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Time-Dependent Differences in the Rat's Motor Response to Amphetamine

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GAYTAN, O., A. SWANN AND N. DAFNY. *Time-dependent differences in the rats motor response to amphetamine.* PHARMACOL BIOCHEM BEHAV **59**(2) 459–467, 1998.—The dose-related motor effects of *d*-amphetamine given at the beginning of the light and dark cycle of rats were investigated using a computerized activity-monitoring system that recorded five different motor behavior indices. After 7 days of acclimatization and 2 days of baseline monitoring, rats were randomized into either 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) either 1 h into the light phase (0800) or another five groups at 1 h into the dark phase (2000) of day 3. The time control group exhibited a stable baseline level of activity for the length of the experiment. All doses (0.6, 1.25, 2.5, and 10 mg/kg) significantly elevated ($p < 0.01$) locomotor activity compared to baseline at both times of administration, but not all motor indices followed the same pattern of response. At both injection times, the maximum increase over baseline generally occurred following the 1.25 mg/kg dose of amphetamine ($p < 0.001$). The duration of the drug effect also increased with each dose. The stereotypic effects produced by high doses of AMP (10 mg/kg) was different when applied at the light phase compared to the dark phase, but the amphetamine effect on locomotor behavior remained the same regardless of the difference in motor activity baseline between the activity phases. © 1998 Elsevier Science Inc.

d-Amphetamine Dose response Psychomotor stimulant Motor activity Behavior Chronopharmacology

THE stimulatory and behavioral effects of the psychomotor stimulant amphetamine (AMP) were reported as early as 1932 (4). The locomotor activity response to AMP 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, while high doses elicit a different behavioral pattern that is characterized by early and late phases of increased locomotor activity. As the dose increases, these two phases of hyperactivity 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 (6,16,25,31). Over the last 2 decades, the effects of stimulants on motor behavior has been the focus of numerous studies (17,28). Yet, in most of these studies very little attention has been given to the timing of drug administration, even though many drugs, including AMP, have been shown to vary in their pharmacokinetics and their efficacy throughout the day (29,30,34). Consequently, variation within and between laboratories regarding the time at which a drug is administered may possibly contribute to a variability in effects of both acute, and chronic administrations of stimulants.

Scheving et al. (30) reported that the LD_{50} of AMP, which is considered an indirect dopamine agonist, varies throughout the day. Additionally, circadian fluctuations in dopamine levels and receptor density, as well as in α, and β-adrenergic receptor densities in the rat brain have also been reported (1,5,11,12,18,19). These circadian fluctuations in the neurotransmitter levels by which AMP exerts its behavioral effects may cause differences in its effects on locomotor activity throughout the day. Furthermore, tolerance to the stimulatory effects of continuously infused AMP during the light phase, but not during the dark phase, has been reported (10).

The present study was initiated to investigate whether differences in the time of drug administration influences the locomotor and/or stereotypic responses to AMP. For this purpose the effects elicited by AMP at two different times were investigated under conditions designed to minimize variabil-

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ity between studies. A comprehensive investigation of the dose–response relationship for locomotor activity immediately and for 24 h after a single AMP injection at the beginning of the rat's inactive phase (i.e., light cycle), and at the beginning of the active phase (i.e., dark cycle), were performed. The initial studies focused on: (a) determining whether the motor indices used in monitoring a time control group display a stable hourly and daily baseline of activity over a prolonged period of time (8 days); (b) the advantages/disadvantages of monitoring and evaluating more than one motor index following different doses of AMP; (c) comparison between the dose–response relationship obtained during the light phase (rest period of rat) to the dark phase (active period); and (d) whether there are any persistent effects or alterations in the normal circadian pattern of locomotor activity after a single administration of AMP in the beginning of the light or dark phase.

METHODS

Male Sprague–Dawley rats (n = 92) 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 12L:12D schedule (light on at 0700 h) for a minimum of 5 to 7 days before experimentation in order to internally synchronize their neuroendocrine systems; food pellets and water were supplied ad lib. On the last day of acclimatization, rats were weighed and individually housed in the experimental cages with ad lib food and water, and allowed a minimum of 12 h of accommodation to the test cages before recording of locomotor activity.

Apparatus

Omnitec Digiscan RXYZM (16) DVA computerized animal activity monitoring (CAAM) system cages were used. The CAAM system has been described in detail before (3,8). 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 was recorded as an activity score. Interruptions 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, and organized into 22 different locomotor indices.

Due to the similarities in response of the 22 indices the CAAM system provides, only the following representative indices were chosen for further analysis to characterize the different effects of drug administration: (a) total distance (TD), and (b) vertical activity (VA), which measure the amount of forward ambulation and rearing, respectively, and were used to assess those two specific locomotor effects of AMP; (c) stereotypic activity (SA), which measures the repeated interruptions of the same beam(s) from any of the three sensor arrays; (d) number of stereotypic movements (NOS), which measures the number of different episodes of stereotypic activity with at least 1-s interval before the beginning of another episode. SA and NOS assessed the effect of drug treatment on general stereotyped behavior (i.e., repetitive behaviors such as grooming); and (e) horizontal activity (HA), which measures the overall motor activity in the lowest tier and was used to assess the amount of spontaneous motor activity, which is a summation of both locomotor and stereotypic effects of AMP and random movements.

Time Control and Treatment Groups

A time control group (n = 12) was monitored continuously throughout the 24 h cycle for 8 consecutive days while the treatment groups were monitored for 4 days. For the treatment groups, the first two recording days were used to obtain a control measure of baseline activity for each rat, followed by AMP injection (day 3), and posttreatment monitoring (day 4). On day 3, each rat was weighed and randomly assigned to one of the following groups: five groups of eight rats each received SC injections (0.8 cc) of 0.9% saline containing 0, 0.6, 1.25, 2.5, or 10 mg/kg of AMP sulfate (Sigma Chemicals) 1 h into the light cycle (i.e., at 0800). An additional five groups (each n = 8) received the same treatment regimen, except that they received their injections 1 h into the dark cycle (i.e., at 2000). In all groups, data acquisition was stopped during injections, and resumed immediately after injection for 36 to 48 h (day 3–4).

Data Analysis

The acute effect of AMP was tested for significant change of hourly activity after injection, compared with each rat's own averaged baseline hourly activity (days 1–2) at the same time of the day by the paired t-test. The two times of administration were compared using a two-factor ANOVA (dose × time of administration) followed by a least-squares difference to test for differences in the absolute increases over baseline in area under the time curve for the 5 h immediately following injection. The dose–response relationship for both injection times was analyzed by linear regression and response surface analysis to determine the dose that caused the greatest increase over the total 5 h following injection for each of the motor indices. To determine the long-term effects of AMP one way ANOVA of pretreatment and posttreatment dark and light cycles were conducted on all treatment groups. One-way ANOVA was also conducted on the time control group. Significance was set at an $\alpha = 0.05$.

RESULTS

Time Control

The total distance (cm) traveled in the light phase and dark phase during the 8 days of recording, as well as the hourly pattern of activity of three randomly selected days are shown in Fig. 1, with similar observations obtained for the other motor indices (HA, VA, SA, and NOS). Baseline activity was stable over the light or dark phase (Fig. 1A–B). In the hourly histogram (Fig. 1C), a clear difference in activity between the rats' inactive (light phase) and active periods (dark phase) is seen, producing definite circadian patterns of activity with slight hourly variation occurring within the inactive and active periods. In summary, the uninjected time control group displayed a stable daily baseline of activity in all the indices sampled for the length of the study, and exhibited different activity between the active and inactive periods.

The difference in the average hourly counts between the light and dark phases for all five motor indices are displayed in Fig. 2. HA, SA, and NOS each showed about a threefold increase in activity during their active period (dark phase). There was a sevenfold and 10-fold increase in the average TD and VA, respectively, between the inactive and the active period for each rat.

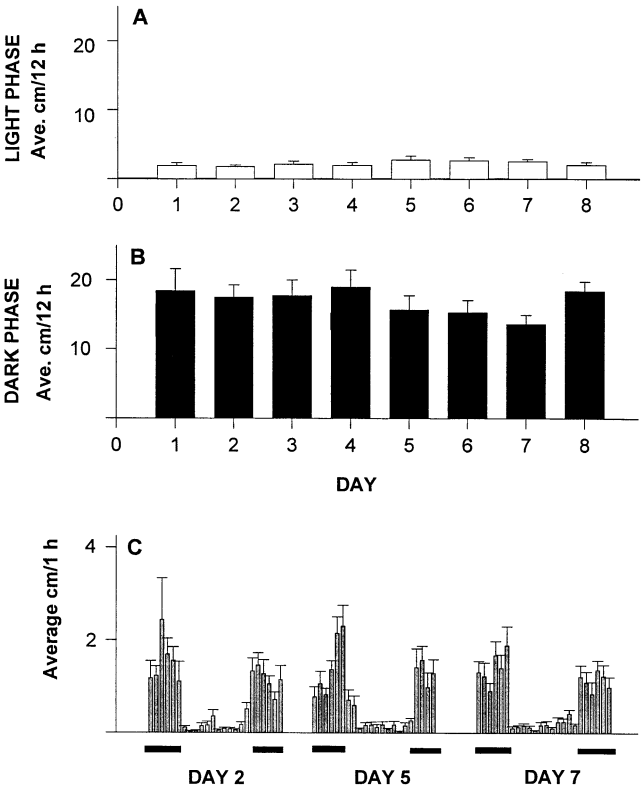

FIG. 1. Total distance in centimeters for the time control group (n = 12), which was not handled for the course of the experiment, are displayed as mean ± SEM for the following: (A) the average total distance (cm) traveled during the 24 h of days 1–8. (B) The average hourly total for days 2, 5, and 7, organized as 6 dark cycle h (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. One-way ANOVA revealed no significant difference among days. Numerical values represent original value divided by a factor of 1,000.

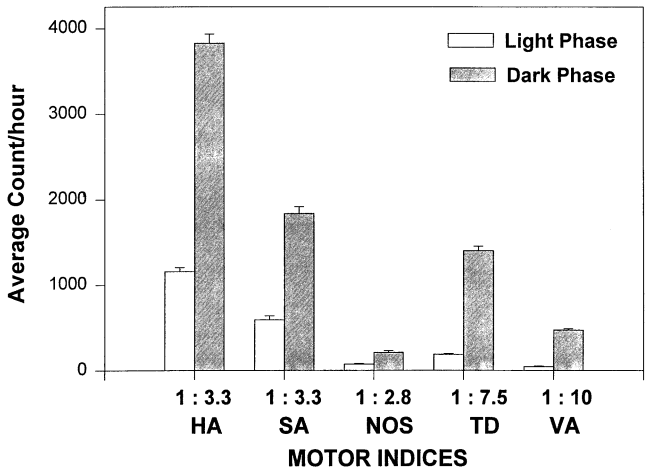

FIG. 2. Average hourly activity counts during the light and dark cycle for all five motor indices in the time control group (n = 12). The ratio of change in activity between the light and dark cycle are presented along the bottom abscissa, along with the five motor indices: horizontal activity (HA); stereotypic activity (SA); number of stereotypic movements (NOS); total distance (TD); and vertical activity (TD).

Saline Control

Immediately after saline injection, animals moved around the cage for several minutes before returning to their preinjection level of activity. When the hourly samples were analyzed for the five locomotor indices and compared to their baseline values, however, rats injected with saline displayed no significant alteration at either time of administration (0800 or 2000). There was no significant effect of handling, insertion of the needle, or volume of injection at either time point for hourly, light and dark cycle, or the total daily activity counts.

Dose Response at 0800 and 2000 injections

Four days of recording were obtained, with baseline activity (days 1 to 2), day of injection (day 3), and post drug activity (day 4); i.e., each rat had 2 days of recording, before administration of drug to establish baseline levels of activity. The baseline hourly activity levels (days 1 and 2) of the AMP dose groups were comparable to those in the time control group (Fig. 2) for all indices studied, and each rat could therefore serve as its own control. Any increase in activity can be compared to the baseline values presented in Fig. 2 to obtain the proportionate rise in activity at the time of administration. The change in motor activity during drug treatment was the difference between the postinjection-treated values on day 3 and the average of its time-matched baseline values on days 1 and 2.

Comparison of the five motor indices to their own baselines revealed that all AMP doses (0.6, 1.25, 2.6, and 10 mg/kg) significantly increased motor activity at both times of administration, as determined by the paired t-test. For an example of the five motor indices, the time course of effect on HA during both times of administration is graphically represented in Fig. 3, and shows that the different doses had similar time courses at both times.

The absolute change over baseline after administration of the lowest dose of AMP, 0.6 mg/kg, reached its maximum effect in the first hour post injection (Fig 3A and B.; $p < 0.01$) and returned to baseline levels by 3 h at both injection times (0800 and 2000).

At 0800, the 1.25 mg/kg dose caused the greatest increase in activity (21,576 counts) in the first hour ($p < 0.001$) when compared to any of the other doses. AMP's effect dropped by 50% in the second hour, but activity was still significantly elevated over baseline ($p < 0.001$). Motor activity returned to baseline levels by 4 h post injection (Fig. 3A). The effect of 1.25 mg/kg of AMP during the rat's active dark phase (2000) was similar to that obtained during the light phase (Fig. 3B).

Following the 2.5 mg/kg AMP injection at 0800, HA did not increase as much in the first hour compared to the increased activity following 1.25 mg/kg, but it still reached its maximum increase of 13,363 counts ($p < 0.001$) in the first hour, as observed with the lower doses. HA remained significantly elevated ($p < 0.001$) in the second hour, and the increase in activity 3 h after injection ($p < 0.01$) was still approximately half of the first hour increase. Activity returned to baseline levels by the fourth hour, 1 h later than the lower AMP doses (0.6 and 1.25 mg/kg). The 2.5 mg/kg dose given at 2000 elicited a larger increase in locomotor activity than did the same dose administered at 0800, and was, therefore, as effective in increasing HA in the first hour ($p < 0.001$) as 1.25

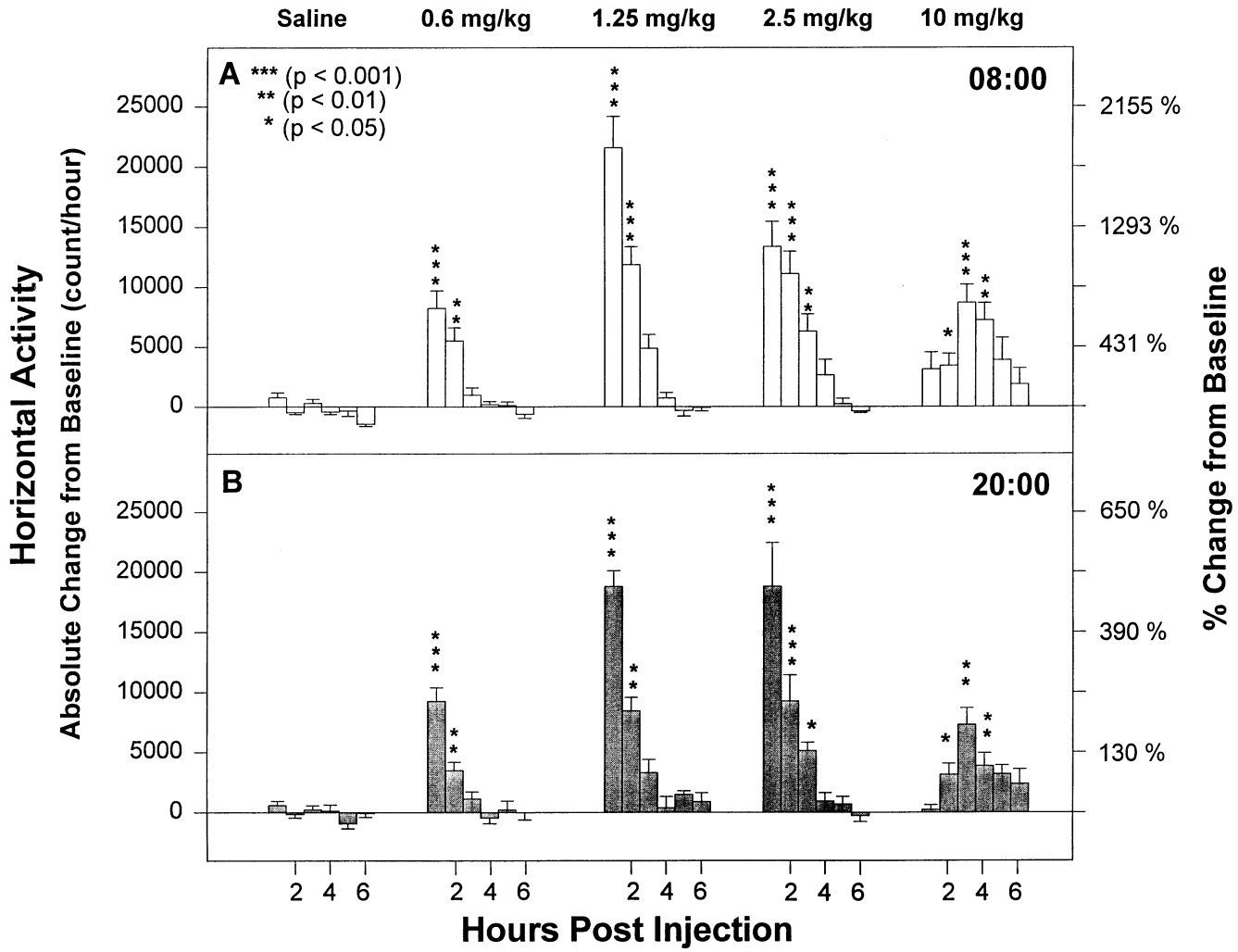

FIG. 3. Time course of the response to single SC administration of saline ($n = 8$) and amphetamine (0.6, 1.25, 2.5, 10 mg/kg; each $n = 8$). (A) One hour into the light cycle; 0800, and (B) 1-h into the dark cycle; 2000. Horizontal activity data are presented as the mean ± SEM of the average increase 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 or dark cycle. Each bar represents 1-h blocks of data.

mg/kg was (Fig. 3B). This increase in activity also lasted until the fourth hour postinjection.

After administration of 10 mg/kg at both times (0800 and 2000), HA was increased significantly during the initial 2 h ($p < 0.05$), but unlike the previous AMP doses, the peak increase in locomotor activity occurred 3 h postinjection ($p < 0.001$; i.e., delayed peak compared to the previous three dosages). HA returned to baseline activity by 5 h postinjection (Fig. 3A and B). In summary, the time course of effect on HA was similar whether the drug was injected in the light phase (0800) or at the dark phase (2000).

Time Course of Other Motor Indices

SA exhibited similar dose response characteristics as data shown in Fig. 3 for HA. With VA, however, the maximum increase in activity counts at both times of administration (2000 and 0800) was clearly induced with the 2.5 mg/kg dose rather than 1.25 mg/kg as in all other indices. Further differences between the motor indices occurred following administration of the largest dose of AMP, 10 mg/kg, especially between the motor indices of TD and NOS. Therefore, TD and NOS following the 0800 and 2000 administration of 10 mg.kg is shown in Fig. 4. Unlike HA (Fig. 3), TD was slightly decreased (−159 cm), and significantly decreased (−856 cm; $p < 0.05$), respectively, in the first hour postinjection, before increasing to its maximal effect which occurred during the third hour after administration at 2000, and in the fourth hour at 0800 (Fig. 4, left). During the initial hour after injection, when TD was decreased, NOS was significantly elevated ($p < 0.01$), and remained at this increased level for all 4 h of the drug effect, with little difference between the hours (Fig. 4, right). Although the increase in NOS was noticeably lower in amplitude at 2000 compared to 0800, NOS was significantly elevated over baseline in the first hour postinjection, indicating that stereotyped behavior was occurring predominantly while forward ambulation was being depressed at both times of administration in the initial hour.

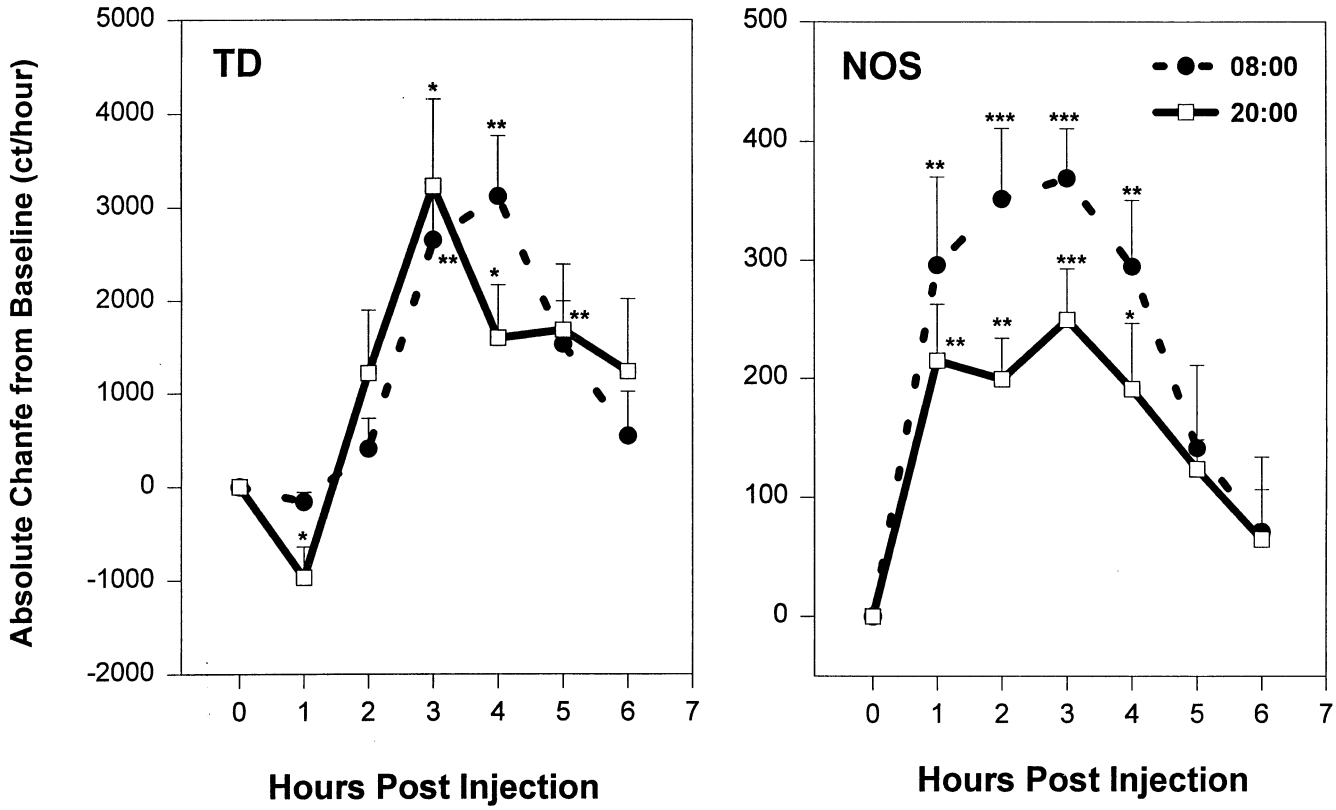

FIG. 4. Time course of the effect on total distance (TD) and number of stereotypic movements (NOS) following 10 mg/kg of AMP given at 0800 and at 2000 (each $n = 8$). The data are presented as the mean ± SEM/h of the average increase in counts of each rat on the day of treatment (day 3), relative to their own corresponding baseline values (days 1 and 2). Baseline values were arbitrarily set at 0, and the data presented are fractions of the original value. For differences in hourly baseline values for each index at the two times of administration, please see Fig. 2. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

Comparison Between Treatment Effect and Time of Administration (0800 vs. 2000)

The activity under the curve (AUC) were calculated for (a) the first 5 h after injection (saline or AMP) on day 3 of the experiment; and (b) the time-matched values of baseline days 1 and 2 averaged into a single value, using the trapezoidal rule.

The absolute change in AUC counts for all doses and motor indices at both times of administration is displayed in Fig. 5. All AMP doses significantly elevated motor activity over that of saline ($p < 0.01$) for all five motor indices. The dose–response relationship of all motor indices, except NOS, fit a quadratic equation ($r^2 = 0.391$ for HA; $y = -1655.7x^2 + 18418x + 7370.7$) consistent with an inverted U-shaped relationship. TD, VA, and SA all had similar fits as HA. The AMP dose of 1.25 mg/kg caused the largest increase in AUC for HA, TD, and SA at both injection times, while VA was increased most by the dose of 2.5 mg/kg. NOS, however, appeared more asymptotic than quadratic, with little difference between the higher doses (Fig. 5).

A significant effect for dose ($p < 0.001$) was obtained for all five motor indices; however, only NOS was significantly different between the times of injection ($F = 2.70, p < 0.05$ for two-factor ANOVA). The significant interaction of dose × time of administration for NOS indicates the presence of a circadian rhythmicity in the stereotypic effects of a single administration of AMP.

Long-Term Effects of Single AMP Injection

The baseline activity (days 1 and 2) and posttreatment activity (day 4; i.e., the data gathered 11 to 36 h postinjection) of both the light and dark periods were compared using one-way ANOVA. The posttreatment HA levels for the light, dark, and daily periods were not significantly altered from those before treatment. All other indices behaved similarly for both times of AMP administration, except for the motor index of TD after injection at 0800. Comparison of baseline and posttreatment values are displayed in Fig. 6. The TD traveled after administration of 2.5 mg/kg AMP was significantly decreased only in the dark cycle of day 4 ($F = 8.04, p < 0.01$; Fig. 6C) while TD after the 10 mg/kg AMP dose was significantly decreased for both the light cycle and the dark cycle on the day after injection ($F = 8.54, p < 0.01$; $F = 4.8, p < 0.05$, respectively). All the other indices returned to baseline activity levels on the day after treatment for both times of administration. This observed long-term effect of a single SC injection of 10 mg/kg on TD did not occur with any dose when injected at the beginning of the dark cycle (2000), and therefore, appears to be dependent on the time of drug administration.

DISCUSSION

Most studies investigating the effects of stimulants of motor behavior have been conducted during the light phase (i.e.,

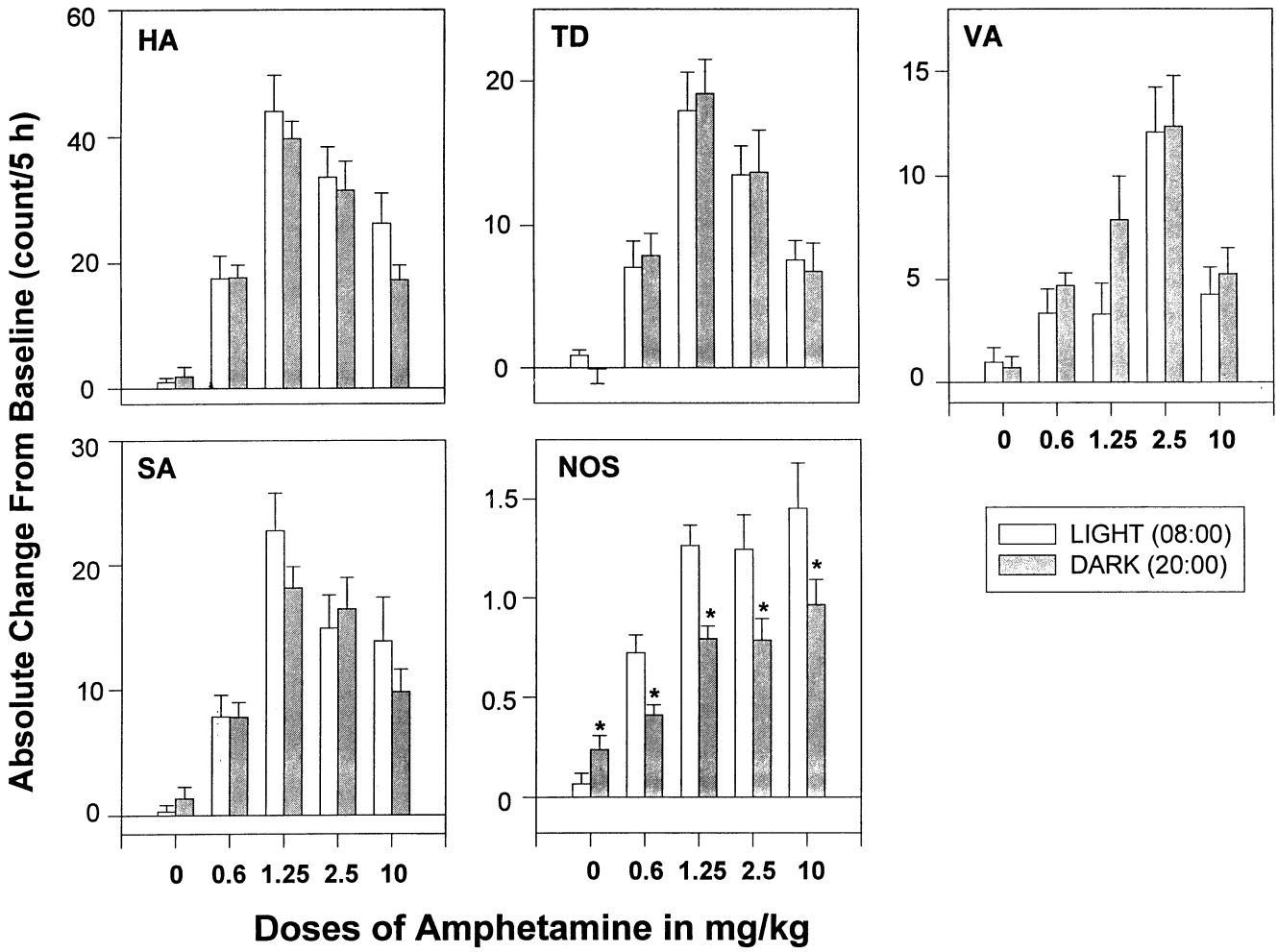

FIG. 5. Average changes at both times in the area under the activity time curve for 5 h after SC administration of saline (n = 8) and amphetamine (0.6, 1.25, 2.5, 10 mg/kg; each n = 8) relative to their own corresponding baseline values (days 1 and 2). Data are presented as the mean ± SEM in counts/5 h for all five parameters studied with baseline values arbitrarily set at 0. Data presented are fractions of original data, so for differences in hourly baseline values for each index at the two times of administration, please see Fig. 2. Statistical significance was determined using the two-factor ANOVA. Numerical values represent the original value divided by a factor of 1,000.

rest period) of the nocturnally active rat. The objective of this study was to ascertain whether there is a difference in the dose–response pattern following AMP administration given during the rat's active period (dark phase) compared to that given during their rest period (light phase). The only previous comparison of AMP effects in the light and dark phase used continuous, rather than single or multiple administration, and found that tolerance to AMP's motor effects occurred during the light phase, but not the dark phase (10). The main finding in the present study is that the stereotypic effects produced by AMP, as well as the ability of a large dose of AMP to produce long-term effects, appeared to differ between the two injection times (light and dark phase). Yet, despite the great difference in baseline activity between the light and dark phase, the effect of AMP on locomotor activity was similar at both times of administration.

Due to large differences in the level of spontaneous motor activity between the active and inactive periods, the protocol design needed to address the following issues before comparing and interpreting the effects of AMP on locomotor activity at the beginning of the light and dark cycle: (a) to identify and minimize factors that could lead to variability; and (b) to check for stability of baseline motor activity. The study was therefore designed as follows: (a) data collection was based on objective computerized recording of motor behavior (3,8), circumventing problems of direct human observation that include inconsistent behavioral definitions, inter- and intra-observer reliability, and fatigue (2,5,7,26,27); (b) data were recorded for prolonged periods of time throughout the light and dark cycle to establish conditions necessary for a more reliable and stable baseline, rather than over a brief 1–3 h period (15,16,20); (c) in addition each animal served as its own control, thus providing comparison of drug effect to a time-matched average baseline of the same animal, rather than comparison between two separate groups of rats or brief pretreatment observations of the same group; (d) multiple indices of locomotor activity, because effects of stimulants on exploratory and stereotyped behavior are complex (2,24); (e) comparison between the dose–response characteristics obtained at different times of AMP administration, because the

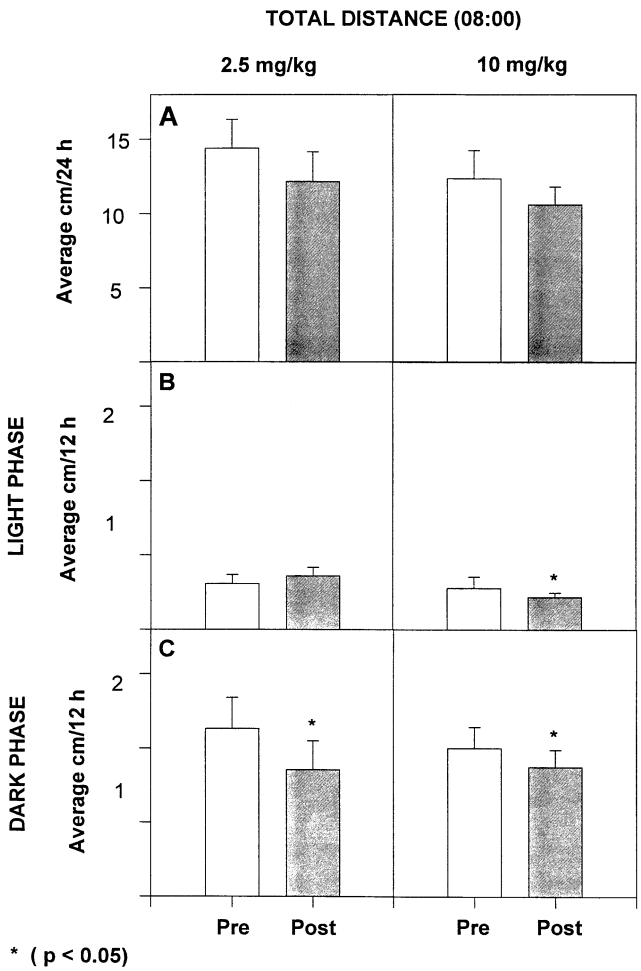

FIG. 6. The total distance in centimeters traveled after amphetamine injection at 0800 by each rat for (A) daily (24 h), (B) light phase, and (C) dark phase activity values for the pretreatment (Pre) cycles (days 1 and 2 averaged into one baseline value), and the posttreatment (Post) cycle, i.e., day 4. Significance of effect was determined using one-way ANOVA. Numerical values represent the original value divided by a factor of 1,000.

efficacy and pharmacokinetics of many drugs, including AMP, are known to vary throughout the day (3,4); and (f) male rats were housed in the experimental room for 8 days prior to experimentation to allow for synchronization to the light/dark cycle at our facility. The AMP doses were chosen based on previous descriptions of the range of motor and stereotypic effects of *d*-amphetamine (21).

The first part of the present study entailed the measurement of baseline activity levels of intact control rats for a prolonged period (8 days), and the determination of whether the motor indices measured are stable enough from hour to hour and day to day to allow conclusions about acute and chronic drug effects. The baseline locomotor activity was stable over 8 days in all the five indices studied during the light and dark phase, without significant hourly variation. All indices measured exhibited a consistent circadian pattern of activity (Fig. 1) as has been described previously (8,22), with high locomotor activity during the dark phase and low motor activity during the light phase. Therefore, the effects of a drug can be compared within each rat to its own time-matched baseline value (light and/or dark phase) for each specific motor index. Additionally, changes in the pattern of 12 h (i.e. light/dark phase) or 24 h locomotor activity counts can be considered an effect of the drug rather than random fluctuations over time.

The five motor indices exhibited distinct dose response characteristics at both times of administration that correlated well with previous reports of AMP's effects (14,17,28). All motor indices, except NOS, followed an inverted U-shaped relationship, with the maximum increase at 1.25 mg/kg in the first hour after injection. With the larger doses of 2.5 and 10 mg/kg, the emergence of a phase of focused stereotypy behavior caused a decrease in the maximum increase in counts of all locomotor indices except VA. The maximum increase in VA occurred at 2.5 mg/kg, rather than at 1.25 mg/kg, which may have reflected the emergence of the stereotypic effect of AMP on rearing.

Administration of 10 mg/kg elicited a decrease from baseline in TD during the initial hour after injection at 0800 and even more at 2000 ($p < 0.05$). In this initial hour after administration, however, all other motor indices were increased ($p < 0.01$) instead of decreased. This initial drop in TD in the first hour with subsequent increases in activity in the later hours, corresponds to the focused "stereotype phase" and subsequent "after" hyperactivity phase that has been previously described (33).

Increases in locomotor activity for the first five hours after AMP injection (Fig. 5), and the time course of effect for each dose (Fig. 3), differed only slightly between the day and nighttime AMP administration, despite differences in neurotransmitter concentrations, receptor density, and motor activity levels between the active (night) and inactive (day) phase. The effect of the lower doses of AMP (0.6 and 1.25 mg/kg), which predominantly increase forward ambulation and rearing, were identical whether given in the active or rest period of the subjects. Therefore, there appears to be no difference in the locomotor response to AMP at these two times of administration for the two lower doses.

Differences between these two times of administration (0800 and 2000) occurred only at the two highest doses (2.5 and 10 mg/kg) indicating that the stereotypic behavior induced by AMP, unlike its locomotor effects, differ throughout the day. The amount of stereotyped behavior produced by AMP administration during the dark phase appears to be less than those obtained in the light phase, as is indicated by the significantly lower magnitude of the dose response for NOS observed at 2000 (Fig. 5). The ability of high doses of AMP to produce a focused stereotypy phase, however, was not impaired, because TD in the initial hour after administration of 10 mg/kg at 2000 was significantly decreased ($p < 0.01$; Fig. 4) below baseline levels. It is important to note that the decrease in TD in the initial hour at 0800 was not significant, but that this lack of significance is due, not to a greater ability of AMP to depress TD at 2000, but to the fact that rats are not ambulating during the light phase (0800) to begin with (Fig. 2). This explanation is supported by observation that 10 mg/kg AMP produces similar amounts of SA at both times of administration (Fig. 5). The duration of the frozen stereotypy phase appears to be shorter at 2000 when compared to 0800, because the peak created by the poststereotypy hyperactivity phase occurred in the third hour after administration at 2000, while the same dose injected at 0800 produced its maximum increase in activity in the fourth hour (Fig. 4). This finding indicates that the stereotypic effect, if not decreased at 2000, is at least shortened at this time. Because the effect of 10 mg/kg

AMP on SA was not different between the two times of administration (Fig. 5), it leaves open the possibility that the decreased NOS at 2000 (Fig. 4 and Fig. 5) may reflect stereotypy that is more continuous and less interrupted by forward locomotion than at 0800.

Additionally, there was a difference in the time course of effect elicited by 2.5 mg/kg between 0800 and 2000. The change in the maximum increase in locomotor activity elicited by 2.5 mg/kg given at 2000 cannot be explained as a shift to the right on the dose–response curve during the dark phase, because the time course of all the other doses remained the same at both times (Fig. 3B). It may, however, indicate that a higher dose was required to produce a focused stereotypy phase during the dark cycle, and therefore, there was no reduction in the forward ambulation response. TD and HA, which are the motor indices most affected by the amount of forward amublation, were only ones of the five locomotor indices to display the change in the time course of 2.5 mg/kg at 2000 when compared to its effects at 0800. Therefore, rats appear to either have a lower susceptibility to the stereotypic effects of AMP during the active period, or, alternatively, a different pattern of stereotyped behavior may be exhibited during the dark phase that does not limit the amount of forward ambulation. Qualitative descriptive techniques will be necessary to completely characterize any differences in the stereotypic response before a conclusion can be reached whether the stereotypic response to AMP is lesser, greater, or different during the dark phase compared to the light phase.

Finally, the long-term effects of single AMP injections was different at the two times of administration. The amount of TD traveled on day 4 was decreased during the light and dark phase compared to baseline values when rats were injected with largest doses of AMP at 0800 (Fig. 6), but not at 2000. This may indicate a greater sensitivity at 0800 for AMP administration to cause a disturbance in what would otherwise be a stable baseline level of activity (i.e., such as with the time control group). One could postulate that the poststimulant depression in dark cycle activity subsequent to chronic repeated administration of AMP during the light cycle, as has been reported by other investigators (22,32), may be less likely to occur with administration of AMP during the dark.

Despite the slight differences between the two times of AMP administration discussed above, the majority of AMP's effects do not appear to be influenced strongly by the state of the baseline level of motor activity at the time of administration. One explanation is that there are two separate systems controlling locomotor activity: one that determines basal motor activity, and a second system that controls the response of the motor system to stress, novel environment, or drug challenges.

Dopamine and other chatecholamine transmission are reported to be essential in the expression of locomotor activity, and the stereotypic effects of stimulants such as AMP have been associated with the dopamine system of substantia nigra and the striatum, while the locomotor effects of stimulants are associated with the nucleus accumbens (13,14,28). Moreover, because dopamine and other cathecolamine levels in these areas (i.e., extrapyramidal system) have been found to display distinct circadian rhythm (1,11,12,18,23), one might expect different motor responses when AMP is introduced at different times of the light/dark cycle. Yet, this was not the case to a large degree, with only slight differences in the stereotypic, but not the locomotor, response during both times. A possible explanation for the slight difference in sensitivity to the time of drug administration for these behaviors may arise from Paulson and Robinson's (23) observation that the concentration of dopamine and its metabolites as measured by on-line microdialysis increased significantly during the dark cycle in the striatum, but that dopamine levels in the nucleus accumbens did not significantly change throughout the day. However, the increased dopamine levels in the dorsal striatum are only weakly correlated with increased spontaneous motor activity during the dark cycle, while there is a positive correlation between the decrease in hypothalamic levels of norepinephrine and depression of spontaneous motor activity during the dark cycle following chronic treatment with AMP (22,35, 36). Therefore, the increased levels of dopamine, and other neurotransmitters in brain regions affected by acute administration of AMP, may not necessarily lead to differences in the response to a motor stimulant throughout the day.

The similar response to AMP at the two times of its administration, 12 h apart, does not rule out the possibility of different responses at other times. Yet the LD_{50} for AMP is different at these two times, suggesting that differences in its pharmacokinetics may also not influence the motor response to an acute challenge (30). Studies of the acute response to AMP injection at other times of the day, and possibly with other stimulants, are needed before the effect of different timing of stimulant administration on motor activity can be elucidated.

In summary, this study revealed a stable baseline level of activity for the 8 days of the study in intact control animals. The effects of high doses of AMP on general stereotypic behavior, as well as their ability to produce long-term/persistent effects, are dependent on the time of drug administration. However, the locomotor effects elicited by lower doses of AMP, when injected during the light or dark phase, are similar. The difference between diurnal effects on locomotor and stereotypic responses to acute administration of AMP, and the adaptation to chronic treatment remain to be determined.

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