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Effects of amphetamine at the beginning of the light cycle on multiple indices of motor activity in the rat

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Abstract

The motor effects of a single dose of *d*-amphetamine on internally synchronized male Sprague Dawley rats and its dose response relationship at the beginning of the light cycle was investigated using a computerized monitoring system. After 7 days of acclimatization to light/dark cycle and 2 days of baseline monitoring, rats were randomized to a no-treatment time control group ($n = 12$) or to receive 0 (vehicle), 0.6, 1.25, 2.5, or 10 mg/kg *d*-amphetamine ($n = 8$ each) 1 h into the light cycle of day 3, and monitored for an additional post-treatment day 4. In the time control group, there was a stable baseline level of activity for both light and dark phases. All doses (0.6, 1.25, 2.5, and 10 mg/kg) significantly elevated ($P < 0.01$) locomotor activity compared to baseline, but not all activity parameters (horizontal activity, total distance, vertical activity, stereotypic activity, and number of stereotypic movements) followed the same pattern of response. The maximum increase in all parameters, except vertical activity, occurred at 1.25 mg/kg ($P < 0.001$). The duration of drug effect increased with dose, with increased activity lasting until the fifth hour after injection of 10 mg/kg. ANOVA revealed no consistent long term effects, with all parameters returning to baseline levels on the day after treatment. The range of variables and the establishment of baseline values at the time of injection for each rat provides the potential to characterize circadian patterns of locomotor activity and chronopharmacologic effects of drugs on motor activity, including sensitization and tolerance.

Keywords: *d*-Amphetamine; Dose response; Psychomotor stimulant; Motor activity; Behavior

1. Introduction

The stimulatory and behavioral effects of the psychomotor stimulant amphetamine were reported as early as 1932 (Downs and Eddy, 1932). Response to amphetamine in the rat is dose related. Low doses increase overall locomotor activity, such as forward ambulation, spontaneous movements, rearing, and intermittent sniffing. Larger doses induce a multiphasic behavioral response pattern characterized by an early rise in locomotor activity that is interrupted by a period of focused stereotyped movements expressed by head bobbing, licking, continual sniffing, and licking the cage floor, and followed by a late phase of increased locomotion (Ernst and Smelik, 1966; Kolta et al., 1985; Randrup and Munkvad, 1975; Schiorring, 1971; Segal and Mandell, 1974). Repeated administration of stimulants can result in behavioral sensitization, with an enhanced response to the locomotor effects, and some, but not all, of the stereotypic effects during subsequent exposure (Eichler et al., 1980; for reviews see Kalivas et al., 1993; Robinson and Becker, 1986). The conditions producing sensitization have varied among laboratories. These inconsistencies in producing similar results may be due in part to the fact that, until recently, most studies have assessed the effects of amphetamine using behavioral observation rating scales, which concentrated mainly on qualitative measurements by human observers of stereotypic behavior and exploratory activity over only a short period of time after treatment (Ellinwood and Balster, 1974; Kolta et al., 1985; Naylor and Costall, 1971). Additionally, very little attention has been given to the time of injection throughout the light phase (i.e., the circadian timing of drug administration), with most investigators working under the presumption that response to stimulants is the same throughout the day. The resultant wide variation regarding the time at which a drug is administered may contribute to the variability in effects of acute and chronic administration of stimulants. Circadian fluctuations in neurotrans-

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mitter levels such as dopamine, as well as in α,β-adrenoceptors and dopamine receptors in the rat brain have been reported (Kafka et al., 1981, Kafka et al., 1985; Lemmer et al., 1985; Bruinink et al., 1983). These fluctuations in the pool of neurotransmitters by which amphetamine exerts its effects may cause differences in rat's motor response to the drug throughout the day.

Investigation of the motor effects of *d*-amphetamine under conditions designed to minimize factors that could lead to variability between studies was conducted. Rats were allowed to internally synchronize their neuroendocrine system to the light/dark cycle (12:12 h) in the test room for 7 days before conducting a comprehensive investigation of the dose-response relationship for motor activity immediately and for 24 h after a single injection of amphetamine. All rats received their injections at the same time of the light cycle. A computerized animal activity monitoring system (Dougherty et al., 1990), was used to measure over 22 different indices of locomotor activity continuously in the rats' home cages. Our initial studies focused on: (1) establishment of conditions for a stable hourly and daily baseline of locomotor activity parameters; (2) the dose-response relationships for the activity parameters at the beginning of the light cycle; (3) whether the integration of more than one variable can yield a more detailed analysis of the rats' locomotor response to amphetamine that is consistent with direct observation, and can be readily compared between and within laboratories; and (4) persistent effects on locomotor activity after a single administration of amphetamine.

2. Materials and methods

Male Sprague Dawley rats ($n = 52$) weighing 150–170 g were housed in the experiment room in groups of four at an ambient temperature of 21 ± 1°C and relative humidity of 37–42%. Animals were maintained on a 12:12 h light/dark schedule (light on at 07:00 h) for a minimum of 7 days prior to housing; food pellets and water were supplied ad libitum. On the last day of acclimatizaton to the light/dark cycle all rats were weighed and individually housed in Omnitec Digiscan RXYZM (16) DVA computerized animal activity monitoring (CAAM) system cages with ad libitum food and water, and allowed at least 12 h of accommodation to the test cages before recording began. Rats were then monitored continuously throughout the 24 h cycle for 4 days. Data were obtained in 1 h samples and downloaded for later evaluation. Data on pre-treatment days 1–2 were collected to obtain a control measure of baseline activity for each rat.

On day 3, each rat was weighed and randomly assigned to one of the following groups. The time control group ($n = 12$) was not handled during eight experimental days. Five groups of eight rats each received s.c. injections (0.8 ml) of 0.9% saline containing 0, 0.6, 1.25, 2.5, or 10 mg/kg of *d*-amphetamine 1 h into the light cycle (08:00 h). Data acquisition was resumed immediately after injection for at least 24 more hours (day 3–4). Horizontal activity, total distance, vertical activity, stereotypic activity, and number of stereotypic movements were used in the analysis.

2.1. Drugs

d-Amphetamine sulfate (Sigma Chemical Co.) was mixed with 0.9% saline and all drug weights were based on the salt.

2.2. Apparatus

The CAAM system has been described in detail (Dougherty et al., 1990). In short, the activity chambers consist of clear acrylic open field boxes (40.5 × 40.5 × 31.5 cm) with two 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 is recorded as an activity score. Interruptions of two or more consecutive beams separated by at least 1 s without beam interruptions was recorded as a movement score. Repeated interruptions of the same beam(s) from any of the two sensor arrays were measured as stereotypic activity; and different episodes of stereotypic activity with at least a 1 s interval before the beginning of another episode was recorded as number of stereotypic movements. Data were downloaded into OASIS data collection program and organized into 22 different locomotor parameters. Due to the homogeneous response of the numerous parameters, only the following five representative parameters were chosen to characterize the different effects of drug administration. The first level of sensors record the overall horizontal motor behavior; the specific parameters analyzed from this sensor were horizontal activity and total distance. The second level of sensors monitor the vertical motor activity behavior, i.e., sitting and rearing, and the specific parameter monitored and analyzed was vertical activity. In addition, the stereotyped behavior parameters mentioned previously (i.e., stereotypic activity and number of stereotypic movements) were also analyzed.

2.3. Data analysis

The five locomotor parameters were analyzed with respect to acute (hourly), or long term (24 h) effects of *d*-amphetamine. The acute effect was tested for significant change of hourly activity after injection from each rat's own averaged baseline hourly activity (days 1–2) at the same time of the day by the paired *t*-test. The intermediate effect was checked for increases in the area under the time

curve for averaged pre-treatment cycle (days 1–2) vs. treatment cycle (day of treatment). To determine the long term effects of *d*-amphetamine one way ANOVA of pre-treatment, treatment, and post-treatment dark and light cycles were conducted on all treatment groups. One way ANOVA was also conducted on the time control group.

3. Results

3.1. Time control

Five locomotor parameters (horizontal activity, total distance, vertical activity, stereotypic activity, and number of stereotypic movements) were recorded and analyzed for 8 days and are summarized in Table 1. In all parameters there was a definite circadian pattern of activity with clear difference in activity levels between the inactive (light phase) and active periods (dark phase). Fig. 1 shows the pattern of hourly, light/dark phase, and 24 h counts of horizontal activity. The average horizontal activity count per hour during the light phase was 1160 ± 48 (S.E.) counts, with over a 3-fold increase in horizontal activity during the active period (dark phase) and an average of 3829 ± 107 counts per hour. The average total distance traveled per hour by each rat during the light phase was 188 ± 10 cm, with over a 7-fold increase in total distance traveled during the dark phase, to 1404 ± 52 cm per hour. There was a 10-fold increase in average vertical activity count per hour between the inactive and the active period (44.8 ± 5.78 counts to 471.8 ± 17.9 counts). The average stereotypic activity during the light phase was 593 ± 47 counts, and there was over a 3-fold increase in activity in the dark phase count of 1837 ± 82. All five parameters displayed stable patterns whether averaged over the light phase, dark phase, or the entire 24 h (Table 1). In summary, the uninjected time control group displayed a stable daily baseline of activity in all the parameters sampled for the length of the study (Fig. 1).

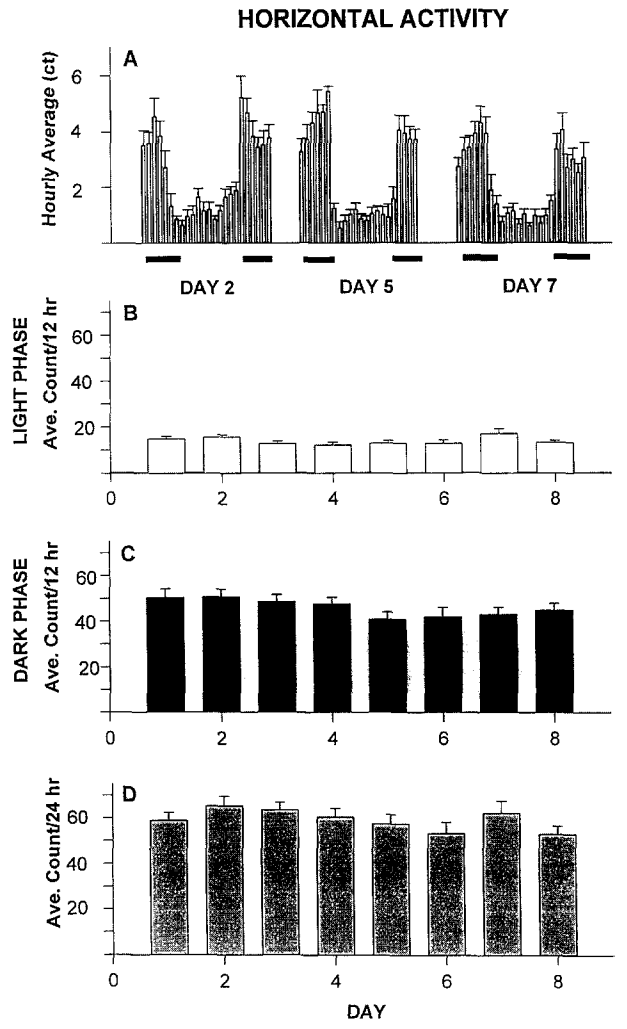

Fig. 1. Horizontal activity counts (means ± S.E.M.) for the untreated time control group ($n = 12$) are displayed for the following: (A) the average hourly count for days 2, 5, and 7, organized as 6 dark cycle hours (black bars indicate lights off), 12 light cycle hours, and the first 6 h of the next dark cycle; thereby creating a clear circadian pattern of activity. (B) The average hourly count during the 12 h light cycle of days 1–8. (C) The average hourly count per rat during the 12 h dark cycle of days 1–8. (E) The average daily (24 h) count per rat for days 1–8. One way ANOVA revealed no significant difference between days.

Table 1
Data for the time control Group A ($n = 12$) is presented as the means ± S.E. for the total daily counts, the light and dark phase total counts, and the ratio of the light to dark activity for the following parameters: horizontal activity (HA), total distance (TD), vertical activity (VA), stereotypic activity (SA), and number of stereotypic movements (NOS)

Parameter	Daily total (24 h)	Light phase (12 h total)	Dark phase (12 h total)	Ratio L:D
HA	59245 ± 151 cts	13918 ± 572 cts	45943 ± 1284 cts	1:3.3
TD	18621 ± 624 cm	2254 ± 124 cm	16965 ± 621 cm	1:7.5
VA	5879 ± 330 cts	538 ± 69 cts	5661 ± 215 cts	1:10
SA	29894 ± 1222 cts	7113 ± 566 cts	23245 ± 991 cts	1:3.3
NOS	3411 ± 318 cts	886 ± 85 cts	2525 ± 256 cts	1:2.8

Values are given as means ± S.E.M.

3.2. Saline control

Immediately after saline injection, animals were observed to move around the cage for several minutes before returning to their pre-injection level of activity. When the hourly samples were analyzed for the five locomotor activity parameters and compared to their own baseline values, rats injected s.c. with saline displayed no significant alteration. That is to say, there was no significant effect of handling, insertion of the needle, and the volume of the injection for the light cycle or total daily activity counts, and the saline control was, therefore, indistinguishable from the time control.

3.3. Dose response

Each experimental group had 2 days of recording, before administration of drug, to determine baseline levels of activity. Therefore, each rat could serve as its own control. The increase in motor activity following drug treatment was the difference between the post injection treated values on day 3 of each rat and its own corresponding hourly baseline values on days 1 and 2. The baseline activity levels of treatment groups (days 1 and 2) were comparable to those in the time control group (Table 1) for all parameters studied. Therefore, any changes in activity can be compared to values presented for the time control group to obtain an idea of proportionate rise in activity at the time of administration. Fig. 2 shows the time course of motor activity after injection of *d*-amphetamine or saline. Comparison of all five locomotor activity parameters to their own baseline revealed that all doses (0.6, 1.25, 2.5 and 10 mg/kg) significantly increased motor activity, as determined by the paired *t*-test. The absolute change over baseline (delta) in horizontal activity after administration of the lowest dose of amphetamine, 0.6 mg/kg, reached its maximum effect in the first hour post injection (PI) with a delta of 8229 counts (Fig. 2A; $P < 0.01$), and returned to baseline levels by 3 h. The 1.25 mg/kg dose exhibited the largest increase in response to amphetamine of 21 576 counts in the first hour ($P < 0.001$), dropping by 50% ($P < 0.001$) in the second hour, and finally returning to baseline levels by 4 h post injection. At 2.5 mg/kg, horizontal activity reached its maximum increase of 13 363 counts ($P < 0.001$) in the first hour as at lower doses, and remained significantly elevated ($P < 0.001$) in the second hour. The increase in activity 3 h after injection ($P < 0.01$) was still approximately half of the first hour increase, and returned to baseline by the fourth hour. After 10 mg/kg, horizontal activity was increased significantly ($P < 0.05$) for the first 2 h, but the peak activity was delayed compared to the previous three dosages. Horizontal activity did not reach its maximum increase until 3 h post injection (8682 counts; $P < 0.001$), and did not return to baseline activity until 5 h post injection (see Fig. 2A). Stereotypic activity and number of stereotypic movements exhibited similar dose response characteristics (see Fig. 2B–E). With vertical activity, however, the largest increase was induced with the 2.5 mg/kg dose rather than 1.25 mg/kg as in all other parameters. The increase in motor activity was delayed in rats given 10 mg/kg, with the total distance in the first hour slightly decreased (−159 cm), followed by an increase in activity that reached its maximum in the third hour.

3.4. Intermediate effect

The area under the activity time curves (AUC) were calculated for: (a) the pre-treatment periods (light phase of

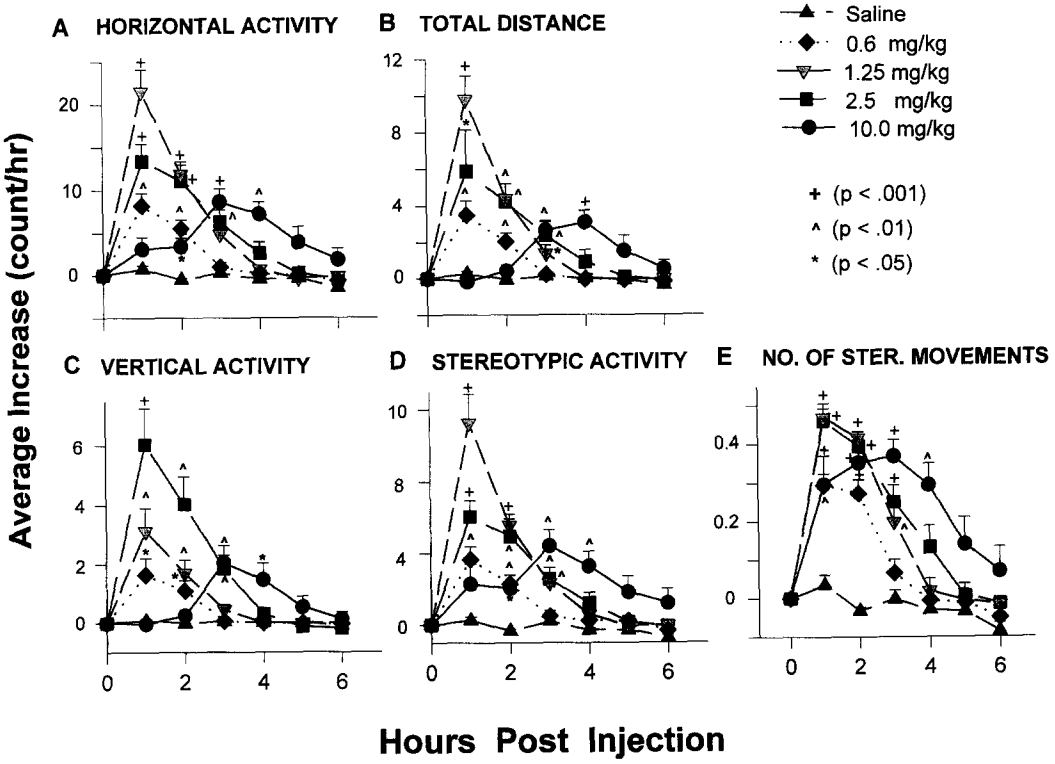

Fig. 2. Time course of the response to single s.c. administration of saline ($n = 8$) and amphetamine (0.6, 1.25, 2.5, 10 mg/kg; each $n = 8$) 1 h into the light cycle; i.e. at 08:00. Data are presented as the means ± S.E.M. of the average increase in activity of each rat on the day of treatment (day 3), relative to their own corresponding baseline values (days 1 and 2) at the same hour of the light cycle. All five parameters are presented above for 6 h after administration. *Numerical values represent the value × a factor of 1000.*

days 1 and 2) and averaged into a baseline value, and (b) the treatment period (light phase) using the trapezoidal rule. Change in AUC compared to the baseline activity time curve is represented as the absolute change (delta) in AUC associated with treatment.

Paired *t*-test of the treatment cycle compared to its own baseline revealed that saline injection had no effect, but that each dose of *d*-amphetamine significantly increased ($P < 0.05$–0.001) the AUC for all five parameters during the treatment cycle (Fig. 3). The horizontal activity AUC of the treatment cycle was increased by $13\,426 \pm 4006$ counts ($P < 0.05$) at 0.6 mg/kg, by $35\,944 \pm 6039$ counts at 1.25 mg/kg, and remained at the same level for the higher doses (Fig. 3A). Total distance and stereotypic activity displayed a pattern similar to that with horizontal activity. The increase in vertical activity, however, peaked with the administration of 2.5 mg/kg ($P < 0.001$) while the 1.25 and 10 mg/kg doses increased vertical activity to only 50% of the 2.5 mg/kg dose (Fig. 3C). The number of stereotypic movements had a more linear dose-response effect with the largest increase after administration of 10 mg/kg.

3.5. Long term effects

The pre-treatment (days 1 and 2), treatment (day 3; 1–23 h post injection), and post-treatment light and dark periods (day 4; 24–36 h post injection) were compared using one way ANOVA. This analysis revealed that only the two highest doses (2.5 and 10 mg/kg) caused significant elevation ($F = 5.3$, $P < 0.01$; $F = 15.2$, $P < 0.001$, respectively) during the treatment light cycle. There was no significant difference in horizontal activity for the dark period (11–23 h after injection) that followed the treatment light period. The post-treatment horizontal activity levels for the light, dark, and daily periods were not significantly altered from those before treatment. All other parameters displayed similar results, except for total distance. The total distance traveled by the 2.5 mg/kg group in the dark cycle of both the day of injection and the day after injection was significantly decreased ($F = 8.54$, $P < 0.01$; $F = 8.04$, $P < 0.01$, respectively; Fig. 4). The total distance traveled by the 10 mg/kg group was also significantly decreased for both the light cycle and the dark cycle on the day after injection ($F = 8.54$, $P < 0.01$; $F = 4.8$,

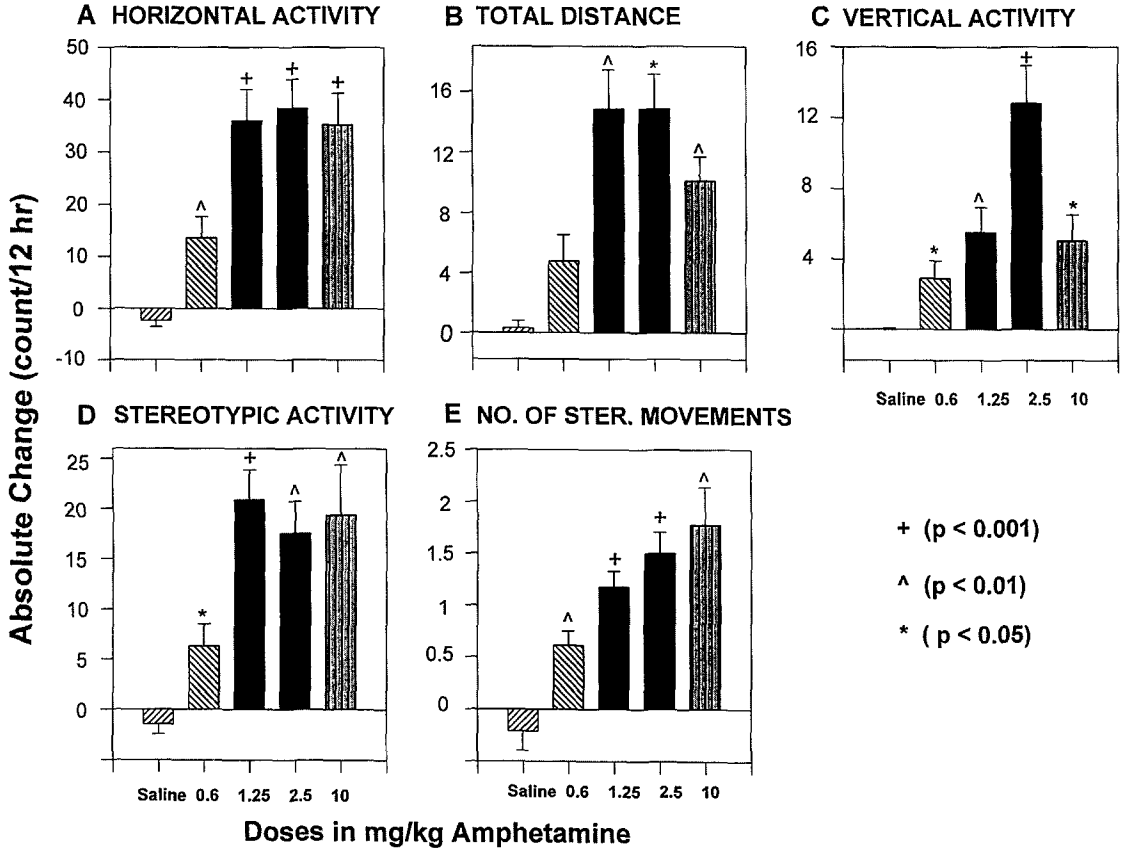

Fig. 3. Average changes in the area under the activity time curve (AUC) after s.c. administration of saline ($n = 8$) and amphetamine (0.6, 1.25, 2.5, 10 mg/kg; each $n = 8$). Data are presented as the means ± S.E.M of the absolute change (delta) in AUC caused by treatment for all five parameters studied, with baseline values arbitrarily set at 0. Statistical significance was determined using the paired *t*-test analysis. *Numerical values represent the value × a factor of 1000.*

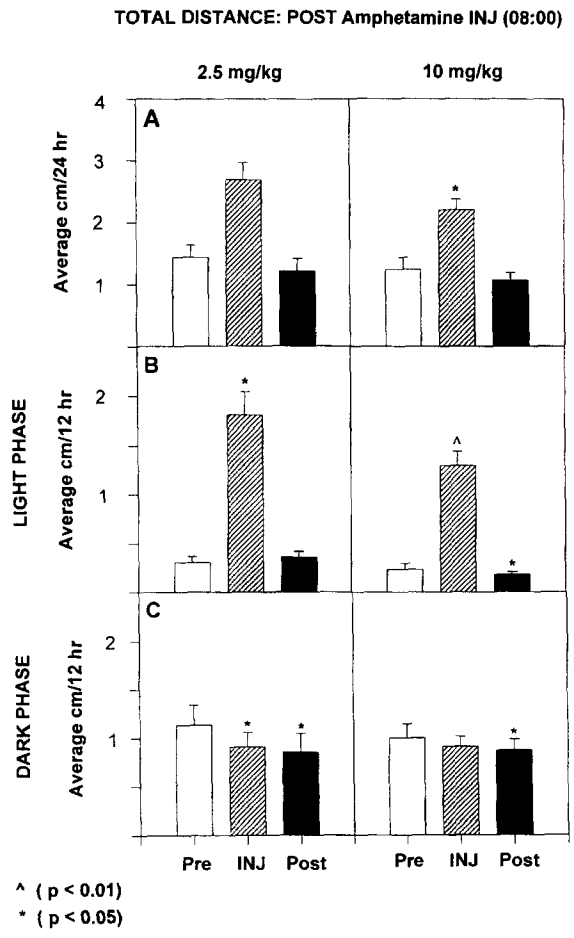

Fig. 4. The total distance in centimeters traveled by each rat for (A) daily (24 h), (B) light phase, and (C) dark phase activity values for the pre-treatment (Pre) cycles (days 1 and 2 averaged into one baseline value); the treatment cycle (INJ), i.e., day 3; and the post treatment (Post) cycle, i.e. day 4. Significance of effect was determined using one way ANOVA. *Numerical values represent the value × a factor of 1000.*

$P < 0.05$, respectively). All other parameters returned to baseline activity levels on the day after treatment.

4. Discussion

The behavioral observation rating scales used in most previous studies of amphetamine have disadvantages including inconsistent definitions, inter and intra observer reliability, fatigue, and loss of concentration by the human observers (Fray et al., 1980; Rebec and Bashore, 1984; Robbins, 1977). The quantification of activity in a form that can be more reliably compared between laboratories is a difficult task for a human observer, and human fatigue limits the amount of time after injection that the drug effects can be assessed. With computerized automatic instruments investigators can collect more complex measurements of motor behavior for longer periods (Donat, 1991; Dougherty et al., 1990). More recent studies have begun to concurrently use automated devices and rating scales to quantify and qualify the increase in locomotor activity produced by a single and/or chronic administration of amphetamine (Kalivas and Weber, 1988; Kolta et al., 1985; Paulson et al., 1991).

These studies, however, usually allow only a short time for acclimation to test chambers before experimentation, and may thereby add the variable of increased exploratory activity to a novel environment (Paulson et al., 1991). Measurements of motor activity were usually limited to 1–3 h after injection, leaving open the possibility that amphetamine may have some long-term effects on locomotor activity after a single injection (Segal and Mandell, 1974). In addition, saline or time controls were established either via a separate group of rats injected concurrently with the treatment groups, or by measurement of the treatment group for a short time preceding injections. Thus introducing potential variability in the assessment of handling effect and dose response characteristics. Since activity levels exhibit hourly fluctuations (Fig. 1A), the use of baseline recording for an hour before treatment may cause more variation in the assessed response to drugs than would the use of each rat's corresponding-in-time baseline values.

Additional variability in the assessment of a drug's locomotor effects by automated devices may also arise from the use of only one locomotor parameter. Since psychostimulants elicit increases in varying aspects of motor behavior such as forward locomotion, rearing, and/or stereotyped movements, the use of a single measurement such as horizontal activity to study such complex changes is limited. Therefore, it becomes important to analyze multiple parameters of motor behavior when using automated devices to study the pharmacological effects of drugs, and thereby, improve the qualitative value of measurements collected by such devices (Donat, 1991).

Furthermore, very little attention has been paid to correlate the time of drug administration both within and between laboratories. This does not take into consideration that many drugs, including psychostimulants, have been shown to vary in their pharmacokinetics and their efficacy throughout the day, and even small differences in the acute effect can accumulate into larger differences during chronic treatment (Smolensky and D'Alonzo, 1993).

Any of the above variables could lead to the inconsistencies found when comparing one drug sensitization study to another. With all this in mind, the above study was designed to minimize as many of these variables as possible, and establish a protocol with more reproducible dose response relationships that could then be used to study chronopharmacological differences (study in progress) or the effects of chronic administration.

The automated system and experimental protocol in this study address an aspect of locomotor activity that has received limited attention in the past. The establishment of prolonged baseline activity levels (8 days) of untreated and unhandled rats, and the determination of whether the motor

activity parameters measured by automated systems are stable enough from hour to hour and day to day to allow conclusions to be made about the acute and chronic effects of psychomotor stimulants. Although activity varied slightly from hour to hour, there was a stable baseline level of activity (Fig. 1) in all the parameters studied over either the light or dark phase. The effects of a drug on the locomotor indices collected by this system can therefore be compared within each rat to its own respective baseline, and any changes in 12 h (i.e., light/dark phase) or 24 h activity counts can be considered an effect of the drug and not of random fluctuations over time.

In an attempt to correlate behavioral measurements by automated devices to behavioral observations, five locomotor variables were chosen to analyze the effect of four amphetamine doses (0.6, 1.25, 2.5, and 10 mg/kg). The doses were chosen based on previous descriptions of the range of motor and stereotypic effects of *d*-amphetamine (Paulson et al., 1991; Segal and Mandell, 1974).

Values for the change from baseline were established for all five motor indices for their maximum effect (E_{max}) in the first hours after injection as well as for the change in area under the time curve (AUC) for the treatment cycle (12 h post injection). In general, the lower doses (0.6, 1.25 and 2.5 mg/kg) displayed increases in all indices studied that peaked in the first hour post injection, while rats given 10 mg/kg exhibit the peak effect in the third hour post injection. The duration of drug effect increased as the dosage increased, from 2 h following 0.6 mg/kg to 5 h after injection of 10 mg/kg. The dose response characteristics established for E_{max} and the AUC revealed an inverted U-shaped relationship with a maximal increase at 1.25 mg/kg. However, the greatest increase in E_{max} and AUC of vertical activity, occurred at 2.5 mg/kg rather than at 1.25 mg/kg. This implies that different parameters measure different aspects of motor behavior.

Horizontal activity appears to be a measure of general activity, while total distance measures forward ambulation, explaining their maximum increase with the lower doses of amphetamine. Vertical activity measures the amount of rearing, and the peak effect of 2.5 mg/kg on vertical activity may reflect the addition of the stereotypic effect of higher doses to the increased ambulatory effect of lower doses of amphetamine. Interestingly, as stereotyped behavior became more focused with the 10 mg/kg dose, the parameters that measure this behavior did not increase as would be expected (Fig. 2D–E), except for the change in AUC for the number of stereotypic movements which appeared to increase as the dosage increased (Fig. 3E). Change in AUC in number of stereotypic movements is, therefore, a more reliable measure of the stereotypic effects of amphetamine than stereotypic activity.

Total distance exhibited a slight decrease in the first hour after 10 mg/kg *d*-amphetamine while the other parameters showed increases that were not quite significant. The initial drop in total distance in the first hour with subsequent increases in overall locomotor activity in the later hours correlates well with the direct observation of Segal and Mandell (1974), who described a frozen 'stereotype phase' which lasts for about 90 min after injection of 7.5 mg/kg or larger dose of amphetamine; followed by an 'after phase' with increases in locomotion for another few hours.

Significant decreases in total distance traveled following the two highest amphetamine doses (Fig. 4) raise the possibility that forward ambulation is the specific parameter of locomotor activity that was reported to decrease by Segal and Mandell (1974). All other parameters had returned to baseline levels within 5 h of drug administration. Therefore, there appear to be no clear cut long term effects on the measures used in this study after a single administration of amphetamine.

Values for the E_{max}, duration of effect, and AUC can be compared with corresponding data after multiple injections, and the process of sensitization, or tolerance, can then be investigated for each locomotor parameter. Due to diversity of stereotyped behavior, quantification of the process of sensitization may be difficult for the higher doses of amphetamine. This automated system may be better suited for the assessment of mainly locomotor activity. Total distance, however, can reveal the appearance of intense focused stereotypic activity. This method of behavioral data acquisition may potentially delineate the process of sensitization to the locomotor and stereotypic behaviors and establish them as separate processes.

In conclusion, this automated system provided a quantitative analysis of the effects of a single administration of *d*-amphetamine on different parameters of motor activity, with each rat serving as it's own baseline control. This method provides the potential to analyze, and consistently reproduce, the effects of single and multiple injections, with a clearer characterization of sensitization or tolerance to effects of stimuli on different aspects of locomotor activity. These methods can also be used to establish the circadian response patterns of locomotor activity, and the differences in effects of drugs on motor activity at different times of administration. In addition, the use of internally synchronized animals and the same circadian timing of drug administration allows for the possible minimization of variability within and between studies.

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