

Evaluation of light/dark cycle in anxiety- and depressive-like behaviors after regular treatment with methylphenidate hydrochloride in rats of different ages

Avaliação do ciclo claro e escuro no comportamento relacionado à ansiedade e à depressão em ratos de diferentes idades após tratamento crônico com hidrocloreto de metilfenidato

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Abstract

Objective: Methylphenidate hydrochloride is the most widely used medication for treatment and management of attention-deficit hyperactivity disorder. However, the chronic effects of methylphenidate hydrochloride on anxiety- and depressive-like rat behaviors remain poorly investigated. In this context, the present study evaluated the effects of treatment with methylphenidate hydrochloride on anxiety- and depressive-like behaviors using young and adult rats during the light and the dark cycle. **Method:** Male Wistar rats (25 or 60 days old) received a once-daily (in either the light or dark cycle) methylphenidate hydrochloride (2mg/kg) or saline intraperitoneal injection for 28 days. We performed elevated plus maze and forced swimming test two hours after the last injection. **Results:** The light/dark cycle was a significant factor in the anxiety-like behaviors; however, no significant interaction between all three factors (cycle, age and methylphenidate hydrochloride) was found. Nevertheless, we observed a nominally significant interaction between the light/ dark cycle and age in the forced swimming test. **Conclusion:** Our results have shown that age and the light/dark cycle are more significant modulators of anxiety- and depressive-like behaviors than methylphenidate hydrochloride treatment.

Descriptors: Attention; Behavior; Anxiety; Rats, Wistar; Methylphenidate

Resumo

Objetivo: Hidrocloreto de metilfenidato é a medicação preferida para o tratamento e manutenção do transtorno de atenção e hiperatividade. No entanto, os efeitos do tratamento crônico com hidrocloreto de metilfenidato em diferentes idades e ciclos sobre o comportamento relacionado à ansiedade e à depressão ainda não está claro. Neste contexto, o presente estudo teve como objetivo avaliar os efeitos do tratamento com hidrocloreto de metilfenidato sobre o comportamento relacionado à ansiedade e à depressão em diferentes idades e no ciclo claro e escuro. **Método:** Foram utilizados ratos Wistar machos jovens e adultos que receberam uma vez ao dia (ciclo claro e escuro) hidrocloreto de metilfenidato (2mg/kg) ou salina com injeção intraperitoneal, durante 28 dias. Após duas horas da última injeção, os animais foram submetidos ao testes de labirinto em cruz elevada e natação forçada. **Resultados:** A fase do ciclo claro e escuro foi um fator significativo para o comportamento relacionado à ansiedade. Além disso, não houve interação significativa entre os ciclos claro e escuro, idade e metilfenidato no comportamento relacionado à ansiedade e à depressão, mas foi observada uma interação significativa entre ciclo claro e escuro e idade no teste de natação forçada. **Conclusão:** Nossos resultados mostraram que a idade e o ciclo claro e escuro são moduladores significativos de ambos os comportamentos quanto do tratamento com hidrocloreto de metilfenidato.

Descritores: Atenção; Comportamento; Ansiedade; Ratos Wistar; Metilfenidato

Introduction

Attention-deficit hyperactivity disorder (ADHD) is the most commonly diagnosed neuropsychiatric disorder in childhood,

characterized by excessive levels of inattentiveness, impulsivity and hyperactivity. Most ADHD patients benefit from treatment

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with methylphenidate hydrochloride (MPH), which effectively reduces symptoms in up to 70% of child patients. Moreover, recognition that ADHD persists into adulthood has led to an increased use of MPH in adult patients.¹ Age and circadian rhythms are modulators of MPH response in rats.² Biological rhythms, particularly circadian rhythms, are an essential part of the temporal order of living systems and their disruption has been related to some neuropsychiatric disorders.²

MPH has effects on behavioral tasks associated with anxiety and depressive symptoms, although the findings are controversial.^{2,3} It has been shown that drugs that can modulate anxiety-like symptoms, such as benzodiazepines, present age- and diurnal-dependent results in rats.^{4,5} Notably, antidepressants are known to affect the circadian timing system in rodents.^{5,6} Therefore, we hypothesized that the effects of MPH on the elevated plus maze (a test that can access anxiety-like behaviors) and the forced swimming test (a test that can access depressive-like behaviors) might be altered according to age and the light/dark cycle.

Experimental procedures

1. Animals

Young (postnatal day PD25; 75-85g) and adults (PD60; 250-300g) male Wistar rats obtained from our breeding colony were housed five to a cage with food and water available *ad libitum* and maintained on a 12-hour light/dark cycle (lights on at 7:00 a.m.). We performed the behavioral tasks during either the light (7:00-10:00a.m.) or the dark (7:00-10:00p.m) cycle. All experimental procedures were in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals and the Brazilian Society for Neuroscience and Behavior recommendations for animal care. The Ethics Committee of Universidade do Extremo Sul Catarinense approved this study before we conducted the experiments.

2. Drugs and treatments

Rats were divided into four groups: Saline-light; MPH-light; Saline-dark and MPH-dark ($n = 30$ per group; $n = 15$ per test), and received once a day (at 7:00 a.m. if light, or 7:00 p.m. if dark) either 2mg/kg racemic dextro- and levo-MPH mixture dissolved in saline solution (Ritalin, Novartis), or a saline intraperitoneal injection, for 28 days. We performed the treatment between PD25 - PD53 (in the case of the young rats) or PD60 - PD88 (for the adult rats). The selection of the MPH dose was based on previous findings that MPH doses of less than 5mg/kg intraperitoneal injection better reflect those used clinically in patients.⁷ We submitted animals to behavioral tests in a different room from the one in which they were injected, two hours after the last injection.

3. Behavioral procedures

The elevated plus maze apparatus was made of wood and consisted of two opposed open arms (50 x 10 x 2cm) and two opposed closed arms (50 x 10 x 40cm), all facing a central platform (10 x 10cm), elevated 45cm from the floor. Each rat was placed in the center of an elevated plus maze facing one of the closed arms (n

= 15 for each test condition). During a 5-minute test performed in a dark room illuminated with red light, we registered the number of entries into each arm and the time spent there.

The forced swimming test involved two individual exposures to a cylindrical tank with water in which rats could not touch the bottom of the tank or escape. The tank is made of transparent Plexiglas, 80cm high and 30cm in diameter, and filled with water (22-23°C) to a depth of 40cm. The water in the tank was changed after each rat. For the first exposure, rats were placed in the water for 15 minutes (the pre-test session). Twenty-four hours later, rats were placed in the water for 5 minutes (test session) and the length of time for which they remained immobile was recorded in seconds. We performed this test in a light room after 30 minutes' acclimatization. The animals had different behaviors during the light or dark cycle as expected (i.e. they were more active during the dark cycle).

4. Statistical analysis

The effects of cycle, treatment and age were analyzed using three-way analysis of variance (ANOVA) (equal variance and normality tests $p > 0.05$). The model included as factors: cycle (light or dark), treatment (saline or MPH) and age (young or adult) followed by Holm-Sidak post-hoc test. We used Kruskal-Wallis one-way ANOVA on Ranks (because normality test result $p < 0.05$) to verify differences between experimental groups. Relevant mean differences were subsequently assessed using Dunn's multiple-comparison procedure. All analyses were performed using SigmaStat 3.1, and $p < 0.05$ indicated nominally statistical significance. The graph was presented in mean $\pm 1.96 \cdot SE$

Results

The time spent in each arm on elevated plus maze was presented as a ratio (time spent ratio = time spent in the open arm/ time spent in the closed arm) - Figure 1. Three-way ANOVA revealed that cycle was a significant factor ($F = 8.326$; $p = 0.005$), while treatment ($F = 0.019$; $p = 0.891$) and age ($F = 2.879$; $p = 0.093$) were not significant. Holm-Sidak multiple comparison procedure showed cycle as a significant factor (difference of means = 0.288; $t = 2.885$; $p = 0.0049$). Kruskal-Wallis one-way ANOVA on Ranks showed differences in the mean values across the groups ($H = 33.482$; $p = 0.00002$). Therefore, we applied Dunn's test as a multiple comparison procedure. The Dunn's result indicated significant differences between adult rats treated with MPH during the light and the dark cycles (difference on Ranks = 36.291; $Q = 3.417$); and between young and adult rats during the light cycle after saline (difference on Ranks = 50.333; $Q = 4.140$) or MPH (difference on Ranks = 43.576; $Q = 3.645$) treatment (Figure 1).

The number of entries into each arm on elevated plus maze is presented as a ratio (number of entries ratio = number of entries into the open arm/ number of entries into the closed arm). Most of the groups presented ratios under 1.0, indicating that they entered the closed arms more often (the exception were adult rats during the dark cycle, whether treated with saline or MPH). Three-way ANOVA revealed that cycle was a significant factor

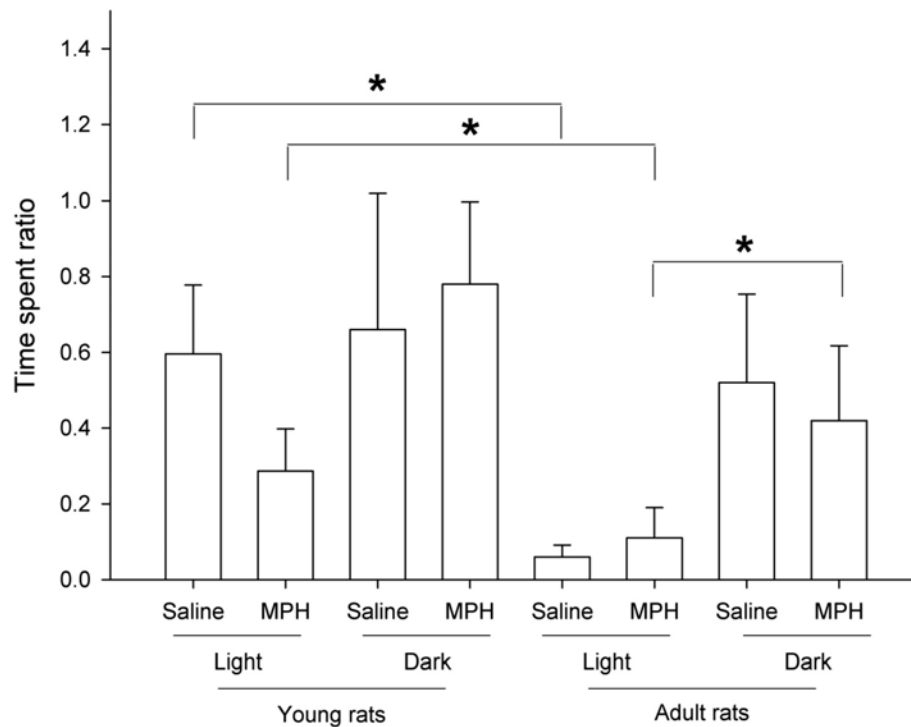


Figure 1 – Open/close time spent ratio in elevated plus maze test.

Figure represents the time spent ratio among time spent in each arm. Bars represent mean \pm 1.96

* SE of 15 rats at least.

* $p < 0.05$.

($F = 9.060$; $p = 0.003$), while treatment ($F = 0.017$; $p = 0.895$) and age ($F = 0.869$; $p = 0.353$) were not significant. Holm-Sidak multiple comparison procedure showed cycle as a significant factor (difference of means = 0.508; $t = 3.010$; $p = 0.0034$). Kruskal-Wallis one-way ANOVA on Ranks showed differences in the mean values across the groups ($H = 18.987$; $p = 0.00823$). Dunn's test was applied as a multiple comparison procedure, and the Dunn's test result indicated no significant differences between specific groups ($p > 0.050$) (data not shown).

The forced swimming test immobility time showed cycle ($F = 2.373$; $p = 0.126$), treatment ($F = 0.289$; $p = 0.591$) and age ($F = 1.499$; $p = 0.223$) as non-significant factors after three-way ANOVA, although we did find a significant interaction between cycle and age ($F = 4.055$; $p = 0.046$). Kruskal-Wallis one-way ANOVA on Ranks showed no significant differences ($H = 9.660$; $p = 0.20867$) (data not shown).

Discussion

Our results have shown light/dark cycle to be an important factor in the anxiety-like behavior results. However, no major effects of the administration of MPH were seen on anxiety- and depressive-like behaviors, nor was any significant interaction among these three factors found in anxiety-like behavior. We observed a nominally significant interaction between light/dark cycle and age in the forced swimming test. A recent article has reported no differences in the elevated plus maze results after intraperitoneal MPH (2.0mg/kg, twice daily) administration.⁸

Changes in anxiety- and depressive-like behaviors in rats were seen after MPH treatments with the same dose (2.0mg/kg) twice daily⁵ or with 5.0mg/kg twice daily.⁹ Differential MPH effects observed across rat strains could explain these controversial findings.¹⁰

There is convincing evidence that the effects in rats of the light/dark cycle undergo dramatic changes in the course of the animal's life, particularly during early ontogenetic development and in old age.¹¹ The light/dark cycle was a significant factor in the elevated plus maze results, while age and methylphenidate hydrochloride were not. Not many reports have evaluated the age-dependent expression of anxiety-related behaviors, and even fewer results are available regarding the influence of diurnal cycles. Both elevated¹² and no changes¹³ have been shown to produce anxiety-like behaviors in older animals when compared with younger.

We have found a nominally significant interaction between light/dark cycle and age in the forced swimming test results. Kelliher et al. have explored diurnal influences on the forced swimming test results with no melatonin administration, and reported fewer depressive-like behaviors during the light phase in male Sprague-Dawley rats.¹⁴ Results from studies using melatonin receptor agonists and melatonin administration have been controversial.¹⁵ We administered MPH in the dark cycle at 7:00 p.m. It is relevant to mention that the peak of melatonin occurs at around 3 a.m. and the corticosterone peak occurs in the evening (around 7:00 p.m.). Both hormones may interfere with MPH behavioral effects.

In conclusion, our study evaluated together three important factors (light/dark cycle, age and MPH treatment) for anxiety- and depressive-like behaviors in rats. Our results have shown age and light/dark cycle as the most significant modulators of both behaviors. Further experiments are needed to shed more light on the role of those factors and the pharmacological mechanisms linked to anxiety- and depressive-like behaviors in rats. We would also like to reinforce the idea that the age of the rat, and the time of the day that experiments are performed, may very well alter the behavioral results of these two tests.

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Disclosures

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* Modest

** Significant

*** Significant: Amounts given to the author's institution or to a colleague for research in which the author has participation, not directly to the author.

Note: UNESC = Universidade do Extremo Sul Catarinense; CNPq = Conselho Nacional de Pesquisa e Desenvolvimento; FAPESC = Fundação de Amparo à Pesquisa do Estado de Santa Catarina.

For more information, see Instructions for Authors.

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