Effects of acute topiramate dosing on open field behavior in mice

Efeitos da administração aguda de topiramato sobre o comportamento de camundongos em campo aberto

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ABSTRACT

Although topiramate (TPM) has been used to treat human disease, there are few studies of its effects on the behavior of animal models. **Objective.** This study aimed to assess the effect of acute TPM administration on the behavior of mice undergoing the open-field test. **Method.** The animals were divided in two groups: the treatment group (n = 10), which received 10 mg/kg TPM intraperitoneally, and the control group (n = 10), which received saline. 30 minutes after drug administration, the animals were assessed for 5 minutes in the open-field. The following parameters were analyzed: number of squares explored, immobility time, central area permanence time, peripheral apparatus permanence time, rearing frequency and time, grooming frequency and time, rearing frequency during the last minute, number of fecal boli, and estimated speed. **Results.** The treatment group had a higher number of squares explored (p = 0.02) and greater estimated speed (p = 0.01). **Conclusion.** The results suggest that acute TPM administration increases the locomotor activity of mice without interfering with learning, anxiety, stress, and exploratory behavior.

Keywords. Mice, Locomotor Activity, Topiramate, Behavior.

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RESUMO

Embora o topiramato (TPM) seja utilizado para tratar doenças em humanos, poucos são os estudos de seus efeitos sobre o comportamento em modelos animais. **Objetivo.** Este estudo teve como objetivo avaliar o efeito da administração aguda de TPM sobre o comportamento de ratos submetidos ao teste de campo aberto. **Método.** Os animais foram divididos em dois grupos: o grupo tratamento (n = 10), que recebeu 10 mg/kg intraperitoneal de TPM e o grupo controle (n = 10), que recebeu solução salina. Após 30 minutos de administração da droga, os animais foram avaliados durante 5 minutos no teste de campo aberto. Os seguintes parâmetros foram analisados: número de quadrados explorados, tempo de imobilidade, tempo de permanência na área central, tempo de permanência na área periférica, tempo de permanência em pé, frequência e tempo de grooming, tempo em pé durante o último minuto, número de bolos fecais e a velocidade estimada. **Resultados.** O grupo tratamento teve um maior número de locais explorados (p = 0.02) e maior velocidade estimada (p = 0.01). **Conclusão.** Os resultados sugerem que a administração aguda de TPM aumenta a atividade locomotora de camundongos sem interferir com a aprendizagem, ansiedade, estresse e comportamento exploratório.

Unitermos. Camundongo, Atividade Locomotora, Topiramato, Comportamento.

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INTRODUCTION

Topiramate (TPM) is a drug that inhibits some ion channels, reducing the frequency of reactivation of calcium channels and voltage-activated sodium channels. It is excitatory of the GABA-A receptors, leading to a neuronal chloride ion influx and generating inhibitory post-synaptic potentials. It also antagonizes the ability of the kainate sub receptor to activate the AMPA receptor subtypes of the glutamate excitatory neurotrasmitter, with a possible additional effect on the activity of the NMDA-type glutamatergic receptors[1].

TPM has found several uses in human disease. Initially developed to treat epilepsy[2], its use has been extended to other clinical situations such as bipolar disorder[3], mood disorders[4], neuropathic pain[5], kleptomania[6], migraine[7], several chemical addictions[8], obesity[9], and ischemic stroke[10]. In a few studies, TPM was associated with cognitive impairment when compared with other drugs, this reinforces the need for basic research to the use of topiramate[11].

Yet studies of TPM on animal models are scarce in spite of the existence of adequate apparatuses for the assessment of several emotional parameters (chiefly in rodents) such as stress, anxiety, fear, and locomotion. The open-field test is one of the most validated and used tools for emotionality assessment[12].

This study aimed to assess the behavior of acutely TPM-treated mice in the open field test.

METHOD

Animals

Adult male Swiss mice (age 90 days and weight approximately 40g) from the Central Bioterium of the Juiz de Fora Federal University (UFJF) were used. The animals were kept in 41 X 34 X 16cm polypropylene cages (10 animals in each cage) lined with wood shavings in the Cognitive Neurophysiology Laboratory of the UFJF.

In order to guarantee well being and avoid environmental influences on test performance, the cages were kept on a ventilated bookcase with controlled temperature (22±1ºC) and humidity (50±5%), in 12-hour light/12-hour dark cycles (lights on from 7 a.m.). The animals were initially kept in the laboratory for 2 weeks, with free access to water and ration, being handled only during cage cleaning, by the same person and at the same time. The experimental protocol followed the guidelines of the NIH Guide for the Use and Care of Laboratory Animals (National Academy Press, USA) and was approved by the local Ethics Committee of Federal University of Juiz de Fora, MG.

Drug

After being weighed on an electronic scale, the animals were divided in control and treatment groups, each one with 10 animals. Controls received 0.9% saline and the other animals received TPM (Janssen Cilag Farmacêutica, Brazil), diluted in 0.9% saline, so that each animal received 10mg/kg. Intraperitoneal administration was achieved with an insulin syringe fitted with an ultrafine needle, during the light-hours phase of the cycle. 30 minutes after administration the animals underwent the open-field test.

Test apparatus

The open field used in this experiment consisted of a 45cm-long square wooden apparatus, closed with 15cm-high walls[13]. The floor, made of rough glass to make locomotion easier, was painted with 36 7.5cm-long squares to enable the number of explored squares to be counted. The apparatus was kept in a room under dim light (22±1 lux) and the tests were undertaken during the lights-on phase of the cycle, between 10 a.m. and 4 p.m. In order to remove any olfactory cues, the apparatus was cleaned with 70% isopropylic alcohol after each test.

Experimental procedure

After TPM or saline injection, each animal was returned to the cage and, after 30 minutes, was gently placed at the center of the open field and filmed for 5 minutes with a high resolution webcam (1300Kb), which obtained the images and sent them straight to

the ANY-maze software (Stoelting Co., Wood Dale, IL, USA). The software analyzed the following parameters: number of squares explored (counted as the animal crossed the dividing line with the four limbs), immobility time (period during which the animal stayed still or in a rearing/grooming position), central area permanence time (16 squares in the central area), time in the apparatus periphery (20 peripheral squares adjacent to the walls, where the animal would have thigmotaxic cues), frequency and duration of rearing episodes (exploratory behavior in which the animal stands on the rear limbs), frequency and duration of grooming (self-cleaning behavior in which the animal explores the body with the snout or the head with the front limbs), and number of fecal boli. Anxiety behavior was assessed through the time spent in the 16 squares non-adjacent to the apparatus walls[14]. In order to better assess the locomotor activity, the estimated speed (pictures/minute) of each animal, that is the number of squares crossed divided by the actual locomotion time (5 minutes minus immobility time), was measured. The frequency of rearing during the last minute of the test was also measured, so as to compare habituation between the groups[15].

Statistical analysis

After descriptive analysis and verification of sample normality and equivalent variance assumptions, the t-test was used to assess any significant difference between the means from both groups. Pearson`s correlation test was also used to assess concordance for rearing and grooming behaviors. The results of descriptive statistics are presented as means ± standard error. 5% significance level was adopted, and the version 7 Statistica packgae (StatSoft Inc., Tulsa, OK, USA) was used for data analysis.

RESULTS

Results of t-test were not statistically significant for the following parameters: time spent in the center (t = 0.80, df = 18, p = 0.43), time spent in the periphery (t = -0.80, df = 18, p = 0.43), rearing frequency (t = -1.01, df = 18, p = 0.33), rearing frequency during the last minute (t = 0.24, df = 18, p = 0.81), rearing time (t = -0.69, df = 18, p = 0.50), grooming frequency (t = 1.18, df = 18, p = 0.25), grooming time (t = 0.48, df = 18, p = 0.64), and immobility time (t = -0.06, df = 18, p = 0.96) (Tab. 1).

Table 1
Parameters for the control group (n = 10) and TPM-treated group (n = 10) in the open field. Data are expressed as means ± standard error

	Control	Topiramate	*p*
Number of squares	287.3±25.1	429.1±51.2	0.02*
Time at the center (s)	63.4±6.8	76.1±14.3	0.43
Time at the periphery (s)	236.6±6.8	223.9±14.3	0.43
Rearing frequency	19.9±5.9	13.2±3.1	0.33
Rearing frequency (last minute)	3.3±1.2	2.8±1.7	0.81
Rearing time (s)	21.0±6.2	16.2±3.1	0.50
Grooming frequency	2.6±0.9	7.6±4.2	0.25
Grooming time (s)	10.6±4.1	14.7±7.5	0.64
Immobility time (s)	31.6±8.2	30.9±9.5	0.96
Speed (squares/min)	59.8±5.1	90.9±10.4	0.01*
Number of fecal boli	3.6±0.4	1.4±0.5	< 0.01*

p < 5%

The following parameters had statistically significant (α = 0.05) differences: number of explored pictures (t = 2.49, df = 18, p = 0.02), estimated speed (t = 2.69, df = 18, p = 0.01), and number of fecal boli (t = -3.12, df = 18, p < 0.01). The treatment group had fewer fecal boli and greater locomotor activity as ascertained by the larger number of pictures explored and higher estimated speed (Fig. 1).

There was strong positive correlation on Pearson's test between rearing time and frequency (r = 0.926, p < 0.01) as well as between grooming time and frequency (r = 0.924, p < 0.01).

DISCUSSION

The data showed increased locomotor activity and reduction of fecal boli in the TPM-treated group.

Although locomotor activity is generally assessed through the number of pictures explored[13], we introduced the estimated speed parameter, which showed consistent results, pointing to its possible use as a new open-field parameter, as a variable that takes into account the immobility time, which might be affected by stress, fear, or anxiety[12].

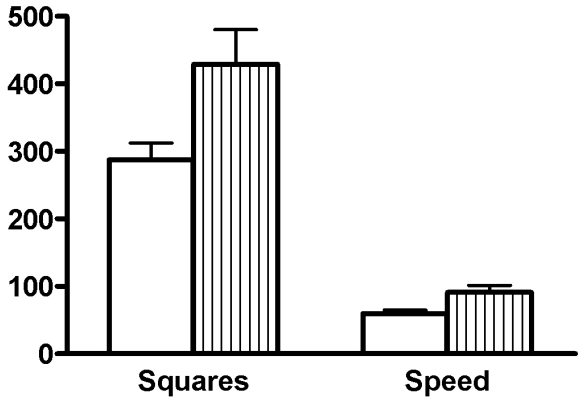

Fig. 1. Comparison of means ($p < 0.05$) and standard error of locomotor activity (explored squares and estimated speed) in the control group (blank bars) and TPM-treated group (lined bars).

The fact that TPM-treated animals had greater locomotor activity could be accounted for by a reduction of the habituation-linked learning process, and thus by an impairment of short term memory acquisition in the hippocampus through changes in the glutamatergic tonus[16], once deterioration of human cognitive function with the drug has been reported[17].

However, such a fact does not seem to have occurred in our study, once there was no difference of rearing frequency during the last minute between the groups. This parameter has good accuracy for habituation analysis in the open field[18]. Similar results were found in another study[19].

Another possible explanation for the increased locomotor activity could be an increase in the anxiety levels of TPM-treated animals, although our data did not show any difference between the time spent in the periphery or in the center. It is generally accepted that anxious animals seek safer environments, represented by the squares adjacent to the walls in the open field, an area where there are more thigmotaxic cues[14]. This suggests that the two groups had equivalent anxiety levels.

Thus TPM-treated animals might have had greater locomotor activity by a direct action of TPM over structures responsible for motor patterns such as the striatum and prefrontal cortex, once a close relation between locomotor activity and dopaminergic tonus in the basal nuclei has been shown[20,21].

NMDA glutamatergic and dopaminergic receptors from various central nervous system sites have been shown to interfere with locomotor activity. An interaction of the two neurotransmitter systems in the nucleus accumbens, prefrontal cortex, and basal nuclei has been hypothesized[22].

TPM acts in the corticomesolimbic pathway[23] and might increase dopamine release from the medial prefrontal cortex[24]. TPM also acts in the glutamatergic tonus, chiefly over the AMPA and kainate receptors, and also possibly in the NDMA receptors[25,26]. These neurochemical alterations might thus account for the increased locomotion seen in the TPM-treated animals in our study.

The reduction in the number of fecal boli in the TPM-treated animals might suggest lower stress levels, although anxiety alterations that commonly follow stress alterations in the open field[12] were not evidenced here. Therefore, the reduction in the fecal boli may have been due to a direct action of TPM over gastrointestinal motility[27].

Our results suggested that the increase in the locomotor activity was independent from the increase in the exploratory activity, once when the latter increases, the animals exhibit greater rearing and grooming time or frequency[18]. This fact stresses the idea that TPM may have acted through motor-related circuitry, once exploratory behavior is an adaptive and evolutionary mechanism involving motivational circuitry[28]. Most studies assessing the exploratory behaviors rearing and grooming have used both time and frequency of these parameters. We found a strong positive correlation be-

tween time and frequency of rearing and also of time and frequency of grooming, suggesting that measuring just one parameter (time or frequency) may suffice. Such an approach may simplify the open field test.

One drawback of our study was the use of a single dose of TPM (10mg/Kg). Although other authors have managed to use higher doses, a pilot study we conducted showed higher doses to produce ataxia and sedation. We did not test the effects of chronic administration or the effects more than 30 minutes after acute TPM administration. Further studies should assess different doses at different times.

CONCLUSION

Our results suggest that acute TPM administration increases the locomotor activity of mice without affecting other parameters such as learning, anxiety, stress, and exploratory behavior.

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