Published in final edited form as:

J Neural Transm. 2013 December ; 120(12): 1717–1731. doi:10.1007/s00702-013-1063-4.

Adult Female Rats Altered Diurnal Locomotor Activity Pattern Following Chronic Methylphenidate Treatment

T. Trinh, S Kohllepin, P.B. Yang, K.D. Burau, and N. Dafny

Abstract

Methylphenidate (MPD) is one of the most prescribed pharmacological agents and also used as cognitive enhancement and for recreational purposes. The objective of this study was to investigate the repetitive dose-response effects of MPD on rhythm locomotor activity pattern of female WKY rats and compare to prior study done on male. The hypothesis is that change in the circadian activity pattern indicates a long-lasting effect of the drug. Four animal groups (saline control, 0.6, 2.5, and 10.0 mg/kg MPD dose groups) were housed in a sound-controlled room at 12:12 light/dark cycle. All received saline injections on experimental day 1 (ED 1). On EDs 2-7, the control group received saline injection; the other groups received 0.6, 2.5, or 10.0 mg/kg MPD, respectively. On ED 8-10, injections were withheld. On ED 11, each group received the same dose as EDs 2-7. Hourly histograms and cosine statistical analyses calculating the acrophase (ϕ), amplitude (A), and MESOR (M) were applied to assess the 24-hour circadian activity pattern. The 0.6 and 2.5 mg/kg MPD groups exhibited significant ($p < 0.05$) change in their circadian activity pattern on ED 11. The 10.0 mg/kg MPD group exhibited tolerance on ED 11 and also a significant change in activity pattern on ED 8 compared to ED 1, consistent with withdrawal behavior ($p < 0.007$). In conclusion, chronic MPD administration alters circadian locomotor activity of adult female WKY rats and confirms that chronic MPD use elicits long lasting effects

1.0 Introduction

The circadian rhythm regulates many aspects of mammalian physiology and behavior and is an integral part of life for all bodily functions. It is seen in a range of life forms from the subcellular organelles to the complex human body (Welsch et al. 1995, Moore 1983). The suprachiasmatic nucleus (SCN) is widely accepted as the master clock (Moore 1983, Reid and Burgess 2005, Sujino et al. 2003, Yamazaki et al. 2000) and expresses several clock genes such as *Per1*, *Per2*, *Cry1*, *Cry2*, *bmal1*, *Rev-erb* (Klein et al. 1991, Reppert and Weaver 2002). This master clock also dictates the circadian rhythm and synchronizes multiple internal clocks governing physiological processes such as hormonal release, heart rate, blood pressure, eating pattern, and sleep/wake cycle (Bittman et al. 2003, Janssen et al. 1994, Mistlberger 2005) by entraining to the light/dark cycle via the retinohypothalamic tract (Benstaali et al. 2001, Cermakian and Sassone-Corsi 2002, Johnson et al. 1988, Moore and Lenn 1972). In addition to environmental cues, pharmacological agents such as psychostimulants have been shown to have effects on diurnal activity differences. For example chronic EtOH alters *Per1* and *Per2* gene expression in hypothalamus and *Per2* and

Per3 in SCN (Chen et al. 2004, Manev and Uz 2006) causing sleep-wake abnormalities and depression (Vitaterna et al. 2001). Methamphetamine alters striatal Per1 and Per2 genes causing a shift from nocturnal to diurnal rhythms (Iijima et al. 2002). Methylphenidate has been shown to delay the SCN electrical activity, resulting in altered sleep and circadian rhythmicity (Antle et al. 2012). Disruption of circadian rhythm can result in short term conditions, such as jet lag, fatigue, insomnia, and contribute to long-term pathologies such as diabetes mellitus, heart disease, and hypertension (Bray and Young 2007, Filipowski et al. 2002, Karlsson et al. 2001, Lamont et al. 2007). In addition, changes in circadian rhythmic activity serve as experimental markers in animals to correlate with long-term effects of psychostimulant in humans (Algham et al., 2009; Bergheim et al., 2012; Glaser et al., 2012; Lee et al. 2011).

Methylphenidate hydrochloride is a central nervous system stimulant resembling in structure to dextroamphetamine (Kallman and Isaac 1975, Patrick and Markowitz 1997). The d-enantiomer is pharmacologically active but the l-enantiomer does not cross blood-brain barrier contrary to prior belief (Ding et al. 2004). When MPD is given intravenously it binds to DA transporter (DAT) and prevents the reuptake of DA to the presynaptic terminal, resulting in the accumulation of DA in the synaptic cleft (Izenwasser et al. 1999, Massello and Carpenter 1999, Patrick and Markowitz 1997, Volkow et al. 1999b). Cross sensitization between MPD and cocaine was reviewed by Gatley et al. (1999) citing 26 animal studies supporting the similarity between the two stimulants in terms of drug discrimination, self-administration, locomotor activity, and other specific behaviors while 6 opposing animal studies were also reviewed. MPD, or better known through its trade name Ritalin, is currently the most prescribed treatments for Attention Deficit and Hyperactive Disorder (ADHD) according to the 2011 Center for Disease Control report. Moreover, MPD is gaining popularity among college students as well as middle-age adults without the diagnosis of ADHD as a cognitive enhancement and for recreational usage (Greely et al. 2008, Stix 2009). A 2005 survey of US college-age students shows that 7% have used stimulant for “nonmedical” reason at least once (McCabe et al. 2005).

This experiment aims to study the dose-response effect of long term effect of acute and chronic MPD exposure on adult female Wistar-Kyoto (WKY) rats. Female rats have mainly been excluded from studies due to their more complex reproductive system than males. It is reported that responses to morphine, amphetamine, and cocaine are stronger in females than males in animal models and in humans (Brecht et al. 2004, Hernandez-Avila et al. 2004, Lynch 2008). It is reported that gender and strain affect the responses to psychostimulants (Chelaru et al. 2012, Dafny and Yang 2006, Kelly et al. 1999, Melnick and Dow-Edwards 2001). To our knowledge, no prior studies have been done on adult female WKY rats. This strain was chosen as the study subject in contrast to other strains, such as Sprague-Dawley (SD) or Spontaneously Hypertensive rats (SHR), for genetic variation. The immediate effects of MPD on WKY, SHR, and SD rats were reported (Amini et al. 2004, Yang et al. 2003); however, none was reported on the long term effect of MPD on WKY rats. This study will close the gap. The hypothesis is that alteration in the circadian activity pattern after chronic drug exposure indicates that the drug does exert long-term effect. The objective of this study is to confirm the above hypothesis by recording the effect of acute and chronic MPD on circadian activity pattern of adult WKY female rats for 11 consecutive days.

2.0 Methods

2.1 Animals

Female adult WKY rats (N=32) weighing from 200-250 grams were housed as 4 rats per cage. The cages were in a sound controlled room with ambient temp of 21 +/- 2°C and a relative humidity of 37-42%. In addition food pellets and water were supplied ad libitum throughout the experiment. The experimental room was maintained on a 12:12 light/dark schedule (light on at 06:00). Animals were housed in the same test cages for 7 days for acclimatization in order to internally synchronize their neuroendocrine systems. On the last day of the naturalization, rats were weighed and randomly divided into one control group and three treatment groups. The groups are as follow: saline (control, n=8), 0.6 mg/kg MPD (n=8), 2.5 mg/kg MPD (n=8), and 10.0 mg/kg MPD (n=8). The animals were individually housed in the test cages (these test cages became their home cages), and an additional 24h of accommodation was allowed before locomotor activity recording was begun.

2.2 Experimental Cages

The experimental protocol has been described in detail (Gaytan et al., 1997, Yang et al., 2003, 2011). In short, open field boxes (40.5 × 40.5 × 31.5 cm each) were used, which were equipped with two levels of 16 infrared beams with their motion sensors, which were placed 6 and 12 cm from the cage floor, respectively. A computerized animal activity monitoring (CAAM; AccuScan Instruments, Inc., Columbus Ohio) system checked each sensor beam at a frequency of 100 Hz to determine whether beams were interrupted. The interruption of any beam was recorded as an activity score; cumulative counts were compiled and downloaded every 10 minutes into OASIS data collection program that differentiated these events into various locomotor activity indices.

2.3 Drugs

Methylphenidate (MPD) hydrochloride was dissolved in 0.9% saline and dosages calculated as freebase. The animals were injected intra-peritoneally (i.p.) equally with a volume of 0.8 cc with 0.9% saline. There are no universally recognized MPD dosage guidelines or blood levels to achieve optimum treatment. A study of 289 patients treated with MPD (White and Yadao 2000) reported that the range of doses was from 0.06 to 29.3 mg/kg with the majority being 1.0 to 3.0 mg/kg (Crutchley and Temlett 1999, Kollins 2001, Solanto 1998 and 2000). Drug effects in rodents often require higher doses than in humans because they metabolize MPD more rapidly than humans (Gatley et al. 1999). Doses between 0.5 to 3.5 mg/kg MPD given intraperitoneally in rodents were reported to promote plasma peak concentration within the typical clinical range (Kuczenski and Segal, 2002). The range of 5 to 10 mg/kg MPD is considered moderate dosage and above 10 mg/kg as a high dosage (Bowman and Kuhn 1996, Brandon and Steiner 2003, Kollins et al. 2001, Solanto 1998 and 2000). The three treatment dosages 0.6 mg/kg, 2.5 mg/kg, and 10.0 mg/kg of MPD-D chosen in this study were based on our previous dose response studies. Chronic treatment with 2.5 mg/kg and, in a few cases, also the 0.6 mg/kg MPD dose elicited behavioral sensitization (Gaytan et al. 1996a, 1997a, 2000a, 2000b; Lee et al. 2008; Wanchoo et al. 2009; Yang et al. 2000a, 2001, 2000b, 2003, 2006a, 2006b, 2007). The doses in the present study correlate to low,

medium, and high doses, respectively (Dafny and Yang 2006). Injections were made between 06:30h and 07:00h each day according to protocol (Table 1).

2.4 Procedure

Locomotor activity was recorded non-stop for 11 days using the CAAM system except during animal handling. The horizontal activity (HA), which measures the overall motor activity in the lowest sensor, was evaluated in this present study. 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

2.5 Data Analysis

Data analysis was performed to assess dose response effects of 3 different doses of MPD on the hourly total HA counts for EDs 1, 2, 7, 8, and 11 (Table 2). In addition to the three dosage groups a control group was also used to ensure uniformity throughout the experiment (Table 1). The effect of MPD was divided to four phases of drug activity (Podet et al. 2010; Yang et al. 2006 & 2011). The four phases are: (1) Acute phase – comparing effect after MPD injection on ED 2 to ED 1, the baseline post-saline injection, to observe whether a single (acute) injection is able to change the locomotor diurnal rhythm activity pattern. (2) Induction phase – the activity pattern on ED 7, the last day of the drug maintenance treatment, was compared to that of ED 2 to observe whether 6 days of MPD treatment induced any changes in activity pattern. (3) Washout phase (also known as withdrawal phase) – the activity patterns of ED 8, 9, 10 were compared to ED 1 to observe whether the 6 days of MPD treatment (ED 2-7) alter the baseline activity levels of days without treatment (ED 8-10). (4) Expression phase, the activity pattern of ED 11 was compared to ED 1 to observe whether the effect of chronic MPD exposure during ED 2-7 is expressed after 3 days without treatment (ED 8-10).

Two forms of data analysis were performed to evaluate the dose response effects of three MPD doses. The 10-minute activity counts were summed to hourly histograms and their mean and standard deviation was calculated. The hourly histograms with their mean and standard deviation were used to evaluate visually any changes in circadian rhythm pattern compared to the control histogram. The second evaluation used the 10-minute counts to calculate the 24-hour rhythms for each of the four phases using the cosine curve statistical analysis (CCSA) test (Bingham et al. 1982). This test analyzed the data using three parameters: MESOR (average activity represented by the curve and its peak activity), Amplitude (distance from the MESOR to highest point of the approximating curve) and Acrophase (time at which the maximum/amplitude occurs). The estimate of these three parameters provides the ability to test for statistically significant changes in HA pattern regard to time and intensity within the 24 h experimental period. It is this parameterization that allows for accurate representation to find out whether a significant shift in HA circadian rhythm activity pattern occurred in the acute, induction, washout, or expression phases.

3.0 Results

3.1 Control (Saline Injection)

Locomotor activity of 8 adult female WKY rats injected i.p., with 0.8 cc 0.9% saline serve as the control group. Each animal was injected every day between 06:30 h and 07:00 h for 7 consecutive days and then a period of three days with no injection followed by another saline re-challenge injection on ED 11 (Table 1). Recording of motor activity began after the first injection at about 07:00 h nonstop until 24h after the last injection of ED 11. Figure 1A shows a summary of hourly HA counts for ED 1, 2, 7, 8, and 11 of the saline control group, which shows the (control) rhythmic activity of a nocturnal animal; an increase in locomotor activity during the dark phase (night) and decreased motor activity during the light period (day). During ED 1, 2, 7, 8 and 11, the HA followed this general pattern with minor changes that were deemed insignificant (Fig 1A). Figure 1B provided the superimpose 10-minute counts of HA and the calculated CCSA test of all 4 groups on ED 1 post-injection demonstrating that all the locomotor activity exhibits the same general locomotor activity pattern with only minor insignificant fluctuations, i.e. all animal groups have similar baseline activity pattern. Based on these findings, one can conclude that significant deviations from the locomotor activity obtained on ED 1 could be attributed to the drug (MPD) effect (Algahim et al. 2009; Lee et al. 2011; Bergheim et al, 2012). Due to the observation that HA obtained at ED 1 was the same as all other EDs in the control group and that all 4 groups have similar activity pattern post-injection on ED1, it follows that the activity of ED 1 after saline injection in each MPD group can and was used as the control for the drug effect of that dose group.

3.2 MPD Acute Effects (Comparing ED 2 to ED 1)

The 0.6 mg/kg MPD dose showed an initial increase in activity on ED 2 following MPD injection compared to ED 1 (Fig 2A – 0.6 mg/kg dose), and return to base line level for the requiring period. The CCSA shows no significant alteration in the HA pattern activity on ED 2 compared to ED 1 (Fig 2B – 0.6 mg/kg and Table 2). The 2.5 mg/kg MPD injection caused an increase in activity during the initial post injection hours and between 18:00 and 20:00, i.e. from the 11th-13th hour past injection (Fig 2A – 2.5 mg/kg). However, the CCSA test (Fig 2B – 2.5 mg/kg) found this increase in activity at ED 2 compared to ED 1 to be of no statistical significance. The acute 10 mg/kg MPD dose exposure resulted in an increase in HA within the first three hours post-injection (Fig 2A and Fig 2B – 10 mg/kg). This immediate high-activity response to MPD injection exerted an exaggerated effect on the 24-hour fitted CCSA test, ie. “pulling” the curve “up” at the far left at approximately hour zero (Fig 2B – 10 mg/kg). In order to appropriately parameterize the long-term effects of MPD on the locomotor activity patterns, these first three hours of data were excluded from the CCSA test, resulting in a cosine curve more accurately representing the long-term effects of the entire time post injection pattern of ED 2. These data were used for the analysis of this paper. The 10 mg/kg MPD dose also exhibited an increase in activity between 6th-8th, 9th-12th, and 13th-16th hours past injection (Fig 2A – 10 mg/kg). The CCSA showed statistical significance decrease in the Acrophase ($p = 0.013$) (Fig 2C – 10 mg/kg and Table 2).

3.3 MPD Induction Phase (Comparing ED 7 to ED 2)

The 0.6 mg/kg MPD injection at ED 7 elicited the same increase in activity post MPD injection as ED 2. However, this increase in activity was prolonged and lasted from 0-5th hours post-injection. The activity also increased during the 7th-10th and 11th-16th hours after injection on ED 2 (Fig 4A – 0.6 mg/kg dose). The statistical comparison using the CCSA test (Fig 4B – 0.6 mg/kg and Table 2) showed that these changes in locomotion are not statistically different (Table 2). The locomotor activity following 2.5 mg/kg MPD at ED 7 exhibited an increase in activity that lasted from 0 to 11th hour after injection. In addition, an increase in activity was seen again from 13th-16th hour after injection (Fig 4A – 2.5 mg/kg dose). However, the CCSA test showed that these changes in locomotive activity pattern are statistically insignificant (Fig 4B – 2.5 mg/kg & Table 2), i.e., 6 days of 2.5 mg/kg MPD have no effects on the HA circadian activity patterns. The 10 mg/kg of MPD exposure on ED 7 compared to ED 2 locomotor activity only exhibited an increase in activity between the 1st-5th hours after injection (Fig 4A – 10 mg/kg). The CCSA test for this group shows the change to be statistically insignificant (Fig 4B – 10 mg/kg & Table 2).

3.4 Washout Phase (Comparing ED 8 to ED 1)

On ED 8, MPD injections were held to determine whether withdrawal was induced by the six daily administration of MPD. The 0.6 mg/kg MPD dose group exhibited increased activity from 0-2nd hours post-injection, at the 5th hour, and 10-12th hour post injection (Fig 5A – 0.6 mg/kg). The CCSA test of ED 8 vs. ED 1 locomotor activity was found to be statistically insignificant (Fig 5B – 0.6 mg/kg & Table 2). The 2.5 mg/kg MPD dose group exhibited an increase of activity from 0-2nd, 5th-7th, 9th-12th, and 13th-17th hours post-injection (Fig 5A – 2.5 mg/kg dose curve). However, the CCSA test shows that these increases to be statistically insignificant (Fig 5B – 2.5 mg/kg & Table 2). The 10 mg/kg MPD dose group also exhibited an increase in locomotor activity from 0-4th and 9th-17th hours post-injection (Fig 5A – 10 mg/kg). The CCSA test showed statistical significance in regard to the Acrophase ($p = 0.007$) (Fig 5B – 10 mg/kg & Table 2). At the beginning of ED 8, all of the experimental animals showed an increase in activity at the time that they had received the MPD injections during the previous 6 days. This suggests that the animals were expecting to get the next dose of the MPD drug or that they are expressing behavioral withdrawal.

3.5 MPD Expression Phase (Comparing ED 11 to ED 2)

To determine whether the changes induced by repetitive MPD administration were transient or long-lasting, the locomotor activity of ED 11 were compared to ED 2. The 0.6 mg/kg MPD groups exhibited an increase in activity of during the 3rd and 12th to 17th hours post MPD injection (Fig 6A – 0.6 mg/kg). The CCSA test shows a significant increase in amplitude ($p=0.041$) (Fig 6B – 0.6 mg/kg & Table 2). The 2.5 mg/kg MPD groups exhibited an increase in activity from 0-5th hours post-injection, followed by an increase in activity between 8th-12th hours post-injection, as well as 13th-17th hours post-injection (Fig 6A – 2.5 mg/kg dose). The CCSA test of the ED 11 activity compared to ED 2 activity post MPD exposure exhibit significant different in the MESOR ($p=0.027$) (Fig 6B and Table 2 – 2.5 mg/kg). The 10 mg/kg MPD group at ED 11, i.e. after 6 daily MPD exposure and 3 washout

days, showed an initial increase in locomotor activity when compared to ED 2 between the 0-4th hr post-injection followed by an increase in activity from 11th-13th hr post-injection (Fig 6A – 10.0 mg/kg dose histogram). The CCSA test shows statistically insignificant difference between ED 11 and ED 2 (Fig 6B – 10 mg/kg cosine curve and Table 2).

4.0 Discussion

The neurotransmitter dopamine facilitates multiple critical facets of human physiology such as locomotion, cognition, emotion and reward. Thus, it is prudent that a pharmacological agent that increases the extracellular concentrations of dopamine in synaptic clefts be further investigated, especially when the prescribed and non-prescribed usage of such agent is on the rise. It would also stand to reason that animal models of a known subpopulation that use this substance be specifically studied since using human subjects for this purpose in a prospective manner would be impractical and unethical.

4.1 Three major findings of this study

(1) The 0.6 mg/kg dose group shows an increased amplitude at ED 11, the expression phase, that was statistically significant ($p=0.041$) (Table 2). This change in locomotor activity after a washout period implies that chronic 0.6 mg/kg MPD exposure altered the circadian rhythm locomotor activity pattern of the animals and/or that the animals were sensitized to 0.6mg/kg MPD. (2) The 2.5 mg/kg dose group demonstrated MESOR trending up over the 11 ED's and statistically significant ($p=0.041$) at expression phase (Table 2 and Fig 7). The chronic exposure of 2.5mg/kg MPD was able to establish a new baseline locomotor activity pattern. This is in contrast to the MESOR of the 0.6mg/kg dose group, which also trended up but the p -value did not reach statistical significance (Table 2 and Fig 7). Perhaps the 0.6mg/kg MPD dose was too low and not enough MPD and, subsequently, DA accumulated in the synaptic clefts to affect a change in locomotor activity as the 2.5 mg/kg dose group. Perhaps more time is needed for this change in locomotor activity to manifest as statistical significance. Of note, comparing the change in amplitude of the 2.5mg/kg dose group from ED8 to ED11 (235 → 320 average counts/10 min) to the change in amplitude from ED2 to ED7 (220 → 248 average counts/10 min), it is observed that the change in amplitude was 4 times greater at the expression phase than the induction phase (Table 2 and Fig 7). One could not help but wonder perhaps a degree of sensitization is at play. (3) The 10mg/kg dose group shows the acrophase shifted approximately 2.5 hour earlier on ED 2 ($p=0.013$) (Table 2). This change in locomotor activity pattern was affected by a single 10.0 mg/kg MPD injection and persisted through 6 daily injections, 3 days of washout, and upon MPD rechallenger at ED 11 which suggests that, indeed, a new baseline of locomotor activity has been established. The change in acrophase also highlights the fact that it is important to analyze the data and trends fully despite the numerical analysis suggesting statistical insignificance, ie acrophase of ED 11 vs. 2 was $p=0.697$ while the acrophase was actually shifted ~2.5 hours earlier from EDs 2-11. While the change in the amplitude of circadian rhythm activity pattern from ED 11 vs. 2 did not show a statistical significance, it may be due to the expression of tolerance, i.e. the 10 mg/kg MPD dose did not alter the activity pattern but induced tolerance which is also a long-term effect of the drug.

4.2 Psychostimulants affecting neuroadaptation

Neuroadaptation by mesocorticolimbic dopaminergic pathway which includes the ventral tegmental area (VTA), nucleus accumbens (NAc), and the prefrontal cortex (PFC) is a major factor in development of behavioral sensitization (Chen et al 2009, Chong et al. 2012, Claussen et al. 2012, Everitt and Robbins 2005, Kalivas and Stewart 1991, Nestler 2001, Podet et al. 2010, Robinson and Becker 1986, Salek et al. 2012,). Neuroadaptation is mediated by neuronal structural plasticity manifesting by changes in size of cell bodies or changes in spine morphology such as arborization and the density of dendritic spines (Robinson and Kolb 2004, Russo et al. 2010, Sklair-Tavron et al. 1996). Chronic psychostimulant exposure leads to an increased density and complexity of spines of medium-sized spiny neurons in the NAc (Lee 2006), which result in long-term potentiation (Nagerl et al. 2004, Okamoto et al. 2004, Matsuzaki et al. 2004). MPD initially interacts with dopamine and glutamine in dendritic spines of medium-spiny neurons (MSN) in ventral and dorsal striatum (Robinson and Kolb 2004). This study shows that the 0.6 mg/kg MPD dose failed to elicit effect following the initial MPD exposure while elicits increased the locomotor activity amplitude only after repetitive MPD exposure at the expression phase. Perhaps silent receptors were recruited (Boudreau et al. 2007, Boudreau and Wolf 2005, Conrad et al. 2008, Huang et al. 2009, Malenka and Nicoll 1999, Marie et al. 2005) following repetitive exposure of the drug, and the behavioral response manifested as the increased amplitude.

Different brain region exhibit different levels of DA receptor types (Wirz-Justice 1987). Both D1 and D2 receptors are found in high concentration in NAc (Girault and Greengard 2004) with excitatory and inhibitory roles, respectively (Einhorn et al. 1988, Ruskin et al. 2001, Shi et al. 1997). D1-like DA receptors mediate extracellular signal-kinase (ERK) which upregulates FosB, a marker of increased sensitivity to behavioral effects and increased motivation for the drug (Nestler 2001, Nestler and Malenka 2004) and is reported to induce long-term neuroadaptations in brain (Shi and McGinty 2010, Zhang et al. 2004). When D1 receptors are inhibited, there is an increase in phosphorylation of cAMP response element binding protein (CREB) which leads to homeostatic negative feedback adaptation, inhibiting sensitivity, ie behavioral tolerance (Chao and Nestler 2004, Nestler and Malenka 2004). On the other hand, D2-like DA receptors are reported to exert inhibitory effect (Ferguson et al. 2010, Kreitzer and Malenka 2008, Zhang et al. 2004). Of note, in this experiment, the 10.0 mg/kg MPD dose group showed MESOR trending down during the acute phase (366.37 → 358.61 average counts/10 min), further down during induction (358.61 → 303.11 average counts/10 min), up during washout (303.11 → 338.16 average counts/10 min), and, finally, down during the expression phase (338.16 → 334.97 average counts/10 min). This trend suggests that chronic exposure of 10.0 mg/kg MPD exerts an attenuation effect on the animals' locomotor activity. Perhaps at this dosage, D2 receptors were preferentially activated. Furthermore, the magnitude of decrease in MESOR during the expression phase was less than during the induction phase, and behavior tolerance may be the underlying reason for this observation. Since chronic treatment of MPD showed an increase in locomotion, one might assume that psychostimulants not only inhibit uptake of DA by blocking the DAT, but also may increase production of D1 and/or D2 receptors [Kleven et al., 1990].

The chronic MPD effects observed in this study suggest that MPD, like cocaine, is capable of inducing both tolerance and sensitization [Izenwasser and French 2002]. Sensitization was observed in this study at the 0.6 and 2.5 mg/kg MPD while tolerance was observed at the 10.0 mg/kg MPD. Cocaine and amphetamine may not only inhibit uptake of DA but may have also induced the production of D1 and D2 receptors [Kleven et al., 1990]. Therein, the question arises as to whether chronic administration of MPD would also induce both D1 and D2 receptors, which may account for the simultaneous manifestation of opposing behavioral responses, sensitization and tolerance.

5.0 Conclusion

After chronic treatment of MPD and a washout period, all three dose-groups manifested changes in diurnal locomotor activity indices upon rechallenge with the drug. These changes in locomotor activity pattern are interpreted as long-term effect exerted by MPD. Perhaps the behavioral changes resulted from alteration of the clock genes, regulation of transcription factors, or preferential activation of receptors. Further investigation into the mechanism of change in circadian rhythm pattern exerted by MPD thereby affecting the various homeostatic functions within the body is warranted.

Acknowledgments

We would like to thank Mallinckrodt for their donation of methylphenidate. This research was supported in part by NIH R01 DA027222.

References

- Abarca C, Albrecht U, Spanagel R. Cocaine sensitization and reward are under the influence of circadian genes and rhythm. *Proc Natl Acad Sci USA*. 2002; 99(13):9026–903. [PubMed: 12084940]
- Algahim MF, Yang PB, Wilcox VT, Bureau KD, Swann AC, Dafny N. Prolonged methylphenidate treatment alters the behavioral diurnal activity pattern of adult male Sprague-Dawley rats. *Pharmacol Biochem Behav*. 2009; 92:93–99. [PubMed: 19028517]
- Amini B, Yang PB, Swann AC, Dafny N. Differential locomotor responses in male rats from three strains to acute methylphenidate. *Int J Neurosci*. 2004; 114:1063–1084. [PubMed: 15370174]
- Antle MC, van Diep HC, Deboer T, Pedram P, Pereira RR, Meijer JH. Methylphenidate modifies the motion of the circadian clock. *Neuropsychopharmacology*. 2012; 37:2446–2455. [PubMed: 22763623]
- Beitner-Johnson D, Nestler EJ. Chronic morphine impairs axoplasmic transport in the rat mesolimbic dopamine system. *Neuroreport*. 1993; 5:57–60. [PubMed: 7506589]
- Beitner-Johnson D, Guitart X, Nestler EJ. Neurofilament proteins and the mesolimbic dopamine system: common regulation by chronic morphine and chronic cocaine in the rat ventral tegmental area. *J Neurosci*. 1992; 12:2165–2176. [PubMed: 1376774]
- Benstaali C, Mailloux A, Bogdan A, Auzeby A, Touitou Y. Circadian rhythms of body temperature and motor activity in rodents their relationships with the light-dark cycle. *Life Sci*. 2001; 68:2645–2656. [PubMed: 11400908]
- Bergheim M, Yang PB, Bureau KD, Dafny N. Adolescent rat circadian activity is modulated by psychostimulants. *Brain Res*. 2012; 1431:35–45. [PubMed: 22146676]
- Bingham C, Arbogast B, Guillaume GC, Lee JK, Halberg F. Inferential statistical methods for estimating and comparing cosinor parameters. *Chronobiologia*. 1982; 9(4):397–439. [PubMed: 7168995]

- Bittman EL, Doherty L, Huang L, Paroskie A. Period gene expression in mouse endocrine tissues. *Am J Physiol Regul Integr Comp Physiol*. 2003; 285:561–569.
- Boudreau AC, Reimers JM, Milovanovic M, Wolf ME. Cell surface AMPA receptors in the rat nucleus accumbens increase during cocaine withdrawal but internalize after cocaine challenge in association with altered activation of mitogen-activated protein kinases. *J Neurosci*. 2007; 27:10621–35. [PubMed: 17898233]
- Boudreau AC, Wolf ME. Behavioral sensitization to cocaine is associated with increased AMPA receptor surface expression in the nucleus accumbens. *J Neurosci*. 2005; 25:9144–51. [PubMed: 16207873]
- Bowman BP, Kuhn CM. Age-related differences in the chronic and acute response to cocaine in the rat. *Dev Psychobiol*. 1996; 29:597–611. [PubMed: 8911774]
- Brandon CL, Steiner H. Repeated methylphenidate treatment in adolescent rats alters gene regulation in the striatum. *Eur J Neurosci*. 2003; 18(6):1584–1592. [PubMed: 14511337]
- Bray MS, Young ME. Circadian rhythms in the development of obesity: potential role for the circadian clock within the adipocyte. *Obes Rev*. 2007; 8:169–181. [PubMed: 17300281]
- Brecht ML, O'Brien A, van Mayrhauser C, Anglin MD. Methamphetamine use behaviors and gender differences. *Addict Behav*. 2004; 29:89–106. [PubMed: 14667423]
- Cermakian N, Sassone-Corsi P. Environmental stimulus perception and control of circadian clocks. *Curr opin Neurobiol*. 2002; 12:359–365. [PubMed: 12139981]
- Chao J, Nestler EJ. Molecular neurobiology of drug addiction. *Annu Rev Med*. 2004; 55:113–132. [PubMed: 14746512]
- Chelaru MI, Yang PB, Dafny N. Sex differences in the behavioral response to methylphenidate in three adolescent rat strains (WKY, SHR, SD). *Behav Brain Res*. 2012; 226:8–17. [PubMed: 21889544]
- Chen JC, Chen PC, Chiang YC. Molecular mechanisms of psychostimulant addiction. *Chang Gung Med J*. 2009; 32:148–154. [PubMed: 19403004]
- Chen CP, Kuhn P, Advis JP, Sarkar DK. Chronic ethanol consumption impairs the circadian rhythm of pro-opiomelanocortin and period genes mRNA expression in the hypothalamus of the male rat. *J Neurochem*. 2004; 88(6):1547–1554. [PubMed: 15009656]
- Chong SL, Claussen CM, Dafny N. Nucleus accumbens neuronal activity in freely behaving rats is modulated following acute and chronic methylphenidate administration 2 204. *Brain Res Bull*. 2012; 87:445–456. [PubMed: 22248440]
- Claussen C, Dafny N. Acute and chronic methylphenidate modulates the neuronal activity of the caudate nucleus recorded from freely behaving rats 4 207. *Brain Res Bull*. 2012; 87:387–396. [PubMed: 22040860]
- CDC. 2011 report Division of Human Development, National Center on Birth Defects and Developmental Disabilities. Centers for Disease Control and Prevention; www.cdc.gov/ncbddd/index.html. Page last updated: December 12, 2011
- Conrad KL, Tseng KY, Uejima JL, Reimers JM, Heng LJ, Shaham Y, Marinelli M, Wolf ME. Formation of accumbens GluR2-lacking AMPA receptors mediates incubation of cocaine craving. *Nature*. 2008; 454:118–121. [PubMed: 18500330]
- Crutchley A, Temlett JA. Methylphenidate (Ritalin) use and abuse. *S Afr Med J*. 1999 Oct; 89(10): 1076–1079. [PubMed: 10582063]
- Dafny N, Yang PB. The role of age, genotype, sex, and route of acute and chronic administration of methylphenidate: a review of its locomotor effects. *Brain Res Bull*. 2006; 68:393–405. [PubMed: 16459193]
- Ding YS, Gatley SJ, Thanos PK, Shea C, Garza V, Xu Y, Carter P, King P, Warner D, Taintor N, Park D, Pyatt B, Fowler JS, Volkow N. Brain Kinetics of Methylphenidate (Ritalin) Enantiomers After Oral Administration. *Synapse*. 2004; 53:168–175. [PubMed: 15236349]
- Einhorn LC, Johansen PA, White FJ. Electrophysiological effects of cocaine in the mesoaccumbens dopamine system: studies in the ventral tegmental area. *J Neurosci*. 1988; 8:100–112. [PubMed: 3339402]
- Everitt BJ, Robbins TW. Neural systems of reinforcement for drug addiction: from actions to habits to compulsion. *Nat Neurosci*. 2005; 8:1481–1489. [PubMed: 16251991]

- Ferguson SM, Eskenazi D, Ishikawa M, Wanat MJ, Phillips PE, Dong Y, et al. Transient neuronal inhibition reveals opposing roles of indirect and direct pathways in sensitization. *Nat Neurosci*. 2010; 14(1):22–24. [PubMed: 21131952]
- Filipski E, King VM, Li X, Granda TG, Mormont MC, Liu X, Claustrat B, Hastings MH, Levi F. Host circadian clock as a control point in tumor progression. *J Natl Cancer Inst*. 2002; 94:690–697. [PubMed: 11983758]
- Gatley SJ, Volkow ND, Gifford AN, Fowler JS, Dewey SL, Ding YS, Logan J. Dopamine-transporter occupancy after intravenous doses of cocaine and methylphenidate in mice and humans. *Psychopharmacol*. 1999; 146:93–100.
- Gaytan O, Al-Rahim S, Swann A, Dafny N. Sensitization to locomotor effects of methylphenidate in the rat. *Life Sci*. 1997; 61:PL101–PL107. [PubMed: 9275016]
- Gaytan O, Nason R, Alagugurusamy R, Swann A, Dafny N. MK 801 blocks the development of sensitization to the locomotor effects of methylphenidate. *Brain Res Bull*. 2000a; 51:485–492. [PubMed: 10758338]
- Gaytan O, Swann AC, Dafny N. Effects of amphetamine at the beginning of the light cycle on multiple indices of motor activity in the rat. *Eur J Pharmacol*. 1996; 300(1-2):1–8. [PubMed: 8741158]
- Gaytan O, Yang P, Swann A, Dafny N. Diurnal differences in sensitization to methylphenidate. *Brain Research*. 2000b; 864:24–39. [PubMed: 10793183]
- Girault JA, Greengard P. The neurobiology of dopamine signaling. *Arch Neurol*. 2004; 61:641–644. [PubMed: 15148138]
- Glaser AM, Reyes-Vazquez C, Prieto-Gomez B, Burau K, Dafny N. Morphine administration and abrupt cessation alter the behavioral diurnal activity pattern. *Pharmacol Biochem Behav*. 2012; 101:544–552. [PubMed: 22386964]
- Greely H, Sahakian B, Harris J, Kessler RC, Gazzaniga M, Campbell P, Farah MJ. Towards responsible use of cognitive-enhancing drugs by the healthy. *Nature*. 2008; 456:702–705. [PubMed: 19060880]
- Hernandez-Avila CA, Rounsaville BJ, Kranzler HR. Opioid-, cannabis-, and alcohol-dependent women show more rapid progression to substance abuse treatment. *Drug Alcohol Depend*. 2004; 74:265–272. [PubMed: 15194204]
- Huang YH, Lin Y, Mu P, Lee BR, Brown TE, Wayman G, Marie H, Liu W, Yen Z, Sorg BA, Schluter OM, Zukin RS, Dong Y. In vivo cocaine experience generates silent synapses. *Neuron*. 2009; 63:40–47. [PubMed: 19607791]
- Iijima M, Nikaido T, Akiyama M, Moriya T, Shibata S. Methamphetamine-induced, suprachiasmatic nucleus-independent circadian rhythms of activity and mPer gene expression in the striatum of the mouse. *Eur J Neurosci*. 2002; 16:921–929. [PubMed: 12372028]
- Izenwasser S, Coy AE, Ladenheim B, Loeloff RJ, Cadet JL, French D. Chronic methylphenidate alters locomotor activity and dopamine transporters differently from cocaine. *Eur J Pharmacol*. 1999; 373:187–193. [PubMed: 10414438]
- Izenwasser S, French D. Tolerance and sensitization of the locomotor-activating effects of cocaine are mediated via independent mechanisms. *Pharmacol Biochem Behav*. 2002; 73:877–882. [PubMed: 12213534]
- Janssen BJA, Tyssen CM, Duindam H, Rietveld WJ. Suprachiasmatic lesions eliminate 24-h blood pressure variability in rats. *Physiol Behav*. 1994; 55:307–311. [PubMed: 8153170]
- Johnson RF, Moore RY, Morin LP. Loss of entrainment and anatomical plasticity after lesions of the hamster retinohypothalamic tract. *Brain Res*. 1988; 460:297–313. [PubMed: 2465060]
- Kalivas PW, Stewart J. Dopamine transmission in the initiation and expression of drug- and stress-induced sensitization of motor activity. *Brain Res Rev*. 1991; 16:223–244. [PubMed: 1665095]
- Kallman WM, Isacc W. The effects of age and illumination on the dose-response curve for three stimulants. *Psychopharmacology (Berlin)*. 1975; 40:313–318.
- Kalsbeek A, Foppen E, Schali J, Van Heijningen C, van der Vliet J, et al. Circadian control of the daily plasma glucose rhythm: an interplay of GABA and glutamate. *PLoS One*. 2008; 3:e3194. [PubMed: 18791643]

- Karlsson B, Knutsson A, Lindahl B. Is there an association between shift work and having a metabolic syndrome? Results from a population based study of 27,485 people. *Occup Environ Med.* 2001; 58:747–752. [PubMed: 11600731]
- Kelly SJ, Ostrowski NL, Wilson MA. Gender differences in brain and behavior: hormonal and neural bases. *Pharmacol Biochem Behav.* 1999; 64:655–664. [PubMed: 10593187]
- Klein, DC.; Moore, RY.; Reppert, SM. *Suprachiasmaic nucleus: the mind's clock.* New York: Oxford UP; 1991. isbn-13: 978-0195062502
- Kleven MS, Perry BD, Woolverton WL, Seiden LA. Effects of repeated injections of cocaine on D1 and D2 dopamine receptors in rat brain. *Brain Res.* 1990; 532:265–270. [PubMed: 2149299]
- Kollins SH, MacDonald EK, Rush CR. Assessing the abuse potential of methylphenidate in nonhuman and human subjects: a review. *Pharmacol Biochem Behav.* 2001; 68(3):611–627. [PubMed: 11325419]
- Kreitzer AC, Malenka RC. Striatal plasticity and basal ganglia circuit function. *Neuron.* 2008; 60(4): 543–554. [PubMed: 19038213]
- Kuczenski R, Segal DS. Exposure of adolescent rats to oral methylphenidate: preferential effects on extracellular norepinephrine and absence of sensitization and cross-sensitization to methamphetamine. *J Neurosci.* 2002; 22(16):7264–7271. [PubMed: 12177221]
- Lamont EW, James FO, Boivin DB, Cermakian N. From circadian clock gene expression to pathologies. *Sleep Med.* 2007; 8:547–556. [PubMed: 17395534]
- Lee KW, Kim Y, Kim AM, Helmin K, Nairn AC, Greengard P. Cocaine-induced dendritic spine formation in D1 and D2 dopamine receptor-containing medium spiny neurons in nucleus accumbens. *Proc Natl Acad Sci USA.* 2006; 103:3399–3404. [PubMed: 16492766]
- Lee MJ, Swann AC, Dafny N. Methylphenidate sensitization is prevented by prefrontal cortex lesion. *Brain Res Bull.* 2008; 76:131–140. [PubMed: 18395622]
- Lee MJ, Yang PB, Wilcox VT, Bureau KD, Swann AC, Dafny N. Repetitive methylphenidate administration modulates the diurnal behavioral activity pattern of adult female SD rats. *J Neural Transm.* 2011; 118:285–298. [PubMed: 21057965]
- Lynch WJ. Acquisition and maintenance of cocaine self-administration in adolescent rats: effects of sex and gonadal hormone. *Psychopharmacology.* 2008; 197:237–246. [PubMed: 18066534]
- Malenka RC, Nicoll RA. Long-term potentiation – a decade of progress? *Science.* 1999; 285:1870–4. [PubMed: 10489359]
- Manev H, Uz T. Clock genes: influencing and being influenced by psychoactive drugs. *Trends Pharmacol Sci.* 2006; 27(4):187–189.
- Marie H, Morishita W, Yu X, Calakos N, Malenka RC. Generation of silent synapses by acute in vivo expression of CaMKIV and CREB. *Neuron.* 2005; 45:741–752. [PubMed: 15748849]
- Massello WI, Carpenter DA. A fatality due to the intranasal abuse of methylphenidate (Ritalin). *J Forensic Sci.* 1999; 44:220–221. [PubMed: 9987890]
- Matsuzaki M, Honkura N, Ellis-Davis GC, Kasai H. Structural basis of long-term potentiation in single dendritic spines. *Nature.* 2004; 429:761–766. [PubMed: 15190253]
- McCabe SE, Knight JR, Teter CJ, Wechsler H. Non-medical use of prescription stimulants among US college students: prevalence and correlates from a national survey. *Addiction.* 2005; 100:96–106. [PubMed: 15598197]
- Melnick SM, Dow-Edwards DL. Differential responses to chronic amphetamine in adult male and female rats exposed to postnatal cocaine treatment. *Pharmacol Biochem Behav.* 2001; 69:219–234. [PubMed: 11420089]
- Mistlberger RE. Circadian regulation of sleep in mammals: role of the suprachiasmatic nucleus. *Brain Res Brain Res Rev.* 2005; 49:429–454. [PubMed: 16269313]
- Moore RY. Organization and function of a central nervous system circadian oscillator: the suprachiasmatic hypothalamic nucleus. *Fed Proc.* 1983; 42(11):2783–2789. [PubMed: 6135628]
- Moore RY, Lenn NJ. A retinohypothalamic projection in the rat. *J Comp Neurol.* 1972; 146:1–14. [PubMed: 4116104]

- Nagerl UV, Eberhorn N, Cambridge SB, Bonhoeffer T. Bidirectional activity-dependent morphological plasticity in hippocampal neurons. *Neuron*. 2004; 44:759–767. [PubMed: 15572108]
- Nestler EJ. Molecular basis of long-term plasticity underlying addiction. *Nat Rev Neurosci*. 2001; 2:119–128. [PubMed: 11252991]
- Nestler EJ, Malenka RC. The addicted brain. *Sci Am*. 2004; 290:78–85. [PubMed: 14981881]
- Okamoto KI, Nagai T, Miyawaki A, Hayashi Y. Rapid and persistent modulation of actin dynamics regulates postsynaptic reorganization underlying bidirectional plasticity. *Nat Neurosci*. 2004; 7:1104–1112. [PubMed: 15361876]
- Patrick KS, Markowitz JS. Pharmacology of methylphenidate, amphetamine enantiomers, and penoline in attention deficit/hyperactivity disorder. *Hum Psychopharmacol*. 1997; 12:527–546.
- Podet A, Lee MJ, Swanda AC, Dafny N. Nucleus accumbens lesion modulates the effects of methylphenidate. *Brain Res Bull*. 2010; 82:293–301. [PubMed: 20470871]
- Reid K, Burgess H. Circadian rhythm sleep disorders. *Primary Care*. 2005; 32(2):449–473. [PubMed: 15935195]
- Reppert SM, Weaver DR. Coordination of circadian timing in mammals. *Nature*. 2002; 418:935–941. [PubMed: 12198538]
- Robinson TE, Becker JB. Enduring changes in brain and behavior produced by chronic amphetamine administration: a review and evaluation of animal models of amphetamine psychosis. *Brain Res*. 1986; 396:157–198. [PubMed: 3527341]
- Robinson TE, Gorny G, Mitton E, Kolb B. Cocaine self-administration alters the morphology of dendrites and dendritic spines in the nucleus accumbens and neocortex. *Synapse*. 2001; 39:257–266. [PubMed: 11169774]
- Robinson TE, Kolb B. Alterations in the morphology of dendrites and dendritic spines in the nucleus accumbens and prefrontal cortex following repeated treatment with amphetamine or cocaine. *Eur J Neurosci*. 1999; 11:1598–1604. [PubMed: 10215912]
- Robinson TE, Kolb B. Structural plasticity associated with exposure of drugs of abuse. *Neuropharmacology*. 2004; 47(Suppl 1):33–46. [PubMed: 15464124]
- Ruskin DN, Bergstrom DA, Shenker A, Freeman LE, Baek D, Walters JR. Drugs used in the treatment of attention-deficit/hyperactivity disorder affect postsynaptic firing rate and oscillation without preferential dopamine autoreceptor action. *Biol Psychiatry*. 2001; 49:340–350. [PubMed: 11239905]
- Russo SJ, Dietz DM, Dumitriu D, Morrison JH, Malenka RC, Nestler EJ. The addicted synapse: mechanisms of synaptic and structural plasticity in nucleus accumbens. *Trends Neurosci*. 2010; 33:267–276. [PubMed: 20207024]
- Salek RL, Claussen CM, Perez A, Dafny N. Acute and chronic methylphenidate alters prefrontal cortex neuronal activity recorded from freely behaving rats. *Eur J Pharmacol*. 2012; 679:60–67. [PubMed: 22306242]
- Shi WX, Zheng P, Liang XF, Bunney BS. Characterization of dopamine-induced depolarization of prefrontal cortical neurons. *Synapse*. 1997; 26:415–422. [PubMed: 9215600]
- Shi X, McGinty JF. D1 and D2 dopamine receptors differentially mediate the activation of phosphoproteins in the striatum of amphetamine-sensitized rats. *Psychopharmacology*. 2010; 214(3):653–663. [PubMed: 21057772]
- Sklair-Tavron L, Shi WX, Lane SB, Harris HW, Bunney BS, Nestler EJ. Chronic morphine induces visible changes in the morphology of mesolimbic dopamine neurons. *Proc Natl Acad Sci USA*. 1996; 93:11202–11207. [PubMed: 8855333]
- Solanto MV. Neuropsychopharmacological mechanisms of stimulant drug action in attention deficit hyperactivity disorder: a review and integration. *Behav Brain Res*. 1998; 94(1):127–152. [PubMed: 9708845]
- Solanto MV. Clinical psychopharmacology of AD/HD: implications for animal models. *Neurosci Biobehav Rev*. 2000; 24(1):27–30. [PubMed: 10654657]
- Stix G. Turbocharging the brain. *Sci Am*. 2009; 301(4):46–49. 52–55. [PubMed: 19780452]

- Sujino M, Masumoto KH, Yamaguchi S, van der Horst GT, Okamura H, Inouye ST. Suprachiasmatic nucleus grafts restore circadian behavioral rhythms of genetically arrhythmic mice. *Curr Biol*. 2003; 13:664–668. [PubMed: 12699623]
- van Oosterhout F, Lucassen EA, Houben T, vanderLeest HT, Antle MC, Meijer JH. Amplitude of the SCN clock enhanced by the behavioral activity rhythm. *LoS ONE*. 2012; 7(6):e39693.10.1371/journal.pone.0039693
- Vitaterna MH, Takahashi JS, Turek FW. Overview of circadian rhythms. *Alcohol Res Health*. 2001; 25:85–93. [PubMed: 11584554]
- Volkow ND, Wang GJ, Fowler JS, Fischman M, Foltin R, Abumrad NN, Gatley SJ, Logan J, Wong C, Gifford A, Ding YS, Hitzemann R, Pappas N. Methylphenidate and cocaine have a similar in vivo potency to block dopamine transporters in the human brain. *Life Sci*. 1999a; 65:L7–L12.
- Volkow ND, Wang GJ, Fowler JS, Logan J, Wong C, Hitzemann R, Pappas NR. Reinforcing effects of psychostimulants in humans are associated with increases in brain dopamine and occupancy of D2 receptors. *J Pharmacol Exp Ther*. 1999b; 291:409–415. [PubMed: 10490931]
- Wanchoo SJ, Swann AC, Dafny N. Descending glutamatergic pathway of PFC are involved in acute and chronic action of methylphenidate. *Brain Res*. 2009; 1301:68–79. [PubMed: 19747456]
- Welsh DK, Logothetis DE, Meister M, Reppert S. Individual neurons dissociated from rat suprachiasmatic nucleus express independently phased circadian firing rhythms. *Neuron*. 1995; 14:697–706. [PubMed: 7718233]
- White SR, Yadao CM. Characterization of methylphenidate exposures reported to a regional poison control center. *Arch Pediatr Adolesc Med* Dec. 2000; 154(12):1199–1203.
- Wirz-Justice A. Circadian rhythms in mammalian neurotransmitter receptors. *Prog Neurobiol*. 1987; 29:219–259. [PubMed: 3037606]
- Yamazaki S, Numano R, Abe M, Hida A, Takahashi R, Ueda M, Block GD, Sakaki Y, Menaker M, Tei H. Resetting central and peripheral circadian oscillators in transgenic rats. *Science*. 2000; 288:682–685. [PubMed: 10784453]
- Yang PB, Amini B, Swann AC, Dafny N. Strain differences in the behavioral responses of male rats to chronically administered methylphenidate. *Brain Res*. 2003; 971(2):139–52. [PubMed: 12706230]
- Yang PB, Beasley A, Swann A, Dafny N. Valproate modulates the expression of methylphenidate (Ritalin) sensitization. *Brain Res*. 2000a; 874:216–220. [PubMed: 10960607]
- Yang PB, Cuellar DO III, Swann AC, Dafny N. Age and genetic strain differences in response to chronic methylphenidate administration 103. *Behav Brain Res*. 2011; 218:206–217. [PubMed: 21111006]
- Yang PB, Singhal N, Modi G, Swann A, Dafny N. Effects of lithium chloride on induction and expression of methylphenidate sensitization. *Eur J Pharmacol*. 2001; 426(1-2):65–72. [PubMed: 11525772]
- Yang PB, Swann A, Dafny N. NMDA receptor antagonist disrupt acute and chronic effects of methylphenidate. *Physiol and Behav*. 2000b; 71:133–145. [PubMed: 11134695]
- Yang PB, Swann A, Dafny N. Dose response characteristics of methylphenidate on locomotor behavior and on sensory evoked potentials recorded from VTA, NAc and PFC in freely behaving rats. *Behav and Brain Function*. 2006a; 4:25–36.
- Yang PB, Swann AC, Dafny N. Acute and chronic methylphenidate dose-response assessment on three adolescent male rat strains. 2006b; 71(1-3):301–10. Epub.
- Yang PB, Swann AC, Dafny N. chronic administration of methylphenidate produces neurophysiological and behavioral sensitization. *Brain Res*. 2007; 1145:66–80. [PubMed: 17335781]
- Zhang L, Lou D, Jiao H, Zhang D, Wang X, Xia Y, et al. Cocaine-induced intracellular signaling and gene expression are oppositely regulated by the dopamine D1 and D3 receptors. *J Neurosci*. 2004; 24(13):3344–3354. [PubMed: 15056714]

Figure 1.

Baseline activity pattern **A** – Hourly histogram of the control group demonstrating consistent rhythm of the average horizontal activity counts per hour of EDs 1, 2, 7, 8, and 11. The arrows indicate time of injection (saline) and shaded areas indicate dark period of 23 h experimental period. Only experimental day 1 begins at 06:00 h whereas all other days thereafter begin at 00:00 h. The rhythmic activity of a nocturnal animal, i.e. increased locomotor activity during the dark phase (night) and decreased motor activity during the light period (day), is observed. **B** – A 24-hour cosine curve statistical analysis of horizontal activity circadian rhythm pattern in counts per 10 minutes, post-injection, for all treatment groups on ED 1. The locomotor activities of the four animal groups exhibit the same general locomotor activity pattern with only minor insignificant fluctuations. Time 0 = 07:00 hours and the area shaded indicates a dark period of the 24 hour experimental period. CCSA-cosine curve statistical analysis.

Figure 2.

Acute phase response in the 3 experimental dose groups. **A** – Histogram representation of HA counts/hour of experimental days 1 and 2. Arrow indicates time of injection (saline on ED1 and MPD on ED2); **B** – Cosine curve comparing experimental days 1 and 2 over a 23 hour period post-drug injection. Time 0 = 07:00 hours and the area shaded indicates a dark period of the 24 hour experimental period. The CCSA test shows no significant alteration in the HA pattern activity on ED 2 compared to ED 1 for the 0.6 and 2.5 mg/kg dose groups; the 10.0 mg/kg dose group exhibited a statistical significant decrease in acrophase.

Figure 3.

Induction phase effect in the 3 experimental dose groups. **A** – A histogram representing HA counts/h of experimental days 2 and 7. Arrows indicate time of injection (MPD). **B** – Cosine curve comparing experimental days 2 and 7 over a 23 h period post-drug injection; Time 0 = 07:00 hours and the area shaded indicates a dark period of the 24 hour experimental period. The CCSA test shows no significant alteration in the HA pattern activity for all 3 dose groups.

Figure 4.

Washout phase effect in the 3 experimental dose groups. **A** – Histogram representation of HA counts/h of experimental days 1 and 8; Arrow indicates time of injection (saline on experimental day 1). At the beginning of ED 8, all of the experimental animals showed an increase in activity at the time that they had received the MPD injections during the previous 6 days **B** – Cosine curve comparing experimental days 1 and 8 over a 23 h period. Time 0 = 07:00 hours and the area shaded indicates a dark period of the 24 hour experimental period. The CCSA test showed statistical significance in regard to the acrophase of the 10.0 mg/kg MPD dose group.

Figure 5.

Expression phase effect in the 3 experimental dose groups. **A** – Histogram representation of HA counts/h of experimental days 2 and 11. Arrows indicate time of injection (MPD). **B** – Cosine curve comparing experimental days 2 and 11 over a 23 h experimental period. Time 0 = 07:00 hours and the area shaded indicates a dark period of the 24 hour experimental period. The CCSA test shows statistical significance in amplitude of the 0.6mg/kg MPD group and MESOR of the 2.5mg/kg MPD group.

Figure 6. MESOR and amplitude of the 3 experimental dose groups throughout the experimental days.

Table 1

Experimental Protocol – All 4 groups rats received saline injection on ED 1. On EDs 2-7, each group received saline, 0.6mg/kg MPD, 2.5mg/kg MPD, or 10.0 mg/kg MPD injections, respectively. EDs 8-10 were washout days. On ED 11, each group was rechallenged with the same injection as during ED 2. The MPD challenge dose groups were injected at approximately 07:00. ED-experimental day. MPD-methylphenidate.

	Control (n=8)	0.6 mg/kg MPD (n=8)	2.5 mg/kg MPD (n=8)	10.0 mg/kg MPD (n=8)
ED 1	Saline	Saline	Saline	Saline
ED 2-7	Saline	0.6 mg/kg MPD	2.5 mg/kg MPD	10.0 mg/kg MPD
ED 8-10	No injection	No injection	No injection	No injection
ED 11	Saline	0.6 mg/kg MPD	2.5 mg/kg MPD	10.0 mg/kg MPD

Statistical Data of the Experimental Groups – MESOR, amplitude and acrophase of EDs 1, 2, 7, 8, 11 and statistical analysis of the acute phase (ED2 vs. 1), the induction phase (ED7 vs. 2), the washout phase (ED8 vs. 10, and the expression phase (ED11 vs. 2) are shown.

Table 2

	Experimental days & phases	MESOR	p-value	F-statistic	Amplitude	p-value	F-statistic	Acrophase	p-value	F-statistic
0.6 mg/kg	ED1	342.51			192.33			15.67		
	ED2	337.50			153.01			16.30		
	Acute: ED2 vs1		0.822	0.051		0.366	0.821		0.474	0.515
	ED7	354.34			200.80			15.52		
	Induction: ED7 vs2		0.540	0.377		0.274	1.202		0.339	0.919
2.5 mg/kg	ED8	378.40			195.11			16.18		
	Washout: ED8 vs1		0.222	1.499		0.951	0.004		0.520	0.416
	ED11	376.15			242.88			16.56		
	Expression: ED11 vs2		0.162	1.966		0.041	4.207		0.730	0.119
	ED1	358.06			207.58			15.64		
10.0 mg/kg	ED2	366.57			220.33			16.16		
	Acute: ED2 vs1		0.778	0.079		0.783	0.076		0.480	0.501
	ED7	384.53			248.98			14.72		
	Induction: ED7 vs2		0.622	0.244		0.621	0.245		0.073	3.24
	ED8	392.07			235.30			15.74		
10.0 mg/kg	Washout: ED8 vs1		0.311	1.032		0.587	0.296		0.898	0.016
	ED11	443.20			320.65			15.27		
	Expression: ED11 vs2		0.027	4.979		0.067	3.377		0.193	1.706
	ED1	366.37			153.97			17.72		
	ED2	358.61			171.22			15.14		
	Acute: ED2 vs1		0.732	0.117		0.721	0.128		0.013	6.223

Experimental days & phases	MESOR	p-value	F-statistic	Amplitude	p-value	F-statistic	Acrophase	p-value	F-statistic
ED7	303.11			201.85			14.47		
Induction: ED7 vs2		0.114	2.519		0.582	0.303		0.485	0.49
ED8	338.16			186.82			15.27		
Washout: ED8 vs1		0.306	1.052		0.455	0.56		0.007	7.317
ED11	334.97			174.22			14.74		
Expression: ED11 vs2		0.497	0.463		0.957	0.003		0.697	0.152