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Methylphenidate: diurnal effects on locomotor and stereotypic behavior in the rat

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Abstract

The dose–response relationship and time course of effect on motor activity after a single dose of methylphenidate given at different times of the light/dark cycle was investigated using a computerized infrared activity analysis system. After 5 to 7 days of acclimation and 2 days of baseline activity recording, rats received a single subcutaneous injection of vehicle (saline) or of 0.6, 2.5, 10 or 40 mg/kg methylphenidate at 08:00, 14:00, 20:00, or 02:00. Recording was then resumed for an additional 36 to 48 hours. The locomotor indices analyzed were horizontal activity, total distance, vertical activity, stereotypic activity, and number of stereotypic movements. Saline and 0.6 mg/kg did not alter motor activity, but 2.5, 10 and 40 mg/kg significantly increased ($P < 0.01$) motor activity. The time to the maximum effect and the duration of effect increased with dose. Ten mg/kg had the most robust effect on locomotor activity, while the largest dose, 40 mg/kg, elicited a more focused stereotyped activity that limited the amount of forward ambulation. A single injection of methylphenidate had only transient effects. The locomotor stimulating effects of the lower doses were similar whether given during the light or dark phase, despite the large diurnal variations in baseline activity between the activity phases. The stereotypic effects of the highest dose of methylphenidate, however, varied between the light and dark phase, with a smaller stereotypic effect during the dark phase when compared to administration during the light phase. © 1997 Elsevier Science B.V.

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1. Introduction

Methylphenidate (Ritalin) is the most widely prescribed psychomotor stimulant, and due to the relatively long duration of treatment, most of the research conducted on methylphenidate (MPD) in humans has centered on its possible abuse potential, and its side effects [6,14,30]. Of the MPD studies conducted in animals [10,33,34,45], only a few have investigated the effects of acute and chronic exposure to this drug on motor behavior [1,31,48]. Chronic intermittent administration of other psychomotor stimulants, such as amphetamines and cocaine, can produce both behavioral sensitization [19,32,37,41] and tolerance [8,11] to their locomotor and stereotypic effects in animals.

Moreover, most studies of the behavioral effects of acute and/or chronic administration of stimulants in the rat have been conducted during the light cycle (i.e., the sleep time of the rat) with little attention given to other times of the day (24 h), even though motor behavior varies considerably throughout the light/dark cycle [13,15,35]. Many drugs, including stimulants, have been shown to vary in their pharmacokinetics and their efficacy throughout the day [42,43,49]. Even the neurotransmitters involved in the motor effects of stimulants exhibit circadian variations, with fluctuations in dopamine levels as well as in dopamine, α, and β-adrenergic receptor densities in the rat brain [2,16–18,28,29], which may result in differences in the motor response of animals to stimulants throughout the day. Consequently, variation within and among laboratories regarding the time at which a drug is administered may lead to variability in acute effects, as well as differences in the outcome of chronic administration of stimulants.

The present study was initiated to investigate whether differences in the time of acute MPD administration may cause changes in its effect on motor activity. The effect of MPD on locomotor and stereotypic behavior at the beginning and middle of the light and dark phase was investigated under conditions designed to minimize variability

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between studies. A computerized animal activity monitoring (CAAM) system [5,13] was used to measure multiple indices of locomotor activity continuously in the rats' home cages. The initial studies focused on: (1) determining whether the motor indices used in monitoring the animals display a stable hourly and daily baseline of activity over the course of the experiment (4 days); (2) investigating relationships between locomotor and stereotypic behavior throughout the light/dark cycle during the normal state and after drug administration; (3) comparing the dose–response relationship for MPD at the four different times of the day; and (4) determining whether there are any persistent alterations in the circadian pattern of locomotor activity after a single administration of MPD.

2. Materials and methods

Male Sprague–Dawley rats ($n = 172$) weighing 180–225 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 5 to 7 days before experimentation in order to internally synchronize their neuroendocrine systems. On the last day of acclimatization, 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

The CAAM system has been described in detail [5,13]. In short, the activity chambers consist of clear acrylic open field boxes ($40.5 \times 40.5 \times 31.5$ 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 minutes 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: (1) total distance (TD), and (2) vertical activity (VA), which measure the amount of forward ambulation and rearing, respectively, and were used to assess those two specific locomotor effects of MPD; (3) stereotypic activity (SA), which measures the repeated interruptions of the same beam(s) from any of the three sensor arrays; (4) number of stereotypic movements (NOS), which measures the number of different episodes of stereotypic activity with at least a one second interval before the beginning of another episode. SA and NOS assessed the effect of drug treatment on general stereotyped behavior (i.e., repetitive behavior); and (5) 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 MPD and random movements throughout the drug effect.

2.2. Injection and recording protocol

After 5 to 7 days of acclimation, and 12 h of accommodation to test cages, motor activity was recorded continuously and summed in 10 min bins throughout the 24 h 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, 2.5, 10, or 40 mg/kg of MPD hydrochloride (Research Biochemicals Inc., Natick, MA) at 08:00; 14:00; 20:00; or 02:00. Recording was then resumed for an additional 36 to 48 h, which included a post-treatment period (day 4).

2.3. Data analysis

All locomotor indices were analyzed for acute and persistent (≥ 12 h) effects of MPD. The acute effect was considered as the difference between activity during the five hours immediately after injection and the same rat's average baseline (days 1 and 2) at the same time of day. These differences were then used to calculate the effect maximum (E_{max}), and time to maximum effect (T_{max}) for each dose and motor index. The E_{max} was defined as the largest change from baseline in a 10 min sample period during the first 5 h following drug administration for each rat. The four times of MPD administration were compared using two factor ANOVA (dose $\times$ time of administration) followed by a least squares difference to test for differences in the E_{max}, T_{max}, and in the absolute increases over baseline in the area-under-the-activity time curve (AUC) for the five hours immediately following injection. The persistent effect (12–36 h) of MPD 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

The horizontal activity during the light phase (12 h) and dark phase (12 h), as well as the hourly pattern of activity

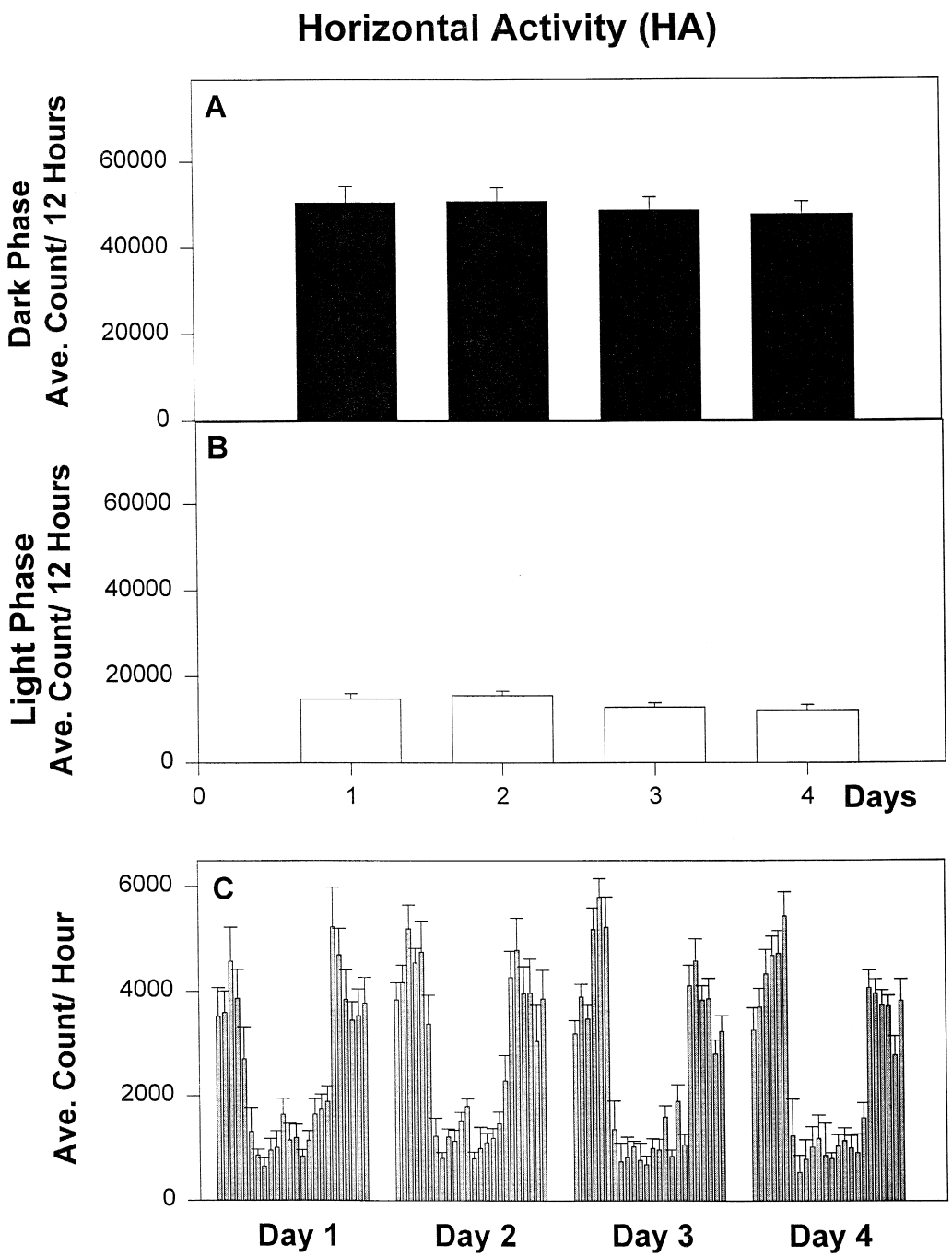

Fig. 1. Horizontal activity for the untreated time control group ($n = 12$) is displayed as mean $\pm$ S.E.M for the following: (A) The average total activity counts (12 h) during the dark cycle of Days 1–4. (B) The average total activity counts (12 h) during the light cycle of Days 1–4. (C) The average hourly activity counts for days 1 to 4 organized as 6 dark cycle hours, 12 light cycle hours, and the first 6 h of the next dark cycle; thereby creating a circadian pattern of activity. One way ANOVA revealed no significant difference between days.

(24 h) are shown in Fig. 1. Similar observations were obtained for the other indices (TD, VA, SA, and NOS). Baseline activity was stable during both the light and dark phase (Fig. 1A and B). The hourly histogram (Fig. 1C) revealed a clear difference in activity between the rats' inactive (light phase) and active (dark phase) periods, with a consistent circadian rhythm of activity.

The differences 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 three-fold increase in activity during the active period (dark phase). There was a seven-fold and ten-fold increase in TD and VA, respectively, between the light and dark phase.

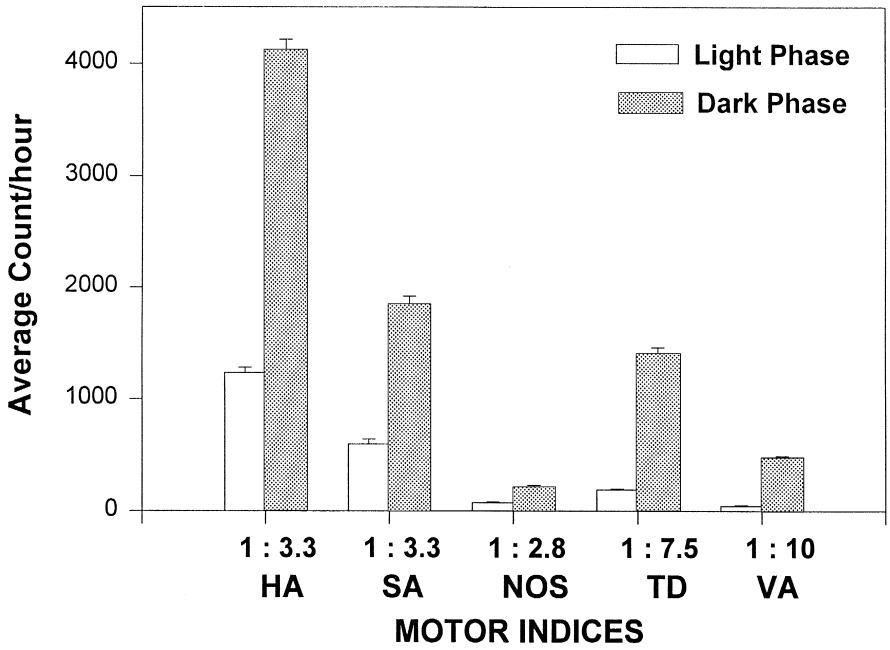

Fig. 2. Average hourly activity counts during the light and dark cycle for all five motor indices in the time control group ($n = 12$). The ratio of change in activity between the light and dark cycle are presented along the bottom abscissa, along with the five motor indices: horizontal activity (HA);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

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 recorded over the length of the study.

3.2. Time course of behavioral effects of MPD given at 08:00

The effect of a single dose of MPD (10 mg/kg) on HA at the four times of administration is displayed in Fig. 3. The baseline activity levels of days 1 and 2 were comparable to those in the time control group (Fig. 1) for all indices studied, and were stable from one day to the next. Therefore, the data from days 1 and 2 were averaged to obtain the baseline levels of activity throughout the day for each rat that were used for statistical analysis. Data collected immediately after injection were compared to time-matched averaged baseline values of days 1 and 2 to obtain the absolute change in activity during drug treatment for each motor index. Although the amount of baseline activity differed between the four injection times, it is clear that the increase in activity caused by MPD (10 mg/kg) was similar throughout the light/dark cycle.

Saline had no effect on the motor indices, except a transient rise in activity during the initial 10 min after injection during the light phase (08:00 and 14:00), but not during the dark phase (20:00 and 02:00). The dose–response characteristics of the four different doses of MPD given at 08:00 are presented in Fig. 4. Fig. 4A shows how the lowest dose of methylphenidate, 0.6 mg/kg, did not elevate motor activity; it was comparable in effect to saline (not shown in figure) for all motor indices and times of injection studied. The other 3 doses, however, significantly elevated motor activity.

After the administration of 2.5 mg/kg MPD, TD was immediately elevated, reaching a maximum increase (i.e., E_{max}) of 1350 cm/10 min ($P < 0.001$) at 17 ± 9 min ($T_{max;}$ i.e., time to the peak effect) after administration, and remained significantly elevated for 120 min post injection (Fig. 4B). The motor indices of HA, VA, SA, and NOS behaved similarly after administration of 2.5 mg/kg at 08:00. The time course of effect following 2.5 mg/kg at all other times of administration did not significantly differ from the effect presented in Fig. 4B.

The intermediate dose of 10 mg/kg significantly elevated ($P < 0.001$) TD immediately after injection, and reached its maximum increase (E_{max}) of 1836 cm/10 min ($P < 0.001$) above baseline at 53 ± 11 min after injection (T_{max}). Activity returned to baseline levels by 170–180 min after injection (Fig. 4C). All other indices behaved similarly, and there was little difference in the time course of effect for this dose at any of the other times of administration.

The effect of the highest MPD dose (40 mg/kg) exhibited a different pattern of response on TD (Fig. 4D). After an initial increase ($P < 0.001$) in TD during the first 20–30 min after injection, activity returned to baseline levels for the following 120 min, before increasing again to a second peak of 1138 cm/10 min above baseline at 183 ± 15 min after injection. The second phase of increased activity lasted until 280 min after injection, and the E_{max} and T_{max} were calculated for this period.

Unlike the lower doses, the effect of 40 mg/kg of MPD at 08:00 varied between the different motor indices, so the effects of this dose on VA, NOS, SA, and HA are presented in Fig. 5. Unlike TD (Fig. 4D), the initial phase of increased VA lasted for a longer time (60 min), yet the second phase of increase in VA began 10 to 20 min earlier compared to TD (Fig. 5A compared to Fig. 4D). The remainder of the drug effect was similar for both indices. The time course for NOS was different from TD and VA, with an initial increase in activity that started immediately after MPD injection and persisted for 300 min (Fig. 5B). The time course of SA (Fig. 5C) and HA (Fig. 5D) were similar, and remained significantly elevated from 70–150 min compared to TD and VA (Fig. 4D and Fig. 5A), which

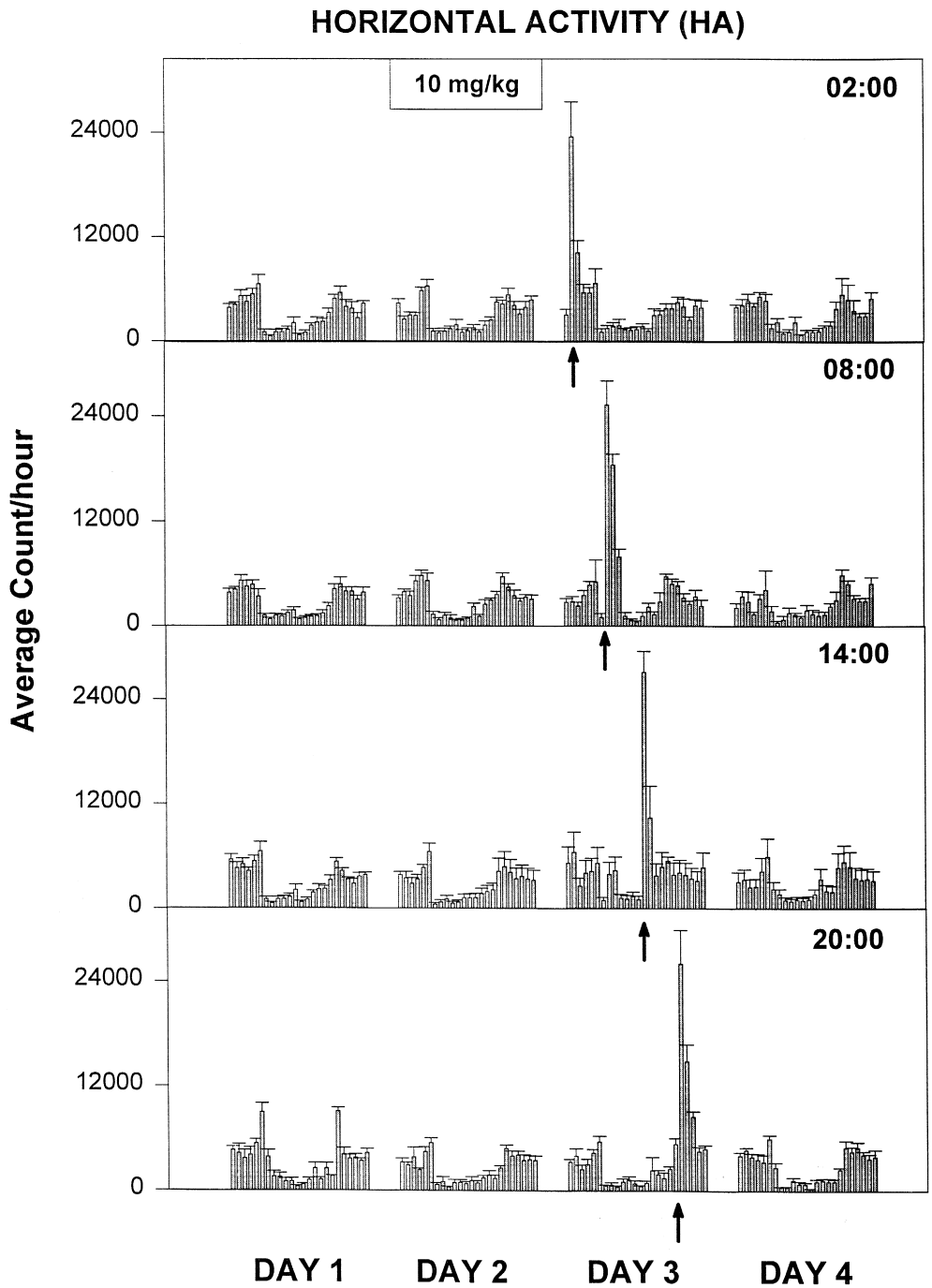

Fig. 3. The average horizontal activity count is displayed as the mean ± S.E.M per h for the four experimental days of the treatment groups (each $n = 8$) receiving 10 mg/kg at either 02:00, 08:00, 14:00, or 20:00. Arrows indicate the time of drug injection.

were indistinguishable from baseline during that time period (i.e., stereotyped activity occurring during absence of forward ambulation). Motor activity during this time was, therefore, dominated by general stereotyped behavior.

The response characteristics/pattern to 40 mg/kg MPD also varied at the four times of administration, as shown for TD in Fig. 6. During the light phase, there were two phases of increased activity, interrupted by a period of inactivity resulting from focused stereotypy (Fig. 6A and B). However, the initial phase of increased activity at 14:00 lasted about 50 min longer than at 08:00. This complex response pattern was less evident during the dark phase. Moreover, the overall increase in activity appeared to be less during the dark phase than during the light

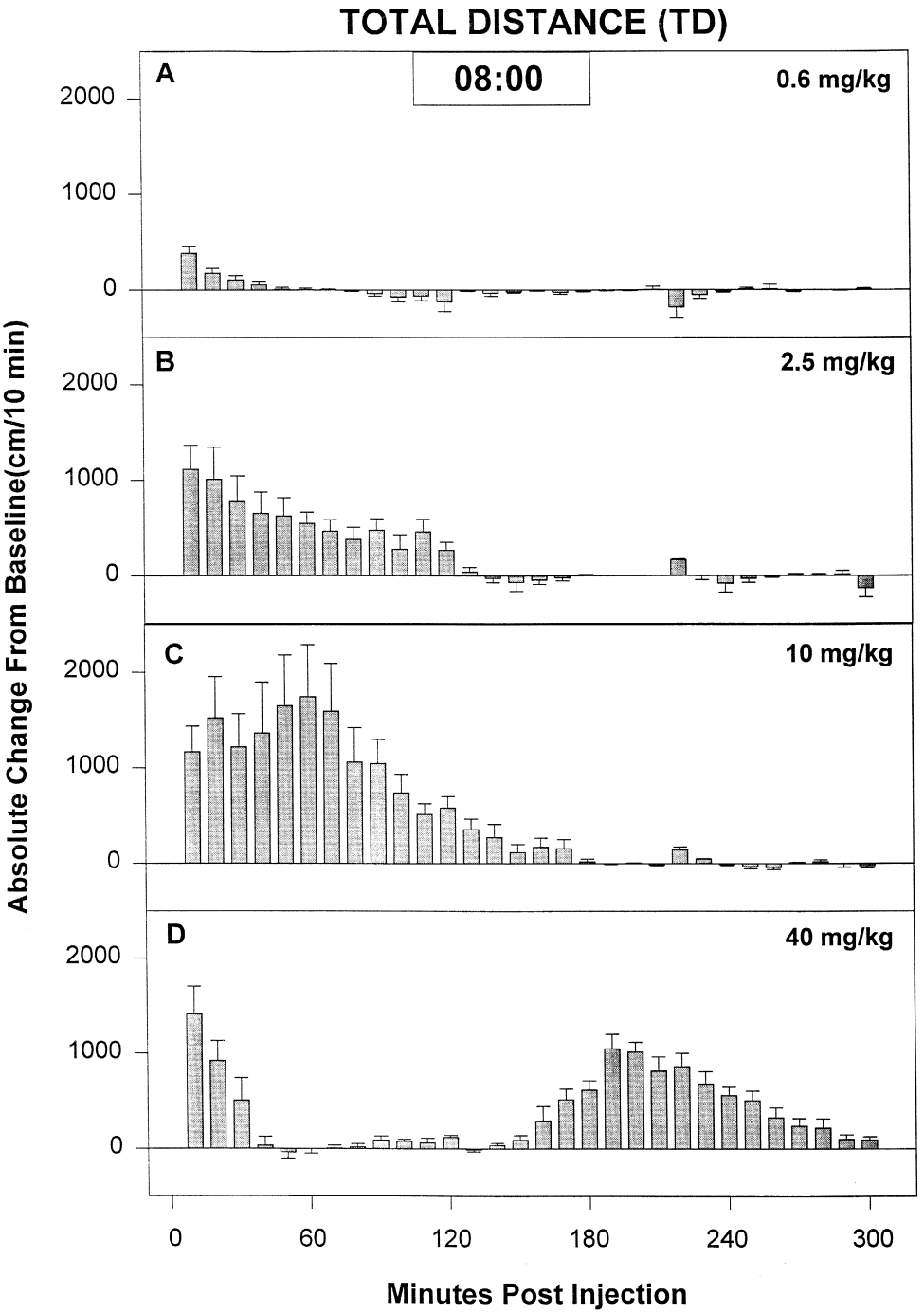

Fig. 4. Time course of effect on total distance for all four doses of methylphenidate (in mg/kg): 0.6 (A), 2.5 (B), 10 (C), and 40 (D); each $n = 8$, given one hour into the light cycle; 08:00. Total distance is presented as the mean ± S.E.M. per 10 min of the average increase in activity of each rat on the day of treatment (Day 3), relative to their own corresponding baseline values (Days 1 and 2).

phase, especially at 02:00 (Fig. 6C and D). The time course of effect for 40 mg/kg therefore appears to vary with time of administration.

3.3. Comparison between dose-related effects and time of administration

The largest increase in motor activity (E_{max}) above baseline (i.e., peak effect) was at 10 mg/kg (Fig. 4C) for each motor index except for NOS. The peak effect (E_{max}) for all four doses of MPD was the same at each time of administration for HA, TD, VA, and SA. By contrast, the E_{max} for NOS varied significantly across the times of administration ($F = 13.2$; $P < 0.001$), with a significant dose × time interaction ($F = 2.39$; $P < 0.05$). Post-hoc analysis revealed that the effect of MPD at 20:00 or 02:00 was less than that at 08:00 or 14:00 ($P < 0.001$). However, the time to the maximum effect (T_{max}), which increased

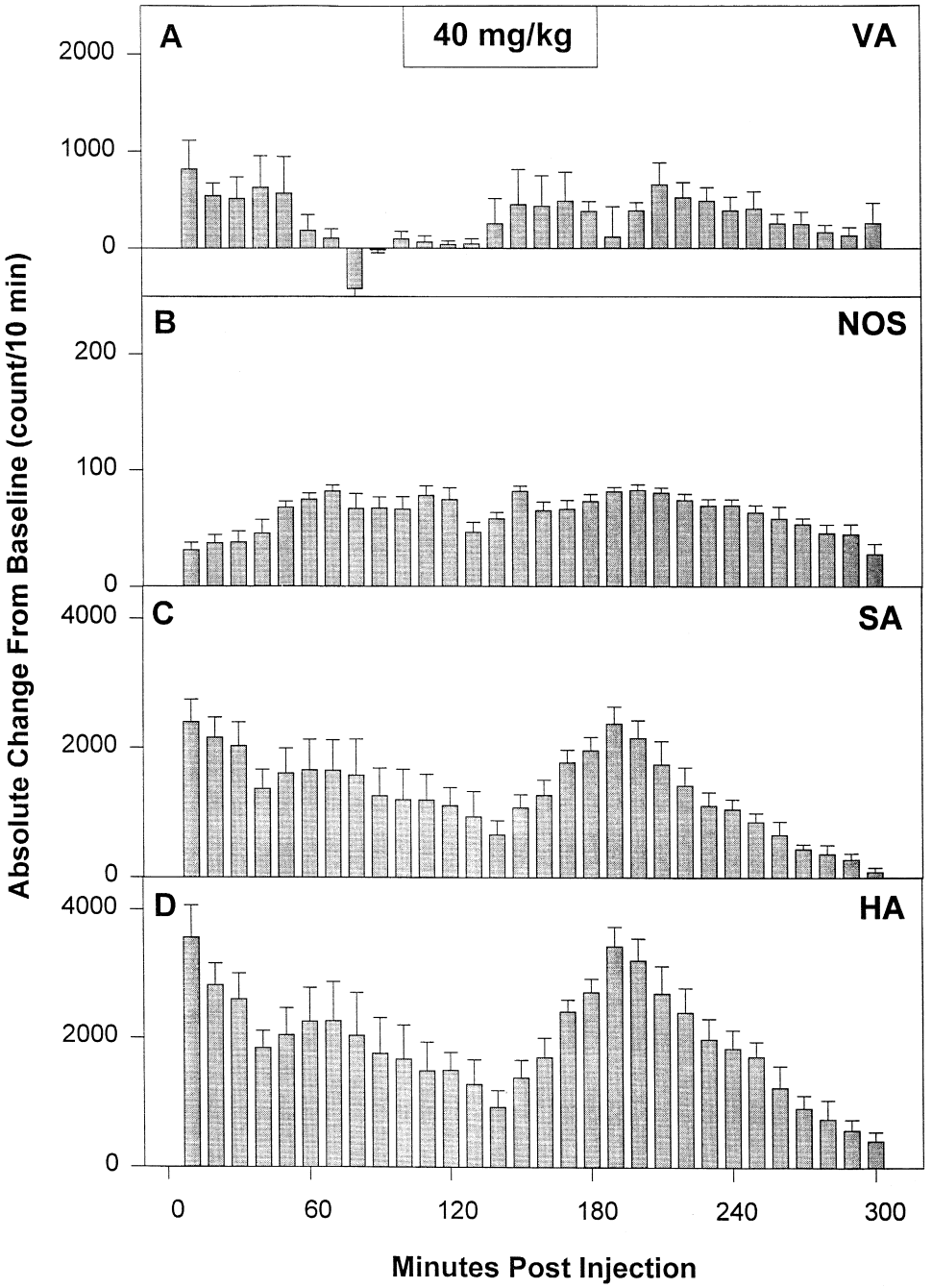

Fig. 5. Time course of the effect for 40 mg/kg of methylphenidate given at 08:00 ($n = 8$) on the indices of: (A) vertical activity (VA), (B) number of stereotypic movements (NOS), (C) stereotypic activity (SA), and (D) horizontal activity (HA). The data are presented as the means ± S.E.M. per 10 min 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).

with increasing dose as described earlier, was the same at all times of administration; i.e., each dose took the same amount of time to reach its peak effect at each time of administration.

The dose–response relationship of the absolute change in AUC (5 h) at the four times of administration is displayed in Fig. 7. In general, the dose–response for the AUC of MPD over the 5 h after drug administration displayed a linear, or exponential, relationship for all motor indices (HA, VA, SA, and NOS), except for TD, due to its longer duration of activity. HA, SA, and NOS (Fig. 7A–C) had a significant interaction between the effect of dose and time of its administration ($F = 2.97$; $F = 3.25$; and $F = 3.8$, $P < 0.001$, respectively for HA, SA, and NOS), indicating that the dose–response relationships changed throughout the day. Post-hoc analysis re-

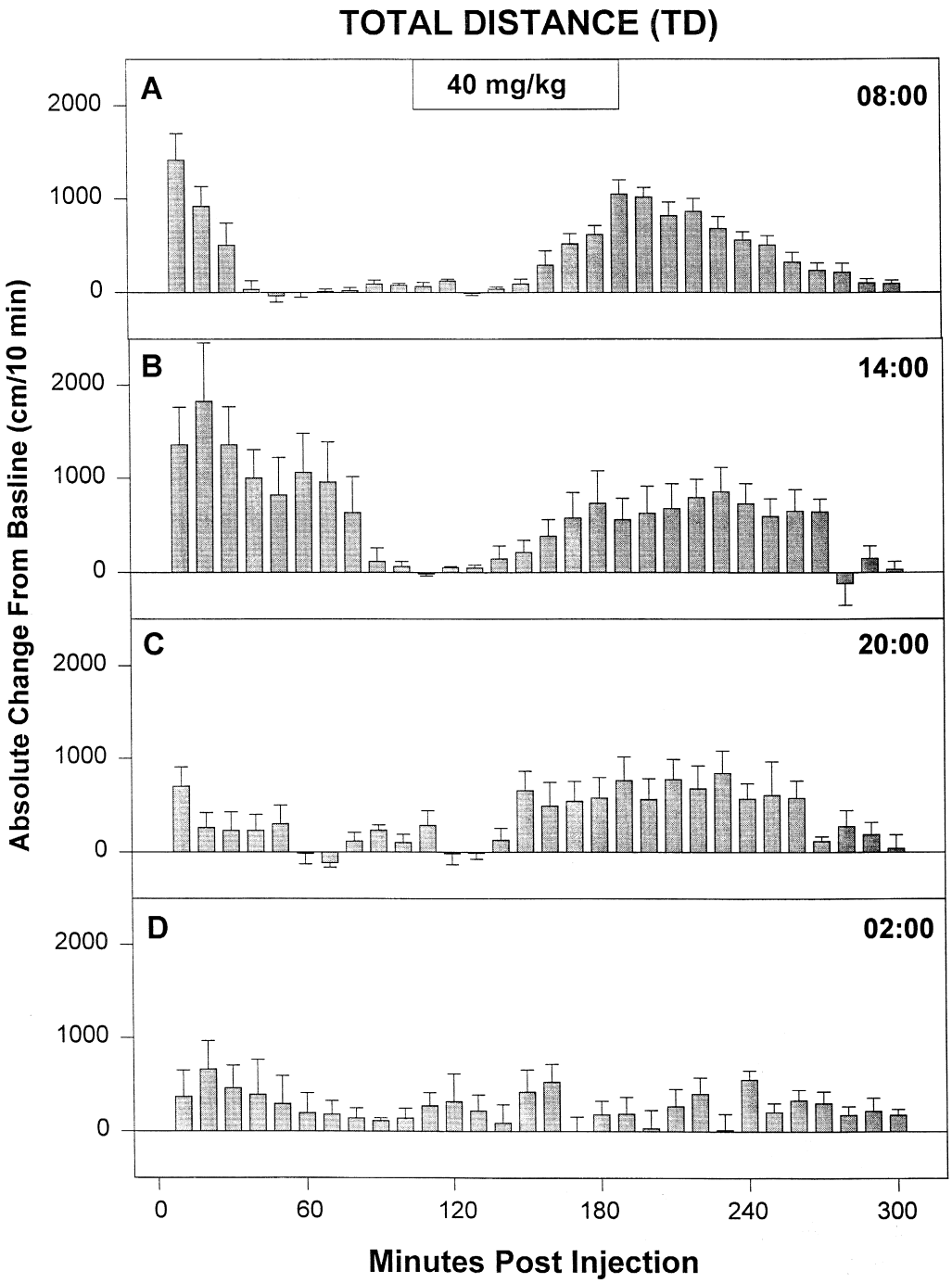

Fig. 6. Time course of effect on total distance for treatment groups given 40 mg/kg of methylphenidate at either (A) 08:00, (B) 14:00, (C) 20:00, or (D) 02:00 (each $n = 8$). The data are presented as the means ± S.E.M. per 10 min of the average increase in activity of each rat on the day of treatment (Day 3), relative to their own corresponding baseline values (Days 1 and 2).

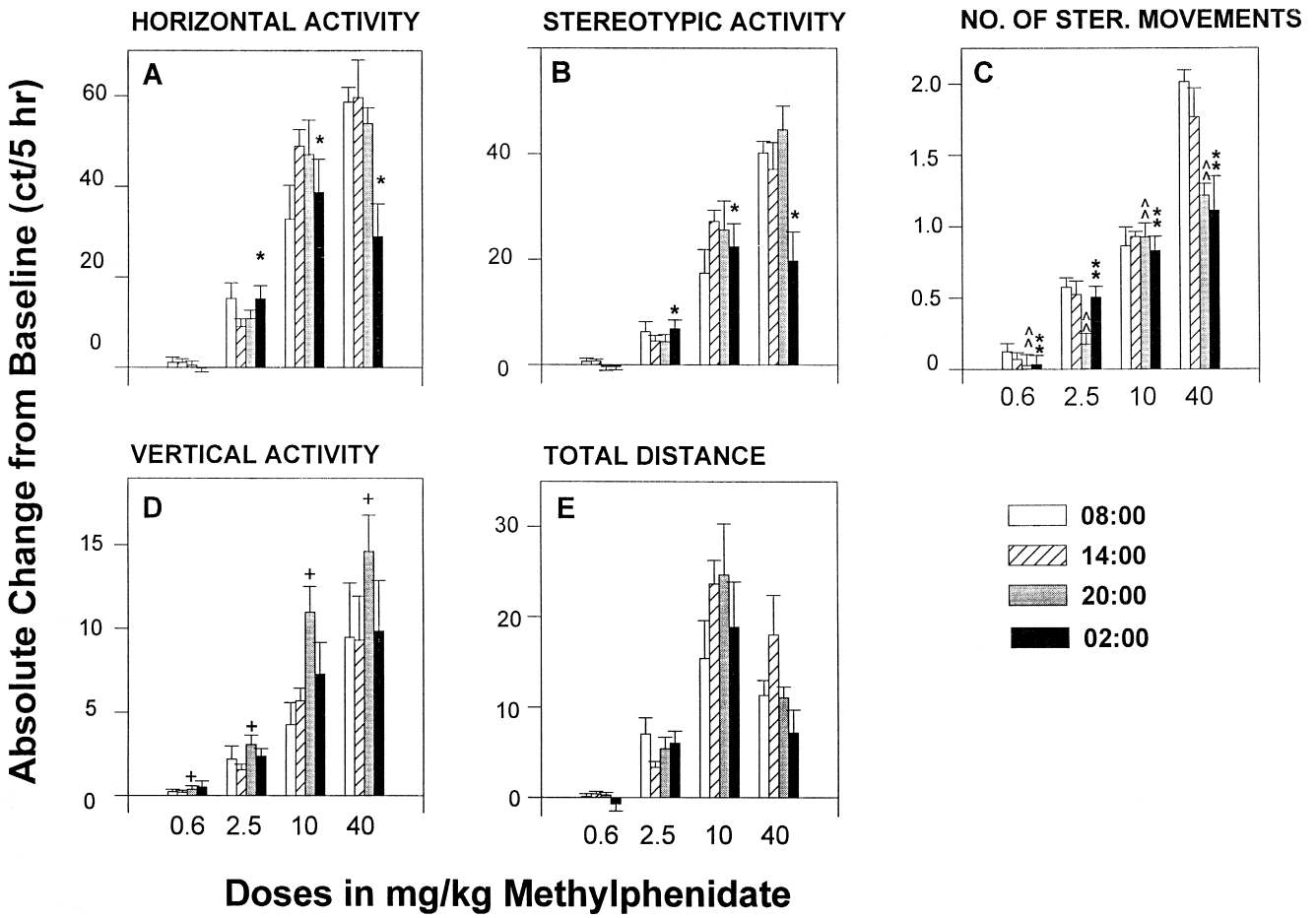

Fig. 7. The dose–response at all four times of administration in the area under the activity time curve for five hours after s.c. administration of methylphenidate (0.6, 2.5, 10, and 40 mg/kg; each $n = 8$) relative to their own corresponding baseline values (Days 1 and 2). Data are presented as the means ± S.E.M. in counts per 5 h for all five indices studied with baseline values arbitrarily set at 0. Significant differences in the absolute effect between one time group and the other three times of administration was evaluated using least square difference for pairwise comparison. * = ($P < 0.05$; 02:00 vs. other times); ** = ($P < 0.01$; 02:00 vs. 08:00 and 14:00); ^^= ($P < 0.01$; 20:00 vs. 08:00 and 14:00); + = ($P < 0.01$; 20:00 vs. other times for VA). Numerical values represent the original values divided by a factor of 1000.

vealed significantly lower dose-related increases for all three motor indices when MPD was given at 02:00 compared to the effect of MPD at the three other times of administration ($P < 0.01$). The dose–response relationship became more quadratic than linear at 02:00 due to the lower effect of 40 mg/kg at 02:00, and this probably accounted for the significant interaction between dose and time of administration (Fig. 7A–C). NOS was also different at 20:00 ($P < 0.01$) (Fig. 7C). VA showed a significant difference relative to time of administration ($F = 3.47$, $P < 0.05$), but there was no significant dose × time interaction (Fig. 7D). Post-hoc analysis revealed that the magnitude of effect was significantly ($P < 0.01$) greater at 20:00 than at any other time of administration. There was no difference in the dose–response of TD at any of the times of administration (Fig. 7E).

3.4. Persistent effect

Single factor ANOVA with repeated measures of pretreatment (days 1 and 2) and post treatment periods (day 4) was carried out to identify any persistent changes in activity caused by administration of MPD. This analysis revealed that none of the motor indices was significantly affected by any dose given at any time of administration. Therefore, the activity levels of the light and dark cycles of day 4 were indistinguishable from the baseline days for all the treatment groups.

4. Discussion

The main objective of this investigation was to determine whether the dose-related effects of MPD are different during the active (dark phase) and rest (light phase) periods. The only previous comparison of stimulant effects in the light and dark phase was done with amphetamine, and used continuous, rather than acute, drug administration [16].

Stimulants, including MPD, increase two different as-

pects of motor activity: locomotor and stereotypic behavior [9,37,46]. Some investigators have proposed that the stimulant-induced alterations of these two motor behaviors are competitively related, with an absence of forward locomotion and rearing during periods of intense stereotypy [44,46]. Distinct brain regions have been implicated in the locomotor [22,23,47] and stereotypic effects of amphetamine and related stimulants [3,24,25]. This study shows that the locomotor and stereotypic effects of stimulants can be differentiated further on the basis of their sensitivity to changes in the time of drug administration. The general stereotypic effects produced by MPD injection appeared to be time dependent and differed between the light and dark phase. Yet, despite the great difference in baseline activity between the light and dark phase, the locomotor effect of MPD was similar at each time of administration.

Due to the large differences in the level of spontaneous motor activity between the active and inactive periods, it was necessary to minimize factors that could lead to variability. This study was, therefore, designed as follows: (1) data collection was based on computerized recording of motor behavior [5,13], circumventing problems of direct human observation that may include inconsistent behavioral definitions, inter and intra observer reliability, and fatigue [4,7,12,39,40]; (2) Data were recorded for prolonged periods (i.e., 2 days) throughout the light and dark cycle to establish a stable and reliable baseline, rather than over a 'brief' 1–3 hour period [21,26,31]; (3) each animal served as its own control, thus providing comparison of treatment effect to a time-matched average baseline for the same animal, rather than comparison between two separate groups of rats or brief pre-treatment observations of the same group; and (4) multiple indices of locomotor behavior were used, since effects of stimulants on motor behavior are complex [5,36].

All five motor indices of activity studied in this experimental protocol displayed consistent baseline levels and circadian patterns of activity over the course of the study (Fig. 1). The effects of a drug can therefore be compared within each rat to its own time-matched baseline, and any changes can be considered an effect of the drug and not of fluctuations over time. The time control group revealed that the ratio between the motor behavior during the inactive (light) to the active (dark) period of the rat is not the same for all the motor indices studied. The increase in forward locomotion and rearing (i.e., TD and VA) during the dark cycle is greater than that of the other motor indices measuring general stereotyped behavior (i.e., SA and NOS; Fig. 2). Therefore, the relative contribution of stereotypic behavior (SA and NOS) to spontaneous motor activity (HA) is greater during the light than during the dark cycle. This indicates that during the light phase, where the episodes of sleep can be clearly seen (Fig. 1C), minimal forward ambulation and rearing are occurring, and the occasional increases in activity which are seen during the light phase (i.e., interruptions of sleep) are more likely caused by repetitive behavior such as grooming.

Saline and the lowest dose of MPD studied had no effect on motor activity. All other MPD doses exhibited dose–response characteristics at 08:00 that were similar to those reported for other stimulants given during the light cycle [20,27,46]. Despite the large difference in the level of spontaneous motor activity before drug administration, the locomotor activating doses of MPD (2.5 and 10 mg/kg) showed no change in their effect throughout the day. Therefore, the locomotor effects of low MPD doses are not dependent on the time of administration.

Comparison of the dose–response relationships of the E_{max} and the 5 h AUC of MPD at all times of administration, however, revealed differences in the stereotypic effect between the light and dark cycles. Four observations support this conclusion. First, only the motor indices affected by stereotyped activity (i.e., NOS, SA, and HA) displayed significantly altered dose–response characteristics when the drug was given at 02:00. The diminished effect of 40 mg/kg of MPD given at 02:00 created a more quadratic relationship, as opposed to the linear relationship of these indices at all the other times of administration (Fig. 7A–C). Second, administration of MPD had a lower maximal effect (E_{max}) on NOS when given at 02:00 or at 20:00. Third, the time course of effect of 40 mg/kg on TD was different between the light and dark cycle, with the focused 'stereotypy phase' less apparent during the dark cycle (Fig. 6). Finally, if the data from the 40 mg/kg dose groups is removed from the two-factor ANOVA, the significant difference between times of administration is lost. Therefore, stereotypic response to MPD appears to have a circadian rhythmicity, with a smaller effect during the dark cycle than during the light cycle, especially at 02:00. Although speculative, differences in the effects of MPD throughout the day point to the possibility that differences in the time of drug administration may play a role in the amount or type of sensitization produced by repeated administrations of stimulants. Studies testing this hypothesis are warranted.

These results allow for the separation of MPD's effect on forward ambulation, rearing, and general stereotyped behavior, based on their susceptibility to changes in the time of drug administration. A possible explanation for this difference in time dependencies arises from the combination of previous lesion experiments with a recent microdialysis study on the levels of extra cellular dopamine in different brain regions in the spontaneously active rat. Lesion studies have shown that the stereotypic effects of stimulants are associated with substantia nigra and striatum, while locomotor effects involve the nucleus accumbens [3,22–25,47]. Moreover, Paulson and Robinson [35] 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, the change in the stereotypic effect and rearing may be related to the change in dopamine levels throughout the day in the striatum, while the consistency of MPD's effect on forward ambulation may reflect the lack of change in dopamine levels in the nucleus accumbens.

Another possible explanation of these findings might be a ceiling effect on locomotor stimulation by MPD. Yet, the increase caused by 10 mg/kg of MPD at 20:00, when baseline activity was highest, was exactly the same as after administration during the light cycle. Furthermore, the amount of activity after drug administration was much higher than in the untreated rat. A ceiling effect cannot, therefore, explain why the locomotor effect of MPD is the same at each time of administration.

If stereotypic and locomotor effects are competitively related [44,46], the observation that 40 mg/kg MPD injected during the dark phase elicits less stereotyped behavior than the same dose given during the light phase could be explained by the proportionately greater increases in the level of locomotor versus stereotypic behavior during the dark cycle. The competitive nature of the focused stereotypy phase and forward ambulation was clearly apparent in the multiphasic response pattern of TD following 40 mg/kg MPD injection at 08:00, but the same cannot be said for MPD's effect on TD during the dark phase, especially at 02:00 (Fig. 6). It is important to keep in mind, however, that a different stereotyped response pattern may be occurring after administration of 40 mg/kg at 02:00 that is not competitively related to forward ambulation, and these results only suggest that there may be a lower stereotypic effect at 02:00. Studies using qualitative descriptive techniques are now warranted and will be necessary to completely characterize the differences in the stereotypic response during the dark cycle.

The relationship between stereotyped behavior and rearing is less clear, because rearing is part of both the locomotor and stereotypic effect of stimulants [38,46]. The fact that the dose–response of AUC for VA was linear (Fig. 7D) while that of TD was quadratic (Fig. 7E), along with the shorter duration of focused stereotypy in the time course of VA vs. TD at 08:00 (Fig. 5A and Fig. 4D), show clearly that, at least with MPD, focused stereotypy is not as inversely related to rearing as it is to forward ambulation. Moreover, the absolute magnitude of MPD's effect on rearing was significantly greater at 20:00 ($P < 0.01$) than at any other time (Fig. 7D), and this increase in VA was not accompanied by a change in the other motor indices. While the explanation for this is not clear, this finding weakens the possibility that the lower stereotypic effect during the dark cycle is caused by competition between stereotypic and locomotor behavior.

There were no significant persistent effects (i.e., 12–36 h post injection) on the motor indices studied, regardless of the dose used or the time of drug administration. Therefore, a single injection of MPD did not appear to influence the activity levels, or the circadian pattern of locomotor activity, of rats on the day after injection.

In summary, this study revealed that the effects of high doses of MPD on general stereotypic behavior were dependent on the time of administration, with a lower stereotypic effect during the dark phase than during the light phase. However, the locomotor effects elicited by lower doses of MPD were similar throughout the day. Whether these differences in acute effect throughout the day will lead to differences in the process of sensitization, or other adaptations to stimulant treatment, remains to be determined.

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