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Behavioral daily rhythmic activity pattern of adolescent female rat is modulated by acute and chronic cocaine

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Abstract

Cocaine is one of well-known drugs of abuse, and many children experience early exposure to cocaine. Because of an immature neuronal system in adolescents, they may react differently to repeated cocaine administration compared to adults. Most of the published papers report the effect of cocaine on adult male rats and this paper focused on the effects of cocaine on the 24 h locomotor activity rhythm patterns activity of adolescent Sprague Dawley (SD) female rats. Changes in the locomotor activity rhythm patterns could indicate that cocaine elicits long-term changes in the clock genes of the body that regulate different physiological processes. The objective of this study was to investigate whether cocaine in adolescent female rats modulated their daily activity pattern. Animals were divided into control (saline), 3.0, 7.5, 15.0 mg/kg cocaine groups. On experimental day 1 (ED 1), all groups were given saline injection. From ED 2 to ED 7, either saline or cocaine (3.0, 7.5, or 15.0 mg/kg) was given daily. ED 8 to ED 10 were the washout days, where no injection was given. On ED 11, the animals were injected with saline or with the same dose of cocaine as they were treated on ED 2 to ED 7. Each animal's locomotor activities was recorded nonstop following saline or cocaine injection for 11 consecutive days using the open field assay. In conclusion, it was observed that all three groups receiving repeated cocaine administration (3.0, 7.5, and 15.0 mg/kg) displayed significantly altered locomotor activity rhythm patterns.

Keywords

Cocaine; Daily rhythm; Adolescents; Female

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Conflict of interest

The authors declare no conflicts of interest.

Introduction

The Center for Disease Control and Prevention reported in 2009 that, 6.4 % of high school students had previously used cocaine and 2.8 % of high school students stated that they currently use cocaine (Eaton et al. 2010). In 2009, 0.3 % of 12-years-old adolescents reported that they had used cocaine in their lifetime, and this number increases to 6.7 % by age 18 (SAMHSA 2011 National survey on drug use and health). Because of these alarming numbers regarding how early children are exposed to cocaine because most of the cocaine studies to date have been done on adult male animal subjects, it is important to understand the effects of acute and chronic cocaine on the adolescent female animal model.

Psychostimulant drugs, including cocaine, act upon the neurotransmitter systems that undergo maturation, through receptor pruning and neuronal pathway reorganization, during adolescence (Wiley et al. 2011). The exposure of psychostimulants to immature nervous system is thought to be the main reason why adolescents tend to react differently to psychostimulant drugs than the adults do (Izenwasser 2005). In a molecular study involving adolescent rats and cocaine administration, it was shown that cocaine exposure in rats during adolescence causes temporal gene expression pattern changes in prefrontal cortex, one of the main sites involved in psychostimulant drug reactions (Black et al. 2006a). It was also shown that cocaine exposure during adolescence results in molecular changes in the amygdala and decreases fear learning and anxiety behaviors in rats that last to adulthood (Sullivan et al. 2011). Furthermore, some studies suggest that cocaine may actually be more addictive to adolescents than to adults (Estroff et al. 1989).

Several studies reported that the physiology of behavior of living organisms from bacteria to human are controlled by a clock that responds to daily environmental clues (Algham et al. 2009; Bergheim et al. 2012; Klein et al. 1991; Lee et al. 2009, 2011, Moore and Lenn 1972). The endogenous clock is characterized by a cycle of approximately 24 h in duration and it responds to internal or external cues to maintain the homeostatic function by regulating biological and physiological processes such as body temperature, blood pressure, sleep-wake cycles, metabolism, and locomotor activity (Chou et al. 2003; Shin et al. 2008). Projection from light sensitive retinal ganglion ascends to the suprachiasmatic nucleus (SCN) which is considered the master clock that regulates the levels of gene expression, hormones, and protein synthesis to reset and synchronize the rhythms to day/night cycle (Antle et al. 2012; Moore and Lenn 1972). This clock is sensitive also to various pharmacological agents, and drug exposure may alter the amplitude or the phase of the circadian pacemaker (Giorgetti and Zhdanova 2000). Psychostimulants such as methylphenidate were reported to exhibit an effect on the circadian activity pattern of locomotion (Algham et al. 2009, 2010; Antle et al. 2012; Lee et al. 2009, 2011). These changes in the circadian activity rhythm serves as an experimental marker in animals to correlate with the long-term effect of the drug (Algham et al. 2009, 2010, ; Bergheim et al. 2012; Glaser et al. 2012; Lee et al. 2009, 2011; Norrell et al. 2010). Many aspects of mammalian physiology operate on a diurnal or circadian rhythmic pattern that displays approximately 24 h cycle. Numerous clock genes, governed and controlled by the master clock located in SCN, control the levels of gene expression. Disruption of circadian rhythm

can lead to decreased cognitive function and metabolic, and cardiac impairment (Mong et al. 2011). Significant alteration of circadian rhythms with prolonged cocaine exposure could indicate that cocaine exerts long-term effects through circadian clock gene expression (Uz et al. 2005).

Although it is not exactly known, where the difference between male and female daily rhythms comes from, it is clear that there are sex differences in daily rhythms. Studies have shown that women have significantly shorter circadian period when it comes to sleep cycles, endogenous temperature, or melatonin rhythms (Mong et al. 2011). Women also respond differently to cocaine. Women seem to get addicted sooner and show symptoms of dependence sooner than men (Becker et al. 2001; Hernandez-Avila et al. 2004). Yet, not many studies have used female subjects to study the effects of psychostimulants on daily rhythms. In this study, we used the open field assay to assess whether acute and/or chronic cocaine causes behavioral changes in locomotor behavioral daily rhythmic patterns of female adolescent Sprague Dawley rats.

Materials and methods

Animals

Forty-eight young, postnatal day 32 (P-32) to P-35, female Sprague Dawley (SD) rats, weighing 90–110 g upon arrival, were acquired from Harlan, Indianapolis, IN, USA, and housed 4–5 animals per cage. The animals were kept in a sound proof experiment room, on a 12:12 night/dark cycle (lights on at 06:00), at a relative humidity of 37–42 %, and at an ambient temperature of 21 ± 2 °C. Food pellets and water were provided ad libitum. After 5–7 days of acclimation, the animals were randomly divided into four groups: saline/control ($n = 12$), 3.0 mg/kg cocaine ($n = 12$), 7.5 mg/kg cocaine ($n = 12$), and 15 mg/kg cocaine ($n = 12$) groups. Then, they were individually placed in open field testing cages ($40.5 \times 40.5 \times 31.5$ cm) in the same experimental room. These testing cages were used as their home cages throughout the experiment (Table 1).

Apparatus

Locomotor activity was recorded using the computerized open field animal activity monitoring (CAAM; AccuScan Instruments, Inc., Columbus, OH) system. The recordings were stopped only for the daily injections and to add food and water between 06:30 and 07:00 throughout the 11 non-stop recording days. The animals were not disturbed in anyway otherwise. CAAM system consisted of sixteen open field cages with two levels of 16 infrared beams. The interruptions of these beams were counted and compiled by AccuScan Analyzer and downloaded to a PC every 10 min. Then, OASIS software compiled these data into different locomotor movement indices including horizontal activities (HA), total distance traveled (TD), and the number of stereotypic activities (NOS) (Gaytan et al. 1997, 1999, 2000; Yang et al. 2003, 2006, 2010, 2011).

Drugs

Cocaine obtained from Sigma Aldrich (St. Louis, MO) was dissolved in 0.9 % isotonic saline solution (0.9 % NaCl). All the injections (saline/cocaine) were of the same volume

(0.8 cc) to ensure that different responses will not be due to differences in volumes injected. All injections were given between 06:30 and 07:00 intra-peritoneally (i.p.).

Procedure

On experimental day 1 (ED 1), all groups were given a single injection of 0.8 cc 0.9 % NaCl solution, at approximately 07:00 am and the recording started immediately after the injection. On ED 2 to ED 7, daily injections of either saline, 3.0, 7.5, or 15.0 mg/kg cocaine depending on the group assignments were given. On ED 8–10, the locomotor activities were recorded with no injections, and these 3 days were considered as the washout period. A single saline or a re-challenge cocaine injection of identical dose as given in ED 2 through 7, followed on ED 11 (Table 1). All efforts were made to minimize the number of animals used and their suffering. The experiment procedures conformed to the guideline of NIH and the declaration of Helsinki, and approved by our local animal welfare committee.

Data analysis

Two data analyses were done. (1) Hourly histograms with standard errors were produced by summing and averaging six 10-min recording bins. (2) The same 10-min bins were also used for hourly cosine curve statistical analysis (CCSA) test (Bingham et al. 1982) to perform the statistical analysis. The CCSA test used the data points from each 10 min to form a cosine curve that shows the locomotor activity pattern for each ED. It also estimated three parameters, mesor (average activity level), amplitude (distance from mesor to highest point), and acrophase (time at which the maximum amplitude occurs). These three components were then used to statistically compare locomotor activity patterns of different EDs, and to determine whether locomotor activity pattern has been significantly altered (Bingham et al. 1982) from 1 day to another as a result of cocaine exposure. Since the focus of this study is on the long-term effect of the activity patterns and not the immediate effect of the drug, the first 3 h of the data after the cocaine injections were excluded in the CCSA analysis (Algahim et al. 2010; Lee et al. 2011; Bergheim et al. 2012).

Four comparisons were made: (1) acute effect—compared the activity of ED 1 (control recording with saline) to that of ED 2 (the first day of cocaine injection) to observe whether a single (acute) injection can change the locomotor activity daily activity pattern; (2) induction phase—compared the activity of ED 7 (the last day of the drug maintenance treatments) to that of ED 1 to observe whether the 6 days of treatment can induce changes; (3) expression phase—compared the activity of ED 11 (cocaine re-challenge exposure) with that of ED 1 to observe whether the chronic treatment can elicit long-lasting effect; and (4) washout phase—compared the activity of ED 8 to that of ED 1 to observe whether the activity alterations induced during the six maintenance drugs period persist in the absence of daily cocaine injections (Table 1).

Results

Control

Figure 1 shows the hourly HA histogram and the corresponding superimposed CCSA graphs of ED 1, 2, 7, 8, and 11 of the saline control group. The daily recording started immediately

following saline injections each morning. The adolescent SD female rats in control group exhibited similar locomotor activity patterns throughout the 11 EDs. Similar observation of saline injection using identical protocol was observed in other studies using other psychostimulants (Algahim et al. 2009, 2010; Bergheim et al. 2012; Lee et al. 2011). The CCSA test analysis of the activity following saline exposure and the washout days was not significantly different between ED 1 and all the other experimental days (Fig. 1, Table 2), i.e. the activity level and patterns during all the EDs (ED 1–ED 11) were similar with minor, not significant, fluctuations. Since ED 1 exhibited the same activity patterns as all the other EDs, the ED 1 data of the cocaine groups can and was used as control (Algahim et al. 2010; Bergheim et al. 2012; Lee et al. 2011; Norrell et al. 2010). Therefore, any significant changes in the daily activity locomotor patterns between ED 1 and the other EDs of cocaine treated groups (3.0, 7.5, and 15.0 mg/kg cocaine) can be considered as the drug effects.

Acute effect: ED 1 vs. 2

Figure 2 shows the hourly histogram and the corresponding CCSA graphs of circadian HA and NOS patterns of ED 1 and ED 2 of the 3.0 mg/kg cocaine group. The first cocaine injection resulted in decrease in activity during the majority of the light phase, and the CCSA test indicated that these changes in activity are significant difference between the daily HA patterns of the two EDs (Table 3). The NOS activity of ED 2 exhibits some increases in their activities compared to ED 1. The CCSA test indicated that these changes in activities on ED 2 elicited by the drug were significantly different from the activity pattern of ED 1 (Table 2). The CCSA test did not indicate significant differences between the two EDs (ED 2 compared to ED 1) for any of the indices in the group treated with 7.5 mg/kg cocaine. On the other hand, 15.0 mg/kg cocaine group exhibited increased activities in all three indices during most hours of the light and dark phases on ED 2 compared to ED 1, and these differences were indicated as significant according to the CCSA test (Table 2, graphs omitted).

Induction phase: comparing ED 7 vs. 1

The hourly histograms and CCSA graphs comparing the daily TD and NOS patterns of ED 7–1 for 3.0 mg/kg cocaine group are shown in Fig. 3. Both TD and NOS activity at ED 7 indices showed increased activities during the light and dark phases compared to ED 1. The CCSA tests indicated that these changes are significant ($P < 0.05$; Table 3). For 7.5 mg/kg cocaine group, the HA and TD indices exhibited increased activities during the first 2 h of the light phase and during the dark phase, and the CCSA test indicated these increases elicited significant difference between the daily activity pattern of the HA and TD patterns of ED 7 compared to ED 1 ($P < 0.05$; Table 3). All three of the locomotor indices following the 15 mg/kg cocaine exposure group also exhibited increased activities during the first 2 h of light phase and during the dark phase, and the daily locomotor patterns of all three indices of ED 7 were significantly different from those of ED 1, according to the CCSA test ($P < 0.05$; Table 2).

Washout phase: comparing ED 8 vs. 1

During the washout phase, the animals were not treated with any injections and the recordings continued the same as in the previous EDs. The HA, TD and NOS indices exhibited increased activities during the first part of light phase and during the dark phase of ED 8 compared to ED 1 in the 3.0, 7.5 and 15 mg/kg cocaine group, which were significantly different according to the CCSA test ($P < 0.05$; Fig. 4; Table 3).

Expression phase: ED 11 vs. 1

Figure 5 shows the hourly histogram and the CCSA graphs for all three indices of 3.0 mg/kg cocaine group, comparing the ED 11 to ED 1. All three indices showed increased activities during the majority of light and dark phases on ED 11 compared to ED 1, and these differences were significant changes by the CCSA test ($P < 0.05$; Table 3). All the three indices of 7.5 and 15.0 mg/kg cocaine groups also showed similar pattern, i.e., increased activities in majority of hours of ED 11 when compared to ED 1, and these changes were significant according to the CCSA test as well ($P < 0.05$; Table 2).

Discussion

Endogenous neurotransmitter, neuromodulator, hormone levels, and locomotor activity are involved in the biological psychostimulant response and demonstrate a consistent 24 h daily rhythm activity pattern. The possible long-term effects of psychostimulant exposure in adolescence are considered controversial, and thus, the objective of this study was to investigate whether the chronic exposure to the psychostimulant cocaine affects the locomotor daily rhythm activity patterns of female adolescent SD rat. The hypothesis of this study is that change in daily rhythm activity pattern is an indicator of the long-term effect of the treatment. The CCSA test was used to compare the effect of acute and chronic cocaine exposure on the daily activity pattern of four animal groups. This statistical test showed that all doses of cocaine, low, moderate, or high dose, were able to cause changes of locomotor daily activity rhythm patterns. After 1 day (acute) of cocaine injection, all three dose groups displayed significantly different locomotor daily rhythm when compared to the baseline daily rhythm. The last day of maintenance cocaine injection (ED 7) also displayed significant different locomotor daily rhythms from those of baseline daily rhythms in all the three cocaine groups. The same patterns could be also seen during the cocaine first washout day, i.e., withdrawal phase, and during the re-challenge on day (ED 11) as well.

In animal models, the progressive augmentation of locomotor activity following repeated drug administration is considered the expression of behavioral sensitization to the drug, behavioral sensitization is one of the experimental marker indicator signs of drug dependence in animals (Askenasy et al. 2007; Dafny and Yang 2006; Glaser et al. 2012; Norrell et al. 2010; Yang et al. 2003, 2006, 2010, 2011). The neuroadaptation within the mesocortico-limbic dopaminergic pathway, involving ventral tegmental area, nucleus accumbens, and prefrontal cortex, is a major player in the development of behavioral sensitization (Chen et al. 2009; Chong et al. 2012; Claussen et al. 2012; Claussen and Dafny 2012; Podet et al. 2010; Salek et al. 2012; Robinson and Becker 1986; Kalivas and Stewart 1991; Nestler 2002; Everitt and Robbins 2005; Kalivas and O'Brien 2008). Although not

completely understood yet, there seems to be a relationship between behavioral sensitization and circadian clock genes in the brain, (Yuferov et al. 2005; Abarca et al. 2002; McClung et al. 2005) because the degree of sensitization to psychostimulant is time dependent (Abarca et al. 2002). In other words, the degree of behavioral sensitization is dependent on which phase of a daily rhythm the subject is in when cocaine was administered. It has been reported that the most robust response to cocaine happens when the drug is given during the early light phase (Abarca et al. 2002). Furthermore, the mutant mice lacking functional *Clock* gene (a gene that codes for a part of transcriptional activator in the master clock in SCN) showed baseline hyperactive behavior, stronger cocaine-induced locomotor sensitization, and increased drug reward compared to the control subjects (McClung et al. 2005). In addition, *mPer1* gene (a target gene of CLOCK protein) mutant mice showed little to no sensitization to repetitive cocaine exposure while *mPer2* gene (another target gene of CLOCK protein) mutant mice showed hypersensitization to repeated cocaine administration (Abarca et al. 2002). This suggests that the relationship between different circadian genes have different and complex influence on cocaine addiction. On the other hand, repeated cocaine administration has significant effect on the clock gene expressions. Yuferov et al. (2003), reported that circadian gene *Per1* and *Per2* expression increased in caudate-putamen after acute cocaine administration, Lynch et al. (2008) observe that repeated cocaine administration altered several circadian gene expressions in rat striatum as well. In a different study, hippocampal clock gene expression changes were also noted after repeated cocaine administration. (Uz et al. 2005) Other psychostimulants were also shown to have similar effects on these clock genes (Manev and Uz 2006).

Adolescence adds another variable to the relationship between clock genes and cocaine addiction, because their neuronal system is still immature at this age. There are contradictory results; some studies with adolescent rats report that repetitive exposure of cocaine elicited behavioral sensitization, while other studies report that repetitive exposure to cocaine failed to elicit behavioral sensitization in adolescent subjects (Laviola et al. 1995; Niculescu et al. 2005; Collins and Izenwasser 2002; Guerriero et al. 2006). In studies that specifically worked with adolescent rats and gene expressions, it was found that cocaine administration during adolescence caused transient changes in gene expressions in prefrontal cortex and amygdala (Sullivan et al. 2011; Black et al. 2006a). However, the behavioral changes seen in drug-seeking behaviors and learned fear behaviors were present long after the gene expressions and protein levels normalized (Black et al. 2006b). This is most likely due to changes in gene expressions leading to irregularity in axon guidance, synapse strengthening, and synapse formation and causing abnormal neuron circuit formation (Sullivan et al. 2011; Black et al. 2006a). This study observed that repeated cocaine administration alters the locomotor daily rhythm activity patterns in female adolescent rats. The locomotor daily changes following cocaine noted in the adolescent rats in this study can also be a product of transient gene expression change with behavioral changes lasting longer because of changes in synapse formation, but this requires further studies.

Another variable present in the current study is the sex differences in the response to cocaine administration. In adults, hormonal influence may account partially for the differences in response to cocaine between two adult genders. With prepubertal castration, male rats

showed substantial increase in cocaine-stimulated behaviors as adults while female rats showed decrease in cocaine-stimulated behaviors as adults compared to control groups that did not have prepubertal castration (Parylak et al. 2008). Furthermore, stronger response to psychostimulants has been seen in females than males both in animal models and in humans (Brecht et al. 2004; Hernandez-Avila et al. 2004; Lynch 2008). For example, adult women's circadian melatonin and temperature rhythms are significantly shorter than those of adult men in humans as well as in animal models (Duffy et al. 2011; Schull et al. 1989; Davis et al. 1983). Therefore, it is possible that the response to cocaine may have hormonal influence as well as non-hormonal influence. Among adult female rodents, cocaine's stimulating effects seem stronger during proestrus and estrus than during diestrus (Sell et al. 2000). However, sex hormones may not account fully for the differences between the genders. For instance, in a study observing the sensitivity to conditioned reward of cocaine, it was shown that female rats were more sensitive to cocaine than male rats even during adolescence, where sexual hormone influence is limited (Zakharova et al. 2009). None of these studies, however, focused on sex differences in the effects of cocaine specifically on locomotor daily activity rhythm patterns. Current study showed that cocaine is able to change locomotor activity daily rhythms in female adolescent rats before the reproductive hormones kick in. Further studies are needed to compare the results of current studies of locomotor activity daily pattern to studies involving male adolescent rats to see if sexual differences exist during adolescence as well as with adult male and female rats to compare response to cocaine before and after the initiation of regulatory effects of the sex hormones.

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Fig. 1.

Control group. The hourly histogram of horizontal activity (HA) of experimental days (EDs) 1, 2, 7, 8 and 11 and superimposed cosine curve statistical analysis (CCSA) graph of EDs 1, 2, 7, 8, and 11 are displayed. On CCSA graph, each symbol represents different EDs. Similar locomotor activity patterns are seen with minimal fluctuations throughout the EDs (Table 2). Similar results were seen in total distance traveling (*TD*) and number of stereotypic movement (*NOS*). The *shaded area* under the *histograms* indicates the dark periods

Fig. 2. The hourly histograms and CCSA graphs comparing ED 1 and ED 2 for HA and NOS indices of the 3.0 mg/kg cocaine group. *Open circle* ED 1 and *plus symbol* ED 2 in the CCSA graphs

Fig. 3. The hourly histograms and CCSA graphs comparing ED 1 and ED 7 for TD and NOS indices of the 3.0 mg/kg cocaine group. *Open circle* ED 1 and *plus symbol* ED 7 in the CCSA graphs

Fig. 4. The hourly histograms and CCSA graphs comparing ED 1 and ED 8 for TD and NOS indices of the 3.0 mg/kg cocaine group. *Open circle* ED 1 and *plus symbol* ED 8 in the CCSA graphs

Fig. 5.

The hourly histograms and CCSA graphs comparing ED 1 and ED 11 for HA, TD and NOS indices of 3.0 mg/kg cocaine group are shown. *Open circle* ED 1 and *plus symbol* ED 11 in the CCSA graphs

Table 1

Describes the treatment and the experimental protocol for each of the 4 animal groups

Groups	ED 1	ED 2–7	ED 8–10	ED 11
Control	Saline	Saline	Washout	Saline
3.0 mg/kg Cocaine	Saline	3.0 mg/kg cocaine	Washout	3.0 mg/kg cocaine
7.5 mg/kg Cocaine	Saline	7.5 mg/kg cocaine	Washout	7.5 mg/kg cocaine
15.0 mg/kg Cocaine	Saline	15.0 mg/kg cocaine	Washout	15.0 mg/kg cocaine

ED experimental day

All injections were i.p. and equalized to volume of 0.8 cc/animal and were given at about 07:00

Table 2
Statistic calculation using the cosine curve statistical analysis (CCSA) for the control (saline) group comparing across all experimental days

Comparison	LI	Mesor		Amp		Acrotime	
		F-statistics	P values	F-statistics	P-values	F-statistics	P-values
All days (1, 2, 7, 8, 11)	HA	1.209 (4, 680)	0.305	1.428 (4, 680)	0.223	1.561 (4, 680)	0.183
	TD	0.923 (4, 680)	0.450	2.021 (4, 680)	0.090	1.938 (4, 680)	0.102
	NOS	1.719 (4, 680)	0.144	0.151 (4, 680)	0.963	1.198 (4, 680)	0.310

Comparing all the experimental days of control group shows no significant change in circadian rhythm throughout the experiment, i.e., activity obtained on ED 1 following saline was similar to that obtained on ED 2, 7, 8, and 11

Significance was set at $P < 0.05$

LI Locomotor index, *Mesor* mean level of cosine curve, *Amp* amplitude–height of the peak above the mean level, *Acrotime* time at which peak occurs

Table 3

The statistical calculation using the CCSA test for all the cocaine groups comparing between different experimental days

Contrast	LI	Drug conc. (mg/kg)	Characteristic	F-statistic	P-value
Day 1 vs. 2	HA	3.0	Mesor	17.03 (1,254)	0.000
		15.0	Acrophase	5.277 (1,254)	0.022
	TD	15	Acrophase	4.851 (1,254)	0.029
		NOS	3.0	Mesor	6.773 (1,254)
Day 1 vs. 7	HA	15	Acrophase	5.764 (1,254)	0.017
		7.5	Acrophase	4.734 (1,254)	0.031
		15.0	Acrophase	10.27 (1,254)	0.002
	TD	3.0	Mesor	4.017 (1,254)	0.046
		7.5	Acrophase	4.883 (1,254)	0.028
		15.0	Amplitude	5.832 (1,254)	0.016
Day 1 vs. 8	NOS	3.0	Acrophase	8.23 (1,254)	0.004
			Mesor	3.935 (1,254)	0.048
		15.0	Acrophase	8.433 (1,254)	0.004
	HA	7.5	Acrophase	9.83 (1,254)	0.002
		15.0	Mesor	5.466 (1,254)	0.020
		TD	3.0	Acrotine	10.96 (1,254)
Day 1 vs. 11	TD	Mesor	10.07 (1,254)	0.002	
		7.5	Acrophase	6.636 (1,254)	0.011
		15.0	Amplitude	5.26 (1,254)	0.023
	NOS	Acrophase	8.021 (1,254)	0.005	
		3.0	Mesor	6.728 (1,254)	0.010
		7.5	Amplitude	5.821 (1,254)	0.017
Day 1 vs. 11	NOS	Acrophase	7.793 (1,254)	0.006	
		15.0	Acrophase	8.401 (1,254)	0.004
		3.0	Mesor	8.13 (1,254)	0.005
	HA	Amplitude	15.87 (1,254)	0.000	
		7.5	Amplitude	8.265 (1,254)	0.004
		Acrotine	8.87 (1,254)	0.003	

Contrast	LI	Drug conc. (mg/kg)	Characteristic	F-statistic	P-value
		15.0	Mesor	4.502 (1,254)	0.035
			Amplitude	13.28 (1,254)	0.000
			Acrotime	20.26 (1,254)	0.000
	TD	3.0	Mesor	7.601 (1,254)	0.006
			Amplitude	6.843 (1,254)	0.009
			Amplitude	4.608 (1,254)	0.033
		7.5	Acrophase	6.844 (1,254)	0.009
			Mesor	7.838 (1,254)	0.006
			Amplitude	22.45 (1,254)	0.000
	NOS	3.0	Acrophase	8.929 (1,254)	0.003
			Amplitude	5.281 (1,254)	0.022
			Amplitude	5.593 (1,254)	0.019
		15.0	Acrophase	6.098 (1,254)	0.014
			Amplitude	7.198 (1,254)	0.008
			Acrophase	22.19 (1,254)	0.000

The italicized values indicate the significant changes between the two compared experimental days. Only the results with significant differences were shown

Significance was set at $P < 0.05$

LI locomotor indices; Mesor mean level of cosine curve; Amp amplitude–height of the peak above the mean level; Acrotime time at which peak occurs