number of stereotypic movements were used to assess the effect of drug treatment on general stereotyped behavior. Finally, horizontal activity measures the overall motor activity in the lowest tier of the testing cages and was used to assess the overall amount of motor activity, which is a summation of both locomotor and stereotypic effects elicited by amphetamine.

2.2. Injection protocol

After 7 days of acclimation in the experimental room and at least 12 h of accommodation to test cages, motor activity was recorded continuously and summed in 10 min bins throughout the 24 h light/dark cycle for four consecutive days. The first two recording days were used to obtain baseline activity for each rat. On day 3, each rat was weighed and randomly assigned to a time control group ($n = 12$) or to one of sixteen experimental groups (each $n = 8$) that received s.c. injections (0.8 cc) of 0.9% saline containing either 0, 0.6, 1.25 or 10 mg/kg of amphetamine sulfate (Sigma Chemicals) at 08.00, 14.00, 20.00 or 02.00. Recording was then resumed for an additional 36–48 h, which included post-treatment monitoring (day 4).

2.3. Data analysis

All locomotor indices were analyzed for acute and long-term (≥ 12 h) effects of amphetamine. The acute effect was considered as the difference between activity during the 4 h immediately after injection and the activity obtained from the same animal on days 1 and 2 at the same time of day. The 10 min bins were used to test for qualitative changes in the response pattern (i.e. the effect maximum and time to maximum effect) between the times of administration for each dose and motor index. The effect maximum was defined as the largest change from baseline in a 10 min sample. The overall effect in the initial 4 h following injection of amphetamine was assessed by totaling the absolute changes in motor activity over baseline activity in the area-under-the-activity time curve (AUC). Differences between the four different times of amphetamine administration in the 4 h AUC were

determined using two factor analysis of variance, or ANOVA, (dose × time of administration), followed by Scheffe's test for post-hoc comparisons. The long-term effect (12–36 h) of amphetamine was determined using one way ANOVA with repeated measures of pre-treatment and post-treatment dark and light periods for all treatment groups. Significance for comparisons was set at $P < 0.05$.

3. Results

3.1. Time control

Daily activity (24 h), as well as the light and dark phase (12 h) activity levels were stable from day to day for the length of the experiment and the average values for all 4 days are presented in Table 1 for the 5 motor indices studied. The levels of activity, however, differed between the rats' inactive period (light phase) and the active periods (dark phase) and the ratio of change in the average counts between the light and dark phases for all five motor indices are also included in Table 1. Horizontal activity, stereotypic activity and number of stereotypic movements each showed about a three-fold increase in magnitude during their active period, while in total distance and vertical activity there was a sixfold increase between the inactive and the active period for each rat.

Although activity differed between the activity phases, the hourly pattern of activity showed a consistent circadian pattern that did not differ from day to day. In summary, the time control group displayed stable daily baseline levels of activity, as well as a consistent circadian pattern of activity, in all the indices sampled for the length of the study.

3.2. Dose–response of amphetamine given at 14.00

For all groups and indices studied, the baseline activity levels of days 1 and 2 were similar to the time control group. Therefore, the data from days 1 and 2 were averaged to obtain baseline levels of activity throughout the day for each rat. Data collected immediately after injection could then be compared to its time-matched baseline values obtained on days 1 and 2 to determine the absolute change in activity during drug treatment for each motor index.

Fig. 1 shows the effect of the three amphetamine doses given at 14.00 on total distance. Administration of saline had no significant effect, causing only a transient rise in locomotor activity during the initial 10 min following injection (Fig. 1A). The other motor indices behaved similarly and their was no significant handling effect (i.e. volume load and/or insertion of needle) at any time of administration.

Administration of 0.6 mg/kg amphetamine immediately elevated ($P < 0.01$) the index of total distance, which reached a maximum increase ($P < 0.001$) at 20–30 min after administration, and remained significantly elevated for 70 min (Fig. 1B). Horizontal activity, vertical activity, stereotypic activity and number of stereotypic movements behaved similarly to data presented in Fig. 1 after administration of 0.6 mg/kg at 14.00.

The intermediate dose of 1.25 mg/kg significantly ($P < 0.001$) elevated total distance immediately after injection, with the maximum increase ($P < 0.001$) 50–60 min after injection and returned to baseline levels by 160–170 min after injection (Fig. 1C). All other indices studied behaved similarly at 14.00.

The time-course of effect for the highest amphetamine dose (10 mg/kg) on total distance was more complex (Fig. 1D). After an initial increase ($P < 0.001$) in total distance during the first 10–20 min after injection, activity returned to baseline levels (30–110 min), before increasing again to a second peak at 170–180 min after injection. The second phase of increased activity lasted until 240 min after injection.

Unlike the lower doses, the effect of 10 mg/kg of amphetamine at 14.00 varied across motor indices. Vertical activity displayed the same multiphasic response pattern as total distance (Fig. 1D). The time-course for number of stereotypic movements was different from total distance and vertical activity, however, with an initial increase in activity that started immediately after amphetamine injection and persisted for the entire duration of

Table 1
The total daily activity average (24 h), as well as the average activity during the light phase (12 h), dark phase (12 h) presented as mean ± S.E.M. for the time control group ($n = 12$)

Parameter	Daily total (24 h), mean ± S.E.	Dark phase (12 h total), mean ± S.E.	Dark phase (12 h total), mean ± S.E.	Ratio L:D
HA	57037 ± 1389 cts	13741 ± 1140 cts	43298 ± 1504 cts	1:3.1
TD	18291 ± 277 cm	2527 ± 48 cm	15765 ± 347 cm	1:6.2
VA	4148 ± 256 cts	591 ± 75 cts	3357 ± 646 cts	1:6.0
SA	49867 ± 1292 cts	8622 ± 603 cts	24933 ± 646 cts	1:2.9
NOS	3834 ± 48 cts	965 ± 37 cts	2869 ± 54 cts	1:2.9

The ratio of change in activity from the light to the dark phase (L:D) are given for horizontal activity (HA), total distance (TD), vertical activity (VA), stereotypic activity (SA) and number of stereotypic movements (NOS).
L = light; D = dark.

drug effect. Horizontal activity and stereotypic activity also remained significantly elevated from 30–120 min compared to total distance and vertical activity, which were indistinguishable from baseline during that time period. This was consistent with stereotyped activity occurring during absence of forward ambulation and rearing.

3.3. Different time of amphetamine administration

The dose–response relationship of the absolute change in AUC (4 h) at the four times of drug administration is displayed in Fig. 2 for the five motor indices studied. The effect of amphetamine on horizontal activity and stereotypic activity varied significantly by time of administration ($F(3, 78) = 6.01$; $F(3, 78) = 6.76$; respectively, $P < 0.01$) but there was no significant interaction between the effect of dose and time of its administration, indicating that the dose–response relationships remained the same throughout the day (Fig. 2A and B). Post-hoc analysis of both indices showed that the magnitude elicited by amphetamine was greater at 08.00 ($P < 0.01$) than at any other time and that

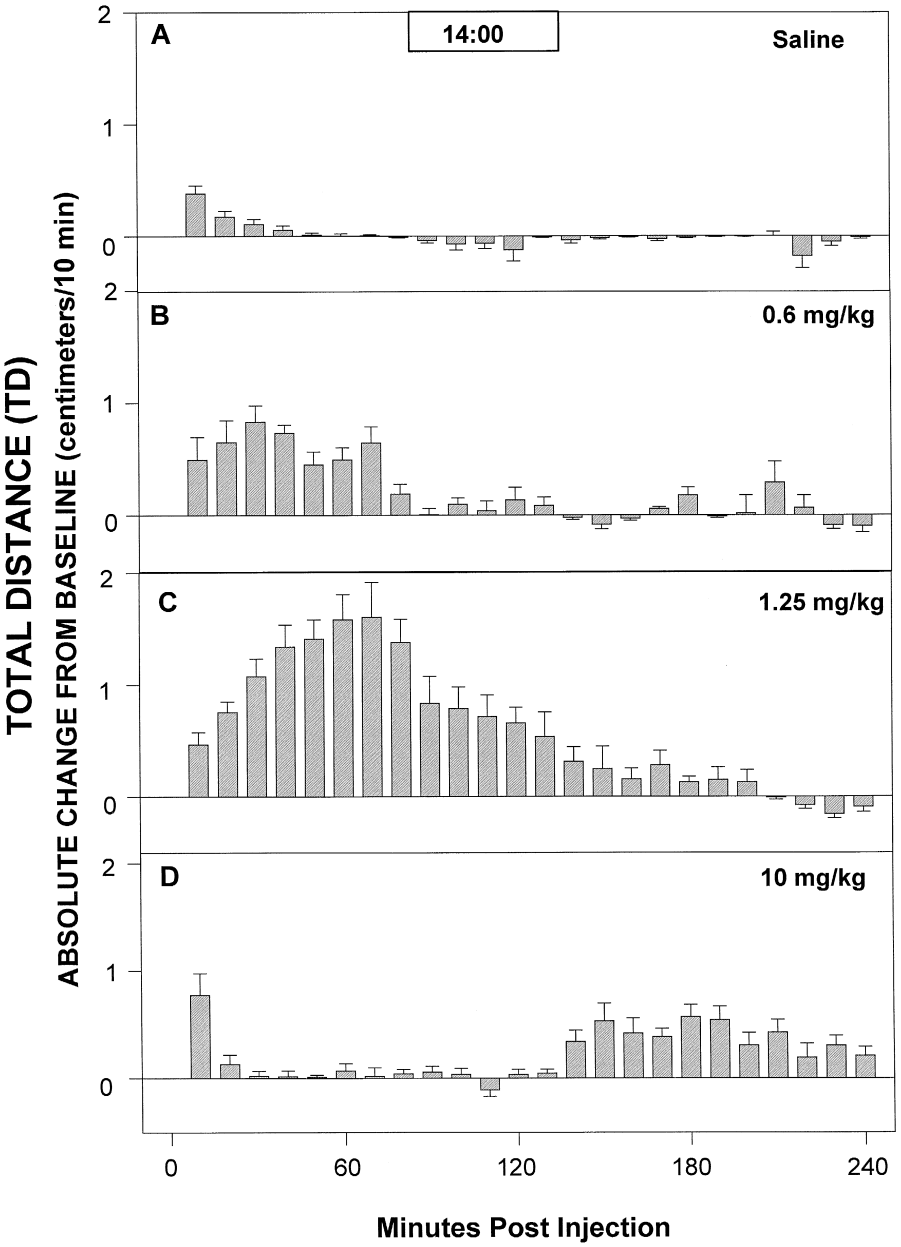

Fig. 1. Temporal response pattern for total distance caused by all three doses of amphetamine and saline given in the middle of the light cycle; 14.00 (each $n = 8$). Total distance is presented as the mean ± S.E.M./10 minutes of the average increase in activity of each rat on the day of treatment (day 3), relative to their own corresponding baseline values (days 1 and 2). Numerical values represent the original value divided by a factor of 1000.

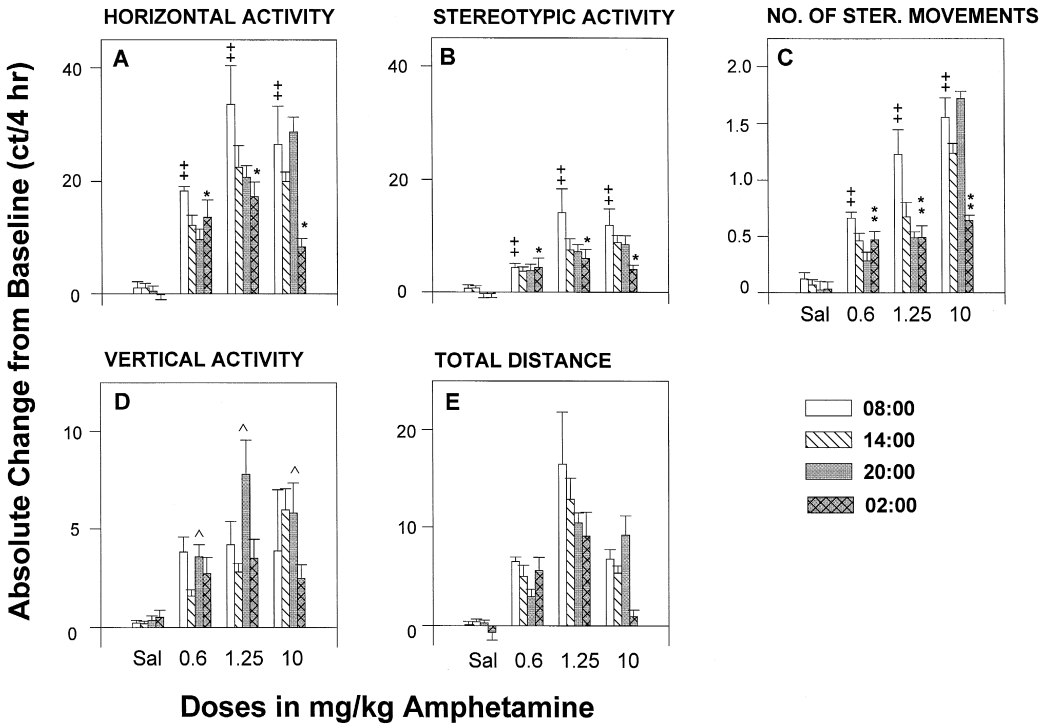

Fig. 2. The dose–response characteristics obtained following all four times of amphetamine administration (saline, 0.6, 1.25 and 10 mg/kg; each $n = 8$) relative to their own corresponding baseline values (average of days 1 and 2). Data are presented as the mean ± S.E.M. in counts/4 h for all five indices studied with baseline values arbitrarily set at 0. All symbols denote significant differences in main effect between times of administration as follows: $^{+}p < 0.05$; $^{++}p < 0.01$; $^{+++}p < 0.001$ for 08.00 versus all other times; $^{*}p < 0.01$ and $^{**}p < 0.01$ for 02.00 versus all other times and $^{\wedge}p < 0.05$ for 20.00 versus all other times. Numerical values represent the original value divided by a factor of 1000.

the effect of amphetamine at 02.00 was significantly lower than at any other time of administration ($P < 0.05$).

The dose-related effects of amphetamine on number of stereotypic movements also differed significantly between the times of administration ($F(3, 78) = 13.8$; $P < 0.0001$), but unlike horizontal activity and stereotypic activity, there was a significant interaction between the dose and the time of administration ($F(6, 78) = 6.49$; $P < 0.0001$; Fig. 2C). The effect of amphetamine was significantly greater at 08.00 ($P < 0.001$). The dose–response relationship become more asymptotic during the middle of the dark phase (Fig. 2C), with a significantly lower response at 02.00 ($P < 0.01$). The dose-related effects of amphetamine on the number of stereotypic movements differs between the dark and light phase.

The effect of amphetamine on vertical activity (Fig. 2D), differed between the times of administration ($F(3, 78) = 3.85$, $P < 0.05$), with no significant interaction between dose and time of administration (Fig. 2D). The magnitude of the effect was greater when amphetamine was injected at 20.00 ($P < 0.05$) than at any other time of administration. Unlike the rest of the indices, the dose–response for total distance did not vary significantly between the different times of administration. However, the effect of 10 mg/kg on total distance (Fig. 2E) shows that the effect of 10 mg/kg appeared lower at 02.00. In summary, the effects of amphetamine on all motor indices except total distance depended on the time of drug administration.

3.4. Temporal patterns of amphetamine's effects

The absolute changes from baseline in 10 min activity counts caused by each amphetamine dose were compared for all four times (08.00, 14.00, 20.00 and 02.00) of drug administration for each motor index and the temporal response patterns of indices that were clearly different between the times of administration are presented in Figs. 3 and 4.

Fig. 3 shows that, although the shape of the response pattern (i.e. time to maximum effect and duration of effect) is similar at all times of amphetamine administration, the amplitude of the effect on each motor index varied differently across the times of administration. For example, the increase in activity following amphetamine injection (1.25 mg/kg) at 08.00 was 60–70% greater than the other three times (14.00, 20.00 and 02.00) of administration (Fig. 3A). The effects of 1.25 mg/kg on horizontal activity and stereotypic activity (not shown) were similar to those of number of stereotypic movements (Fig. 3A). The increase in vertical activity elicited by 1.25 mg/kg amphetamine, however, was similar following injection at 08.00, 14.00 and 02.00, but the increase in vertical activity observed at

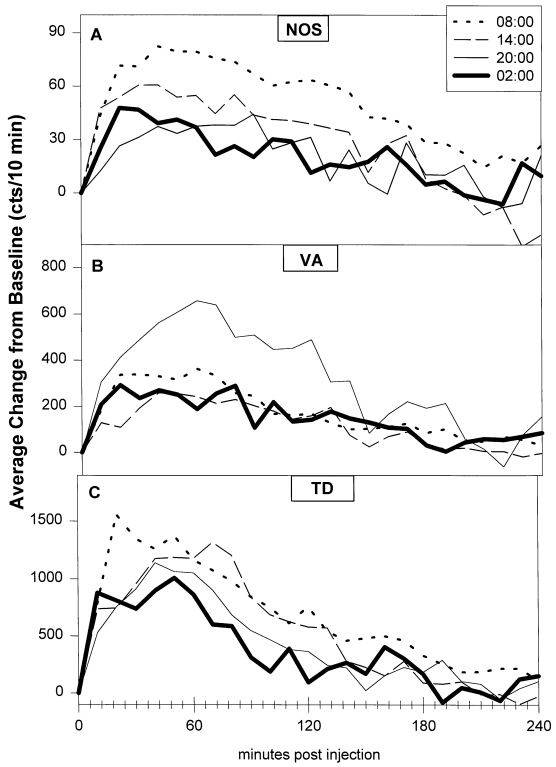

Fig. 3. Temporal response pattern following 1.25 mg/kg of amphetamine given at all four times of administration (each $n = 8$). (A) Number of stereotypic movements, (B) vertical activity and (C) total distance. The data is presented as the mean ± S.E.M./10 minutes of the average increase in activity of each rat on the day of treatment (day 3), relative to their own corresponding baseline values (days 1 and 2).

20.00 was 100–120% greater in amplitude from 30 to 130 min after injection compared to the other three times of administration (Fig. 3B). Finally, the temporal response pattern of total distance following 1.25 mg/kg was similar at all times of amphetamine administration (Fig. 3C). In summary, the effect of 1.25 mg/kg on forward ambulation did not vary throughout the day, but the stereotypic and rearing effects induced by 1.25 mg/kg amphetamine did.

The clearest difference in amphetamine's effect throughout the day was the diminished effect of 10 mg/kg at 02.00 on all five motor indices studied (Fig. 2). The temporal response patterns exhibited by number of stereotypic movements, vertical activity and total distance after administration of 10 mg/kg at 14.00 and at 02.00 are compared in Fig. 4. Response to this amphetamine dose at 08.00 and 20.00 were similar to the response at 14.00, therefore, representative data from 14.00 was compared to the effect during the middle of the dark phase; 02.00 (Fig. 4). The pattern for number of stereotypic movements was similar at all times of administration (10 mg/kg) with an immediate increase after injection that remained elevated with only slight fluctuations throughout the drug effect (Fig. 4, top panel). However, for 10 mg/kg at 02.00, the effect at each 10 min interval is diminished compared to the other three times of drug administration throughout the entire duration of the drug effect (Fig. 4, top panel). By contrast, the vertical activity pattern elicited by 10 mg/kg amphetamine given at 02.00 displayed a multiphasic response pattern, characterized by early and late phases of hyperactivity interrupted by a focused stereotypy phase where no vertical activity occurred (Fig. 4, middle panel) but stereotyped behavior still did (Fig. 4, top panel). However, the focused stereotypy phase appears to last 100 min compared to 80 min (i.e. 20 min longer) when amphetamine was injected at 02.00 compared to the other times (08.00, 14.00 and 20.00). The late phase increase in vertical activity (150–240 min) following 10 mg/kg at 02.00 was slightly diminished compared to the late phase increase after amphetamine injection at 14.00. The effect of 10 mg/kg on total distance initially followed the same multiphasic response pattern when given at 02.00, as it does at the other times of administration, but, after 120

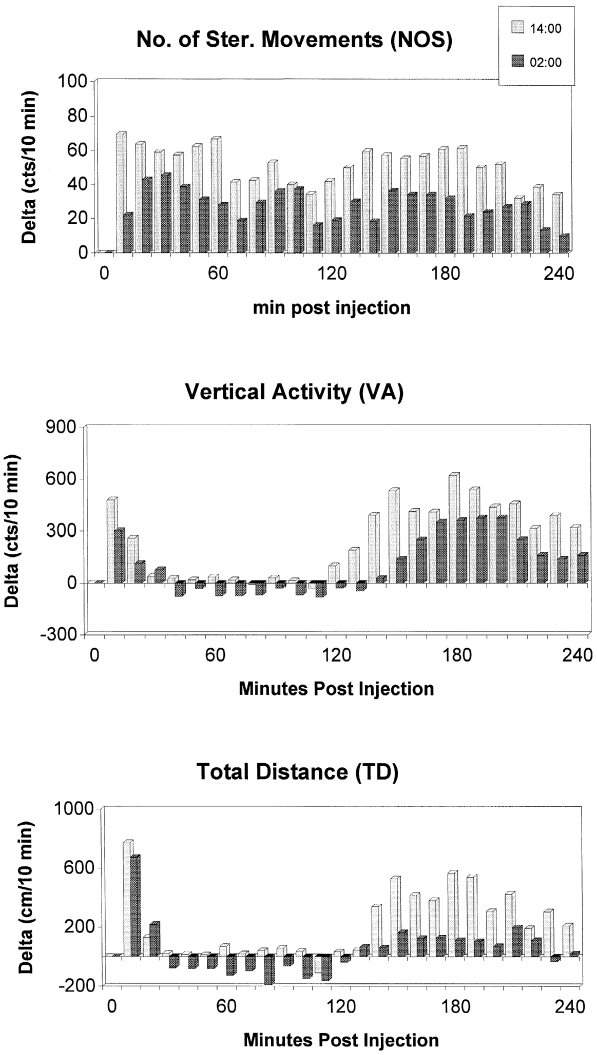

Fig. 4. Temporal response pattern for 10 mg/kg of amphetamine given 14.00 and 02.00 (each $n = 8$). (A) Number of stereotypic movements, (B) vertical activity and C) total distance. The data is presented as the mean ± S.E.M./10 min of the delta, which is the average increase in activity of each rat on the day of treatment (day 3), relative to their own corresponding baseline values (days 1 and 2).

min post-injection there is a complete loss of the second phase of increased activity at 02.00 (Fig. 4, bottom panel). Therefore, the temporal response pattern is completely different for total distance during the middle of the dark phase compared to all other times of administration.

In summary, both the quantitative and qualitative changes show that amphetamine's effects on stereotypic behavior are time dependent, but its effects on locomotor activity are not.

3.5. Long-lasting effects of single amphetamine injection

Comparisons between light and dark phase activity during baseline (days 1 and 2 averaged) and post-treatment (day 4; i.e. the data gathered 23 to 48 h post injection) were analyzed for changes in activity levels of all treatment groups. The administration of 0.6 and 1.25 mg/kg amphetamine had no effect on the activity levels on day 4 for any of the motor indices regardless of amphetamine dose or time of administration. However, administration of 10 mg/kg produced changes in the levels of total distance on day 4, as shown in Fig. 5. Total distance traveled in the post-treatment dark phase of day 4 was significantly decreased following administration of 10 mg/kg at 08.00 ($F(1, 3) = 36.28$, $P < 0.001$) and 14.00 ($F(1, 3) = 17.43$, $P < 0.003$), but activity during the light phase of day 4 remained unaffected. This depressive effect on nocturnal

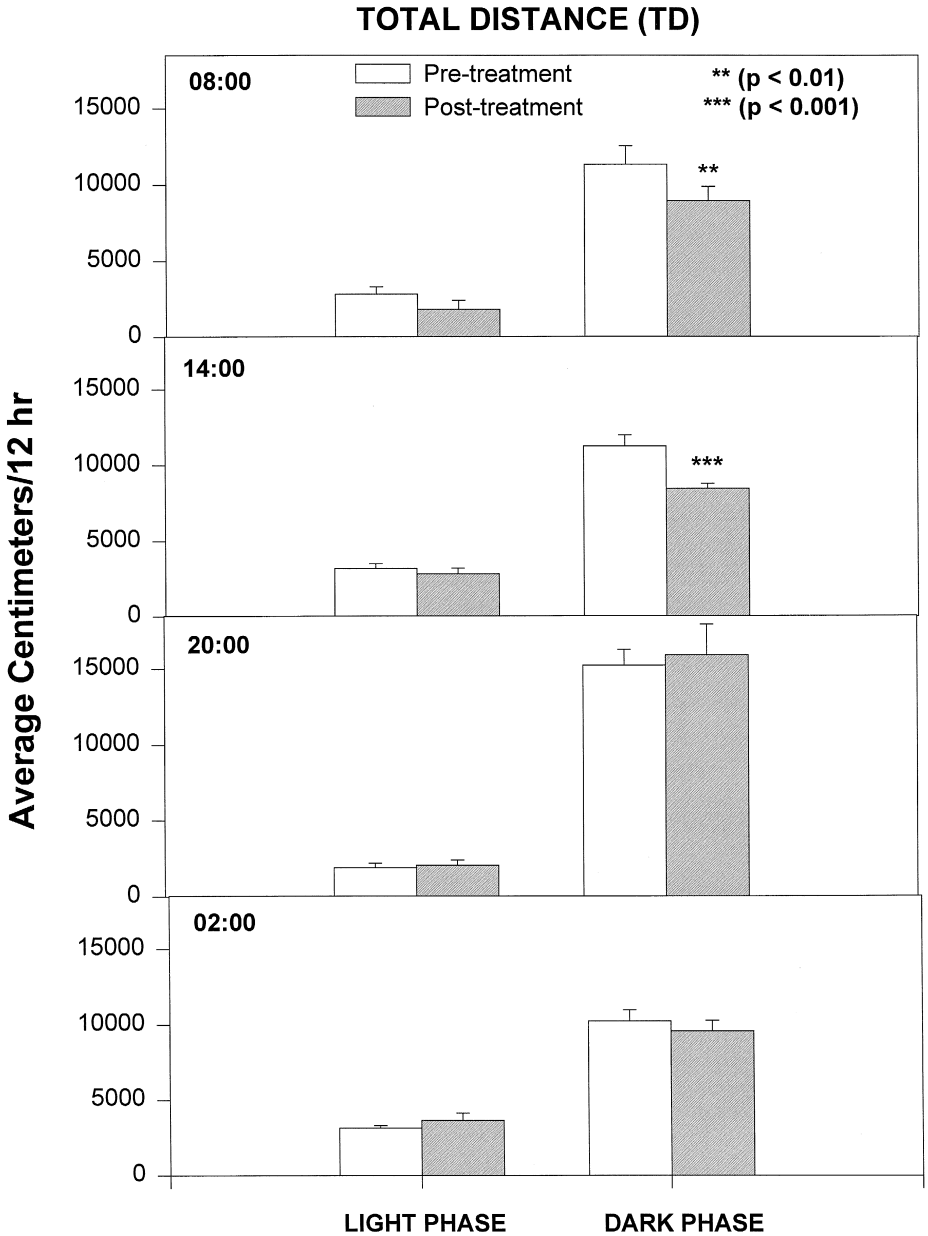

Fig. 5. The total distance in centimeters traveled in the light and dark phase (12 h each) for pre-treatment (days 1 and 2 averaged into one baseline value) and the post treatment (day 4) following administration of 10 mg/kg amphetamine at all four times of administration. Significance of effect was determined using repeated measures ANOVA.

forward ambulation, however, was not observed after administration of 10 mg/kg during the dark phase (20.00 and 02.00; Fig. 5, bottom two panels) and it was only observed for total distance and not the other motor indices. In summary, only the largest dose of amphetamine studied had any long-lasting effects, by causing a decrease in total distance during the subsequent dark phase, which was dependent on the time of drug administration.

4. Discussion

Most studies investigating the effects of stimulants on motor behavior in rats have been conducted during the light phase (i.e. the sleeping period) of the nocturnally active rat. The objective of this study was to determine whether there is a difference in the dose–response characteristics following amphetamine administration during the rat's active period (dark phase) compared to the rest period (light phase). The only previous comparison of amphetamine effects in the light and dark phase used continuous, rather than single or multiple administration, and found that tolerance to amphetamine's motor effects occurred during the light phase, but not during the dark phase (Martin-Iverson and Iversen, 1989). The main findings in the present study are that (1) the stereotypic effect of amphetamine is dependent on the injection time (i.e. time dependent); (2) the ability of a large dose of amphetamine to produce subsequent depression of nocturnal forward ambulation is also time dependent and (3) despite the great difference in baseline activity between the light and dark phase, the locomotor effect of amphetamine was generally similar at each time of its administration.

The large differences in baseline activity levels between the light and dark phase necessitated the use of a protocol that could minimize the variability reported in previous studies. Therefore, to account for the baseline variability this study utilized rats which were housed in the experimental room for seven days prior to experimentation to allow for synchronization to the light/dark cycle at our facility. Moreover, 2 days (48 h) of baseline activity were recorded so each rat could serve as its own control, and treatment effect on day 3 could then be compared to a time-matched average baseline for the same animal, allowing for correction of circadian differences in activity levels prior to drug administration, as well as individual differences in activity levels between rats. Moreover, a computerized monitoring system was used to circumvent limitations of direct human observation (Ellinwood and Balster, 1974; Robbins, 1977; Fray et al., 1980; Rebec and Bashore, 1984; Donat, 1991). Five different motor indices were utilized, since the effects of stimulants on motor behavior are complex and the use of one dimensional index, such as latch-counts, or horizontal activity, etc., might conceal the presence of distinct drug effects (Dougherty et al., 1990; Donat, 1991; Paulus and Geyer, 1993). The amphetamine doses used in this study were chosen based on our previous dose–response characteristics studies of the motor and stereotypic effects of amphetamine (Gaytan et al., 1996b).

The dose–response characteristics of amphetamine injected at 14.00 was consistent with previous reports of amphetamine administration during the light cycle (Robinson and Becker, 1986; Kalivas and Weber, 1988; Kuczenski and Segal, 1988; Kalivas and Stewart, 1991). Despite large differences in neurotransmitter concentrations, receptor density, and motor activity levels between the light and dark phase, the lower doses of amphetamine (0.6 and 1.25 mg/kg) showed no change in their effect on total distance (forward ambulation) throughout the day. The dose-related effects of amphetamine on rearing, which is affected by both locomotor and stereotypic responses, were also similar throughout the day (i.e. no interaction between dose and time of administration), but the magnitude of amphetamine's elicited effect on vertical activity was time dependent, with its greater amplitude in response occurring unexpectedly at the beginning of the dark phase when baseline activity is highest (20.00).

Comparison of the 4 h AUC and the temporal response patterns following all amphetamine doses revealed differences in the stereotypic response between the light and dark phase. Different effects of amphetamine across the four times of administration occurred largely in the indices that assessed stereotyped behavior (i.e. horizontal activity, stereotypic activity and number of stereotypic movements; Fig. 2A, B and C). A distinct circadian rhythmicity was observed in the 4 h AUC of these three indices, especially for number of stereotypic movements. The stereotypic effect was greatest at 08.00 and smallest at 02.00. The decreased stereotypic effect at 02.00 was clearly due to the diminished effect of 10 mg/kg injected at this time. The temporal response pattern of number of stereotypic movements to 10 mg/kg given at 02.00 was similar to the response at the other three times, but the amplitude of the increase caused by this dose was diminished throughout the duration of the drug effect (Fig. 4A). Moreover, although 10 mg/kg elicited a focused stereotypy phase on both total distance and vertical activity when injected at 02.00 (Fig. 4B and C), the second phase of hyperactivity that was present at all other times of administration was diminished in magnitude for vertical activity and, more importantly, there was a complete loss of the expected second phase of hyperactivity for total distance (Fig. 4C). The ability to dissociate the different phases of the temporal response pattern characteristically found at large doses of stimulants has been shown in studies of chronic administration of stimulants (Segal and Kuczenski, 1987; Stewart and Vezina, 1991), but not for single amphetamine doses.

A greater increase in the stereotypic response was observed following 1.25 mg/kg amphetamine at 08.00, which was most apparent for number of stereotypic movements and to a lesser degree for horizontal activity, and stereotypic activity (Fig. 2C). However, this increase in the

response amplitude was not seen for vertical activity or total distance (Fig. 3B and C). It is possible that the stereotypic effect of amphetamine is not necessarily increased at 08.00 and decreased at 02.00, but that amphetamine may elicit different stereotyped response patterns at these two times that may account for the loss of the second phase of hyperactivity for total distance following 10 mg/kg at 02.00 and the increased response amplitude of 1.25 mg/kg amphetamine at 08.00. The results obtained in this study suggest that there are differences in the stereotypic response throughout the day, with the most significant difference between the four times of administration occurring during the middle of the dark phase (02.00). However, we recognize that stereotypic behavior is not readily measured by automated systems and qualitative descriptive techniques will be necessary to completely characterize any differences, if present, in the stereotypic response throughout the day.

The experimental protocol used indicates that amphetamine's effect on forward ambulation, rearing, and general stereotyped behavior, can be separated based on their susceptibility to changes in the time of drug administration. A possible explanation for this difference is based on lesion experiments, which have shown that the stereotypic effects of stimulants are associated with substantia nigra and the striatum, while locomotor effects are associated with the nucleus accumbens (Creese and Iversen, 1974; Kehne et al., 1981; Kelly and Iversen, 1975; Kelly et al., 1975; Kelly, 1977). Moreover, Paulson and Robinson (1994) reported that the concentration of dopamine and its metabolites increased significantly during the dark cycle in the striatum, but that dopamine levels in the nucleus accumbens did not significantly change throughout the day. Therefore, changes in the stereotypic and rearing effects of amphetamine may be related to the diurnal variations in dopamine levels in the striatum, while the consistent response of forward ambulation may reflect the lack of change in dopamine levels in the nucleus accumbens.

Another possible explanation for these results may arise from the observation that the increases in baseline levels of activity between the dark and light phases were greater for forward locomotion and rearing (i.e. total distance and vertical activity) than for the motor indices measuring general stereotyped behavior (i.e. stereotypic activity and number of stereotypic movements; Table 1). Therefore, the relative contribution of locomotor behavior to spontaneous motor activity (horizontal activity) is greater during the dark than during the light phase and may create a ceiling effect, where the higher amphetamine doses are unable to increase forward ambulation any further. However, the increase in total distance following 1.25 mg/kg of amphetamine given at 20.00, when the baseline activity was at its highest level, was similar in magnitude and pattern to administration of the same amphetamine dose during the light cycle, when baseline activity is at its lowest level. Amphetamine administration at 20.00 also elicited increases in total distance that reached higher levels than would normally occur during the dark phase in an untreated rat. Furthermore, the largest increase in rearing caused by amphetamine occurred when the drug was administered during the beginning of the dark phase (20.00). A ceiling effect caused by the differences in baseline levels of activity cannot, therefore, explain why the locomotor effect of amphetamine is generally the same at each time of administration.

Finally, the ability of 10 mg/kg amphetamine to reduce the total distance traveled during the dark phase of day 4 was dependent on the time of amphetamine administration, and only occurred with drug administration during the light phase (Fig. 5). One could postulate that post-stimulant depression in dark phase activity subsequent to repeated administration of amphetamine during the light phase (Segal and Mandell, 1974; Paulson et al., 1991; Segal and Kuczenski, 1994), may be less likely to occur with repetitive administration of amphetamine during the dark phase.

In summary, this study revealed that the effects of a high dose of amphetamine on general stereotypic behavior, as well as the ability to cause nocturnal depression of forward ambulation, were dependent on the time of drug administration. However, the locomotor effects elicited by lower doses of amphetamine were generally similar throughout the four times of administration. Whether these differences in acute effect of amphetamine throughout the day will lead to differences in the development of sensitization following repeated administration of this drug, or other adaptations to stimulant treatment, remains to be determined.

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