Journal of Pharmacological Sciences 127 (2015) 382–390

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Contents lists available at ScienceDirect

Journal of Pharmacological Sciences

journal homepage: www.elsevier.com/locate/jphs

Full paper

Disruption of running activity rhythm following restricted feeding in female mice: Preventive effects of antidepressants

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ARTICLE INFO

Article history:
Received 5 January 2015
Received in revised form
12 February 2015
Accepted 22 February 2015
Available online 4 March 2015

Keywords:
Wheel-running activity
Circadian rhythm
Chronometrical analysis
Antidepressant drugs
Female mice

ABSTRACT

Biological rhythms are critical in the etiology of mood disorders; therefore, effective mood disorder treatments should address rhythm disturbances. Among the variables synchronized with the light–dark cycle, spontaneous activity in rodents is useful for investigating circadian rhythms. However, previous studies have focused only on the increase of wheel-running activity under restricted feeding conditions, while little information is available on circadian rhythm of running activity. In this study, chronometrical analysis was used to assess whether circadian rhythms during wheel-running are altered by restricted feeding and affected by antidepressant drugs. Wheel revolutions were automatically recorded and analyzed using cosinor-rhythmometry in 8-week old ICR albino mice. When feeding was restricted to 1 h per day (21:00–22:00), wheel-running rhythms were reliably disrupted. Female mice exhibited marked alterations in the pattern and extent of wheel-running beginning on day 1. Subchronic treatment with imipramine or paroxetine, as well as tandospirone and (−)-DOI, prevented wheel-running rhythm disruption. Thus, altering the circadian activity rhythms of female mice on a 1-h feeding schedule may be useful for investigating disturbances in biological rhythms.

1. Introduction

Since the concept of the “animal model” was introduced into psychiatric medicine, various experimental models of mental illnesses, including anxiety, depression and dementia, have been developed and used to screen novel psychotropic drugs, and to understand the brain mechanisms underlying behavioral disorders. However, the disruption of rhythmicity in psychiatric patients remains under-researched: the difficulty of devising a valid animal model, and the complicated statistical analysis required to assess circadian rhythms, may have restricted the use of a chronobiological approach in experimental psychopharmacology. However, depressive disorder is accompanied by alterations in the periodic rhythms of daily life. Indeed, the rhythm of the sleep–wake cycle, which is synchronized with the light–dark cycle, is important in mood disorders and desynchronization with the light–dark cycle is a risk factor for depression (1–3). Females suffering from mood disorders are characterized by a high prevalence of sleep disturbances (4) and irregular eating patterns (5). Although pharmaceutical research has focused on time-dependent drug effects and pharmacodynamics (6–8), information concerning the effects of drugs on desynchronized behavioral patterns remains limited.

Among the variables synchronized with the light–dark cycle, spontaneous activity in rodents is particularly instructive for the investigation of circadian rhythms. When mice and rats are individually housed in cages with running-wheels under restricted feeding conditions (1 h per day), they exhibit excessive wheel-running and decreased food consumption, with activity decreasing markedly after reaching peak wheel revolutions, followed by death (9). In early investigations, Routtenberg (10, 11) termed this phenomenon, characterized by novelty and food-deprivation stressors, as “self-starvation”. Páre and Houser (12) emphasized that augmenting running activity itself generated stress, termed by these authors “activity-stress.” In addition to the development of gastric mucosal lesions (13, 14), the following physical disabilities, possibly resulting from emaciation, have also been reported and have included atrophy of the thymus and spleen (15), as well as and adrenal hypertrophy (16). The number of wheel revolutions during the light-phase was greater compared with the dark-phase in nocturnal rats maintained on a 1-h feeding schedule (17). Although obtaining accurate information on wheel-running activity rhythms following

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Peer review under responsibility of Japanese Pharmacological Society.

http://dx.doi.org/10.1016/j.jphs.2015.02.015

restricted feeding is important, chronometric analysis of variations in running activity, plotted against time, has not been reported.

Circadian rhythm disturbances may be the major determinant of mood disorders (3, 18, 19). The prevalence of mood disorders, including sleeping and eating disturbances, is higher in females than in males; the effectiveness of psychotropic drugs also differs between genders (20, 21). Nevertheless, investigators rarely use female animals due to concerns regarding complicated interactions between endocrine changes during the estrous cycle and drug effects. However, it is important to establish that drugs prescribed to females, including antidepressants, are efficacious and safe.

In the present study, a chronobiological approach was taken to assess whether restricting feeding to 1 h per day in female ICR mice desynchronizes wheel-running activity with respect to the light–dark cycle. Because rhythmometrical, spontaneous wheel-running data is limited, we first analyzed three activity rhythm components (midline estimating statistic of rhythm [MESOR], amplitude, and acrophase) in female and male mice under restricted feeding conditions. We then investigated whether antidepressant drug treatment prevents desynchronization in female mice; current evidence suggests that disturbances in circadian rhythms could be normalized by daily treatment with antidepressants (3, 22). Serotonergic nervous activity in the brain appears to contribute to circadian rhythm regulation in mood disorder patients (3), and to depressive-like states in animals (23, 24). We also investigated whether the desynchronization of running activity rhythms, in response to a 1-h feeding schedule, is altered by subchronic treatment with tandospirone, a 5-HT_{1A} receptor agonist, or (−)-2,5-dimethoxy-4-iodoamphetamine (DOI), a 5-HT_{2A} receptor agonist.

2. Materials and methods

2.1. Subjects

ICR albino male and female mice (CLEA, Osaka), 6 weeks of age, were habituated to laboratory conditions for at least 2 weeks in a conventional polycarbonate cage (21 × 31 × 14 cm, 10 animals per cage) before the experiments commenced. Mice had free access to food and water. Cage floors were covered with wood shavings, and were cleaned every 4 days. The temperature of the vivarium was maintained at 23 ± 2 °C; a 12/12-h light/dark cycle was automatically controlled (lights on/off at 07:00 and 19:00, respectively). The experiments were conducted in accordance with National Institutes of Health guidelines and the Guide for Animal Experimentation of the Ehime University School of Medicine.

2.2. Apparatus

The apparatus consisted of a running wheel (20.5 cm in diameter) and adjoining stainless-steel cage (7 × 7 × 8 cm) with a wire-mesh bottom (Muromachi Kikai Co. Ltd., Tokyo). A sliding door separated the wheel from the adjoining cage, and mice could take water freely. Each cage was placed on a small plate with wood shavings, but mice did not touch the wood shavings. Wheel revolutions were automatically recorded, using an interface (MSR128-V5, M-System Co. Ltd., Osaka) connected to a computer system, at 1 min intervals. For analysis purposes, intervals were summed to 60 min.

2.3. General procedure

The estrous stage of 8-week-old female mice was determined twice per day (between 09:00–10:00 and 16:00–17:00) using vaginal smears before the experiment. Vaginal smears were collected using a modified Pasteur pipette filled with 0.9% saline solution. We identified each estrous stage microscopically according to the methods described in Bekku et al. (24). When the mice were in the diestrus stage, they were removed from their home cage and placed into a wheel cage, at 17:00 to measure running activity. All mice were habituated to the apparatus for 4 days without food restriction prior to the onset of the experimental feeding conditions. We measured daily body weight at 17:00.

2.4. Experimental design

With the exception of the first experiment, three separate series of experiments were conducted using female mice. The first experiment examined gender differences; 19 males and 15 females were randomly subdivided into *ad libitum* and 1-h feeding groups. The second experiment assessed whether subchronic treatment with imipramine (at 5 or 10 mg/kg) or paroxetine (at 2.5 or 5 mg/kg) attenuated the circadian rhythm desynchronization induced by 1-h feeding conditions. Forty mice were randomly assigned to five groups. The third experiment investigated whether 5-HT_{1A} or 5-HT_{2A} receptors are involved in attenuating the circadian rhythm desynchronization induced by 1-h feeding, using subchronic treatment with tandospirone (at 2.5 mg/kg or 5 mg/kg) or (−)-DOI (at 0.5 mg/kg or 1 mg/kg). Forty-seven mice were randomly assigned to six groups.

2.5. Drug administration

Each drug or vehicle was administered twice per day (at 09:00 and 17:00). Drug treatments were started the day after introducing each mouse to the apparatus, and continued until the experiment was terminated. Imipramine hydrochloride (WAKO Pure Chemical Industries, Ltd., Tokyo) and paroxetine maleate (Sigma, St. Louis, MO, USA) were administered orally. Tandospirone dihydrogen citrate (Sumitomo Pharmaceuticals Co., Ltd., Osaka) was administered orally, while (−)-DOI (Sigma, St. Louis, MO, USA) was administered subcutaneously. All drugs were dissolved in distilled water, except for (−)-DOI which was dissolved in isotonic saline.

2.6. Statistical analysis

The number of wheel revolutions was collected by an interface connected to a computer system; we analyzed these data using cosinor-rhythmometry. Periodic regression analysis (PRA) obtained the best-fit regression curve to the wheel revolutions of every 60 min period for 24 h. A fundamental rhythm with 24-h harmonics was fitted using Fourier analysis, and was assessed by the coefficient of determination (R^2) using least-squares analysis. We calculated the following three parameters (25): MESOR (24-h mean wheel revolutions about which oscillation occurs); amplitude (the distance between MESOR and the highest value of the curve); and acrophase (timing of the highest point, in degrees). Periodic analysis of covariance (PANCOVA) was used to assess group differences in periodic regression curves, according to wheel-running extent (MESOR) and pattern (amplitude and acrophase). A value of $p < 0.01$ was taken to indicate statistical significance. Differences in either extent or pattern were considered statistically significant according to the method described by Hayashi (26). MESOR, amplitude, and acrophase consistency, between the control and experimental groups, was analyzed using the intraclass correlation coefficient (ICC). All data are expressed as means ± *SE*. Multiple comparisons were performed using Bonferroni's multiple comparison tests following ANOVA or ANCOVA.