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Prolonged methylphenidate treatment alters the behavioral diurnal activity pattern of adult male Sprague-Dawley rats

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ABSTRACT

Methylphenidate (MPD) is becoming a drug of abuse among adult professionals and students, alike. Yet, few studies have investigated its long-term effects on the adult population. We hypothesized that prolonged administration of MPD leads to changes in the diurnal horizontal activity (HA) pattern, an effect persisting beyond acute drug effects. Four groups of adult male Sprague-Dawley rats ($N=32$) were divided into a saline/control, 0.6, 2.5, or 10.0 mg/kg MPD group. Each group was treated with saline on experimental day 1, followed by six consecutive days of designated treatment (days 2–7), then, after three consecutive days of washout (days 8–10), each group was re-challenged with its respective treatment (day 11). Activity was monitored continuously throughout the 11 experimental days. There was a dose-dependent increase in HA in the first hour post-injection. The 0.6 mg/kg MPD group exhibited changes in diurnal activity pattern only during the wash-out period. The 2.5 mg/kg MPD group exhibited the most profound changes in HA after 6 days of continuous injection, washout, and MPD re-challenge ($p<0.05$, $p=0.001$, $p<0.001$) respectively, and the 10.0 mg/kg MPD group exhibited changes during the washout and re-challenge periods ($p<0.01$, $p<0.001$), respectively. In conclusion, prolonged administration of MPD modulated the diurnal HA pattern in a dose-dependent manner.

1. Introduction

Nearly all animals have endogenous clocks that rhythmically synchronize physiological functions with their environments. In mammals, endogenous oscillators regulating their circadian rhythm activities include the suprachiasmatic nucleus (SCN) of the hypothalamus. The SCN is synchronized by external cues (zeitgebers), such as the light/dark cycle, (Bear et. al., 2007; Carpenter and Grossberg, 1985; Minors and Waterhouse, 1986) from the retina via the retino-hypothalamic tract (Moore, 1988; Reppert and Weaver, 2001). This synchronization allows the animal to be more metabolically and physiologically stable; any deviation from its normal circadian rhythm results in pathological conditions that require resynchronization or synchronization to the new rhythm (Carpenter and Grossberg, 1985; Wang et al., 2006). Mammalian circadian rhythm activities are cyclical variations that match physiological and behavioral requirements, maintaining homeostasis, or physiological stability despite variations in internal and external conditions (Ader and Friedman, 1968; Dafny et al., 1973; Luce, 1971).

Psychostimulants may modify circadian rhythm activity ((Marchant and Mistlberger, 1995; Wideman et al., 2005; Wang et al., 2006) by altering expression of clock genes (Abarca et al., 2002; Uz et al., 2003; Schulz, 2006). Chronic treatment with psychostimulants, including cocaine, amphetamine and methylphenidate (MPD), exerts long-term effects including tolerance, withdrawal, and sensitization (Wolf, 1998; McDougall et al., 1999; Izenwasser and French, 2002; Dafny and Yang, 2006; Askenasy et al., 2007). However, effects of MPD on the circadian or diurnal rhythm activity pattern have not been reported. Therefore, we have investigated effects of acute and repeated MPD administration on the diurnal pattern of locomotor activity, and have compared this to its effects on motor activity. The primary hypothesis of the study is that doses of MPD that alter motor activity will also alter its diurnal pattern.

2. Method

2.1. Animals

Thirty-two adult male Sprague-Dawley rats weighing 170–180 g on purchase day, were each housed individually. The home cages before and during the experiment were in a sound-attenuated room with an ambient temperature of 21±2 °C and relative humidity of 37–42%; food pellets and water were provided ad libitum. The room was illuminated on a 12:12 light/dark cycle (light on at 06:00). The initial 5–7 days were used for acclimation. On the last day of the acclimation period, the rats were weighed and randomly divided into four groups: saline/control

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Table 1
Experimental protocol

	Experimental day (s)			
	1	2–7	8–10	11
Experimental group				
Control	Saline	Saline	Washout	Saline
1	Saline	0.6 mg/kg MPD	Washout	0.6 mg/kg MPD
2	Saline	2.5 mg/kg MPD	Washout	2.5 mg/kg MPD
3	Saline	10.0 mg/kg MPD	Washout	10.0 mg/kg MPD

For each experimental group $n=8$. All injections were given in the morning between 06:30 h and 07:00 h. MPD was dissolved in a 0.9% isotonic saline solution and dosages were calculated as free base. Injections were given intra-peritoneally (i.p.) and equalized to a volume of 0.8 cc with 0.9% saline. All of the saline injections consisted of an i.p. 0.8 cc isotonic saline solution (0.9% NaCl) administered i.p.
Abbreviation: MPD-methylphenidate.

($n=8$); 0.6 mg/kg MPD ($n=8$), 2.5 mg/kg MPD ($n=8$), and 10.0 mg/kg MPD ($n=8$). Each rat was then placed back into to its home cage, labeled as to experimental group, and allowed to acclimate for an additional 24 h before initiation of the experimental protocol.

2.2. Drugs

Methylphenidate hydrochloride (MPD) was obtained from Mallinckrot (Hazelwood, MO). MPD was dissolved in a 0.9% isotonic saline solution and dosages were calculated as free base. Injections were given intra-peritoneally (i.p.) and equalized to a volume of 0.8 cc with 0.9% saline, so that the volume of each injection was the same for all animals. Vehicle injections consisted of 0.8 cc isotonic saline solution (0.9% NaCl) administered i.p. Previous dose response experiments in this laboratory showed that MPD doses below 0.6 mg/kg i.p. had no effect on locomotor activity (Gaytan et al., 1997), while doses of 0.6, 2.5 and 10.0 mg/kg elicited no effect, sensitization, and tolerance, respectively (Gaytan et al., 1997; Yang et al., 2001, 2003, 2006). Therefore, 0.6, 2.5, and 10.0 mg/kg of MPD doses were used in this study. In rodents, MPD doses between 0.5 and 3.5 mg/kg i.p. were reported to promote peak plasma concentrations within the typical clinical usage range (Kuczenski and Segal, 2002). The range of 5.0–10.0 mg/kg MPD i.p. is considered moderate; above 10.0 mg/kg i.p. is considered a high dose (Solanto, 1998, 2000; Kollins et al., 2001; Brandon and Steiner, 2003). As rodents metabolize MPD more rapidly than humans do, in the present study 0.6, 2.5, and 10.0 mg/kg MPD correspond to low, medium and high dose, respectively (Dafny and Yang, 2006). Furthermore, in our previous dose-response study of four different injection times indicates that injection in the morning exerts the most significant effects (Gaytan et al., 2000). In this study, all injections were made between 06:30 h and 07:00 h (Table 1).

2.3. Procedure

Sixteen open field cages (40.5×40.5×31.5 cm each) located in the animal/experimental room were used to record the locomotor activity continuously for 11 days, using the computerized animal activity monitoring system (CAAM; AccuScan Instruments, Inc.,Columbus, OH), except during cleaning and animal handling between 06:30 h and 07:00 h. The CAAM system consisted of two arrays of 16 infrared beams (crisscrossing) and their sensors (2.5 cm apart) were placed at 6.0 and 12.5 cm from the cage floor, respectively. Beam interruptions over each 10 min period throughout a 23.5 h period were summed by an Accuscan Analyzer (Accuscan Instruments Inc. Columbus, OH.) and transferred to a PC to be sorted into their respective hours using the Oasis program.

The experimental protocol, as summarized in Table 1, was: On experimental day 1, all animals were injected with saline; on experimental days 2 through 7, each group was injected with a single injection of either saline, 0.6, 2.5, or 10.0 m/kg MPD, respectively, followed by 3 days of washout (experimental days 8–10); then on experimental day 11, a re-challenge injection of saline or MPD was given, similar to the dose given on experimental days 2 through 7 for each experimental group. Each group was treated with the same treatment and volume (0.8 cc) on all the injection days (Table 1) between 06:30 h and 07:00 h. As noted above, the experimental room was opened for approximately one half hour each day and the recording sessions then continued uninterrupted from 07:00 h until 06:30 h the following day. The experiment was carried out in accordance with the guidelines of the NIH and the declaration of Helsinki, and approved by our local Animal Welfare Committee.

2.4. Data analysis

Two forms of data analysis were performed to evaluate the dose response effects of the three MPD doses. The locomotor HA counts

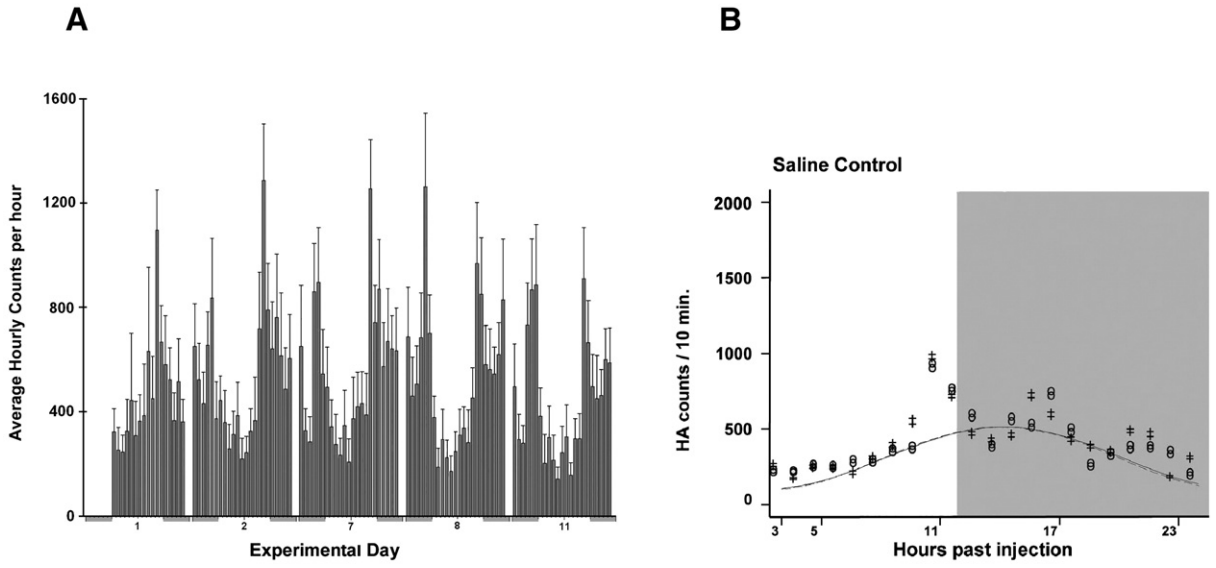

Fig. 1. Horizontal activity counts and Cosine Curve Statistical Analysis graph of control group prior to saline or MPD injection.

Table 2
Cosine Curve Statistical Analysis values of the parameter estimates for horizontal activity during each experimental day

	Experimental day	*N*	MESOR	Amplitude	Acrophase (time)
Control					
Saline	1	139	307.64	200.15	13.59
	2	139	316.89	184.07	13.78
		Significance (*p* value)†	NS	NS	NS
Acute phase					
0.6 mg/kg MPD	1	139	282.50	130.52	12.74
	2	121	288.02	178.30	13.83
		Significance (*p* value)†	NS	NS	NS
2.5 mg/kg MPD	1	139	298.84	108.50	12.01
	2	121	280.50	161.86	12.51
		Significance (*p* value)†	NS	NS	NS
10.0 mg/kg MPD	1	139	341.00	180.15	10.92
	2	121	301.19	198.07	12.00
		Significance (*p* value)†	NS	NS	NS
Induction phase					
0.6 mg/kg MPD	2	121	288.02	178.30	13.83
	7	121	314.95	270.58	13.92
		Significance (*p* value)†	NS	NS	NS
2.5 mg/kg MPD	2	121	280.50	161.86	12.51
	7	121	316.50	220.50	13.90
		Significance (*p* value)†	NS	NS	<**0.05**
10.0 mg/kg MPD	2	121	301.19	198.07	12.00
	7	121	282.44	208.30	12.96
		Significance (*p* value)†	NS	NS	NS
Washout Phase					
0.6 mg/kg MPD	1	139	282.50	130.52	12.74
	8	121	355.17	236.59	14.31
		Significance (*p* value)†	**0.01**	**0.01**	NS
2.5 mg/kg MPD	1	139	298.84	108.50	12.01
	8	121	334.08	251.53	14.39
		Significance (*p* value)†	NS	<**0.01**	**0.001**
10.0 mg/kg MPD	1	139	341.00	180.15	10.92
	8	121	322.10	290.66	13.06
		Significance (*p* value)†	NS	<**0.01**	NS
Expression phase					
0.6 mg/kg MPD	2	121	288.02	178.30	13.83
	11	121	300.98	267.45	14.57
		Significance (*p* value)†	NS	**0.05**	NS
2.5 mg/kg MPD	2	121	280.50	161.86	12.51
	11	121	350.17	237.59	14.73
		Significance (*p* value)†	**0.001**	<**0.05**	<**0.001**
10.0 mg/kg MPD	2	121	301.19	198.07	12.00
	11	121	447.31	185.88	14.00
		Significance (*p* value)†	<**0.001**	NS	<**0.01**

All values of the parameter estimates are means of the *N* value fore the respective experimental day. *N* is the measured horizontal activity per 10 min for the entire experimental day; MESOR is the average daily horizontal activity; amplitude is the peak activity for each day, and the acrophase is the time at which the peak activity occurred for each experimental day. Data in bold are the significant *p* values when the above two values are compared.
Abbreviations: MPD–methylphenidate; MESOR– midline estimate of rhythm, NS– not significant.
† Significance for a difference at alpha <0.05.

were summed every 10 min into 10-min bins using the Accuscan Analyzer; six such bins were summed to their respective hour to produce an hourly histogram with their standard errors (±2 S.E.). The mean and ±2 S.E. were used to analyze the histograms and compared between the experimental days. The second evaluation used the 10 min bins for statistical analysis of daily activity patterns, using the Cosine Curve Statistical Analysis (CCSA) system (Bingham et al., 1982). The CCSA statistically parameterizes the 24 hourly activity means and S.E. into three model parameter estimates. The rhythm is parameterized by estimates of: 1) MESOR (Midline Estimate of Rhythm), measuring the average horizontal activity (HA) per day; 2) Amplitude (distance from the MESOR to highest point of the approximating curve), which measures the peak level of activity in a day; and 3) Acrophase (time of the cosine approximation), which provides the time of each day's peak activity. The parameterization provides accurate representation of any significant shifts in HA diurnal rhythm pattern that may have occurred during the treatment. The HA of the control group was analyzed for consistency throughout the experimental period. The drug effect was divided into four phases by comparison of the HA on each experimental day: 1) The acute phase: data obtained following MPD injection on experimental day 2 was compared to data obtained from experimental day 1, post saline injection; 2) The induction phase: experimental day 7 vs. experimental days 2; and 3) The washout phase: experimental day 8 vs. experimental days 1; and 4) The expression phase: experimental day 11 vs. experimental day 2. Histograms of the mean and standard deviation of the HA of each experimental group were used to observe changes in the diurnal rhythm pattern (Fig. 1A). A second analysis determined whether the pattern of activity differed from the baseline period during any of the MPD treatment phases.

3. Results

3.1. Control

Fig. 1A summarizes the hourly HA counts for experimental days 1, 2, 7, 8, and 11 of the saline control group ($n=8$) and shows, as expected from nocturnal animals, a rhythmic diurnal activity pattern, with an increase in locomotion during the dark period (night) and decreased locomotion during the light period (day). The CCSA indicated that the MESOR, amplitude, and acrophase of the HA pattern for the 24 h during all of the experimental days followed the same general pattern, with minor nonsignificant fluctuations. For the purpose of simplifying data presentation of the control experimental days, and since all days of saline treatment had a similar diurnal pattern of HA, Fig. 1B and Table 2 present the statistical comparison between experimental days 1 and 2 only. Any significant day-to-day deviations in the CCSA parameters during the control days were attributed to MPD. Furthermore, since the activity pattern in animals treated with saline remained consistent across all eleven days of treatment, we assumed that baseline HA was stable across the treatment period, so experimental day 1 (after saline injection) of the 3 MPD dose groups (0.6, 2.5, and 10.0 mg/kg) served as baseline relative to all phases for its respective group.

3.2. Acute phase (comparing experimental day 2 to experimental day 1)

The acute effect of MPD at doses 0.6 mg/kg, 2.5 mg/kg, and 10.0 mg/kg was evaluated by comparing data obtained on experimental day 2, after MPD injection, to that of experimental day 1, saline control (Fig. 2). The acute effects of MPD immediately after injection (first 2 h) as well as the effect on rhytmicity over the entire 23.5 h experimental period post drug injection were evaluated. Fig. 2 and Table 2 present the CCSA findings for the dose effect during the acute phase.

There was a dose-dependent increase in activity immediately after injection. In the 2.5 and 10.0 mg/kg dose groups this effect was profound and lasted as long as three hours. This skewed the statistical parameterization of the CCSA, as the data points were poorly fit using a cosine curve. Therefore, in order to account for the immediate effect of MPD and to create a statistically appropriate cosine curve, the initial three hours of HA aftert MPD injection were eliminated. To maintain consistent parameterization and analysis of the protocol for 23.5 h

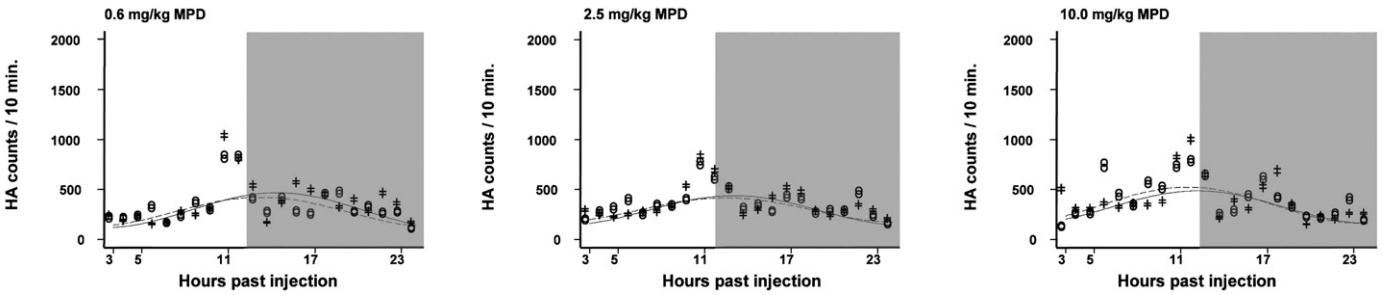

Fig. 2. Acute Phase of all MPD treated groups (Day 1 vs. Day 2).

activity patterns, all subsequent statistical contrasts using the CCSA test were based upon removal of the first three hours after injection of MPD.

After accounting for the immediate drug effect after injection, analysis of the parameter estimates of the CCSA indicated that there was no significant effect on the MESOR, amplitude, and acrophase of the HA pattern on experimental day 2 vs. day 1 at any dose of MPD.

3.3. Induction phase: (comparing experimental day 7 to experimental day 2)

To evaluate the effects of daily MPD treatment, MPD injections over six consecutive days simulated prolonged or repeated drug treatment. Experimental day 7, the last day of MPD maintenance treatment, was therefore compared to experimental day 2, the first day of MPD treatment (Table 1). There was a dose-dependent increase in HA during this period, as shown in Fig. 3 and Table 2.

After six consecutive days of MPD administration, the only significant CCSA effect was alteration of acrophase by 2.5 mg/kg MPD. The acrophase was shifted 1.4 h into the dark phase ($p<0.05$) on experimental day 7 (Table 2) compared to experimental day 2. There were no significant effects of 0.6 or 10.0 mg/kg MPD during this period.

3.4. Washout phase (comparison of experimental days 8–10 to experimental day 1)

HA recordings during the washout period (experimental days 8–10), after the six consecutive days of MPD injection, were compared to the HA recordings of experimental day 1. Fig. 4 and Table 2 present MPD effects during the washout phase and compare experimental day 8 to experimental day 1. Comparisons of experimental days 9 and 10 to experimental day 1 were similar.

CCSA analysis revealed that all three doses of MPD caused significant changes, albeit in different ways. All doses increased the amplitude but effects on timing of activity varied. Comparing experimental day 8 to experimental day 1, 0.6 mg/kg MPD caused a significant increase in the MESOR ($p=0.01$) and a 55% increase in the amplitude ($p=0.01$) on experimental day 8 (Fig. 4, Table 2). MPD at 2.5 mg/kg caused a significant shift in the acrophase (2.4 h into the dark period) and a 43% increase in the amplitude ($p<0.01$, $p=0.001$, respectively) on experimental day 8. 10.0 mg/kg MPD caused only a significant increase in the amplitude (62%, $p<0.01$) and did not significantly affect the MESOR or acrophase of the HA pattern.

3.5. Expression phase (comparison of experimental day 11 to experimental day 2)

The HA pattern on experimental day 11, after MPD re-challenge, was compared to that of experimental day 2, the day of the original MPD injection. Each group was re-challenged with its original dose of MPD. Fig. 5 and Table 2 present the results.

MPD at 0.6 mg/kg caused a significant increase in the amplitude of HA on experimental day 11 (67%, $p=0.05$), but did not cause any significant changes in the MESOR or acrophase. Re-challenge with 2.5 mg/kg MPD caused significant changes in all three CCSA parameter estimates (Fig. 5, Table 2), while at 10 mg/kg there was a significant increase only in the MESOR ($p<0.001$) and the acrophase ($p<0.01$) (Table 2).

CCSA parameters were compared across all phases and dose groups, and the following linear or curvilinear dose-response trends were indicated: 1) A linear decrease in acrophase ($p<0.01$) on the last day of the induction phase, suggesting that as the dose increased, the maximal activity (amplitude) began to occur earlier in the day throughout the six-day period of MPD administration. 2) An increasing curvilinear response for the amplitude ($p<0.05$) on the first day of the washout period implies that the degree of restlessness and increased motor activity was proportional to the dose previously administered. 3) Throughout the 11-days experimental period, HA not only began to increase earlier but was gradually exaggerated during the night period. While these trends may suggest dose-dependent changes, analysis of each phase and dose group revealed that 2.5 mg/kg MPD resulted in the most significant changes across all four phases. Comparing the four experimental phases, Fig. 6 summarizes the gradual and sequential increase in HA

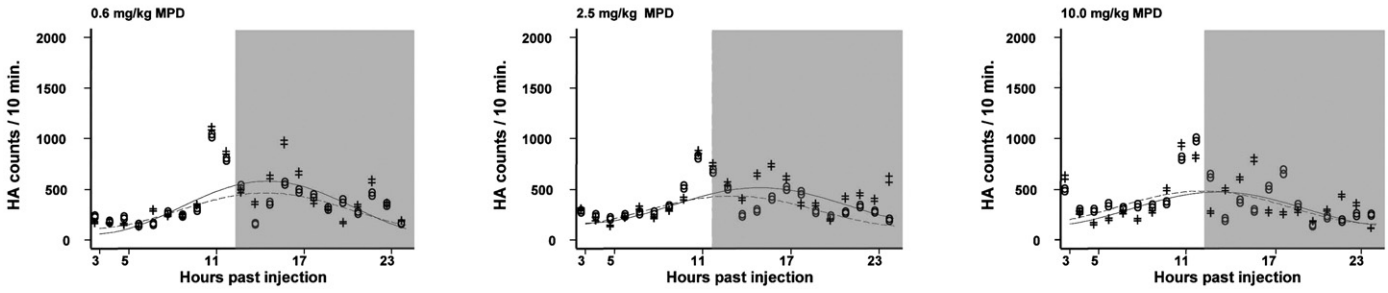

Fig. 3. Induction Phase of al MPD treated groups (Day 2 vs. Day 7).

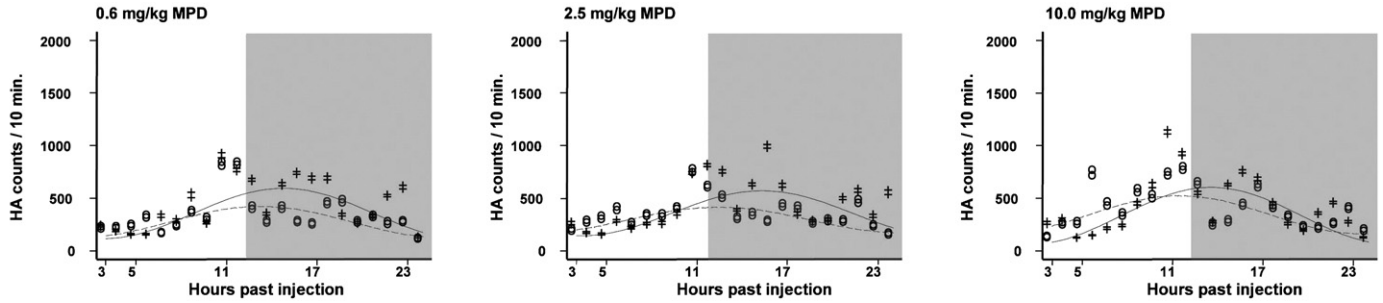

Fig. 4. Washout Phase of all MPD treated groups (Day 1 vs. Day 8).

and the shift in rhythmicity throughout the 11-day experimental period for animals given 2.5 mg/kg MPD.

4. Discussion

We examined the effects of repeated administration of 0.6, 2.5, and 10.0 mg/kg methylphenidate (MPD) on the diurnal horizontal activity pattern in rats. After 6 days of daily treatment, rats receiving only saline exhibited similar locomotor diurnal activity pattern in all experimental days, while the 0.6 mg/kg MPD group exhibited increased locomotor activity during the washout period, without change in the diurnal activity pattern. MPD at 2.5 mg/kg elicited the most profound changes in both the overall activity and its diurnal pattern. This suggests that MPD affects the locomotor activity diurnal pattern in a dose-dependent reverse U-shape pattern. This maximal effect of MPD at 2.5 mg/kg corresponds to the observation that optimal behavioral sensitization to MPD also occurs at this dose.

Considering the potential for over-prescription of MPD to patients with Attention Deficit Hyperactive Disorder (ADHD) (Levin and Kleber, 1995; Sagvolden and Sergeant, 1998; Zito et al., 2000; Accardo and Blondis, 2001; Wilens et al., 2003; Dafny and Yang, 2006; Askenasyet al., 2007) and its potential for abuse by patients with and without ADHD (Wilens et al., 2008), these findings have interesting implications regarding long-term effects of MPD. It will also be useful to understand relationships between effects of MPD and those of other stimulants on circadian rhythm of motor activity.

MPD is in the same class of psychostimulants as cocaine and amphetamine. When administered systemically, potency of MPD is comparable to that of cocaine and amphetamine (Parran and Jasinski, 1991; Massello and Carpenter, 1999). These psychostimulants are indirect dopamine (DA) agonists. MPD and cocaine bind to the DA transporter and inhibit DA reuptake from the synapse, acutely increasing synaptic DA. These drugs therefore potentially enhance the documented effects of DA on locomotion, cognition, emotion, and reward. Increased synaptic DA contributes to acute and long-term effects of MPD and other stimulants, including sensitization, tolerance, and dependence (Wolf, 1998; Laakso et al., 2002; Dafny and Yang, 2006; Askenasy et al., 2007). According to Laakso et al. (2002), all drugs of abuse lead to increases in extracellular concentrations of DA in relevant brain areas. It is likely that, in addition to their parallel effects on behavior, sensitization, and tolerance, stimulants will also share effects on diurnal rhythms involving motor activity.

The biological rhythms of organisms are genetically encoded and are influenced by the expression of specific genes, generally referred to as clock genes (Abarca et al., 2002; Wang et al., 2006). The expression of the clock genes may be modulated by certain drugs; Schulz (2006) describes the effects of prescribed and recreational drugs on the wake-sleep cycle in humans, and suggests that there is a link between pharmacological agents and the alteration of clock gene expression and circadian rhythm activity. Other reports suggest that psychostimulants modify the circadian rhythm activity pattern, resulting in differential expression of clock genes (Marchant and Mistlberger, 1995; Nikaido et al., 2001; Abarca et al., 2002; Uz et al., 2003; Wideman et al., 2005; Schulz, 2006; Wang et al., 2006). These changes in circadian rhythm pattern following drug administration are long-term effects, persisting beyond acute exposure to the drug like behavioral sensitization. Several studies have established a relationship between psychostimulants such as cocaine (Abarca et al., 2002; Uz et al., 2003; Wang et al., 2006) and methamphetamine ((Sokolov et al., 2003), and the expression of clock genes. Considering that MPD is in the same class as cocaine and amphetamine, it is reasonable that its effect on the diurnal HA pattern may be secondary to changes in the expression of clock genes resembling those reported with other psychostimulants.

Our data suggest that daily MPD treatment elicits dose-dependent changes in the diurnal rhythm activity patterns. Whether this redistribution of circadian activity pattern is due to changes in the expression of genes that have primary effects on motor activity or on DA levels remains to be established. In addition, since prolonged treatment of MPD caused a further increase in locomotion or sensitization, similar to cocaine and amphetamine treatment (Gaytan

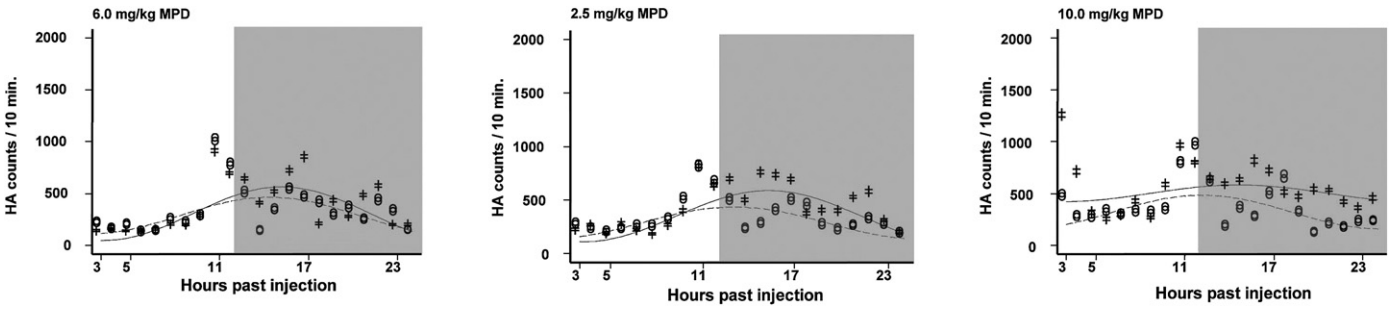

Fig. 5. Expression Phase of all MPD treated groups (Day 1 vs. Day 11).

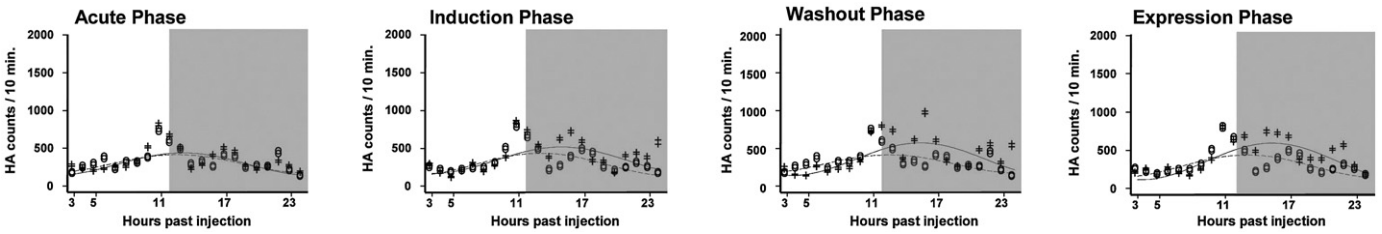

Fig. 6. Effect of the 2.5 mg/kg MPD dose throughout the experimental period.

et al., 1998), it is possible that psychostimulants not only inhibit uptake of DA by blocking the DAT but may have also induced production of the D1 and D2 receptors (Kleven et al., 1990). This increase of DA receptors may have caused the overall elevation in HA, leading to changes in the normal rhythm of motor activity.

Effects at 10.0 mg/kg suggest that MPD, like cocaine, can elicit either tolerance or sensitization, depending on dose. In the case of cocaine, this is influenced by whether the drug is given intermittently or continuously (Reith et al., 1987; Inada et al., 1992; Kunko et al., 1997; Izenwasser et al., 1999; King et al., 1992; Izenwasser and French, 2002), MPD tolerance and sensitization depends more exclusively on the dose administered. Izenwasser and French (2002) reported that tolerance and sensitization can exist simultaneously and support the idea that MPD may express both sensitization and tolerance at 10.0 mg/kg. Kleven et al. (1990) observed that repeated injections of cocaine caused long-term decreases in D1 binding sites and a transient decrease in D2 in some brain areas, while causing the same, but reversed effect, in others. This observation suggests that the decrease in DA receptor binding sites may be related to the induction of tolerance for cocaine as well as for higher doses (10.0 mg/kg) of MPD. Another possible explanation is that repetitive MPD treatment results in molecular or genetic changes of the clock genes. Abarca et al. (2002) and Uz et al. (2003) reported that genetic changes in the expression of the mouse clock gene *period 1* (*mPer1*) occurs following cocaine administration. According to their observations, the level of expression of mPer1 dictates whether sensitization or tolerance develops. Mice with mutant mPer1 lack cocaine sensitization, and also did not develop tolerance. This may also be the case with MPD in high doses, as the rat *mPer1* gene is modified causing tolerance but expression of the gene is restored during the withdrawal period and causes sensitization upon re-challenge.

5. Conclusion

After 6 days of daily treatment with vehicle or MPD at 0.6, 2.5, or 10.0 mg/kg, the saline-control exhibited similar diurnal locomotor activity pattern in all experimental days, while the 0.6 mg/kg MPD group exhibited increased locomotor activity in the washout period, without affecting the diurnal activity pattern. In the 2.5 and 10.0 mg/kg dose groups, the 2.5 mg/kg group exhibited the most profound changes in both the general activity and the diurnal pattern. This suggests that MPD modulates the locomotor activity rhythmic pattern biphasically, in a dose-dependent reverse U-shape pattern. Withholding the drug after 6 daily MPD administrations also exhibited a dose-dependent increase in locomotion in all rats given MPD. At 2.5 mg/kg MPD, there was a shift in the locomotor pattern, suggesting that MPD may have induced a withdrawal effect. Given the widespread human use of MPD, it will be important to determine relationships between these effects and other long-term adaptations to MPD treatment.

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