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Adolescent Rat Circadian Activity is Modulated by Psychostimulants

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Abstract

Circadian pattern of activity regulates many aspects of mammalian physiology and behavior to particular times of the day by entraining the circadian clocks to external environmental signals. Since circadian rhythms are sensitive to many pharmacological agents, it is important to understand if the repetitive use of psychostimulants such as amphetamine will alter the circadian rhythm behavioral activity pattern. The present study uses male Sprague-Dawley rats to study the long-term effects of amphetamine on the locomotor circadian rhythm activity pattern. Rats were randomly assigned to a testing cage that recorded their locomotor activity nonstop for eleven days using the open field assay, as follows: one day of baseline activity was recorded and then the experimental group was injected with amphetamine (0.6 mg/kg) for 6 days, no treatment for 3 days (i.e., washout days) and then re-challenged with amphetamine for one more day while the control group was treated similarly with saline. The Cosine Curve Statistical Analysis (CCSA) test was used to fit a 24-hour curve to activity pattern. Results indicate that repetitive daily amphetamine injections cause behavioral sensitization and a significant change of circadian rhythm of locomotor activity pattern, and elicit behavioral expectation to receive the drug or expression of withdrawal during the washout days. The results suggest that either changes in circadian rhythm caused sensitization and withdrawal or sensitization and withdrawal caused the change in circadian rhythm activity.

Keywords

Acute; chronic; amphetamine; locomotor activity; circadian activity

1. INTRODUCTION

Today, adolescents and young adults around the world are striking deals to buy and sell their prescription psychostimulants for cognitive enhancement, and some newspaper articles contend that —we should welcome new methods of improving our brain function (Greely et. al., 2008). The number of deaths and serious injuries associates with prescription drug use

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rose to record levels recently (Los Angeles Times, 2008). The notion of enhancing mental functions by gulping down a pill that improves attention, memory and planning i.e., cognition is no longer a fantasy (Stix, 2009). Obsession with cognitive enhancers is evidenced in the media and news articles hailing the arrival of what are called ‘smart drug’ or even ‘Viagra for the brain’ (Stix, 2009). The question then surfaces ‘Whether any drug that fiddles with basic brain functions be safe enough to be used at will?’ With the large number of adolescents consuming/abusing amphetamine, it is important to study whether prolonged use of amphetamine in adolescence has any long-term effects. The hypothesis of this study is that significant alteration of circadian rhythmic activity pattern is an experimental marker to indicate that the drug elicits long-term effects and repetitive amphetamine treatment will alter significantly the locomotor circadian activity pattern.

A large body of studies indicate that the physiology of behavior of living organisms from bacteria to humans are controlled by circadian activity rhythms driven by endogenous oscillators and modulated in response to daily environmental cues (Fleur et. al., 1999; Giorgetti and Zhdanova, 2000; Klein et. al., 1991; Lee et. al., 2009; Moore and Lenn, 1972; Reppert and Weaver, 2002; Vitaterna et. al., 2001). The endogenous clock is characterized by a cycle of approximately 24 hours in duration. Alteration of the circadian activity pattern/rhythm leads to behavioral and physiological disorders.

Chronopharmacology discipline was established to study the relationship between the effects of psychostimulants on circadian rhythm in various species including humans (Akhisaroglu, 2004). Circadian rhythm activity is governed by an endogenous clock that maintains the homeostatic functions by regulating biological and physiological processes including body temperature, blood pressure, sleep-wake cycles, locomotor activity, and metabolism (Chou, 2003; Reppert and Weaver, 2002; Shin, 2008).

The suprachiasmatic nuclei (SCN), located in the anterior hypothalamus, is the pacemaker for circadian rhythms. (Klein et. al., 1991; Moore and Lenn, 1972; Nakahata et. al., 2006; Shin, 2008). Projections from the light-sensitive retinal ganglion cells to the SCN reset and synchronize the rhythms to the day-night cycle (Johnson et. al., 1998; Moore and Lenn, 1972; Nakahata et. al., 2006). This photoentrainment results in 24 hour rhythmicity (Reppert and Weaver, 2002).

Circadian body rhythms are sensitive to various pharmacological agents, and drug exposure may alter the amplitude or the phase of the circadian pacemaker (Giorgetti and Zhdanova, 2000). Cocaine, methylphenidate, and methamphetamines was reported to exhibit effect on the circadian rhythm activity by altering feeding and locomotor activity pattern (Algahim et al., 2009; Giorgetti and Zhdanova, 2000; Kita et al 1998; Lee et al., 2009; Uz et al 2002). This change in circadian rhythm activity serves as an experimental marker in animals to correlate with long term effects of psychostimulant in humans. The current literature on altering circadian pattern in response to chronic amphetamine administration is limited. Gaytan, et al. (1996) studied the short-term effects of amphetamines on the circadian rhythm using a computerized locomotor activity monitoring system, which showed that the total distance traveled by the rats decreased after administration of large doses of amphetamines. This decrease in total distance was seen during the day of injection and 24–36 hours post injection, but since this experiment only administered a single dose of amphetamines, it gave no indication of the long-term effects of chronic amphetamine use on the circadian rhythm locomotor activity.

In this experiment, we are examining the long-term (Algahim et al., 2009; Lee et. al., 2009) effects of amphetamine administration in male adolescent Sprague-Dawley rats as determined by the ability of this psychostimulant to elicit change in circadian activity

patterns of locomotor activity using the open field assay. A computerized activity monitoring system recorded 24 hour activity for eleven consecutive days to capture the locomotor behavioral activity rhythm/pattern before and after acute and chronic amphetamine exposure.

2. RESULTS

Saline Control Group

The hourly histogram of all four locomotor parameters (horizontal activity (HA), total distance (TD), stereotypic activity (SA), and number of stereotypic movements (NOS) display a consistent circadian pattern of activity as expected from nocturnal animals; higher activity during the dark period and lower activity during the light period. The locomotor activity levels and its pattern on experimental days 1, 2, 7, 8 and 11 of the saline control group was about the same (Fig. 1). The CCSA test for the five experimental days (experimental days 1, 2, 7, 8 and 11) exhibits similar activity pattern showing that all the experimental days exhibit the same pattern of activity (Table 2 ED 1,2,7,8 and 11).

Activity following Amphetamine injections – Experimental Day 2 compared to Experimental Day 1 (Acute effect)

On the first day of amphetamine injection (experimental day 2 in the amphetamine group and on experimental day 12 on the saline/amphetamine control group-Table 1), the hourly histograms show an increase in HA for the first two hours post amphetamine injection, followed by a decrease in activity for the next three hours (Fig. 2A). The other locomotor indices display similar changes as compared to experimental day 1 baseline levels with an increase in activity for the first 2 hours post amphetamine injection, followed by a decrease in activity for the next 4 hours (Fig. 2B, 2C and 2D). Despite the changes in the hourly histograms in the initial hours post injection, when analyzed by the CCSA test it did not show statistically significant changes in circadian rhythm activity pattern over 24 hours when compared to experimental day 1 baseline for all locomotor indices. Similar observation was obtained from the control group comparing experimental day 12 to experimental day 1.

Activity following amphetamine injections – Experimental Day 2 saline group compared to Experimental Day 2 amphetamine group (acute effect)

When different comparison were made i.e., comparing the effect of the initial amphetamine exposure at experimental day 2 with the saline control group at experimental day 2, the CCSA test showed significant differences in the mesor and the amplitude (Fig. 3 Table 2).

Experimental day 7 saline group compared to experimental day 7 amphetamine group

The locomotor activity following 0.6 mg/kg i.p. amphetamine on experimental day 7 (Table 1) showed an increase in HA, TD, and NOS on the hourly histogram for the first two hours post injection, followed by a decrease in activity for approximately six hours. The hourly histograms also show further increase in all the locomotor indices on experimental day 7 as compared to experimental day 1 of the same group and to experimental day 7 of the saline group with an increase in activity for the first six hours post injection and also for 6 hours during the dark cycle (18:00h – 24:00h). Using the CCSA test to analyze the data, whether the activity rhythmic pattern was modulated by six days of amphetamine exposure it becomes evident that the HA and the NOS showed a significant changes in mesor and amplitude (Fig. 4 and Table 2).

Experimental days 8, 9 and 10 compared to Experimental day 1 of the amphetamine group and to saline group experimental day 8,9, and 10. Locomotor activity in the washout phase

On experimental day 8, neither amphetamines nor saline was given to any group, but in the beginning of the light phase at about 07:00h, the hourly histograms show an increase in HA and TD for 2 hours, followed by a decrease in activity for the next 6 hours as compared to day 1 baseline. The TD and NOS as viewed by the hourly histograms also show an increase for 2 hours followed by a decrease in activity for the next 6 hours. Using the CCSA test to analyze the data as to whether the six daily amphetamine administrations altered the activity rhythmic pattern, only the TD and the NOS showed a significant change ($p < 0.05$) in the mesor and amplitude on experimental day 8, and significant change in the mesor, amplitude and acrophase on experimental day 9 and 10 (Fig. 5 and Table 2).

Comparing activity following amphetamine injection on Experimental day 11 (Expression phase)

The locomotor activity of the amphetamine group on experimental day 11 was compared to its experimental day 1 and to experimental day 11 of the saline control group to find out whether the changes in the activity rhythm pattern was induced after 6 amphetamine injection days, three washout days followed by amphetamine rechallenge at experimental day 11. Amphetamine injections on experimental day 11 showed a significant ($p < 0.01$) increase in HA, TD, and NOS for the first 2 hours post injection, followed by a decrease in activity for the subsequent 4 hours as demonstrated by the hourly histograms. Using the CCSA test to analyze the data, HA, TD, and NOS all showed a statistically significant ($p < 0.01$) change in circadian rhythmic activity pattern respectively as compared to the control recording (Fig. 6 and Table 2).

3. DISCUSSION

Circadian rhythm activities are characteristic of nearly all life forms and observed at all biological levels, including collections of subcellular organelles (Allada et al., 1998; Moore., 2003). Circadian rhythms have been shown to be genetically determined and manifest in biochemical, physiological and behavioral oscillations (Allada et al., 1998; Wirz-Justice, 1987). Circadian rhythms are generated by endogenous clocks and the suprachiasmatic nuclei (SCN) is considered to be the master clock and expresses several clock genes in a periodic and rhythmic fashion (Klein et al., 1991; Reppert and Weaver, 2002). The genes *Period 1* (*Per 1*), *Per2*, *Cryptochrome1* (*Cry1*), *Cry2*, *Bmal1* and *Rev-erb* are regulated by positive and negative gene-expression feedback loops (Reppert and Weaver, 2002). To maintain synchrony with the light/dark cycle, the clock is entrained by light through a monosynaptic pathway from the retina to the SCN via the retinohypothalamic tract (RHT), which originates from a subset of retinal ganglion cells (Johnson et al., 1988; Moore and Lenn, 1972).

Psychostimulants may potentially alter the amplitude or phase of the circadian pacemaker and significantly impact the circadian activity rhythms that regulate homeostasis. It has been reported that psychoactive drugs modulate the CNS neurotransmitter system and in turn modify the brain expression of clock genes (Ammon et. al., 2003; Chen et. al., 2004; Manev and Uz, 2006). Studies also show that both antidepressants and psychostimulants are involved in altering the expression of clock genes in the CNS (Manev and Uz, 2006).

Chronic ethanol consumption for example was reported to alter *Per 1* and *Per2* gene expression rhythms in the hypothalamus and the expression rhythms of *Per2* and *Per 3* in the SCN (Chen et. al., 2004; Manev and Uz, 2006). This disruption of the circadian rhythm from alcohol consumption can lead to sleep-wake abnormalities and depression (Vitaterna

et. al., 2001). Furthermore, chronic morphine, a potent opiate analgesic drug, consumption results in increased expression of many genes including *Per2* (Ammon et. al., 2003; Manev and Uz, 2006). In addition, long-term administration of a psychostimulant, such as cocaine, alters the expression of all the striatal and hippocampal clock genes by blocking the reuptake of dopamine (Manev and Uz, 2006; Uz, et. al., 2005). Another psychostimulant, methamphetamine, alters the expression of Striatal *Per1* and *Per2* genes causing a shift from nocturnal to diurnal rhythms after 6 days of daily injections in male rats (Iijima et. al., 2002). Although methamphetamines and amphetamines are chemically very similar except the methyl group on methamphetamines which makes it more lipid soluble, little is known about the chronic effects of amphetamines on circadian rhythm. In fact, the only study on the effects of amphetamine on circadian rhythm looked at the acute effects after a single dose (Gaytan et. al., 1996) and not after chronic application. This leads us the hypothesis that drugs that induce changes in circadian activity pattern indicate long-term effects of the drug.

Amphetamine has been used for the treatment of attention deficit hyperactive disorder (ADHD), obesity, narcolepsy, chronic fatigue syndrome and Parkinson's disease (Seiden and Sabol, 1993; Mattay et. al., 2003). In humans low dose amphetamine administration produces euphoria, increased energy, decreased appetite, and decreased fatigue (Konradi et. al., 1994). Amphetamines act mainly by stimulating dopamine (DA) release causing over activity of the dopaminergic system through transport-mediated DA release, release from vesicular storage into the cytoplasm, inhibition of DA uptake, and by inhibition of monoamine oxidase (MAO) activity (Nishino et. al.1998; Seiden and Sabol, 1993).

Amphetamines have been known to produce different outcomes when administered to different regions of the brain. Seiden and Sabol (1993) reported that local amphetamines injection in the nucleus accumbens elicited an increase in locomotor activity and when applied on the caudate nucleus amphetamines induced stereotypic movements. Other studies indicate that low to moderate doses of amphetamines elicited behavioral sensitization (Gaytan et. al., 1998, 1999; Perugini and Vezina, 1994; Vezina and Stewart, 1990). One possible explanation for the same drug producing different activities when administered to different brain regions is that the DA receptors in different regions of the brain undergo different 24 hr rhythmic changes in receptor binding and different levels of density of the different DA receptor types (Wirz-Justice, 1987). The changes in receptor binding over 24 hours is mainly due to different second messengers, amine turnover, or function of explicit brain regions; these receptor changes are consistent within brain regions but not for all CNS sites (Wirz-Justice, 1987).

Repeated exposure to psychostimulants can result in progressive augmentation of biochemical and behavioral responses to the drug, characterized as behavioral sensitization (Askenasi et. al., 2008; Dafny and Yang, 2006; Wolf, 1998). It is still up to debate how amphetamines induce behavioral sensitization in adolescent rats, but one possible explanation is that chronic amphetamines elicits stress and prolonged exposure to stress can result in behavioral sensitization and changes in the circadian activity pattern. During adolescence, the brain is still developing and reshaping itself with synaptic pruning and increased myelination causing stronger and faster synaptic connections with the amygdala, the hippocampus, and the prefrontal cortex (Marder, 2007). Several studies suggest that amphetamine exposure during adolescence interferes with proper CNS development (Smith, 2008; Tavares and Silva, 1996). Chronic stress and repeated amphetamine use induce similar changes to the dopamine system that include: (a) an augmented metabolic responses of DA neurons (D'Angio, 1987; Dunn, 1988; Piazza, 1990); (b) changes in the activities of the different DA systems (Blanc et. al., 1980; Piazza, 1990); and (c) an increased behavioral reactivity to psychostimulant administrations (Berke and Hyman, 2000; Piazza, 1990).

Another study reported that during adolescence, a very vulnerable time when the brain is reorganizing itself, the consequences of psychoactive drug exposure results in increased likelihood of developing drug dependence in adulthood (Adriani and Laviola, 2004). However, the concern that psychostimulant use may increase the risk of substance abuse and smoking remains controversial (Witt et. al., 2008).

It was reported that amphetamine-mediated D1 receptor stimulation that results in activation of the CAMP pathway and the protein kinase (PKA) (Konradi et. al., 1994; Monsma et al., 1990). PKA then activates CAMP response element binding protein (CREB) by phosphorylation on its serine (Gonzalez and Montminy, 1989; Konradi et. al., 1994). CREB phosphorylation and Δ FosB expression in the striatum are molecular markers characteristic of repeated amphetamine treatment (Shen et. al. 2008). Furthermore, the CREB protein has been shown to be involved in the transcriptional regulation of many genes including *per 1*, which plays a central role in conveying the light-entraining information of the central clock and involved in resetting the clock (Konradi et. al., 1994; Travnickova-Bendova et. al., 2002). Thus, one possible explanation for the change in circadian pattern of activity after chronic amphetamine use is that amphetamines inadvertently activates CREB which regulates *per 1* gene transcription, resulting in changes to the circadian clock.

The hypothesis of this study is that alteration of locomotor circadian rhythm activity patterns due to repetitive amphetamine exposure indicates that the drug may have long-term effects. The main finding of this study is that a single amphetamine injection (experimental day 2), caused an increase in locomotor and stereotypic activity at the beginning of the light cycle, followed by a decrease in activity during the middle of the light cycle. There were slight changes in locomotor activity after the first day of injection, single drug injection did not change the circadian rhythm activity pattern significantly of the HA while altering the mesor and amplitude of the TD and NOS according to the CCSA test. On the sixth day of amphetamine injections (experimental day 7), there were also increases in the locomotor and stereotypic activity at the beginning of the light cycle and decreased activity during the middle of the light cycle. There was a significant ($p < 0.05$) change in the circadian rhythm activity pattern of all the locomotor indices studied, which suggests that 6 days of 0.6 mg/kg amphetamine elicits long term effect on the neuronal components regulating the circadian activity rhythm in the CNS. Similar experimental protocol in previous studies reported that chronic amphetamine treatment elicits behavioral sensitization with this dose (Gaytan et al., 1997, 1999). Re-challenged amphetamines administered on experimental day 11 after six daily amphetamine exposure, 3 washout days showed further increase in locomotor activity suggesting that behavioral sensitization is expressed during the beginning of the light cycle and decreased activity during the middle of the light cycle. Using the CCSA test indicated that this treatment resulted in significant alteration of the circadian rhythm pattern activity for all the locomotor activities studied. This finding demonstrates that chronic (Lee et. al., 2009) amphetamine treatment in adolescent male SD rats induced a change in circadian activity pattern as well as behavioral sensitization similar to those obtained from adult rats (Gaytan et. al., 1998, 1999). The question is: does the change in circadian rhythm result in sensitization or does the development of behavioral sensitization result in circadian rhythm alteration?

This study also looked at the changes in locomotor and stereotypic activity during the three washout days. Experimental days 8 to 10 (table 1) show the activity recorded 24 to 72 hours after the last repetitive amphetamine administration. In spite of no treatment, these days displayed increased locomotor and stereotypic activity during the beginning of the light cycle at the time of previous administrations of amphetamine on all three days, followed by decreased activity during the middle of the light cycle. The first day of amphetamine removal (experimental day 8), which is 24 hours after last injection, showed a significant

change in circadian pattern of only the TD and the NOS, while the other locomotor indices exhibit increases in activity, but this increase did not display significant changes in circadian activity pattern according to the CCSA test. During the second day of amphetamine removal (experimental day 9), the number of NOS, TD, and SM all displayed significant changes in circadian pattern of activity. On the last day of wash-out (experimental day 10), all four of the locomotor indices showed significant changes in circadian pattern of activity. These significant changes in locomotor activity pattern after amphetamine was abruptly removed (the washout period) may indicate an —expectation response that can also be interpreted as the expression of withdrawal (Algahim et. al., 2009; Lee et. al., 2009). Another study found that amphetamine-induced stereotyped behaviors were altered for 28 days after withdrawal from chronic amphetamine use (Hitzemann et. al., 1977). The cause of the withdrawal symptoms could be due to the changes in circadian gene transcription (*per 1*) that occur during chronic amphetamine use. When chronic exposure to a drug is terminated, some of the neuroadaptations that formed during drug use may remain unopposed and lead to the expression of the withdrawal symptoms (Koob and Bloom 1988). The increased locomotion that occurred on the 3rd day of washout, in comparison to the 1st day of abrupt amphetamine removal needs to be studied further in order to fully understand the reason for this phenomenon.

In summary, our data shows that moderate chronic amphetamine administration in adolescent SD male rats induces sensitization, withdrawal and changes in circadian pattern activity, indicating that repetitive amphetamine administration elicits not only short-term effects, but also long-term effects. Whether the changes in circadian rhythm caused sensitization and withdrawal or if sensitization and withdrawal caused the change in circadian rhythm remains unanswered and requires further study. It is important to recognize that although our experimental data proposes that chronic amphetamine exposure leads to long-term effects in humans, these long-term effects may not necessarily be harmful. This study emphasizes the importance of future clinical trials examining long-term effects of amphetamine to ensure improvements in clinical practice with evidence-based research. Extensive clinical research will help alleviate consumer concerns and public criticism of the increasing use of psychostimulants in our society.

4. Methods

Male adolescent post natal day 34 (P-34) Sprague Dawley rats (n = 20) were purchased and housed in groups of four in a sound proof temperature-controlled room at $21 \pm 2^\circ \text{C}$ with a relative humidity of 37–42%. The animals were maintained under conditions of 12:12 light/dark schedule with lights on at 0600h and off at 1800 for 3 to 5 days prior to experimentation in order to internally synchronize their physiological systems. On the last day of adaptation, the rats were weighed and separated into an amphetamine treatment group and one saline control group at random and individually housed in the test cages, which became their home cages for the rest of the experiment. Prior to initiating locomotor recording, the rats received an additional 24 hours of accommodation/adaptation to their new home cage. Food and water were provided ad libitum.

4.1 Drug

In a previous amphetamine dose-response experiment (Gaytan et. al., 1998, 1999), the dose of 0.6 mg/kg amphetamine given for six consecutive days elicited significant locomotor activation and behavioral sensitization. Therefore, this dose was selected for this study. The dose of 0.6 mg/kg d-amphetamine sulfate (Sigma Chemical Co.) was calculated as free-base and suspended in a 0.9% isotonic saline solution. All injections (saline and d-amphetamine) were 0.8cc in volume and were injected intraperitoneally (i.p.) at about 07:00h, one hour into the light phase.

4.2 Apparatus

Open field cages were used during the recording of locomotor activity. Different indices of locomotor activity were measured utilizing the computerized animal activity monitoring system (CAAM; Accuscan Instruments, Inc.; Columbus, OH) previously described in detail (Dougherty et. al., 1990; Gaytan et. al., 1996; Lee et. al., 2009; Yang et. al., 2003). In short, the locomotor activity was recorded non-stop 24 hours per day over a period of 11 to 12 consecutive days. Each home cage (40.5 × 40.5 × 31.5 cm) consisted of clear open acrylic open field boxes with two levels of infrared motion sensors located at 6 and 12.5cm from the cage floor, respectively. Each sensor consisted of 16 infrared beams spaced 2.5 cm apart. The activity monitoring system checked each of the beams at a frequency of 100 Hz to determine if the beams were interrupted. An activity score was recorded if the beam was interrupted and a movement score indicated the interruption of at least two consecutive beams separated by one or more seconds. Cumulative counts were compiled by the AccuScan Analyzer and downloaded every 10 minutes into the OASIS data collection program and organized into motor indices and transferred to a PC (Algahim et. al., 2009; Gaytan et. al., 1996; Lee et. al., 2009; Yang et. al., 2003). The 10-minute counts were used for the cosine analysis statistical test (Bingham et. al., 1982) and 6 bins of 10 minute locomotor counts were summarized as hourly histograms with their means and standard deviations to represent activity patterns. The following five locomotor indices were analyzed: 1) horizontal activity (HA), which assesses the overall locomotor activity in their lowest sensor and determines the amount of spontaneous motor activity; 2) total distance (TD), which measures the total distance travelled in centimeters; 3) vertical activity (VA), which measures the amount of rearing; 4) stereotypic activity (SA), which measures the repeated interruptions of the same beam(s); and 5) number of stereotypic movements (NOS), which measure the amount of different events of stereotypic activity separated by at least one second prior to the initiation of another episode. The activity monitoring system measured locomotor activity continuously around the clock but was interrupted between 06:00h and 07:00h for either food or water supplementation and administration of saline or amphetamine injections.

4.3 Experimental Protocol

Rats were assigned randomly to the saline control group (N = 8) and to the amphetamine (0.6 mg/kg) group (n = 12). On experimental day 1, at about 07:00h, the recording sessions started immediately post saline injection. On experimental days 2 to 7, the control group received saline and the treatment group received 0.6 mg/kg of amphetamine. Experimental days 8 to 10 were washout days and neither group received injections. Finally, on experimental day 11, the control group was injected with saline to establish a control baseline and to account for animal handling and the injection volume, while the treatment group was rechallenged with 0.6mg/kg of amphetamine. On experimental day 12, both groups were treated with 0.6 mg/kg amphetamine (Table 1).

4.4 Data Analysis

Twenty-four hour activity pattern histograms were plotted (Fig. 1). Each histogram displayed the hour by hour mean activity and standard errors for each day of the study ($2 \pm$ SE difference from control was considered significant). The histograms for the five locomotor indices were visually evaluated for drug effects. The 10 min bins data points were analyzed for 24-hour rhythms by the cosinor curve statistical analysis (CCSA) test (Bingham et. al., 1982), which utilizes the approximation of a cosine curve. The CCSA analyzed the data using three parameters: MESOR, Amplitude, and Acrophase in order to accurately model the cyclic nature of the 24 hour cyclic activity. The mesor (Midline Estimate of Rhythm) parameter measures the average horizontal activity per day. The amplitude parameter (distance from the mesor to the highest point of the approximating curve)

measures the peak level of activity in a day, and the Acrophase (time of the cosine approximation) provides the time of day that peak activity occurs. Cosine curves were adjusted to correlate with each activity level in order to parameterize the 24-hour activity pattern. The above three components were used to make statistical comparisons to determine whether circadian rhythm activity pattern had changed significantly as a result of amphetamine effects. The cosine analysis technique was selected because it best evaluates the potential hour to hour magnitude and timing components of the 24 hour activity with minimal programmer estimation. Histograms contrasting specific treatment days with control days were visually examined to determine whether amphetamine treatment altered the activity pattern. The experimental analysis was divided into 4 phases: 1) The acute effects of amphetamine were studied by comparing experimental day 1 (baseline day) with experimental day 2 (first day of drug administration) of the experimental group and as well with the experimental day 1 of the saline control group. 2) Induction phase consisted of comparison between the observations made after 6 consecutive daily injections (i.e. experimental day 7) with experimental day 1 to see if amphetamine induces changes in activity pattern as well as with experimental day 7 of the saline control group 3) Washout phase recordings were obtained during experimental days 8 through 10 after six daily amphetamine treatments and compared to experimental day 1 in order to determine whether the 6 day amphetamine treatment changes the baseline activity pattern as well as to experimental days 8,9, and 10 of the control group; and 4) The expression phase compared experimental day 1 with experimental day 11 as well as with experimental day 11 of the saline control group in order to examine the changes in activity pattern after the washout days. The results from the treatment days were compared with the control group and analyzed using appropriate 3 F-tests per day per pair to contrasting parameter estimates and then by examining the effects of the treatment changes on the activity patterns (Algahim, et al., 2009; Bingham, et al., 1982, Lee, et al., 2009).

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Research Highlights

1. Single amphetamine exposure increases rat's behavior assessed in open field assay.
2. Chronic amphetamine injections produce behavioral sensitization.
3. Chronic amphetamine injections modulate the rat circadian activity pattern.
4. Chronic amphetamine exposure elicits long-term effect on animal behavior.

Fig. 1.

Horizontal activity counts and cosine curve statistical analysis graph of control group prior to saline or MPD injection.

Control – summarizes ($n = 8$) the hourly histogram of horizontal activity (HA) counts of the control group of experimental days (ED's) 1, 2, 7, 8 and 11 in A and shows similar activity in all the ED's with some minor fluctuation. Under B is the superimposed Cosinar Curve statistical analysis (CCSA) graphs of ED's 1, 2, 7, 8 and 11 of the HA. Each symbol represents one of the EDs (Table 2). The graphs show that according to the CCSA test all the EDs exhibit similar activity levels and similar rhythmic activity pattern i.e., ED 1 activity level and rhythmic activity pattern was similar to all the other ED's (Table 2). Arrows on the histograms indicate the saline injection time. Dark bar under the histograms indicate the dark (night) period. At ED1 recording started in the morning immediately after saline injection.

Fig. 2.

Comparing Day 1 vs. Day 2

summarizes ($n = 12$) the hourly histograms of ED 1 and ED 2 of the horizontal activity (HA) counts, total distance (TD) travelled in cm, number of stereotypic movements (NSM) and stereotypic activity (SA). Arrow indicates the injection time. Dark bar under the histograms marks the dark (night) period. On ED 1, saline was injected while on ED 2, 0.6 mg/kg amphetamine was injected intraperitoneal (i.p.).

Fig. 3A

Fig. 3B

Fig. 3.

summarizes ($n = 12$) and compares the activity pattern using the CCSA test of ED1 with ED2 of the amphetamine group in A and in B the ED2 of the control/saline group with ED2 amphetamine group. The symbol Δ represents the control group, the symbol $+$ represents the ED2 amphetamine group.

Fig. 4. summarizes and compares the activity pattern of ED7 of the control group (symbol +) with the amphetamine group (symbol Δ), using the CCSA data.

Fig. 5A

Fig. 5B

Fig. 5C

Fig. 5. summarizes and compares the three washout days (ED8, ED9, and ED10) of the saline control group with the amphetamine group after six daily amphetamine exposures.

Fig. 6. summarizes and compares the activity pattern of the ED11 saline/control group with the amphetamine rechallenge at ED11.

Table 1

The experimental protocol for each of the two groups used. (ED-experimental day). All injections were equalized to a volume of 0.8 cc and were given at approximately 07:00.

Table 1 describes the experimental protocol. On experimental day 1, all animals were treated with saline. On experimental days 2 through 7, the amphetamine (AMP) group was treated with single daily injections of 0.6 mg/kg AMP i.p., while the control group received single daily saline injections. On experimental days 8 to 10, no treatment was given i.e., washout days. On experimental day 11, the treatment group was given an AMP injection and the control group received saline injection. On experimental day 12, both groups the saline and experimental group received AMP injections. All injections were given at 07:00 in 0.8cc volume.

Post Natal Age	P 40	P 41 – 46	P 47 – 49	P 50	P 51
Experimental Day	1	2–7	8–10	11	12
Treatment Group (n=12)	Saline	AMP	Washout	AMP	AMP
Saline Control Group (n=8)	Saline	Saline	Washout	Saline	AMP

Table 2

The table summarizes the statistical analysis using the Cosine Curve Statistical Analysis (CCSA) test. The table compares the rhythmic activity pattern of the control group with the rhythmic activity pattern of the amphetamine group. All values of the parameters estimated are means of N=131 value for each respective experimental day. Mesor is the average daily activity: amplitude, height of the peak above the mean level. Acrophase is the time at which the peak activity occurred for each experimental day (ED).

Total Distance			Number of Stereotypic			
Parameters	f-statistics	df	p-value	f-statistics	df	p-value
<u>ED1,2,7,8,11</u>						
Mesor	1,529	(4, 215)	0.856	1,347	(4, 215)	0.352
Amplitude	1,757	(4, 215)	0.905	1,405	(4, 215)	0.238
Acrophase	1,287	(4, 215)	0.707	1,374	(4, 215)	0.347
<u>ED2 Acute Phase</u>						
Mesor	10,090	(1,84)	0.002	9,325	(1,84)	0.003
Amplitude	4,801	(1,84)	0.035	0,985	(1,84)	0.058
Acrophase	0,196	(1,84)	0.659	0,720	(1,84)	0.834
<u>ED7 Induction Phase</u>						
Mesor	8,561	(1,84)	0.015	4,148	(1,84)	0.049
Amplitude	4,197	(1,84)	0.042	4,781	(1,84)	0.031
Acrophase	3,876	(1,84)	0.659	3,942	(1,84)	0.054
<u>ED8 Washout Phase</u>						
Mesor	9,020	(1,84)	0.005	4,186	(1,84)	0.032
Amplitude	4,334	(1,84)	0.043	4,023	(1,84)	0.049
Acrophase	0,035	(1,84)	0.850	0,478	(1,84)	0.481
<u>ED9</u>						
Mesor	6,578	(1,84)	0.012	7,601	(1,84)	0.001
Amplitude	4,222	(1,84)	0.042	4,693	(1,84)	0.034
Acrophase	3,896	(1,84)	0.048	3,967	(1,84)	0.043
<u>ED10</u>						
Mesor	8,016	(1,84)	0.006	8,576	(1,84)	0.025

Total Distance			Number of Stereotypic			
Parameters	f-statistics	df	p-value	f-statistics	df	p-value
Amplitude	4,574	(1,84)	0.025	4,891	(1,84)	0.018
Acrophase	3,982	(1,84)	0.008	4,205	(1,84)	0.006
<u>ED11 Expression Phase</u>						
Mesor	11,370	(1,84)	0.001	10,070	(1,84)	0.006
Amplitude	6,171	(1,84)	0.005	5,323	(1,84)	0.009
Acrophase	4,013	(1,84)	0.003	4,551	(1,84)	0.002