Data Article

Circadian rhythms of locomotor activity in rats: Data on the effect of morphine administered from the early stages of embryonic development until weaning

Dominika Pačesová, Veronika Spišská, Jiří Novotný, Zdeňka Bendová*

Department of Physiology, Faculty of Science, Charles University, Prague, Czech Republic

ABSTRACT

The circadian clock generates behavioural and physiological rhythms to maximize the efficacy of organismal functions. The circadian system with a major circadian pacemaker in the suprachiasmatic nucleus of the hypothalamus develops gradually and its proper function in adulthood depends on an appropriate neurochemical milieu during ontogeny [1]. Locomotor activity is under direct control by the circadian clock, and alterations in its rhythmicity indicate changes of circadian clock function. We evaluated circadian parameters of locomotor rhythms of adult male Wistar rats born to mothers that were exposed to a stable dose of 0.1 mg/ml of morphine in drinking water (36 ml water on average/day/each rat) from embryonic day 10 (E10) until weaning at postnatal day 28 (P28). Increasing the dose of morphine in drinking water was used to evaluate the changes in the rhythmic gene expression in the suprachiasmatic nucleus and in the livers of young rats at P20 [2]. At P90, we started measurement of endogenous rhythmicity for 12 days in constant darkness (DD), then we applied a 15 min light pulse at circadian time 15 (CT15) and followed the animals for the next 15 days.
in DD. We evaluated the magnitude of light-induced phase shift and compared the circadian parameters of free-running rhythmicity in the intervals before and after the light pulse. All data were also compared between morphine-exposed animals (M group) and controls (C group) that were not exposed to morphine. An unpaired t-test confirmed a significantly longer light-induced phase delay in M group compared with C group, a prolonged circadian period in M group in the interval after the light pulse, and greater amplitude for C group in the first interval, i.e. before the light pulse. No change in total activity counts between groups was confirmed.

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### Specifications Table

| Subject | Neuroscience: Behavioural |
|---------|---------------------------|
| Specific subject area | Circadian rhythm in locomotor activity |
| Type of data | Graph, Figure |
| How data were acquired | Infrared motion detectors (Mini-Mitter VitalView data-acquisition system) analysed by ClockLab analysis (Version 6, Actimetrics, Wilmette, Illinois) |
| Data format | Raw (supplemental file; the values represent the number of the infrared beam crossing per minute), analysed by ClockLab analysis (Version 6, Actimetrics, Wilmette, Illinois) |
| Parameters for data collection | Wistar rats, pregnant females treated with morphine-containing drinking water (0.1 mg/ml) from gestation day 10 through delivery until weaning (postnatal day 28). After weaning, the offspring were provided with drinking water without morphine. Control animals were provided with drinking water only. |
| Description of data collection | Adult male rats were probed at P90 for the magnitude of phase shift after the light pulse at circadian time 15 (CT15), for the period and amplitude of the circadian rhythm in locomotor activity and total activity. The parameters were compared for the 10-day period before and after the light pulse. |
| Data source location | Institution: Faculty of Science, Charles University |
| | City: Prague |
| | Country: Czech Republic |
| Data accessibility | With the article |
| Related research article | D. Pačesová, V. Spišská, J. Novotný, Z. Bendová, Maternal morphine treatment during pregnancy and lactation affects the circadian clock of rat pups, Brain Res Bull, submitted. |

### Value of the Data

- These data demonstrate the effect of repeated exposure to low levels of morphine in early life development of Wistar rats on circadian rhythmicity in locomotor activity and the magnitude of light-induced phase shift in adulthood.
- These data can be valuable for researchers studying the development of the nervous system in the condition of maternal drug users, or those studying the development of the circadian clock.
- These data provide evidence that exposure to early developmental opioids may affect the function of the circadian system in adulthood, which can be further elaborated in experiments focusing on the principles of defective development of the human circadian clock and neural system.
1. Data Description

We sought to determine whether early life exposure to morphine that leads to neural tube defects and reward circuitry or social behaviour alterations later in life [3–5] also affects the stability of the circadian system and its sensitivity to light pulses in adulthood. We characterized circadian rhythm in locomotor activity in morphine-treated (M group) and control intact animals (C group) 10 days before and 15 days after the light pulse applied at early subjective night (CT15) and the magnitude of phase shift after the light pulse (representative actograms in Fig. 1).

Light is the major signal from the external environment that entrains the circadian clock to solar time. Light pulses that impinge the circadian clock at night phase shift the circadian rhythm [6,7]. The difference between two regression lines fitted to activity onsets were used to calculate phase shift (see Experimental protocol). Our data show that a light pulse applied at CT15 induced a phase delay of about 1 h in C group, and a significantly larger phase delay in M group (Fig. 2).

Although the length of the endogenous period is usually species-specific, it often shows a form of plasticity depending on previous lighting conditions or other previous experience. It is observed frequently that the period length of the endogenous, free-running rhythm can change slightly after a light pulse, which is termed an “aftereffect” [8,9]. We measured the parameters of endogenous rhythmicity in locomotor activity in two time intervals: the first interval was delimited by 10 days before the light pulse (C1 for controls, M1 for morphine group), and the second interval by 10 days after the light pulse, starting the fifth day after the pulse (C2 for controls, M2 for morphine group). The activity was analysed by cosinor analysis using ClockLab analysis software and circadian parameters were compared between C and M groups, and between C1 and C2 or M1 and M2 groups (see Experimental protocol).

One-way ANOVA with Tukey’s multiple comparison test indicated significantly longer period for M2, i.e. for M group between the first and second interval, and also between C and M groups in the second interval (Fig. 3). Furthermore, we found significantly higher amplitude for C1 as compared to M1, but also compared to C2, and significantly lower amplitude for M group in second interval (M2) compared to the interval preceding the light pulse (Fig. 4). The lower amplitude could be associated with lower total activity, but although suggested (Fig. 5), the difference between M and C groups was not statistically confirmed.

![Fig. 1. Representative actograms in ClockLab format of control animal (C) and morphine-treated animal (M). Yellow dots = light pulse administration times. Red and green lines = regression lines fitted to activity onsets of the 10 days before the day of the light pulse and the 10 days after the light pulse.](image-url)
Fig. 2. Magnitude of phase delay after light pulse at CT15 in control rats (C) and rats exposed to morphine in early development (M). An unpaired t-test with Welch’s correction revealed a significant difference between the magnitude of the phase shift between M and C groups ($P = 0.0401$; $t = 2.260$ df = 14.13), as well as significant F test to compare variances ($P = 0.0236$). Data plotted as mean ± SEM, M group: $n = 10$, C group: $n = 8$.

Fig. 3. Comparison of circadian period in locomotor activity rhythm between control rats (C1, C2) and rats exposed to morphine in early development (M1, M2), and between intervals before (C1, M1) and after (C2, M2) light pulse. An unpaired t-test with Welch’s correction revealed a significantly longer circadian period in the M2 group compared to M1 ($P = 0.0031$; $t = 3.579$; df = 13.77) and C2 ($P = 0.0029$; $t = 3.529$; df = 15.43). Data plotted as mean ± SEM, M group: $n = 10$, C group: $n = 8$. 
Fig. 4. Comparison of amplitudes of the circadian rhythm in locomotor activity between control rats (C1, C2) and rats exposed to morphine in early development (M1, M2), and between intervals before (C1, M1) and after (C2, M2) light pulse. An unpaired t-test with Welch’s correction confirmed higher amplitude of circadian rhythmicity in C1 compared to C2 (P = 0.0071; t = 3.620; df = 7.775) and M1 (P = 0.0263; t = 2.737; df = 7.768), and the difference between M1 and M2 (P = 0.0089; t = 3.050; df = 13.58). Data plotted as mean ± SEM, M group: n = 10, C group: n = 8.

Fig. 5. Comparison of total activity counts of locomotor activity between control rats (C1, C2) and rats exposed to morphine in early development (M1, M2), and between intervals before (C1, M1) and after (C2, M2) light pulse. An unpaired t-test with Welch’s correction did not confirm any difference between groups. Data plotted as mean ± SEM, M group: n = 10, C group: n = 8.
2. Experimental Design, Materials and Methods

2.1. Animals

Male Wistar rats (Velaz, Ltd; Koleč, Czech Republic) were kept under a 12/12 h light–dark regime at a temperature of 23 ± 2 °C with free access to food and water for at least two weeks before the experiment. Light was provided by a customized linear daylight white LED source (CCT 5630 K, Spectrasol, Czech Republic) with a dominant wavelength at 459 nm, producing photopic illuminance 60–70 lx (measured on the cage position). Irradiance and spectral characteristics were measured using a calibrated radiospectrometer (GL Spectis 1.0 touch, GL Optics, Poland). The day when the rats were found to be sperm-positive was designated as embryonic day 0 (E0). Date of delivery was designated postnatal day 0 (P0).

2.2. Experimental protocol

Pregnant rats were treated with morphine-containing drinking water (0.1 mg/ml) from E10 through the delivery until weaning. The amount of solution consumed by each female per day was 36 ml on average. To reduce the bitter taste of the solution, noncaloric sweetener (50 mg/l) was added to the drinking water. Control animals received drinking water without morphine. After weaning, both groups of offspring were provided with drinking water. At P90, eleven animals from each group were released into constant darkness (DD), and their locomotor activity was monitored using infrared motion detectors (Mini-Mitter VitalView data acquisition system). After 12 days in DD, animals received a 15-min light pulse at CT15 (i.e., 3 h after the beginning of subjective night). Phase shift was detected from activity onsets using ClockLab analysis (Version 6, Actimetrics, Wilmette, Illinois). Regression lines were fit to activity onsets for the 10 days before the day of the light pulse and the 10 days following the light pulse, omitting the first day. Phase shifts were expressed as the difference between these two regression lines, with a positive value denoting a phase advance and a negative value denoting a phase delay of the activity onset. Total activity level and parameter estimates of the Cosinor analysis for circadian rhythm were analysed for two intervals: the 10-day interval before the light pulse, and the 10-day interval starting on the fifth day following the light pulse. The circadian periods and relative amplitudes were extracted by Chi-squared periodograms with a confidence level of 0.01. Average daily activity (total activity) was calculated by the sum of the counts divided by the total number of days. The differences between the groups were analysed by unpaired t-tests with Welch’s correction in GraphPad Prism version 6.00, with \( P < 0.05 \) required for significance.

Ethics Statement

All experiments comply with the ARRIVE guidelines and were carried out in accordance with the U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines, EU Directive 2010/63/EU for animal experiments, or the National Institutes of Health guide for the care and use of laboratory animals (NIH Publications No. 8023, revised 1978) and the Animal Protection Law of the Czech Republic (licence no. MSMT-23852/2014-14).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships which have, or could be perceived to have, influenced the work reported in this article.
Acknowledgements

This research was funded by the by the Charles University Grant Agency (grant no. 1210217) and the Czech Science Foundation (grant no. 19-032955).

Supplementary Materials

Supplementary material associated with this article can be found in the online version at doi: 10.1016/j.dib.2022.107812.

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