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PROGRAMA DE PÓS-GRADUAÇÃO ENSINO DE CIÊNCIAS: QUÍMICA DA VIDA E SAÚDE

AVALIAÇÃO DO EFEITO DO METILFENIDATO NA DEAMBULAÇÃO E EXPLORAÇÃO DE CAMUNDONGOS EM CAMPO ABERTO

Dissertação de Mestrado

Miriam Moschen Silveira

Orientador: Prof. Dr. Diogo Souza

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A minha família.

“Sem a curiosidade que me move, que me inquieta, que me insere na busca, não aprendo nem ensino”.

Paulo Freire

Resumo

Neste estudo, analisaram-se os efeitos do Cloridrato de Metilfenidato administrado em camundongos em tarefas comportamentais no espaço de campo aberto, utilizando estímulos variados. Quando o estímulo se encontrava no centro do campo aberto, o metilfenidato diminuiu a busca pelo estímulo. Quando o estímulo se encontrava distribuído nos 4 cantos do campo aberto, o metilfenidato aumentou a busca pelo estímulo. Estes resultados sugerem que o metilfenidato tem efeitos diferentes dependendo do estímulo, problematizando o uso do fármaco por crianças e adolescentes de forma generalizada. Este trabalho não questiona o uso do mesmo por pessoas que apresentam o diagnóstico de transtorno de déficit de atenção/hiperatividade (TDAH).

Abstract

In this study, it was evaluated the effect of Methylphenidate (Ritalin®) orally administrated in mice in behavioral open field task, using varied stimulus. When stimulus is placed in the center of the open field, methylphenidate decreased stimulus search. When stimulus was distributed in the four corners of the open field, methylphenidate increased stimulus search. These results suggest methylphenidate has different effects depending on stimulus, problematizing the use of this drug by children and teenagers in a generalized way. This dissertation does not question methylphenidate use for those who show an attention-deficit/hyperactivity disorder (ADHD) diagnostic.

Introdução

O Cloridrato de Metilfenidato é um fraco psicoestimulante do sistema nervoso central, com efeitos mais evidentes sobre as atividades mentais em relação às ações motoras. Reprime as atividades criativas, espontâneas e independentes (Solanto, 2000). A criança ou o adolescente fica dócil, obediente e disposta a realizar tarefas rotineiras e maçantes, como por exemplo "dever de casa". É usualmente utilizado para crianças ou adolescentes diagnosticadas com transtorno de déficit de atenção/ hiperatividade (TDAH). Estas crianças ou adolescentes apresentam problemas de aprendizagem e conhecimento, têm dificuldades no relacionamento com os colegas, são desorganizados, não conseguem cumprir seus compromissos. O transtorno manifesta-se entre 3 e 6 anos de idade. Atualmente 20% das crianças e adolescentes que freqüentam as escolas estão diagnosticadas com o transtorno e utilizam a medicação acima (Riesgo e Rhode, 2004).

Jameson (Jameson, 1984) discorre sobre um "hiperespaço" gerado pela rede global e multinacional de comunicação descentrada, segundo ele este hiperespaço é multifacetado e ultrapassa a capacidade do corpo humano de se localizar, de organizar perceptivelmente o espaço circundante e mapear cognitivamente sua posição em um mundo exterior mapeável, o que nos leva a viver experiências múltiplas e fragmentadas.

A doença social, o pragmatismo, a competitividade, a individualidade, a negação da diversidade, os desencontros da vida de uma criança ou adolescente, e a busca dos pais e professores de uma normalização, rotulam o individuo como hiperativo, buscando a solução na medicalização.

O que me levou a pensar este trabalho é este grande número de crianças e adolescentes que utilizam a medicação. Optei pelo uso de modelos animais.

Estudos utilizando modelos animais têm algumas vantagens inerentes sobre os estudos em humanos (status sócio-econômico, o comportamento padrão e o meio escolar) são fatores a se considerar como influenciáveis em muitos estudos que envolvam pessoas. Modelos animais podem estabelecer efeitos resultantes de tratamentos e exposições documentadas. Além disso, permitem manipulações mais invasivas para investigar alterações neuroquímicas ou neuropatológicas, avaliações para terapêuticas em potencial ou intervenções comportamentais.

Apesar do cérebro dos primatas não-humanos serem mais parecidos com os cérebros humanos que o dos roedores, estes têm as vantagens de possuírem um ciclo de vida curto, são de fácil aquisição e manutenção, o pesquisador pode ter um controle sobre variáveis extras como dieta e meio ambiente.e sua neurobiologia é mais conhecida do que a dos primatas. Os roedores possuem um sistema nervoso mais simples, mas seus mecanismos comportamentais básicos além de serem mais fáceis de se interpretar, são similares aos humanos (Sagvolden et al, 2005).

Modelos animais de ADHD em roedores exibem claramente similaridade com muitos aspectos do transtorno em humanos e devem provar sua utilidade em estudar características específicas.Tarefas comportamentais que modelam aprendizado e memória de curta duração são sensíveis ao ADHD e o Cloridrato de Metilfenidato mostrou normalizar a performance dos animais nas tarefas que envolvem memória de curta duração.(Paule *et al*, 2000).

Aspectos a serem considerados: adaptação do animal ao ambiente do teste, atividade exploratória e deambulação.

Objetivos

- *Problematizar o uso do Metilfenidato por crianças e adolescentes da educação básica, usando modelos animais.*
- *Observar a alteração no padrão de comportamento locomotor e exploratório de camundongos CF-1, machos, adultos jovens (60 dias), frente a estímulos, quando colocados em campo aberto, com ou sem administração do metilfenidato (Ritalina®).*

Materiais e métodos

Os materiais, métodos e resultados dessa dissertação serão apresentados na forma de artigo científico, submetido à revista *Brazilian Journal of Medical and Biological Research*.

Effects of methylphenidate on locomotion and exploratory activities in mice in an open field

Miriam Moschen Silveira[1*], **Marcelo Ganzella**[1], **Eduardo Sörensen Ghisolfi**[2], **Diogo O. Souza**[1], **João Claudio Américo**[1]

[1] Departamento de Bioquímica, ICBS, Universidade Federal do Rio Grande do Sul, Rua Ramiro Barcellos, 2600, Prédio Anexo, Lab. 28, Porto Alegre, RS, Brasil.

[2] Departamento de Bioquímica, Faculdade de Biociências, Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre, RS, Brasil.

Abstract: In this study, we evaluated the effect of orally administrated Methylphenidate (Ritalin®), at dose of 40 mg/kg, in ambulatory and exploratory activities of CF-1 young adult male mice (60 days) in and enriched environment. The environment had objects (stimulus) in an open field box, which attracted the animals. Mice that received methylphenidate showed a decrease in the exploration of objects (stimulus) when it was located in the middle of the open field. In contrast, the exploration of stimulus increased when the stimuli were at the corners of the open field. These results indicate that methylphenidate modified the exploratory activity of mice in different ways, depending on the position of stimulus in an open-field box.

Key Words: Methylphenidate, Open field box, Mice, Attention, ADHD, Learning

Introduction

Methylphenidate (Ritalin®) is a mild CNS stimulant. This drug was first synthesized in 1944 (1) and its pharmacological actions were described in 1954 (2, 3). Nowadays, methylphenidate is used for the treatment of Attention-Deficit Hyperactivity Disorder (ADHD). Patients with ADHD have a clinically heterogeneous syndrome characterized by inattention, hyperactivity, impulsivity and motor disturbance. The disorder appears between the age of 3 to 6, however it is usually noticed when the child starts to frequent the school. (4). ADHD physiopathology is not completely clear; nevertheless it is strongly related to changes in prefrontal cortex functioning, which control functions like attention and planning in human beings (5). Some studies have suggested that a disorganized communication among neurons in this brain region could be an important factor for development of ADHD.

Neurotransmitters, such as dopamine and noradrenaline, are taken up by presynaptic neurons via distinct transporters (6, 7) and this process is fundamental for modulation of dopaminergic and noradrenergic neurotransmission. Literature data have indicated that methylphenidate blocks the reuptake of both neurotransmitters, thus stimulating the catecholaminergic tonus (8).

In 1952, in United States, 52% of the mothers considered that their children were hyperactive and disregarded; today, these behaviors are designed as a syndrome. Nowadays, approximately 6 millions children in United States use methylphenidate. In the same vein, in many schools of Buenos Aires (Argentina), Santiago (Chile), São Paulo, Rio de Janeiro and Porto Alegre (Brazil), about 20% of the children have been identified as getting ADHD (9).

The world of the children is more than his house, his books and his school. When they go to school, they have to face up with a reality that many times is quite different from their previous world. In the school scenario, an ideal student has to be quiet and pay attention to the teacher. Consequently, the classroom environment is always a place that many children cannot stand and they are always marked as "abnormal". They are perceived as inattentive and/or hyperactive, and then they are induced to use a medication, methylphenidate, to adapt to the classroom environment (9).

However, few studies have investigated the possible relation between methylphenidate consumption and general attention. Thus, in this study, by using an experimental animal model (for discussion on animal models aiming investigate behavioral methylphenidate effects see references 10, 11), we evaluated the effects of methylphenidate on mice submitted to different types of stimuli. We aimed to search if the effect of this drug on the exploratory behavior of mice towards stimuli varied with the specificity of each stimulus, or its effect was always the same. By varying the stimuli impact, we aimed to "mimic" a classroom, where the students are submitted to a profusion of stimuli and not only to the teacher ("authorized") stimulus.

Materials and Methods

Animals

CF-1 young adult male mice (60 days), obtained from FEPPS/RS, were habituated in animal house of the Biochemistry Department, ICBS, UFRGS for at least 1 week. One hour before the experiments, the animals were placed in the experimental room for habituation.

Drug and administration

Methylphenidate (Ritalin®): 10 mg tablets were dissolved in saline solution (NaCl 0,9%), just before the oral administration (30 minutes before the open field sessions), using "gavage" needle.

Open field model

Each animal was placed in one of the corners of an open field box (49 cm x 49 cm x 49 cm) facing the center and observed for 10 minutes through a "webcam" (Tort ABL *et al*, 2006), fixed 1 meter above the box. Three concentric squares were virtually delimitated: a big one (30 cm x 30 cm), an average one (15 cm x 15 cm) and a small one (7.5 cm x 7.5 cm) (Fig.1). In the open field box it was evaluated two behaviors: total locomotion and exploratory activity. Animals were exposed only to one open-field session. Between the sessions, the open field was cleaned with alcohol 70%.

Total locomotion was quantified by the distance (in cm) that each animal move along in the session.

The exploratory activity evaluated the time (in seconds) that each animal spent, in contact with one of the object(s) (the stimulus) placed in the open-field box. The objects were black cylinders, 3 cm of diameter and 5 cm height. Two protocols of environment enrichment were used: i) a single central object (one object was place in the center of the box); ii) multiple peripheral objects (four objects were placed at each corner of the box). It was compared the time the animals spent close to the objects with the time that other animals spent in the same area when the objects were not present. This difference in the time was considered for evaluating if the object acted as a stimulus. Further, it was investigated the methylphenidate effect on the interaction between the mice and the object(s) (stimulus).

Groups of animals

To analyze the behavioral effect of object(s)/methylphenidate, the animals were divided in four groups: 1) Control group; 2) Group with the administration of methylphenidate; 3) Group with the presence of the object in the center of the box; 4) Group with the administration of methylphenidate and the presence of the object in the center of the box; 5) Group with the presence of four objects in the corners of the box; 6) Group with the administration of methylphenidate and the presence of four objects in the corners of the box.

Analysis of behavioral responses

Analysis of the animal behavior was performed through the Mouse Tracker 2006 program, developed in our lab (12). Statistical analysis was made in SPSS, by one-way ANOVA test, followed by Tukey post-hoc analysis (Tukey and LSD).

Results

Locomotion

Methylphenidate dose-response curve on mice locomotion

Methylphenidate (1.5; 5; 10 and 40 mg/kg) did not affect the locomotion, in the absence of any object(s) (Fig. 2). Consequently, the dose of 40 mg/kg was used in further experiments.

Effect of object(s)/methylphenidate on locomotion

Methylphenidate administration (40mg/kg) associated to 1 object in the center (Fig. 3a) or to 4 objects in the corners (Fig. 3b) did not affect the total locomotion of the animals.

Exploratory Activity

When the box was in enriched with 1 object in the center, the animals spent more time inside of the concentric squares around the object, compared to animals without the object (Fig. 4), indicating that the object attracted the mice attention. Methylphenidate abolished this effect of the central object. When the box was enriched with 4 objects in the 4 corners, the animals settled less time in the center (Fig. 5b – small square), therefore near the objects (Fig. 5a). Methylphenidate increased the effect of the objects when they were put in the corners (Fig. 5a.b). Thus, methylphenidate changed dramatically the behavior of mice depending on the position and/or the number of the objects.

Discussion

This study investigated the behavioral effects of stimuli/methylphenidate on adult mice in an open field box. It was evaluated locomotion and exploratory activity in different situations: box without objects, with 1 object in the center or with 4 objects (one in each corner), with or without methylphenidate administration. About the effect of objects on the exploratory activity, without methylphenidate, the presence of 1 object in the center or 4 objects in the corners attracted the mice.

As the main purpose of this study was to evaluate the behavioral effects of methylphenidate on mice, it was firstly studied the drug effect on total locomotion. No dose used affected this parameter. Our data is in agreement with literature data, which show that doses between 5 and 20 mg/kg did not affect the locomotion of adult male mice (13). Then, it was used the highest tested dose (40 mg/kg) to evaluate specifically its effect on the exploratory activity towards the stimuli.

When the object (stimulus) was placed in the center, methylphenidate abolished the exploration of the object. However, very interestingly, when the objects were placed in the corners, increased their exploration. These results point that the effect of methylphenidate administration toward the stimuli clearly depended on the nature of the stimulus (in the case, the number and the position of the same stimuli). Trying to "transfer" these observations to the "teacher stimulus" in a classroom, it is important to emphasize that the prescription of methylphenidate, aiming direct their attention to the teacher (the official stimuli) (4), do not take in account the fact that when the students are paying attention on the teacher (the aim of the prescription), they are not being involved with other stimuli, which could be more relevant to them.

There are many studies on the relation between methylphenidate and Attention-Deficit Hyperactivity Disorder (ADHD), a neuropsychiatric syndrome clinically heterogeneous of inattention, hyperactivity and impulsivity, quite common in childhood (14). Those with ADHD are not easily accepted in deuces or groups, their scholar performance is minimal, they do not memorize the class contents and, consequently they do not enjoy the class. ADHD affects 5% of children and teenagers all over the world. It begins at 3 years old, but diagnostic is usually done when the child goes to primary school, where the learning requires periods of concentration and attention. Treatment is done with central nervous system (CNS) stimulants, among them the drug used in this study, methylphenidate, and with a continuous therapeutic strategy of psychosocial therapy involving family and the school. According to Guardiola (15) and Schwartzman (16), ADHD prevalence varied according to the different evaluation criteria, being very frequent wrong diagnosis.

Summarizing, in this study we showed that methylphenidate, a drug commonly recommended to students for guiding attention to official stimuli, affected the attention of mice to objects depending on their location and/or their number in an open field box. A putative correlation with classrooms was speculated.

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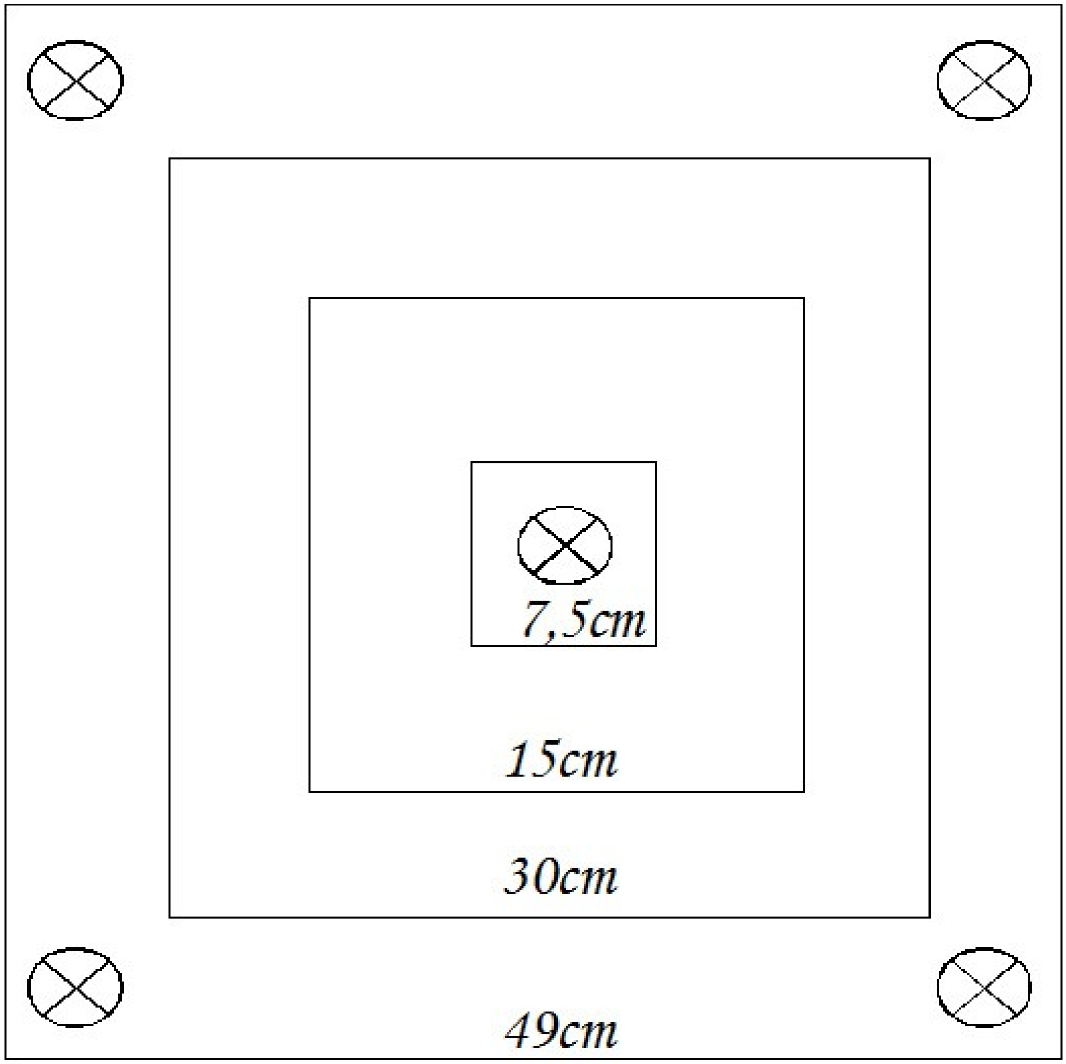

Fig.1: Represents an open field box with a floor virtually divided, delimiting concentric squares. Each square dimensions are indicated above. Circles indicate where the objects were placed.

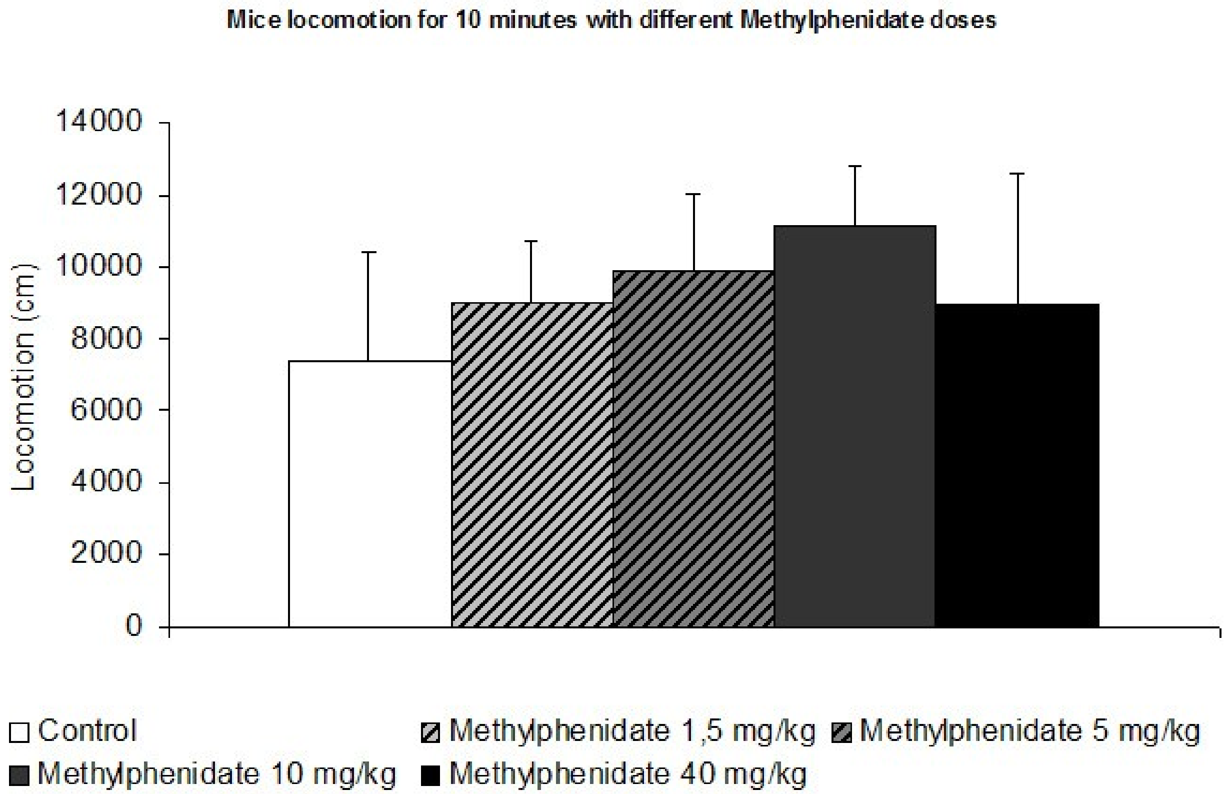

Fig. 2: Effect of methylphenidate on mice locomotion (in cm). Thirty minutes after methylphenidate administration, mice were subject to an open-field (without objects) for 10 min. There was no significant difference among groups. Control = 35, Methylphenidate = 8 for each dose.

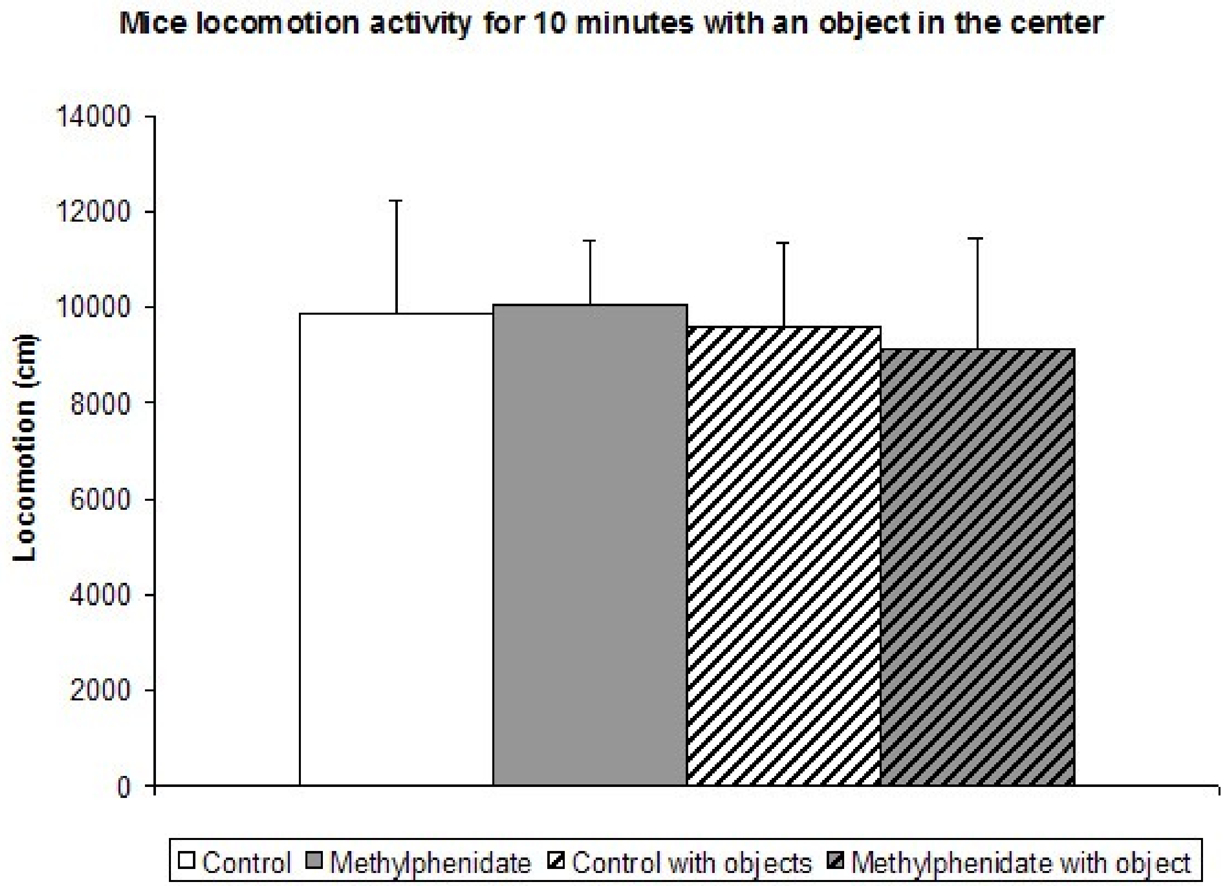

Fig.3a: Mice locomotion (in cm) in the open field box, with 1 object in the center. There was no significant difference among groups. Animals per group: 16.

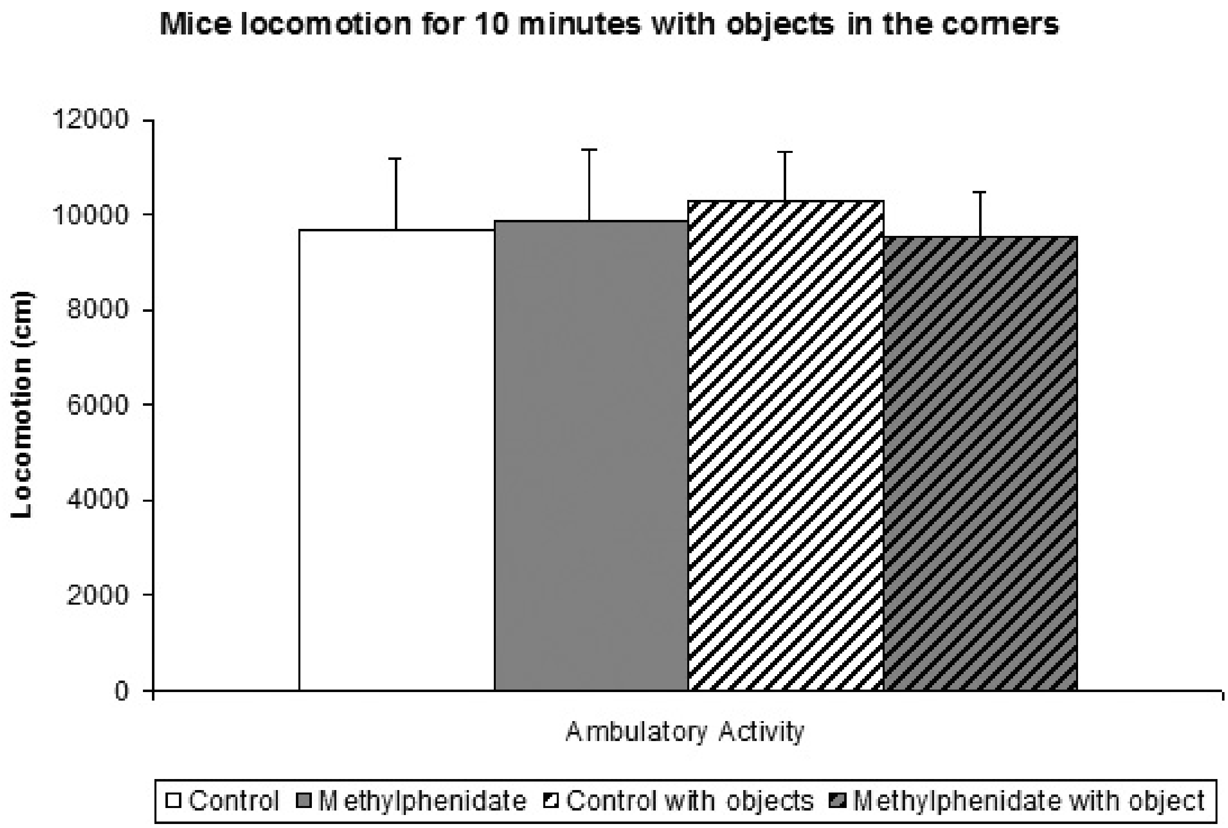

Fig. 3b: Mice locomotion (in cm) in the open field box, with objects in the corners. There was no significant difference among groups. Animals per group: 12.

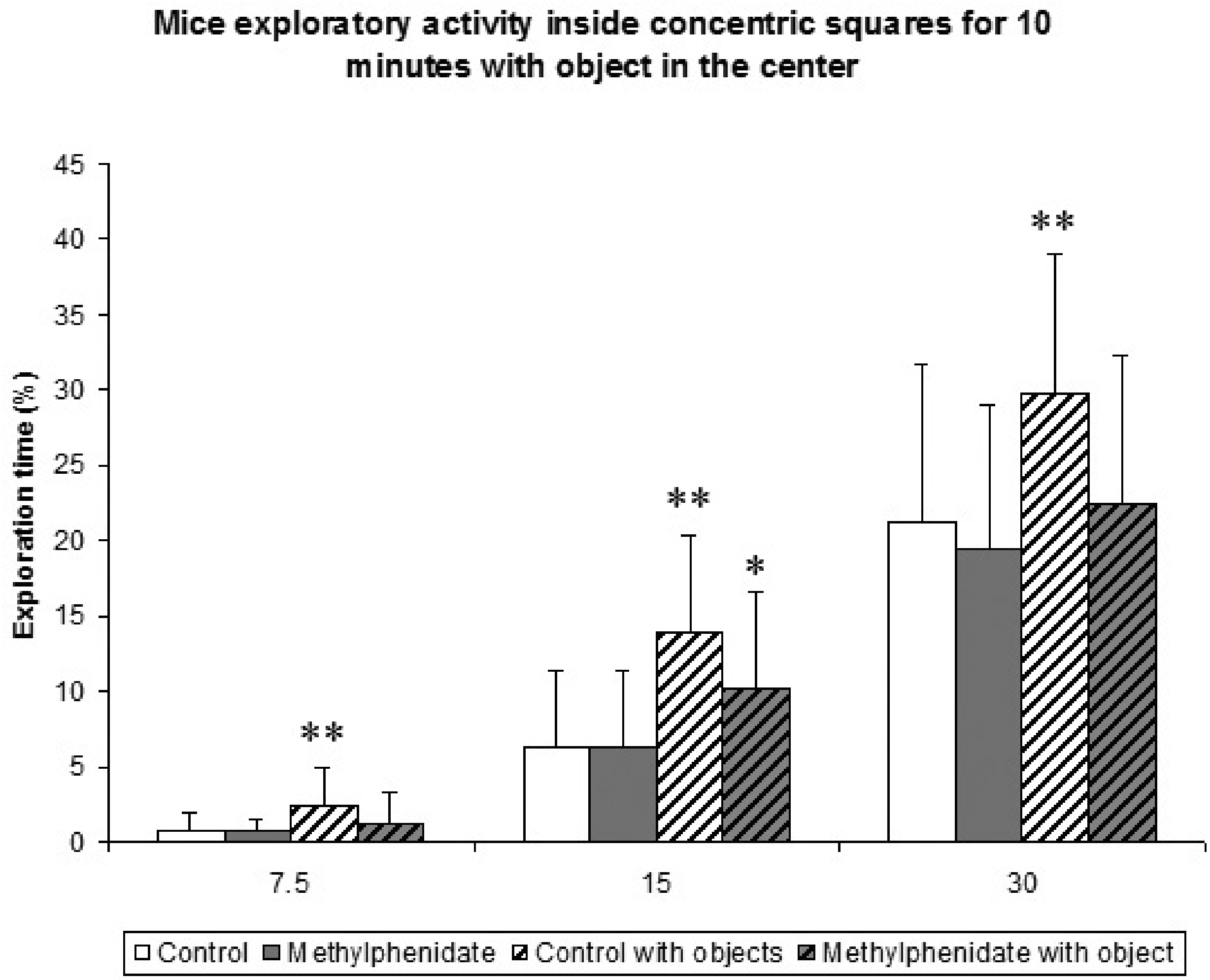

Fig. 4: Exploratory activity in different squares, with or without the object in the center, with or without methylphenidate administration (40 mg/kg) Animals per group: 16. Significance compared to respective control: * - p < 0,05, ** - p < 0,01.

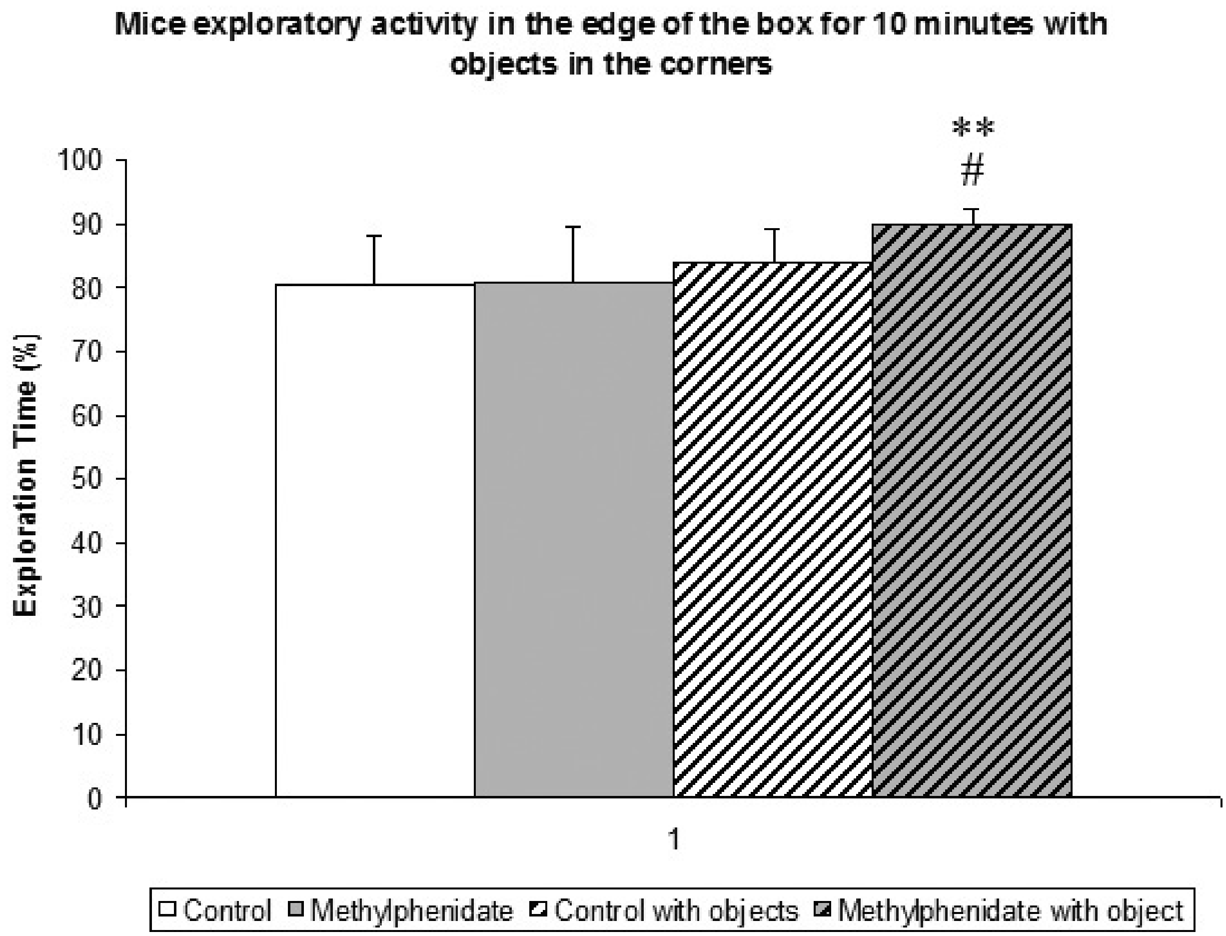

Fig. 5a: Exploratory activity in the edge of the box (out of the biggest square - 30 cm). Animals per group: 12. Significance compared to control: **- $p < 0.01$. Significance compared to methylphenidate group: # $p < 0.05$.

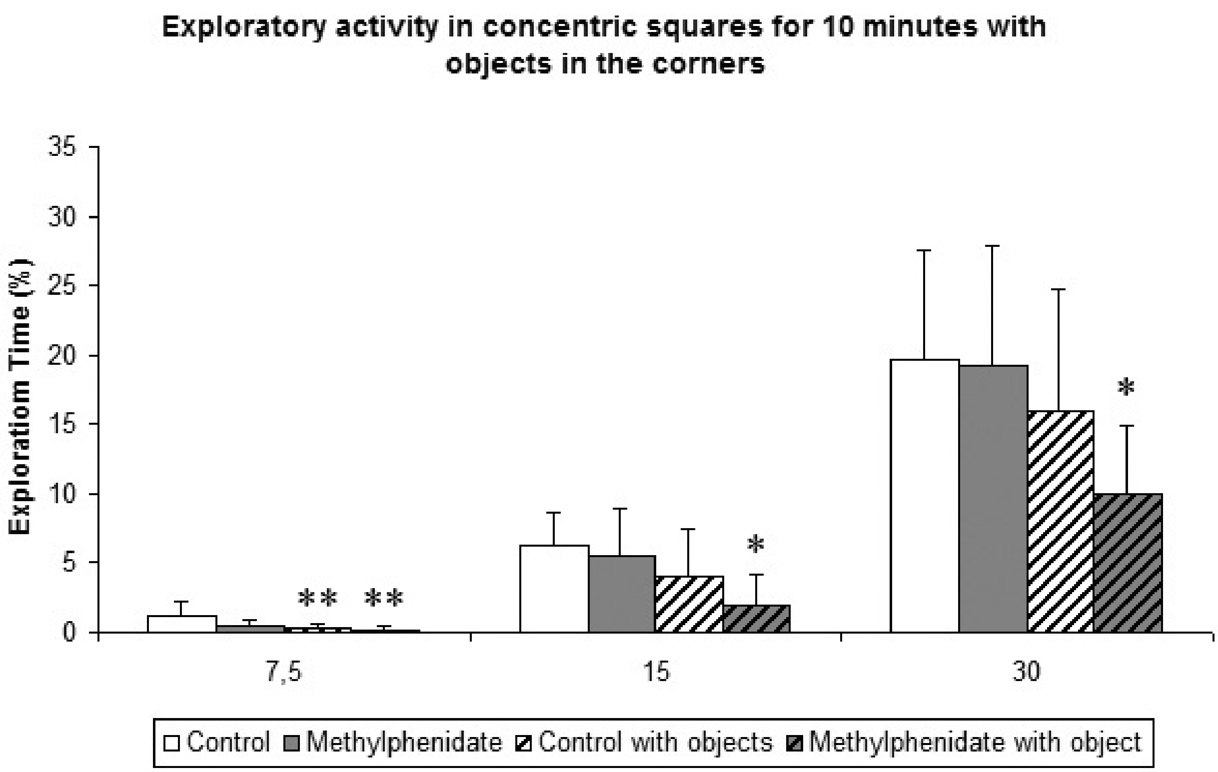

Fig. 5b: Mice exploratory activity inside the virtual delimited squares. Animals per group: 12. Significance compared to control: * $p < 0.05$, ** $p < 0.01$.

Conclusão

A administração do metilfenidato produz diferentes efeitos conforme variam os estímulos, sugerindo que é preciso um olhar mais criterioso quanto ao uso do medicamento em nossas crianças e adolescentes, tão difundido atualmente.

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