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Adolescent and Adult Circadian Rhythm Activity Modulated Differently Following Chronic Methylphenidate Administration

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Abstract

Methylphenidate is one of the most common pharmaceutical treatments for Attention Deficit Hyperactive Disorder (ADHD). It is also gaining popularity as a cognitive enhancement and recreational substance. The current study assesses the long-term effects of methylphenidate (MPD) on the circadian rhythm activity pattern of adolescent and adult male Sprague-Dawley (SD) rats. The experiment lasted for 11 days of non-stop recording, the evaluation was divided into 4 phases: acute, induction, washout, and expression phases. Circadian rhythm changes in each phase were compared between the adolescent and adult rats using the following parameters MESOR (midline estimating statistic of rhythm) or average activity, amplitude (distance from MESOR to the peak activity), and acrophase (time at which peak amplitude occurs). Overall, more significant changes in circadian rhythm pattern among adult rats were observed as compared to adolescent rats. As the circadian rhythm governs the diurnal locomotor activity pattern, changes in the locomotor pattern induced by chronic treatment MPD indicate that the drug exerts a long-term effect on the circadian rhythm.

Keywords

Circadian Rhythm, Adolescent, Adult

1. Introduction

Attention Deficit Hyperactivity Disorder (ADHD) exhibits onset in childhood and lasts into adulthood. This

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disorder is expressed by inattention, hyperactivity, excessive impulsivity, and inability to remain focused or to concentrate for prolonged periods of time [1]. The psychostimulant methylphenidate (MPD), better known by its trade name as Ritalin, is one of the most common pharmaceutical treatments in children and adults with ADHD. Its pharmacotherapy is often utilized for prolonged periods of time [2]. It is structurally similar to dextroamphetamine [3] and binds to dopamine transporter (DAT) thus prevent the reuptake of DA from the synaptic cleft to the presynaptic terminal, resulting in the accumulation of DA in the synaptic cleft [3] [4]. Methylphenidate is steadily gaining popularity among adolescent and young adults as the treatment of ADHD [5]-[7] as well as for cognitive enhancement and for recreation [8] [9].

Chronic exposure to some psychostimulants has been reported to alter the diurnal locomotor activity patterns [10]-[12]. The diurnal locomotor activity pattern is one of many biological rhythms controlled by the master clock located in the suprachiasmatic nucleus (SCN) of the anterior hypothalamus [13]. The SCN is entrained to the 24-hour cycle of external light and dark cues [14]-[16] via projections from the retinohypothalamic tract, and synchronizes the diurnal oscillations of multiple internal clocks regulating many physiological processes such as hormonal release, heart rate, blood pressure, eating pattern, sleep/wake cycle, etc. [16]-[19]. Chronic amphetamine exposure in four different times (08:00, 14:00, 20:00, and 02:00) has been shown to elicit behavioral sensitization around the clock, while following MPD exposure only when the drug was given in the morning *i.e.*, it is dependent on the time of the drug administration [20]. Chronic uses of psychostimulants such as cocaine, MPD and amphetamines have been shown to cause adverse effects such as tolerance, withdrawal and sensitization [2] [20]-[27]. It was reported that intravenous or intranasal administration of MPD has a higher mortality rate than cocaine or amphetamine [1] [28]-[30].

During ontogeny overproduction and subsequent synaptic pruning occurs and the time that these processes occur is correlated to the time frame of the development of ADHD [31] [32]. Moreover, it remains unknown whether chronic exposure of MPD to an adolescent's during the developmental age when overproduction of receptors and synaptic connections and subsequent neuronal pruning occurs, may elicit different changes in the circadian rhythms and homeostasis of the animals as compared to the effect of chronic MPD on adults. The current experiment will explore the possible long-term effects of MPD on the circadian rhythm patterns and will compare the effects between adolescent and adult Sprague-Dawley (SD) male rats. The hypothesis of this study is that change in the behavioral circadian rhythm activity pattern will indicated that chronic MPD treatment causes long lasting effect. Furthermore, the circadian rhythm activity following chronic MPD exposure of adolescent rats will be different from the response to adult rats.

2. Materials and Methods

2.1. Animals

Adolescent and adult male SD rats (n = 72) purchased at approximately 28 and 58 days of age, respectively, from Harlan (Indianapolis, IN, USA). These rats were split up randomly into four adolescent and four adult groups each group consists of nine animals. The rats were individually housed in a Plexiglas cage that was their home and test cage and maintained on a 12-hour light/dark cycle (lights on from 06:00 to 18:00) inside a sound-attenuated room that had an ambient temperature of 21˚C ± 2˚C and relative humidity of 37% to 42%. Rats were supplied with food pellets and water *ad libitum* throughout the study. The animals were habituated in this room for 5 to 7 days prior to group assignment. All experiments were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved by our institutional Animal Welfare Committee. At the beginning of the recording, the animals' ages were at P-40 and P-70 for the adolescent and adults, respectively.

2.2. Drug

Methylphenidate hydrochloride was obtained from Mallinckrodt, Inc. (St. Louis, MO, USA). Three doses of MPD were used (0.6, 2.5 and 10.0 mg/kg), these doses are correlated to low, medium, and high doses, respectively, and were selected based on our previous behavioral dose-response studies [15] [20] [24]-[27] [33]. Eight groups of rats were used. Groups one through four consisted of adolescent rats (n = 35) that were treated with 0.6, 2.5, or 10.0 mg/kg MPD or saline/control (**Table 1**). Groups five through eight consisted of adult rats (n = 37) that were treated with 0.6, 2.5, or 10.0 mg/kg MPD or saline (**Table 1**). The MPD dosages were calculated

Table 1. Experimental protocol.

Phase	Acute	Induction	Washout	Expression
Experimental Day (ED)	1	2 - 7	8 - 10	11
A. Adolescent—Age (days)	40	41 - 46	47 - 49	50
Exp. Group				
1 (n = 8)	Saline	0.6 mg/kg MPD	Washout	0.6 mg/kg
2 (n = 11)	Saline	2.5 mg/kg MPD	Washout	2.5 mg/kg
3 (n = 8)	Saline	10.0 mg/kg MPD	Washout	10.0 mg/kg
4 (n = 8)	Saline	Saline	Washout	Saline
B. Adult—Age (days)	70	71 - 76	77 - 79	80
5 (n = 8)	Saline	0.6 mg/kg MPD	Washout	0.6 mg/kg
6 (n = 13)	Saline	2.5 mg/kg MPD	Washout	2.5 mg/kg
7 (n = 8)	Saline	10.0 mg/kg MPD	Washout	10.0 mg/kg
8 (n = 8)	Saline	Saline	Washout	Saline

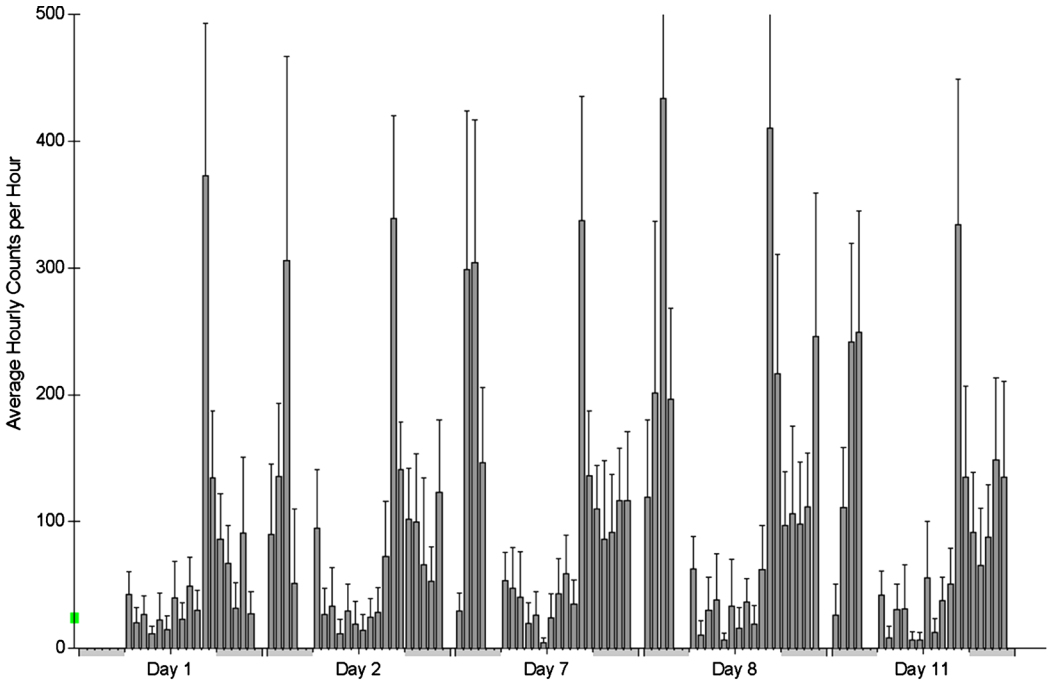
as free-base and dissolved in 0.9% saline. All injections were equalized to a volume of 0.8 ml with 0.9% saline so that the total volume of each injection was the same for all animals. The MPD and saline were administered intraperitoneally (i.p.) between 06:30 hours and 7:00 hours.

2.3. Procedure

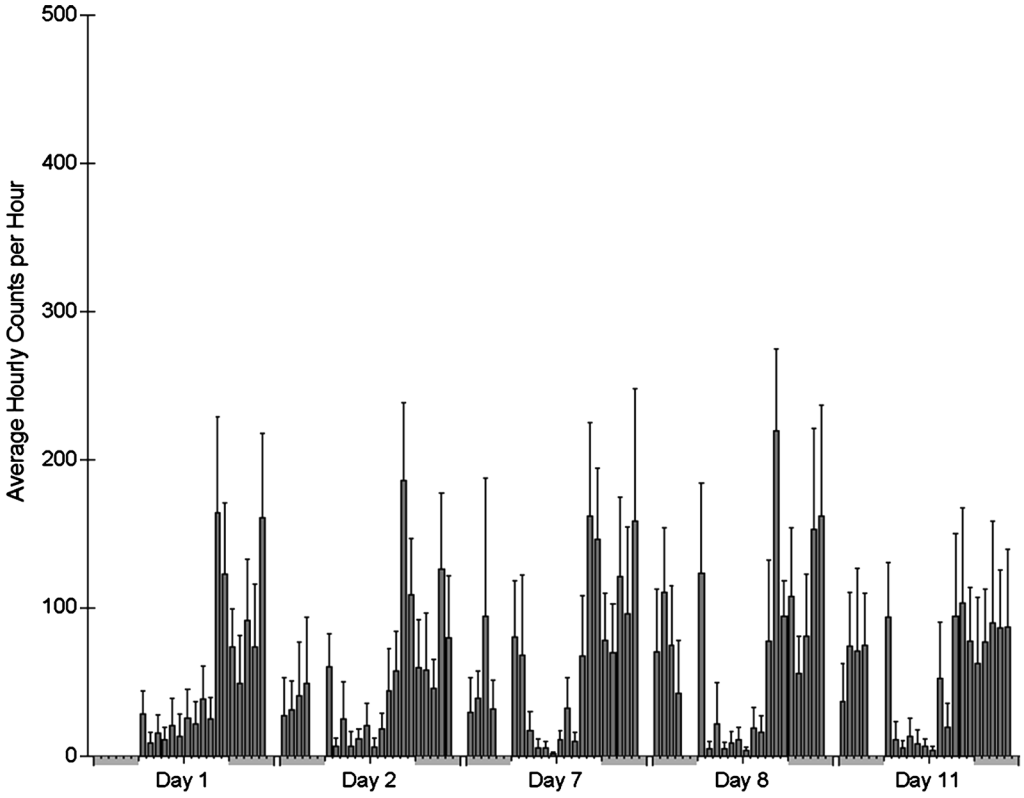
One day prior to recording, each rat was weighed and assigned randomly to their respective group. The computerized, automated activity monitoring (CAAM) system (Accuscan, Columbus, OH, USA) consisted of a clear, acrylic, open-field box (40.5 × 40.5 × 31.5 cm) fitted with two levels of 16 infrared motion sensors located 6.0 and 12.0 cm above the floor of the box. The CAAM recorded interruptions of each infrared beam at a frequency of 100 Hz. Interruptions of any infrared beam were transformed to an activity score. Cumulative counts were compiled automatically by the CAAM system and downloaded every 10 min using the OASIS data collection software (Accuscan) that counted and organized these counts into various locomotor activities indices [15] [20] [24]-[27] [33]. Three locomotor indices were analyzed: total distance traveled (TD), horizontal activity (HA), and number of stereotypic movements (NOS). Total distance traveled measures the amount of forward ambulatory activity in centimeters. Horizontal activity measures the total number of beam interruptions that occurred in the horizontal sensor (lowest tier) during a given sample period and provides total locomotor activity. Number of stereotypic movements measures the number of repetitive episodes with at least 1 second-interval before the beginning of another episode. The number of stereotypic movements is used to assess the effect of drug treatment on general stereotyped behaviors such as sniffing, grooming, and other repetitive behaviors [24]. Since the HA, TD and NOS exhibit similar activity pattern, only the HA data are shown in this study for simplicity purpose. The data collected were automatically downloaded to the computer and summed by the Accuscan analyzer into 10-min bins. Every 6 bins provided 1 hour of activity and was plotted onto 24-hr histograms. Two control groups were used (saline control groups 4 and 8) to verify whether time and animal handling did or did not modulate the locomotor activities (**Figure 1**).

2.4. Data Analysis

The eleven experimental days (EDs) were divided into four phases for data analysis as follow: the acute phase describes the differences in the locomotor activity between ED 2 (the first day of MPD administration) to ED 1 (saline administration/baseline); the induction phase compares activities of ED 7 to that of ED 2 to determine whether 6 consecutive daily MPD treatments would induce alterations in locomotor activity patterns; the washout phase compares the locomotor activity pattern of ED 8 - 10 when MPD was not administered after six



(a)



(b)

Figure 1. Control adolescent and adult animals. The figure summarizes the averages hourly counts of horizontal activity/1hr for experimental day (ED) 1, 2, 7, 8 and 11 and shows similar activity in all the experimental days with minor fluctuations. The histograms show that the adolescent animas are more active than the adult group animals. Each group consist of N = 9.

consecutive daily MPD exposures to ED 1 baseline activity; the expression phase compares activity of ED 11 to ED 2 to determine whether MPD rechallenge at ED 11 would alter the locomotor activity previously seen during the initial (acute) MPD exposure, *i.e.*, whether tolerance or sensitization was expressed.

The activity patterns following saline injection on ED 1 of the adolescent and adult groups and the activity at ED 2 to ED 7 and at ED 11 were the same as ED 1 with minor non significant fluctuations. Thus, the ED 1 activity pattern following saline of each group can and was used as the baseline and control for that group. In doing so, we aim to nullify the possible inter-animal variability.

Two forms of data analysis were used: 1) Hourly histograms and their mean and 2 ± SE, composed from summing of six 10-min counts of locomotor activity, for EDs 1, 2, 7, 8, 9, 10 and 11, were used to compare and analyze for significant changes in activity amplitude and shift between the hourly activities within each experimental phase. 2) Cosine Curve Statistical Analysis (CCSA) test [34] using the 10 min count and organized into three parameters over a 24-hour cycle: MESOR (midline estimating statistic of rhythm) or the average activity represented by the curve, amplitude (distance from MESOR to the peak activity), and acrophase (time at which the amplitude peak occurs) were used. The estimates of these three parameters provide the ability to test for statistical significant change in locomotor activity patterns with respect to time and intensity within the 24 hr of each day. In addition, the CCSA tested parameters independently as well as in combinations for a null hypothesis of no difference. The large numbers of f-tests were used because several aspects of the long-term behavior were independently evaluated. Presence of a change of circadian rhythm in each phase was determined based on an abnormal change of locomotor activity pattern and amplitude from baseline. Furthermore, adolescent and adult rats challenged with MPD were compared to determine if there is a significant change in locomotor activity between rats of different ages challenged with the same doses of acute and chronic MPD exposure. Since the locomotor activity patterns for HA, TD, and NOS were similar among the dose groups and the histograms and CCSA portray similar pattern within each dose group, only the CCSA of HA are shown and discussed here for simplicity.

3. Results

3.1. The Control Groups

Figure 1 shows the hourly HA for control groups 4 and 8. The adolescent and adult rats, were treated with saline on EDs 1 to ED 7, at ED 8 to ED 10 no treatment and at ED 11 saline injection was given. The figure demonstrates the expected baseline locomotor activity pattern from nocturnal animals. The locomotor activity exhibits higher activity during the night and less during the day while showing typical u shape circadian rhythm activity patterns. Following saline administration, both adolescent and adult male rats in the control groups showed similar patterns of activity throughout the 11 EDs. In other words, no alterations of circadian rhythm activity patterns were evident throughout the 11 EDs in the two groups 4 and 8 following repetitive saline injection. The figure shows that adolescents rats are more active than the adult rat group.

3.2. MPD Acute Phase (Comparing ED 2 vs. ED 1) in Adolescent vs. Adult Rats

The adolescent and adult rats exposed to 0.6, 2.5, or 10.0 mg/kg MPD exhibit substantial increases in MESOR and amplitude on ED 2 as compared to activity following saline injection on ED 1, signifying that the drug has relatively immediate effects on locomotor activity upon administration. These increased locomotor indices significantly altered the locomotor activity pattern on the CCSA test. In order to better study the long-term effect of MPD and not the acute effect of the drug, the data from the initial three hours post injection were omitted to eliminate in the calculation the immediate (acute) drug effect, and the remaining nine hours of data were again analyzed. The resulting CCSA test showed that the increased locomotor indices of the acute phase did not reach statistical significant change in HA circadian pattern (**Table 2**).

The acute 0.6 mg/kg MPD failed to alter the locomotor circadian activity pattern of the adolescent group. The acute 0.6 mg/kg MPD exposure in adult male rats exhibited increases in the amplitude while the 2.5 and the 10.0 mg/kg MPD failed to alter the circadian activity pattern of both the adolescent and the adult groups, only the 0.6 mg/kg adult dose group reached statistical significance ($p < 0.026$) (**Table 2**).

3.3. The Induction Phase (Comparing ED 7 vs. ED 2) in Adolescent vs. Adult Rats

The adolescent rats that exposed to 0.6 and 10.0 mg/kg MPD showed increases in all three locomotor indices on

Table 2. Statistical data of the horizontal activity.

	Dose Groups	Parameters	Experimental days							Acute	Induction	Washout		Expression
			1	2	7	8	10	11		ED 2 vs.1	ED 7 vs. 2	ED 8 vs. 1	ED 10 vs. 1	ED 11 vs. 2
ADOLESCENT	0.6 mg/kg MPD	MESOR	55	62	66	56	67	59	p-value	0.478	0.677	0.881	0.268	0.794
									F-stat	0.504	0.174	0.023	1.232	0.068
		Amplitude	30.34	37.73	51.20	48.34	30.12	37.03	p-value	0.588	0.457	0.196	0.989	0.962
									F-stat	0.294	0.556	1.682	0.000	0.002
		Acrophase	14.50	14.42	14.86	15.21	16.62	15.78	p-value	0.952	0.746	0.590	0.240	0.451
									F-stat	0.004	0.105	0.292	0.001	0.569
	2.5 mg/kg MPD	MESOR	65	61	59	58	65	54	p-value	0.691	0.811	0.453	0.946	0.398
									F-stat	0.158	0.057	0.565	0.005	0.716
		Amplitude	38.20	32.93	34.05	46.94	52.40	38.39	p-value	0.722	0.938	0.527	0.330	0.685
									F-stat	0.127	0.006	0.400	0.952	0.165
		Acrophase	14.31	14.21	15.88	15.44	15.88	15.95	p-value	0.941	0.229	0.318	0.173	0.160
									F-stat	0.006	1.452	1.000	1.865	1.989
	10.0 mg/kg MPD	MESOR	65	56	67	56	67	54	p-value	0.433	0.452	0.360	0.791	0.794
									F-stat	0.617	0.639	0.842	0.070	0.068
		Amplitude	35.63	54.24	74.74	54.28	45.34	51.85	p-value	0.254	0.314	0.198	0.533	0.882
									F-stat	1.308	1.016	1.664	0.391	0.022
		Acrophase	13.90	13.10	13.20	13.69	15.94	14.79	p-value	0.545	0.926	0.857	0.132	0.087
									F-stat	0.367	0.009	0.033	2.281	2.945
ADULT	0.6 mg/kg MPD	MESOR	45	58	75	83	86	76	p-value	0.112	0.131	**0.000**	**0.000**	0.111
									F-stat	2.536	2.298	14.320	18.400	2.561
		Amplitude	25.36	52.28	74.27	61.42	72.69	80.04	p-value	**0.026**	0.225	**0.019**	**0.001**	0.124
									F-stat	5.000	1.480	5.548	10.880	2.380
		Acrophase	11.96	13.86	14.12	14.75	15.36	14.97	p-value	0.161	0.787	0.095	**0.036**	0.237
									F-stat	1.980	0.073	2.810	4.422	1.404
	2.5 mg/kg MPD	MESOR	67	58	73	85	88	90	p-value	0.184	0.052	**0.037**	**0.002**	**0.000**
									F-stat	1.774	3.821	4.397	9.451	15.280
		Amplitude	34.53	45.25	86.00	86.40	93.46	83.37	p-value	0.295	**0.001**	**0.000**	**0.000**	**0.004**
									F-stat	1.101	12.000	15.770	31.590	8.415
		Acrophase	12.52	12.81	13.72	14.19	14.41	14.36	p-value	0.750	0.175	0.095	**0.018**	**0.042**
									F-stat	0.101	1.853	2.810	5.651	4.184
	10.0 mg/kg MPD	MESOR	58	56	74	69	76	56	p-value	0.777	0.140	0.183	**0.035**	0.996
									F-stat	0.081	2.189	1.787	4.510	0.000
		Amplitude	45.51	67.17	72.54	76.86	80.65	55.56	p-value	0.104	0.782	**0.020**	**0.010**	0.448
									F-stat	2.668	0.077	5.490	6.729	0.578
		Acrophase	12.63	12.53	13.04	13.09	13.80	13.79	p-value	0.903	0.565	0.581	0.161	0.122
									F-stat	0.015	0.333	0.306	1.978	2.406

ED 7 comparing to ED 2. However, the adolescent 2.5 mg/kg MPD group exhibited decreases in MESOR and amplitude. The three adult groups challenged with 0.6, 2.5, and 10.0 mg/kg MPD uniformly showed increases in MESOR, amplitude and acrophase in the initial two to three hours post-injection on ED 7 as compared to ED 2. The difference between the two cohorts were that only the adolescent 2.5 mg/kg MPD dose group had a decrease in locomotor activity pattern and that only the adult 2.5 mg/kg MPD group reached statistical significance in the MESOR ($p < 0.05$) and in the amplitude ($p < 0.001$) while none was observed in any adolescent group (**Table 2**).

3.4. The Washout Phase (Comparing ED 8 and ED 10 vs. ED 1) in Adolescent vs. Adult Rats

All three adolescent dose groups show an increase in activity between the 07:00 and 08:00 hours, *i.e*., the time that they were treated with MPD in the previous six days. This increased activity for about an hour is dose related characteristics with the 10.0 mg/kg MPD group exhibiting the greatest change. However, the overall changes in locomotor activity patterns of ED 8 and 10 did not reach statistical significance when compared to those of ED 1 (**Table 2**).

In a similar fashion to the adolescent dose groups, all three adult dose groups exhibit increases in MESOR, amplitude and acrophase between 07:00 and 08:00 hours. Besides this similarity, the adult groups' behavior differed starkly from the adolescent groups. The 0.6 mg/kg MPD adult group doubled in MESOR ($p < 0.0001$) and tripled in amplitude ($p < 0.019$) on ED 8 vs. 1; the same increases persisted through ED 10 when compared to ED 1 (**Table 2**). Additionally, the 0.6 mg/kg MPD adult group shifted in acrophase by approximately 3.5 hours into the dark phase on ED 10 ($p < 0.036$) (**Table 2**). The 2.5 mg/kg MPD adult group increased in MESOR by 25% ($p < 0.037$) and amplitude by 250% ($p < 0.0001$) on ED 8, *i.e*. while the animals' horizontal movement that interrupted the infrared beams increased by 25%, the total distance traveling per hour during peak activity was 250% greater than the average (**Table 2**). The changes persisted through ED 10 for the 2.5 mg/kg MPD adult group: MESOR increased 30% ($p < 0.002$), amplitude increased 270% ($p < 0.0001$), and, additionally, acrophase shifted 2 hours into the dark phase ($p < 0.018$). The 10.0 mg/kg MPD adult group increased in amplitude on ED 8 ($p < 0.020$), MESOR on ED 11 ($p < 0.035$), and amplitude on ED 11 ($p < 0.010$ and **Figure 2** washout phase).

3.5. The Expression Phase (Comparing ED 11 vs. ED 2) in Adolescent vs. Adult Rats

The 0.6 mg/kg MPD adolescent group at ED 11 shows almost no changes in MESOR and amplitude when compared to activity of ED 2. The 2.5 and 10.0 mg/kg MPD adolescent groups show decreases in MESOR, amplitude, and acrophase on ED 11 but did not reach statistical significance.

Among the adult groups, only the 2.5 mg/kg MPD group shows statistically significant increases in MESOR ($p < 0.0001$), amplitude ($p < 0.004$), and a 2-hour right shift in acrophase ($p < 0.042$) on ED 11 (**Table 2**). The 0.6 mg/kg MPD adult dose group shows increases in MESOR, amplitude and acrophase while the 10.0 mg/kg MPD adult dose group shows relatively stable mesor, decreased amplitude, and a one-hour right-shifted acrophase at ED 11 (**Figure 2** Expression phase).

4. Discussion

The present study seeks to determine whether there are differences in diurnal activity of adolescent and adult rats after acute and/or chronic MPD treatments. MPD is considered a mild central nervous stimulant that blocks the reuptake of norepinephrine and dopamine into the presynaptic neurons. It also stimulates the cerebral cortex and subcortical structures in a similar fashion as dose amphetamine.

The main findings of this study are as follow: 1) No statistically significant alteration in locomotor activity circadian activity pattern is apparent in all three adolescent MPD-treated groups; 2) The adult rats exposed to repetitive (chronic) 2.5 and 10.0 mg/kg MPD dose groups exhibit altered circadian activity patterns during the induction, washout, and expression phases; 3) The adult 0.6 mg/kg MPD dose group demonstrates altered circadian activity pattern at a later time course, *i.e*., during the washout and expression phases; 4) The 0.6 mg/kg MPD adult group increased locomotor activity drastically during the washout phase; 5) The locomotor activity of the 2.5 mg/kg MPD adult group progressively increased and reached statistical significance during the washout and expression phases; 6) The 10.0 mg/kg MPD adult group increased locomotor activity that reached statistical significance only during the washout phase; 7) The adults receiving the same dosages of MPD as their

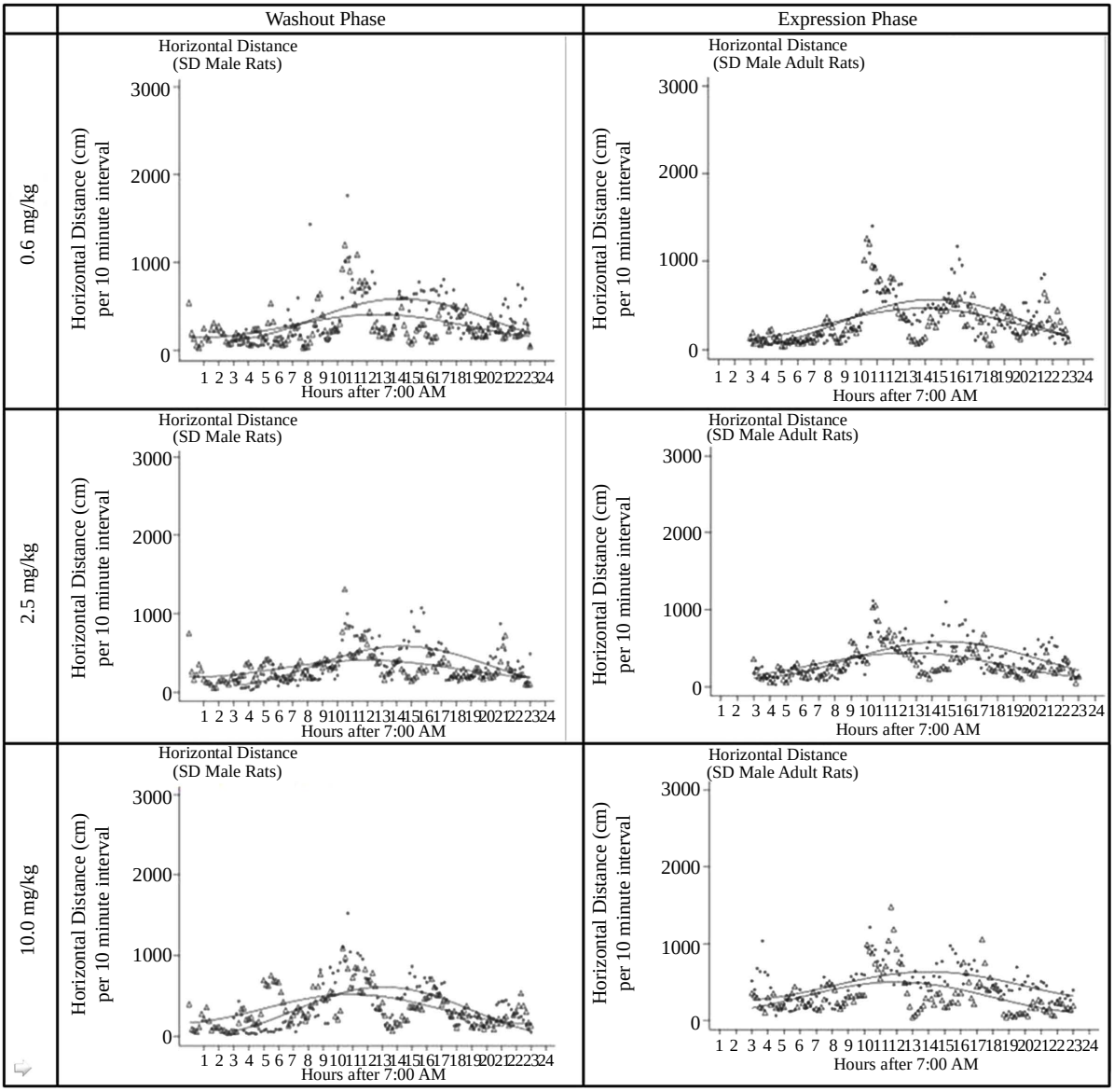


Figure 2. It summarizes (N = 9 per group) and compares the activity pattern using the CCSA test. The dots represent the control (saline) group and the triangles represent the activity of the methylphenidate group. **Figure 3** compares the percentage change from baseline (set arbitrarily at 0%) of adolescent and adult animals during ED 1, 2, 7, 8, 10 and 11.

adolescent counterparts showed a significantly greater increase in activity during the day and night while at the same time exhibiting shifts in peak activity characteristic of circadian rhythm pattern changes. While circadian rhythm pattern changes were present in both adolescent and adult male rats, the severity of the changes were more pronounced in the adult population.

There is a master internal clock in the suprachiasmatic nucleus (SCN) that regulates the circadian rhythm activity [5] [13] [16]. This master clock regulates all the other clocks via a wide variety of clock genes and gene products such as Per1, Per2, Cry1, Cry2, bmal1, Rev-erb [13] [35]. The endogenous circadian rhythm control clocks entrain to the environmental time cues (such as light and dark changes) via the retinohypothalamic tract [13] [16] [18]. In the absence of these cues, the circadian rhythms will vary at a time course slightly more than 24 hours. Patterns governed by circadian rhythms include sleep, temperature control, food consumption, reproductive behaviors, hormone production, heart rate, and blood pressure [18] [19] [35] [36]. These rhythms greatly affect the homeostasis and the well-being of animals. Changes in these rhythms can contribute to changes in the

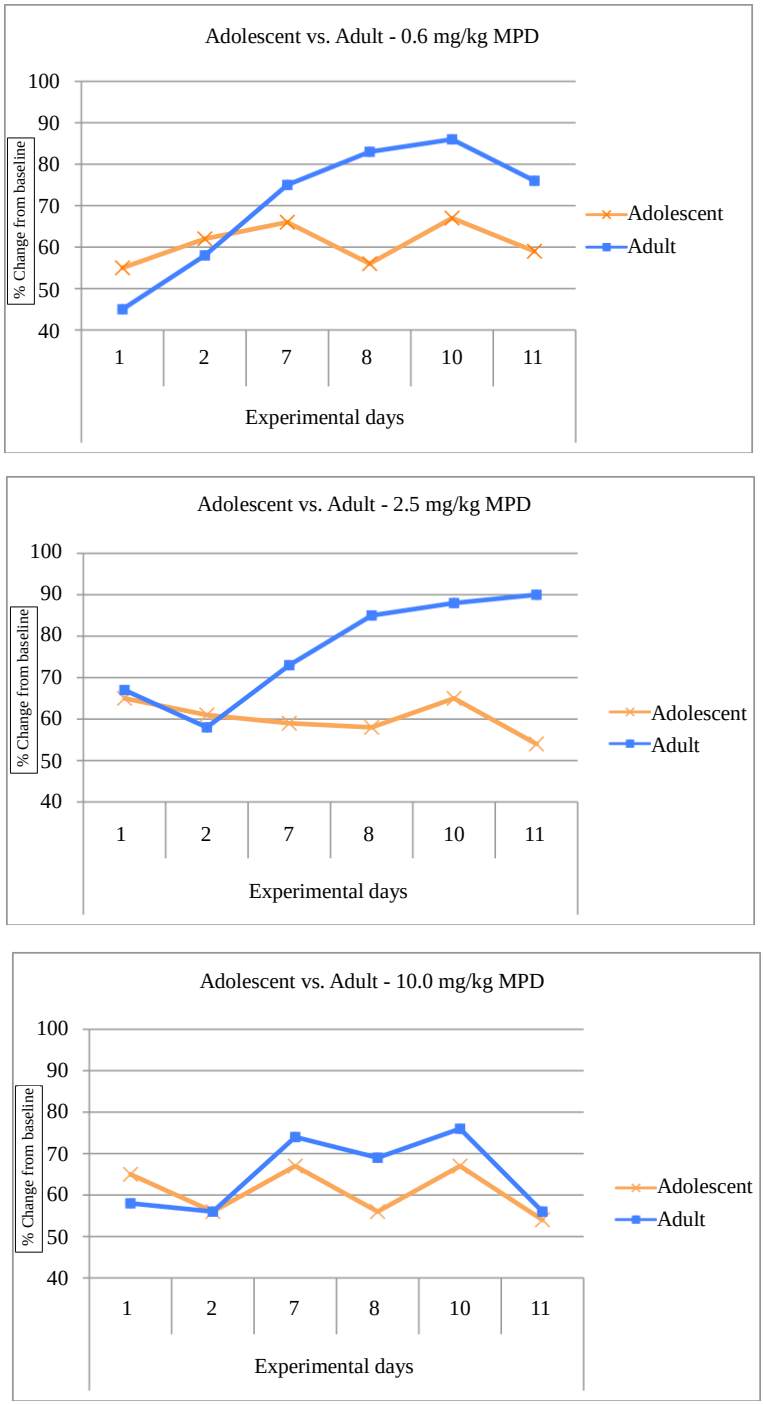


Figure 3. Comparing the percentage change of adult to adolescent.

physiological processes such as jet lag, fatigue, insomnia, or long-term pathologies such as diabetes mellitus, heart disease, and hypertension [17] [18] [36]-[39]. Besides environmental cues, psychostimulants and drugs of abuse also exert an effect on neuroadaptation resulting in altered physiological processes. Chronic morphine exposure decreases the size of DA neurons in the ventral tegmental area and impairs axoplasmic transport, decreases DA transmission, and decreases dendritic branching leading to dysphoria during withdrawal [40] [41]. Chronic cocaine and amphetamine exposure increases dendritic branching and spine density in NAc and PFC [42] [43], which may explain the effect of the drugs on incentive-motivational effects and judgment impairment.

The process of synaptic pruning that occurs during ontogeny may explain the altered response between adolescent and adult rats to MPD. It was demonstrated that the response to psychostimulants varied with age [44]-[47]. During normal development, overproduction of synaptic connections and receptors occurs and follows by their pruning or competitive elimination. The marked over-production and elimination of synapses and receptors during adolescence may serve as a permissive factor for a number of behavioral/psychiatric disorders, including attention deficit hyperactive disorder (ADHD) [48]. Between 5 and 15 years of age in humans, synaptic density in the frontal cortex decreases by approximately 40% [31] [49]. The time-course and nature of ADHD parallel the pattern of overproduction and regressive synaptic elimination described above [49]. A growing body of evidence suggests that exposure to stimulants during ontogeny produces effects on animal behavior and/or biochemical processes that differ from well-known effects on comparable exposure to MPD during adulthood [48]. Some adverse consequences on neuronal development in children were reported [50]. Adolescent rats are affected differently by catecholaminergic agonists when compared with adult rats [46] [51]-[54]. It was reported that adolescent rats exhibited an attenuated behavioral response, while adult rats exhibited an increased behavioral response to psycho-stimulants [46] [53] [55]. Rats and mice exposed to MPD during the period equivalent to human adolescence experienced behavioral changes that endured into adulthood, which suggests that MPD does have a neurobiological effect that lasts into adulthood [14] [48] [56].

The long lasting effects of MPD use may also be attributed to a deficiency or abnormal production of the proteins responsible for controlling human circadian rhythms. The administration of psychostimulants may lead to defective genetic translation of these necessary proteins or possible even direct damage to these genes themselves. A recent study has shown that administration of MPD increased the peripheral index of early DNA damage in young and adult rats, which was more pronounced with chronic treatment and in the striatum compared with the hippocampus [57]. The correlation between increased circadian rhythm pattern changes within the adult rat population as compared to the adolescent rat population be due to the decreased capacity for DNA repair with age. The striatum is one of the richest sources of dopaminergic synapses [58] that are critical for the regulation of striatal function.

With the present study, a greater increase in locomotor activity indicative of circadian rhythm alterations should be noted as a possible side effect of everyday use of these drugs. Even more important is the fact that the psychostimulant used in this study affected adults far more in terms of circadian rhythm changes than it did adolescents. Therefore, it is prudent to advocate further research into the chronic effect of MPD (Ritalin) from the standpoint of chronic use of the drug by our adolescent population and also as a pharmaceutical enhancement and recreational abuse in adults.

5. Conclusion

Figure 1 shows that adolescent rats are more active during the night compared to adult rats. The acute and chronic MPD exposure did not alter the circadian locomotor activity of the adolescent while the circadian locomotor activity of the adult was altered during the induction, washout and expression phase (**Table 2**). **Figure 3** compares the total activity/24 hr of the two age groups (baseline was arbitrarily set as 0%). The figure shows that MPD exposure elicits increased locomotor activity in both groups, however, the adult group exhibited significant ($p < 0.05$) higher activity following 0.6 and 2.5 mg/kg MPD during the washout and the expression phase compared to adolescent rats. During the washout phase in both ages, increased activity was expressed. This increased activity may indicate that the animals are exhibiting behavioral withdrawal. The adolescents exhibited mild withdrawal while the adults exhibited more severe withdrawal signs.

References

[1] Godfrey, J. (2009) Safety of Therapeutic Methyphenidate in Adults: A Systematic Review of the Evidence. *Journal of Psychopharmacology*, **23**, 194-205. http://dx.doi.org/10.1177/0269881108089809

[2] Askenasy, E.O., Taber, K.H., Yang, P.B. and Dafny, N. (2007) Methylphenidate (Ritalin): Behavioral Studies in the Rat. *International Journal of Neuroscience*, **117**, 1-38. http://dx.doi.org/10.1080/00207450600910176

[3] Patrics, K.S. and Markowitz, J.C. (1997) Pharmacology of Methylphenidate, Amphetamine, Enantiomers and Penoline in Attention Deficit/Hyperactivity Disorder. *Human Pharmacology*, **12**, 527-546.

[4] Volkow, N.D., Fowler, J.S., Hitzemann, P. and Wang, G.I. (1996) Neurochemical Mechanism Underlying Responses to Psychostimulants. *NIDA Research Monograph*, **159**, 322-348.

[5] Accardo, P. and Blondis, T.A. (2001) What's All the Fuss about Ritalin? *The Journal of Pediatrics*, **138**, 6-9. http://dx.doi.org/10.1067/mpd.2001.111505

[6] Gerasimov, M.D., Franceschi, M., Volkow, N.D., Gifford, A., Gatley, S.J., Marsteller, D., Molina, P.E. and Dewey, S.L. (2000) Comparison between Intraperitoneal and Oral Methylphenidate Administration: A Microdialysis and Locomotor Activity Study. *Journal of Pharmacology and Experimental Therapeutics*, **296**, 51-57.

[7] Levin, F.R. and Kleber, H.D. (1995) Attention-Deficit Hyperactivity Disorder and Substance Abuse: Relationships and Implications for Treatment. *Harvard Review of Psychiatry*, **2**, 246-258. http://dx.doi.org/10.3109/10673229509017144

[8] Greely, H., Sahakian, B., Harris, J., Kessler, R.C., Gazzaniga, M., Campbell, P. and Farah, M.J. (2008) Towards Responsible Use of Cognitive-Enhancing Drugs by the Healthy. *Nature*, **456**, 702-705. http://dx.doi.org/10.1038/456702a

[9] Stix, G. (2009) Turbocharging the Brain. *Scientific American*, **301**, 46-50. http://dx.doi.org/10.1038/scientificamerican1009-46

[10] Bergheimm, M., Yang, P.B., Burau, K.D. and Dafny, N. (2012) Adolescent Rat Circadian Activity Is Modulated by Psychostimulants. *Brain Research*, **1431**, 35-45. http://dx.doi.org/10.1016/j.brainres.2011.10.027

[11] Glasser, A.U., Reyes-Vasquez, C., Burau, K.D. and Dafny, N. (2012) Continue Morphine Administration and Abrupt Cessation Alters the Normal Locomotor Circadian Activity Pattern. *Pharmacology Biochemistry and Behavior*, **101**, 544-552. http://dx.doi.org/10.1016/j.pbb.2012.02.015

[12] Jones, Z. and Dafny, N. (2013) Acute and Chronic Dose Response Effect of Methylphenidate on Ventral Tegmental Area Neurons Correlated with Animal Behavior. *Brain Research Bulletin*, **96**, 86-92.

[13] Klein, D.C., Moore, R.Y. and Reppert, S.M. (1991) Suprachiasmatic Nucleus: The Mind's Clock. Oxford University Press, New York.

[14] Antle, M.C., Van Diepen, H.C., Deboer, T., Pedram, P., Rodrigues Pereira, R. and Meijer, J.H. (2012) Methylphenidate Modifies the Motion of the Circadian Clock. *Neuropsychopharmacology*, **37**, 2446-2455. http://dx.doi.org/10.1038/npp.2012.103

[15] Gaytan, O., Yang, P., Swann, A. and Dafny, N. (2000) Diurnal Differences in Sensitization to Methylphenidate. *Brain Research*, **864**, 24-39. http://dx.doi.org/10.1016/S0006-8993(00)02117-X

[16] Lee, M.J., Yang, P.B., Wilcox, V.I., Burau, K.P., Swann, A.C. and Dafny, N. (2011) Repetitive Methylphenidate Administration Modultaes the Diurnal Behavioral Activity Pattern of Adult Female SD Rats. *Journal of Neural Transmission*, **118**, 285- 298.

[17] Trinh, N.T., Kohlleppel, S., Wilcox, V.T., Burau, K. and Dafny, N. (2013) Adult Female Rats Altered Diurnal Locomotor Activity Pattern Following Chronic Methylphenidate Treatment. *Journal of Neural Transmission*, **120**, 1717-1731.

[18] Giorgetti, M. and Zhdanova, I.V. (2000) Chronic Cocaine Treatment Induces Dysregulation in the Circadian Pattern of Rats' Feeding Behavior. *Brain Research*, **877**, 170-175. http://dx.doi.org/10.1016/S0006-8993(00)02671-8

[19] Gray, J.D., Punsoni, M., Tabori, N.E., Melton, J.T., Fanslow, V., Ward, M.J., *et al.* (2007) Methylphenidate Administration in Juvenile Rats Alters Brain Areas Involved in Initiation, Motivated Behaviors, Appetite, and Stress. *The Journal of Neuroscience*, **27**, 7196-7207. http://dx.doi.org/10.1523/JNEUROSCI.0109-07.2007

[20] Gaytan, O., Al-Rahim, S., Swann, A. and Dafny, N. (1997) Sensitization to Locomotor Effects of Methylphenidate in the Rat. *Life Sciences*, **61**, PL101-PL107.

[21] Gaytan, O., Al-Rahim, S. and Swann, A. (1998) Diurnal Differences in Rats Motor Response to Amphetamine. *European Journal of Pharmacology*, **345**, 119-128. http://dx.doi.org/10.1016/S0014-2999(97)01558-6

[22] Lee, M.J., Burau, K.D. and Dafny, N. (2013) Behavioral Daily Rhythmic Activity Pattern of Adolescent Female Rats Is Modulated by Acute and Chronic Cocaine. *Journal of Neural Transmission*, **120**, 733-744.

[23] Dafny, N. and Yang, P.B. (2006) The Role of Age, Genotype, Sex, and Route of Acute and Chronic Administration of Methylphenidate: A Review of Its Locomotor Effects. *Brain Research Bulletin*, **68**, 393-405. http://dx.doi.org/10.1016/j.brainresbull.2005.10.005

[24] Kalivas, P.W., Pierce, R.C., Cornish, J. and Sorg, B.A. (1998) A Role for Sensitization in Craving and Relapse in Cocaine Addiction. *Journal of Psychopharmacology*, **122**, 49-53. http://dx.doi.org/10.1177/026988119801200107

[25] Yang, P.B., Behrang, A., Swann, A.C. and Dafny, N. (2003) Strain Differences in the Behavioral Responses of Male Rats to Chronically Administered Methylphenidate. *Brain Research*, **971**, 139-152. http://dx.doi.org/10.1016/S0006-8993(02)04240-3

[26] Yang, P.B., Swann, A.C. and Dafny, N. (2006) Acute and Chronic Methylphenidate Dose-Response Assessment on Three Adolescent Male Rat Strains. *Brain Research Bulletin*, **71**, 301-310. http://dx.doi.org/10.1016/j.brainresbull.2006.09.019

[27] Yang, P.B., Atkins, K.D. and Dafny, N. (2011) Behavioral Sensitization and Cross-Sensitization between Methylphe-

nidate Amphetamine, and 3,4-Methylenedioxymethamphetamine (MDMA) in Female SD Rats. *European Journal of Pharmacology*, **66**, 72-85. http://dx.doi.org/10.1016/j.ejphar.2011.04.035

[28] Hovens, J.G., Cantwell, D.P. and Kiriakos, R. (1994) Psychiatric Comorbidity in Hospitalized Adolescent Substance Abusers. *Journal of the American Academy of Child and Adolescent Psychiatry*, **33**, 476-483. http://dx.doi.org/10.1097/00004583-199405000-00005

[29] Massello, W.I. and Carpenter, D.A. (1999) A Fatality Due to the Intranasal Abuse of Methylphenidate (Ritalin). *Journal of Forensic Sciences*, **44**, 220-221.

[30] Parran Jr., T.V. and Jasinski, D.R. (1991) Intravenous Methylphenidate Abuse: Prototype for Prescription Drug Abuse. *Archives of Internal Medicine*, **151**, 781-783. http://dx.doi.org/10.1001/archinte.1991.00400040119027

[31] Huttenlocher, P.R. (1979) Synaptic Density in Human Frontal Cortex-Developmental Changes and Effects of Aging. *Brain Research*, **163**, 195-205. http://dx.doi.org/10.1016/0006-8993(79)90349-4

[32] Rakie, P. (1986) Development of the Primate Cerebral Cortex. In: Lewis, M., Ed., *Child and Adolescent Psychiatry*, Williams and Wilkins, Baltimore.

[33] Amini, B., Yang, P.B., Swann, A.C. and Dafny, N. (2004) Differential Locomotor Responses in Male Rats from Three Strai to Acute Methylphenidate. *International Journal of Neuroscience*, **114**, 1063-1084. http://dx.doi.org/10.1080/00207450490475526

[34] Alghim, M.F., Yang, P.B., Wilcox, V.T., Burau, K.D., Swann, A.C. and Dafny, N. (2009) Prolonged Methylphenidate Treatment Alters the Behavioral Diurnal Activity Pattern of Adult Male Sprague-Dawley Rats. *Pharmacology Biochemistry and Behavior*, **92**, 93-99.

[35] Reppert, S.M. and Weaver, D.R. (2002) Coordination of Circadian Timing in Mammals. *Nature*, **418**, 935-941. http://dx.doi.org/10.1038/nature00965

[36] Dunlap, J.C. (1999) Molecular Basis for Circadian Clocks. *Cell*, **96**, 271-290. http://dx.doi.org/10.1016/S0092-8674(00)80566-8

[37] Kollins, S.M., MacDonald, E.K. and Rush, C.R. (2001) Assessing the Abuse Potential of Methylphenidate in Nonhuman and Human Species: A Review. *Pharmacology Biochemistry and Behavior*, **68**, 611-627.

[38] Lee, M.J., Yang, P.B., Wilcox, V.T., Burau, K.D., Swann, A.C. and Dafny, N. (2009) Does Repetitive Ritalin Injection Produce Long-Term Effect on SD Female Adolescent Rats? *Neuropharmacology*, **57**, 201-207.

[39] Vitaona, M.H., Takahashi, J.S. and Turek, F.W. (2001) Overview of Circadian Rhythms. *Alcohol Research Health*, **25**, 85-93.

[40] Nestler, E.J. (2004) Molecular Mechanism of Drug Addiction. *Neuropharmacology*, **47**, 24-32. http://dx.doi.org/10.1016/j.neuropharm.2004.06.031

[41] Kim, Y., Taylor, M.A., Baron, M., Sands, A., Nairn, A.C. and Greengard, P. (2009) Methylphenidate Induced Dendritic Spine Formation and ΔFosB Expression in Nucleus Accumbens. *Proceedings of the National Academy of Sciences of the United States of America*, **106**, 2915-2920. http://dx.doi.org/10.1073/pnas.0813179106

[42] Robinson, T.E. and Kolb, B. (1999) Alteration in the Morphology of Dendrites and Dendritic Spines in the Nucleus Accumbens and Prefrontal Cortex Following Repeated Treatment with Amphetamine and Cocaine. *European Journal of Neuroscience*, **11**, 1598-1604. http://dx.doi.org/10.1046/j.1460-9568.1999.00576.x

[43] Robinson, T.E. and Kolb, B. (2004) Structural Plasticity Associated with Exposure to Drug of Abuse. *Neuropharmacology*, **47**, 33-46.

[44] Adriani, W., Chiarotti, F. and Laviola, G. (1998) Elevated Novelty Seeking and Peculiar *d*-Amphetamine Sensitization in Periadolescent Mice Compared with Adult Mice. *Behavioral Neuroscience*, **112**, 1152-1156. http://dx.doi.org/10.1037/0735-7044.112.5.1152

[45] Bowman, B.P. and Kuhn, C.M. (1996) Age-Related Differences in the Chronic and Acute Response to Cocaine in the Rat. *Developmental Psychobiology*, **29**, 597-611. http://dx.doi.org/10.1002/(SICI)1098-2302(199611)29:7<597::AID-DEV4>3.0.CO;2-P

[46] Laviola, G., Wood, R.D., Kuhn, C., Francis, R. and Spear, L.P. (1995) Cocaine Sensitization in Periadolescent and Adult Rats. *The Journal of Pharmacology and Experimental Therapeutics*, **275**, 345-357.

[47] Roffman, J.L. and Raskin, L.A. (1997) Stereotyped Behavior: Effects of *d*-Amphetamine and Methylphenidate in the Young Rat. *Pharmacology Biochemistry and Behavior*, **58**, 1095-1102. http://dx.doi.org/10.1016/S0091-3057(97)00321-3

[48] Andersen, S.L., Arvaniitaglannis, S.A., Pliakas, A.M., LeBlance, C. and Carleron, W.A. (2002) Altered Responsiveness to Cocaine in Rats Exposed to Methylphenidate during Development. *Nature Neuroscience*, **51**, 13-14. http://dx.doi.org/10.1038/nn777

[49] Andersen, S.L. and Teicher, M.H. (2000) Sex Differences in Dopamine Receptors and Their Relevance to ADHD.

Neuroscience Biobehavioral Reviews, **24**, 137-141. http://dx.doi.org/10.1016/S0149-7634(99)00044-5

[50] Insel, T.R. and Charney, D.S. (2003) Research on Major Depression Strategies and Priorities. *Journal of the American Medical Association*, **289**, 3167-3168. http://dx.doi.org/10.1001/jama.289.23.3167

[51] Bolanos, C.A., Glatt, S.J. and Jackson, D. (1998) Subsensitivity to Dopaminergic Drugs in Periadolescent Rats: A Behavioral and Neurochemical Analysis. *Developmental Brain Research*, **111**, 25-33. http://dx.doi.org/10.1016/S0165-3806(98)00116-3

[52] Dell'Anna, M.E., Luthman, J., Landqvist, E. and Olson, L. (1993) Development of Monoamine Systems after Neonatal Anoxia in Rats. *Brain Research Bulletin*, **32**, 159-170. http://dx.doi.org/10.1016/0361-9230(93)90070-R

[53] Spear, L.P. and Brake, S.C. (1983) Periadolescence: Age-Dependent Behavior and Psychopharmacological Responsivity in Rats. *Developmental Psychobiology*, **16**, 83-109. http://dx.doi.org/10.1002/dev.420160203

[54] White, P.J. and Kalivas, P.W. (1998) Neuroadaptations Involved in Amphetamine and Cocaine Addiction. *Drug and Alcohol Dependence*, **51**, 141-153. http://dx.doi.org/10.1016/S0376-8716(98)00072-6

[55] McDougall, S.A., Collins, R.L., Karper, P.E., Watson, J.B. and Crawford, C.A. (1999) Effects of Repeated Methylphenidate Treatment in the Young Rat: Sensitization to Both Locomotor Activity and Stereotyped Sniffing. *Experimental and Clinical Psychopharmacology*, **7**, 208-218. http://dx.doi.org/10.1037/1064-1297.7.3.208

[56] Brandon, C.L., Marinelli, M., Baker, L.K. and White, F.J. (2001) Enhanced Reactivity and Vulnerability to Cocaine Following Methylphenidate Treatment in Adolescent Rats. *Neuropsychopharmacology*, **25**, 651-661. http://dx.doi.org/10.1016/S0893-133X(01)00281-0

[57] Andreazza, A.C., Frey, B.N. and Valvassori, S.S. (2007) DNA Damage in Rats after Treatment with Methylphenidate. *Progress in Neuro-Psychopharmacology, Biological Psychiatry*, **31**, 1282-1288.

[58] Dougherty, D.D., Bonab, A.A., Spencer, T.J., Ranch, S.L., Madras, B.K. and Fischman, A.J. (1996) Dopamine Transporter Density in Patients with Attention Deficit Hyperactivity Disorder. *The Lancet*, **9196**, 2132-2133.

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