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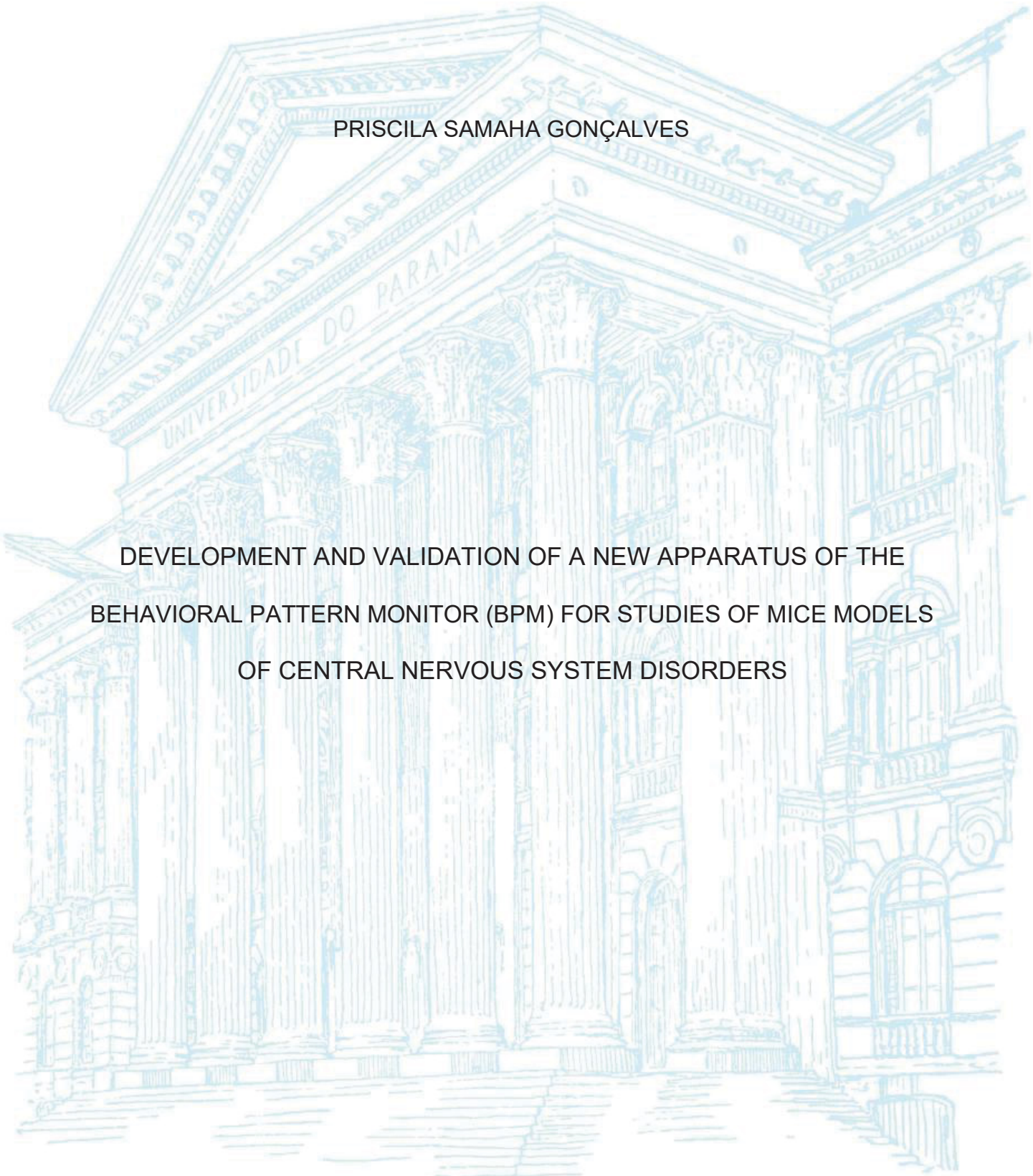

DEVELOPMENT AND VALIDATION OF A NEW APPARATUS OF THE BEHAVIORAL PATTERN MONITOR (BPM) FOR STUDIES OF MICE MODELS OF CENTRAL NERVOUS SYSTEM DISORDERS

CURITIBA
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Tese apresentada como requisito parcial à obtenção do título de Doutor em Farmacologia, no Programa de Pós-Graduação em Farmacologia, Área de concentração Neurociências, Departamento de Farmacologia, Setor de Ciências Biológicas da Universidade Federal do Paraná.

Orientador: Prof. Dr. Roberto Andreatini
Co-orientador: Prof. Dr. Marco Botolato

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TERMO DE APROVAÇÃO

Os membros da Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação FARMACOLOGIA da Universidade Federal do Paraná foram convocados para realizar a arguição da tese de Doutorado de **PRISCILA SAMAHA GONCALVES** intitulada: **DEVELOPMENT AND VALIDATION OF A NEW APPARATUS OF THE BEHAVIORAL PATTERN MONITOR (BPM) FOR STUDIES OF MICE MODELS OF CENTRAL NERVOUS SYSTEM DISORDER**, sob orientação do Prof. Dr. ROBERTO ANDREATINI, que após terem inquirido a aluna e realizada a avaliação do trabalho, são de parecer pela sua APROVAÇÃO no rito de defesa.

A outorga do título de doutora está sujeita à homologação pelo colegiado, ao atendimento de todas as indicações e correções solicitadas pela banca e ao pleno atendimento das demandas regimentais do Programa de Pós-Graduação.

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RESUMO

Mania é a principal característica do transtorno bipolar (TB), comportamento impulsivo, hipersexualidade, irritabilidade e hiperatividade locomotora são alguns de seus principais sintomas. A Síndrome de Tourette (TS) é uma doença neurológica caracterizada por movimentos repetitivos e vocalizações chamados tics. Ambas doenças necessitam de terapias mais toleráveis e eficazes e modelos animais de doenças do SNC são ferramentas essenciais para o seu desenvolvimento. O monitor de padrão de comportamento (BPM), é um aparelho automatizado equipado com sensores capazes de detectar atividade locomotora (número de transições), atividade exploratória (número de holepokes (colocadas do fucinho nos buracos) e rearings (levantamentos)) e padrão de movimentos de camundongos. Ele demonstrou alto poder translacional de modelos animais dopaminérgicos de mania para pacientes maníacos e vice-versa. Estudos mostram que psicoestimulantes são ferramentas farmacológicas eficazes para induzir estados tipo maníacos em camundongos C57BL testados no BPM. Entretanto, esses estudos não foram reproduzidos por outros laboratórios devido ao fato de o BPM não ser disponível comercialmente. Além disso o BPM nunca foi usado para testar outros modelos animais de doenças do Sistema Nervoso Central (SNC). Um dos mais bem caracterizados modelos animais de TS são os camundongos D1CT-7, entretanto, mais estudos são necessários para expandir o perfil comportamental desses camundongos e sua relação com as respostas tipo tic. Esse estudo teve como objetivo desenvolver e validar o BPM 2.0 (baseado no BPM original) para estudos de modelos de doenças do SNC que utilizam camundongos. Atividades locomotora e exploratória, comportamentos de risco e tipo ansioso e vocalizações ultrassônicas (USVs) são possíveis ser examinadas no BPM 2.0. No experimento 1, o BPM 2.0 foi validado para avaliar modelos animais de mania. Camundongos machos C57BL foram tratados com inibidores de DAT (transportador de dopamina): metilfenidato (MPH), GBR 12909 e modafinil intraperitonealmente imediatamente antes de uma sessão teste de uma hora no BPM 2.0. No experimento 2, foi avaliado o perfil comportamental dos camundongos D1CT-7 em comportamento espontâneo e em estresse agudo no BPM 2.0. O experimento 1 mostrou que o BPM 2.0 foi eficaz em detectar aumento da atividade locomotora e comportamento de risco e uma diminuição no comportamento tipo ansioso com todos os inibidores de DAT. Além do mais, o BPM 2.0 foi eficaz para detectar uma diminuição no comportamento exploratório (holepokes e rearings) com o MPH. No experimento 2 os camundongos D1CT-7 em comportamento espontâneo, mostraram aumento na atividade locomotora e rearings e uma diminuição nos holepokes. Por último, foi detectado aumento nos comportamentos repetitivos (rearings e escaladas) durante estresse agudo causado por confinamento espacial dentro no BPM 2.0. A principal limitação do BPM 2.0 foi o microfone de USVs, o qual gravou ruídos na mesma frequência das USVs dos camungondos, dificultando as análises dessas USVs. O presente estudo concluiu que o BPM 2.0 é um equipamento válido para testar modelos de mania e útil para avaliar o perfil comportamental de outros modelos animais de doenças do SNC (ex. TS) que utilizam camundongos.

Palavras-chave: monitor de padrão comportamental, BPM, modelos animais de mania, modelos animais de síndrome de tourette, TS, mania.

ABSTRACT

Mania is the defining feature of Bipolar Disorder (BD) and is characterized by impulsive behavior, hypersexuality, irritability and motor hyperactivity. Tourette Syndrome (TS) is a neurological disorder characterized by multiple repetitive movements and vocalizations called tics. Both CNS disorders need novel therapies with better efficacy and tolerability profiles. Animal models of Central Nervous System (CNS) disorders are essential tools for the development these therapies. The behavioral pattern monitor (BPM) is an automated device equipped with sensors that were capable to detect locomotor activity (number of transitions), exploratory activity (number of holepokes and rearings), and pattern of movements of mice. It demonstrated translational value from dopaminergic animal models of mania to mania patients and reverse translation. Evidence in literature shows that the psychostimulants are pharmacological tool to induce manic-like states in C57BL mice tested in the BPM. However, these studies have not been replicated by other laboratories since the automated BPM apparatus was not commercially available currently. Additionally, the BPM have never been used to test others animal models of CNS disorders. One of the best-characterized animal models of TS is D1CT-7 mice, however, more studies are needed to expand the behavioral profile of these mice and their relations to tic-like responses. This work aimed to develop and validate the BPM 2.0 (based on the original BPM) for studies of mice models of CNS disorders. Locomotor and exploratory activities, risk-taking, anxiety-like behaviors, and ultrasonic vocalizations are possible to be examined in the BPM 2.0. In the experiment 1, the BPM 2.0 was validated to assess mice models of mania. C57BL male mice were treated with DAT (dopamine transporter) inhibitors: Methylphenidate (MPH), GBR 12909 and Modafinil intraperitoneally immediately before one-hour test session. In the experiment 2, it was assessed the Behavioral profile of the D1CT-7 mice in spontaneous behaviour and in acute stress condition. The experiment 1 showed that the BPM 2.0 was able to detect increasing in locomotor activity and risk taking behaviours, and decreasing in the anxiety-like behaviour with all DAT inhibitors treatments. Moreover, the BPM 2.0 was able to detect decreasing in exploratory behaviours (holepokes and rearings) with MPH treatment. In the experiment 2, D1CT-7 mice in spontaneous behaviour, showed increase in locomotor activity and rearings and decrease in holepokes. Lastly, it was detected increase in the repetitive behaviours (rearings and climbings) during acute stress caused by Space Confinement inside the BPM 2.0. The main limitation of the BPM 2.0 was the USV microphone, which recorded noise in the same range frequency of the mice's USV, making difficult the USV analyses. The present study concluded that the BPM 2.0 is a valid apparatus to test mice models of mania and useful to assess the Behavioral profile of other mice models of CNS disorders (e.g., TS).

Key-words: behavioral pattern monitor, BPM, animal models of mania, animal models of Tourette Syndrome, TS, mania.

SUMMARY

1. INTRODUCTION

1.1 Behavioral Pattern Monitor (BPM)

Evaluation of the spontaneous activity of rodents models of CNS disorders have been frequently used to assess the behavioral effects of drugs or other manipulations, either as strict measures of locomotor activity or as measures of other behaviors such emotions or exploration. Most such measures allow concluding only that a given manipulation increases, decreases, or produces no change for quantitative activity. They provide little or no information regarding qualitative changes in the animals' behavior. The use of visual observations to supplement or replace automated measures provides qualitative information, but such measures are time-consuming and often criticized as being subjective and difficult to quantitate. In order to minimize these challenges, Geyer and collaborators developed the Behavioral Pattern Monitor (BPM) for rats, to study the behavioral alterations to a novel environment, which were differentially affected by stimulant drugs. This automated measurement system was designed to assess both the quantity and several aspects of the quality of the behavioral activity of rats and it provided simultaneous monitoring of several different responses as they occur in sequence and time (GEYER *et al.,* 1986).

The BPM developed by Geyer and collaborators, was an automated device equipped with sensors (photo beams) that were capable to detect hyperactivity (number of transitions), exploratory activity (number of holepokes and rearings), and pattern of movements (called spatial d) of rats during about one-hour session test (GEYER *et al.,* 1986).

In 2006, the BPM was developed for mice (RISBROUGH *et al*., 2006), and it demonstrated translational value from dopaminergic animal models of mania to manic patients and reverse translation (YOUNG *et al.,* 2007; PERRY *et al.,* 2009; MINASSIAN *et al.,* 2011). Thus, the BPM for mice has high translational potential for screening novel antimanic-like agents; however, it has never been tested to evaluate other animal models of CNS disorders. In addition, there was only one laboratory in the world conducting experiments in the BPM using mice model of mania (KWIATKOWSKI *et al*, 2019) and currently this apparatus was not available commercially.

1.2 Reproducibility in neuroscience

Reproducible research is the foundation where the advances are built, and the inability to reproduce research findings is probably a long-standing problem (BEGLEY, *et al.,* 2015). This problem has recently been highlighted in editorials and surveys of scientists, with failures in large-scale efforts to replicate findings in the fields of cancer biology and psychology (KWIATKOWSKI *et al.*, 2019). In 2016, the Nature magazine conducted a survey of 1576 researchers and more than 70% of them reported attempting and failing to reproduce another scientist's experiments. Pressure to publish and selective reporting of results were two factors that the majority of respondents (more than 60%) identified as always or often contributing to reproducibility problems (BAKER, 2016).

Determining reproducibility rates in certain scientific fields is underway, and results so far underscore the need for improved reproducibility standards. Methodological differences make it difficult to compare results across laboratories, and further complicate reproducibility issues in the field (KWIATKOWSKI *et al.,* 2019). Among the most common recommendations to increase the reproducibility in the pharmacology area, they cited; blinding of outcome assessment, randomized allocation of animals, power calculation to determine sample size, use of positive and negative controls, determination of dose-response, replication in different models, independent replication (HENDERSON, *et al.,* 2013) and the use of automated devices in the pre clinical research (GEYER *et al.,* 1986).

In the neuroscience field, the problem became more complicated because many psychiatric disorders are complex and challenging to model. These difficulties arise for two main reasons: 1) psychiatric disorders are comprised of diagnostic categories based on mainly subjective heterogeneous symptoms, and 2) the general knowledge of underlying etiology and pathophysiology of these disorders remains limited (KWIATKOWSKI *et al.*, 2019).

As mentioned earlier, there are only one laboratory conducting experiments in the BPM using mice models of mania (KWIATKOWSKI *et al.*, 2019). Given the cross-species relevance of the BPM and the reproducibility issues present in psychiatric research, it is important the realization of new studies using other animal models of mania in the BPM conducted by other laboratories. Such reproducibility would increase the internal validity of using different model of mania-like behavior and contribute to

current global efforts addressing issues of reproducibility in psychiatry research.

1.3 Bipolar Disorder - Mania (BD mania)

BD is a serious CNS disorder that is chronic and debilitating. BD typically consists of both manic and depressive episodes that are normally separated by periods of euthymia (GOODWIN and JAMISON, 2007; AMERICAN PSYCHIATRIC ASSOCIATION 2013). Approximately 45 million people worldwide are affected by BD annually (WORLD HEALTH ORGANIZATION 2018), with direct healthcare costs in the United States of nearly $30 billion and indirect costs of above $120 billion (DISALVER, 2011). The estimated total annual national economic burden of BD was more than $195 billion (BESSONOVA, *et al.,* 2020).

The features of manic episodes include hyper-sexuality, increased reward-seeking behaviors, excessively raised mood, psychomotor agitation, little need for sleep and impulsivity (AMERICAN PSYCHIATRIC ASSOCIATION 2013). Previous studies revealed that recurring manic episodes, might lead to neuroprogression in BD that is clinically reflected as a greater likelihood for subsequent episodes, treatment-resistance, as well as cognitive and functional impairments (SHARMA *et al.,* 2016). About 30% of BD patients attempt suicide, and the associated mortality rate from suicide attempts is high in this population (NOVICK *et al.,* 2010).

1.3.1 Pharmacological treatment of BD mania

Carbamazepine, valproate, lithium, and antipsychotics have been the main treatments for acute mania (CIPRIANI *et al.,* 2011). Although some anticonvulsants (sodium valproate/valproic acid/divalproex and carbamazepine) effectively treat mania, others (topiramate and lamotrigine) did not show a better response than placebo as a monotherapy (CIPRIANI *et al.,* 2011; YILDIZ *et al.,* 2015). However, none of these treatments completely stabilize the behavior or completely restore cognitive functioning in all patients (VAN ENKUIZEN *et al*., 2015a). Thus, more effective treatments with less side effects than the current pharmacotherapies are needed. The development of well validated animal models of mania has an important role for this goal.

1.3.2 Animal models of BD mania induced by psychostimulants

Psychostimulant-induced hyperactivity is the most frequently animal model used to study mania (YOUNG *et al.,* 2010a, 2010b, 2011), with d-amphetamine (AMP)-induced hyperlocomotion in rodents currently being the one most frequently used

(LOGAN and MCCLUNG, 2016; WENDLER *et al.,* 2016). Hyperactivity is observed in 90% of patients during manic episodes and it is frequently used as a primary outcome to assess the validity of animal models of mania (EINAT, 2006; GOODWIN and JAMISON, 2007; YOUNG *et al.,* 2011; MIRANDA *et al.,* 2017, BASTOS, *et al*., 2018, DE MIRANDA, *et al*., 2020). Although hyperactivity is the main readout of manic-like behavior, the inclusion of other mania-like features could refine these models. Thus, exploratory activity, risk-taking behavior, movement patterns, aggressive behavior, distractibility, increase sexual drive, and ultrasonic vocalizations have been proposed to improve animal models of mania (EINAT 2006; 2007; YOUNG *et al.,* 2011; WENDLER *et al.,* 2016; WÖHR, 2021).

1.3.2.1 Mania-like behavior induced by MPH, GBR 12909 and Modafinil in C57BL mice in the BPM.

A dysregulation of dopaminergic homeostasis may contribute to the BD mania profile (VAWTER et al., 2000; MANJI *et al*., 2003). Abnormal expression of the dopamine transporter (DAT) has been implicated in the neuropathophysiology of BD through genetic linkage studies (KELSOE *et al.*1996; GREENWOOD *et al.,* 2001, 2006;), with the functional consequences of an observed DAT mutation leading to reduced cell surface DAT expression (HORSCHITZ *et al.*2005; VAN ENKHUIZEN, *et al.,* 2015b).

The psychostimulants methylphenidate (DAT and norepinephrine transporter (NET) inhibitor), GBR 12909 (potent DAT inhibitor) and Modafinil (weak DAT inhibitor) have been reported as a pharmacological tool to induce hyperactivity, hyper exploration and others manic-like states (e.g. risk-taking behavior, alterations in pattern of movements, etc.) in C57BL mice (YOUNG, *et al.,* 2010a and b; VAN ENKHUIZEN, *et al.,* 2013; SOUZA, *et al.,* 2016; BASTOS, *et al*., 2018; DE MIRANDA, *et al*., 2020). The BPM was validated to evaluate the effects of GBR12909 and amphetamine (DAT and NET inhibitor), administration to C57BL/6 mice on the manic-like behavior (YOUNG, *et al*., 2010b) and modafinil (YOUNG, *et. al*., 2010a, 2011). However, these studies have not been replicated by other laboratories since the automated BPM apparatus was not commercially available currently.

1.4. Tourette Syndrome (TS)

TS(TS) is a neurological disorder characterized by multiple repetitive movements and vocalizations called tics for a duration longer than one year. Tics, as formally defined in the Manual and Statistical Manual of Mental Disorders, 5th edition (DSM-5; APA,

2013), are “sudden, rapid, recurrent, no rhythmic motor movements or vocalizations, generally preceded by urge” (APA, 2013). Since there are several tic syndromes, the DSM-5 defines that TS patients must have multiple motor and vocal tics present at same time with the disorder beginning before age 18 and lasting more than one year. Moreover, the tics are not secondary to a physiological effect of substance or other neurological disorder (APA, 2013).

TS is associated with both simple and complex tics. Simple motor tics are restricted to a small group of muscles in the body and simple vocal tics to small sounds including throat clearing or sniffing. Complex tics involve several muscle groups or purposeful movements including touching objects as well as the use of words or phrases. Complex tics can include echolalia (repeated vocalizations), palilalia (repetition of words or phrases), echopraxia (repeated actions), palipraxia (repeating the last act), self-injurious behaviors, complex vocalizations (eg, animal sounds), coprolalia (swearing), copropraxia (inappropriate touching) etc. (EAPEN and ROBERTSON, 2015).

Tic disorders are often comorbid with other neuropsychiatric disorders, such as obsessive-compulsive disorder (OCD), attention deficit hyperactivity disorder (ADHD), and anxiety (HIRSCHRITT *et al.,* 2015). For these reasons, the characterization of behavioral phenotypes of animal models of tic disorders should always include some tests to capture abnormalities relevant to each of these disorders.

1.4.1 Pharmacological Treatment of TS

Due to the general paucity of more robust evidence, current treatment recommendations are often based on the personal experience of skilled clinicians and the variable regional availability of particular medications (KURLAN, 2014). Alpha-2-adrenergic agonists and antipsychotics are first-line pharmacologic choices. Guanfacine and clonidine are recommended in the United States practice guidelines (PRINGSHEIM *et al.,* 2019). A couple of small trials showed equal efficacy between clonidine and risperidone and another between clonidine and haloperidol. However, there is some evidence that clonidine may only be effective in children with comorbid ADHD. Sedation is by far the most common reason children do not tolerate the alpha-2-adrenergic agonists. Other side effects include orthostatic hypotension, bradycardia, or irritability (JONES, et. al., 2022).

Another treatment currently prescribed is a local injection of botulinum neurotoxin, these injections not only reduce motor tics, but also sensory tics (KURLAN, 2014; PRINGSHEIM *et al.,* 2019).

It should be recognized that cannabinoids appear to have efficacy also for tics in TS, and while this therapy has been mostly done outside the medical arena, the use of these agents is coming into the main stream (PICHLER, *et al.,* 2019).

These therapies, however, have inconsistent efficacy, and often lead to serious side effects that can reduce quality of life and therapeutic compliance of TS patients. In addition to the several adverse effects caused by the medicines prescribed in TS (e.g. tardive dyskinesia, prolongation of Q-T interval, sedation, headache, dizziness, irritability, etc), with optimal pharmacotherapy and others treatments (e.g. behavioral therapy, deep brain stimulation surgery), tics not are improved in all patients, causing problems in daily functioning for patients who do not respond to treatments (ROBERTSON, 2012). Better therapies are needed to improve the quality of life of these patients so, the development of well validate animal models of TS is fundamental.

1.4.2. D1CT-7 mice as a model of TS

D1CT-7 mice express an intracellular form of cholera toxin (CT) that chronically activates stimulatory G-protein (Gs) signal transduction and cAMP synthesis, under the control of the D1 promoter. This specific construct was found to lead to the potentiation of specific populations of D1-positive neurons inside of the somatosensory and piriform cortices, and the amygdala (BORTOLATO and CADEDDU, 2022). These mice also exhibit a variety of compulsive behavioral abnormalities that strongly resembled human cortical-limbic–induced compulsive disorders such as obsessive–compulsive disorder (OCD). These compulsive behaviors included episodes of perseverance or repetition of any of all normal behaviors (e.g. climbing, rearing, grooming, etc.), repetitive nonaggressive biting of siblings during grooming, and repetitive leaping (CAMPBELL *et al.,* 1999).

Furthermore, tic-like behaviors in D1CT-7 mice are exquisitely sensitive to acute environmental stressors. For example, spatial confinement in a cylinder within the home cage evokes a dramatic increase in repetitive behaviors and tic-like responses, as well as pre-pulse inhibition (PPI) deficits (GODAR *et al.,* 2016). The same study documented that the same enhancement can be observed following frustration stress, based on the sudden discontinuation of the administration of palatable food following lever pressing. The responsiveness to these stressors is in alignment with evidence showing that both hypostimulation and anxiety (arguably triggered by spatial confinement) and frustrations are common triggers of tic exacerbation in TS patients (GODAR et al, 2016).

Even with these striking features of face validity, the value of D1CT-7 mice was

questioned due to the artificial nature of their genetic origin (SWERDLOW and SUTHERLAND, 2005). However, further discoveries on the neurobiological substrates of TS have shown that the underpinnings of tic-like behaviors in D1CT-7 mice are in close alignment with the involvement of the somatosensory cortex in TS, bringing these models back into the spotlight as a valuable model of TS. The D1CT-7 model of sensorimotor cortical neuropotentiation suggests that these mice may be valuable to study the biology of TS. In line with this possibility, a study documented sensorimotor alterations in these animals (FOWLER *et al.,* 2017). However, more studies are needed to fully test this possibility by expanding the characterization of the sensory characteristics of these mice and their relations to tic-like responses (BORTOLATO and CADEDDU, 2022).

Considering that TS is a neurological disorder characterized by multiple repetitive movements ,vocalizations and associated comorbidities (OCD, ADHD and Anxiety), evaluating D1CT-7 mice in the BPM can be a useful and practical tool to widen the behavioral profile of these mice in a single test. In addition, these results will be usefull to better understand how these different genetic manipulations can alter the behaviors assessed in the BPM paradigm.

1.5 Ultrasonic Vocalizations (USVs) and mice

Social and communication disorders emerge in multiple psychiatric or neurological disorders, preventing patients as well as their relatives from maintaining a normal social life and contributing to societal well-being. It is therefore crucial to see whether mouse models for psychiatric disorders with social dysfunction also exhibit communication alterations, which can be evaluated through vocal communication. This should contribute to improve face validity of the models, and this can be used as an index of treatment efficacy (GRANON, et. al., 2018).

In rodents, few vocalizations are in the human audible frequency range as most of them are above this range, which are called ultrasonic vocalizations (USVs). Mice produce USVs to convey information related to positive or negative emotional states and to mediate social interactions. Communication is intensely linked to social behavior and for these reasons, USVs study has become a valid assay in behavioral readout and monitoring in this context (GRANON, et. al., 2018).

These USVs were extensively studied in pups as a response to maternal separation (SCATTONI, et. al., 2009) and stressor exposure (BOLLEN *et al.,* 2007) and in adults in response to mating (HOLY & GUO, 2005), encounter of a conspecific

(CHABOUT *et al.,* 2012), and stressful or pleasurable events (BRANCHI, et. al., 1998). Quantitative and qualitative alterations in USVs are reported in numerous mouse models of neurodevelopmental disorders (NDDs) and autism spectrum disorders (ASD). USVs produced from several genetic strains of mice are different, being influenced by genetic background of animal (SUGIMOTO *et al.,* 2011). In particular, variations in number, duration, frequency and other quantitative parameters have been found. In addition, also qualitative differences in calls typology were detected between control and NDDs/ASD mouse models (ROY *et al.,* 2012; HODGES *et al.,* 2017; PREMOLI *et al.,* 2019).

However, there are no published studies evaluating USVs in animal models of TS. Considering that TS is characterized by multiple repetitive movements and vocalizations, to assess USVs in animal models of TS becomes an important tool to expand the behavioral profile of these models.

1.6 First Systel version of the BPM (BPM 1.0)

In 2018, in a partnership between UFPR (Federal University of Parana – Curitiba-Brazil) and a private company (Systel Information Technology, Curitiba, Brazil), the BPM 1.0 was developed based on BPM for mice developed by Young and collaborators (YOUNG *et al.,* 2007; PERRY *et al.,* 2009; YOUNG *et al.,* 2011). Locomotor activity, exploratory behavior, and risk-taking behavior were examined in the BPM. The device consisted of a light- and sound-attenuating wooden outer box with openings in the cover for allow internal ventilation. This box was painted black inside, with an internal white light (300 lux in the center, 90 lux in the four box corners). The internal box was a Plexiglas arena (30.5 cm × 61 cm × 38 cm) with eight holes in the walls (three in each long wall and one in each short wall; 1.25 cm diameter, 1.9 cm above the floor) and three-floor holes with the same size. Each hole contained an infrared beam to detect holepokes. Two sets of photo beams were used to detect activity every 0.1 s. One set of sensors was located 1 cm above the floor (2.5 cm apart) and used to record transitions.

The other set of the 16 sensors (2.0 cm apart) was located 10 cm above the floor and used to record rearings. The position of the mouse was defined across nine unequal regions (four corners, four walls, and center; Fig. 1). The session began by placing the mouse in the bottom left corner of the arena. Each session lasted 60 min. A GoPro7white

camera (WETECH INDUSTRIAL CORP., Ltd., Shenzhen, Guangdong China) recorded all the sessions. The four BPM readout assessments were (1) number of transitions (locomotor activity), (2) number of rearings, which included both on- and off-wall rearings (exploratory behavior), (3) number of holepokes (exploratory behavior), and (4) center entries (number of entries) in the center square of the arena (risk-taking behavior).

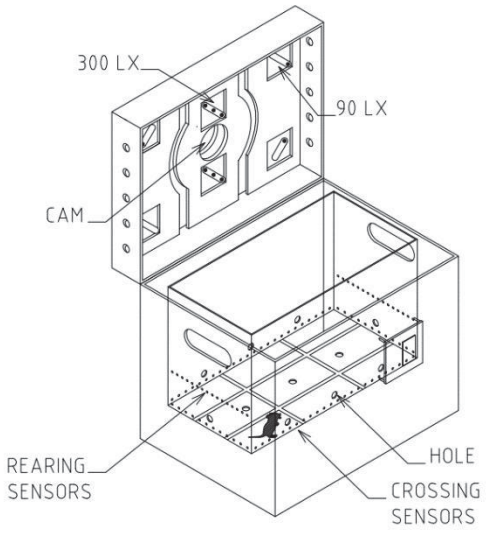

Figure 1. First Systel Version of Behavioral Pattern Monitor (BPM 1.0) for mice (Systel Information Technology/ UFPR, Curitiba, PR, Brazil).

The BPM 1.0 indicated the good positive and negative predictive validity of acute MPH-induced manic-like behaviors of Swiss mice. Although the BPM 1.0 apparatus showed some limitations, it was validated to search new antimanic-like drugs (GONÇALVES, et. al., 2022- Attachment 1)

1.7. Second version of the Systel BPM (BPM 2.0)

In 2020, the BPM 2.0 was developed with the aim of solving some limitations observed in the first version. This new device was developed with important innovations. Locomotor and exploratory activities, risk-taking, anxiety-like behaviors, and ultrasonic vocalizations are possible to be examined in the BPM 2.0. The BPM 2.0 consists of a light- and sound-attenuating outer box. This box is coated with soundproof material and external light with an internal white light (300 lux in the center, 90 lux in the four box

corners). The internal box is a Plexiglas arena (30.5 cm × 61 cm × 38 cm) with eight holes in the walls (three in each long wall and one in each short wall; 1.25 cm diameter, 1.9 cm above the floor) and three-floor holes with the same size. Each hole contains an infrared beam to detect holepokes. Two sets of photobeams is used to detect activity every 0.1 s. One set of sensors is located 1 cm above the floor (2.5cm apart) and used to record transitions. The other set of the 16 sensors (2.0 cm apart) is mobile to adapt of the animal size and used to record rearings. The position of the mouse is defined across nine unequal regions (four corners, four walls, and center (Fig. 2).

The session starts by placing the mouse in the bottom left corner of the arena. Each session last 60 min. All of the sessions will be recorded by an integrated camera and vocalizations will be recorded by ultrasonic vocalization microphone (ULTRAMIC 250K DODOTRONIC). The internal temperature is controlled by a thermometer integrated into the device.

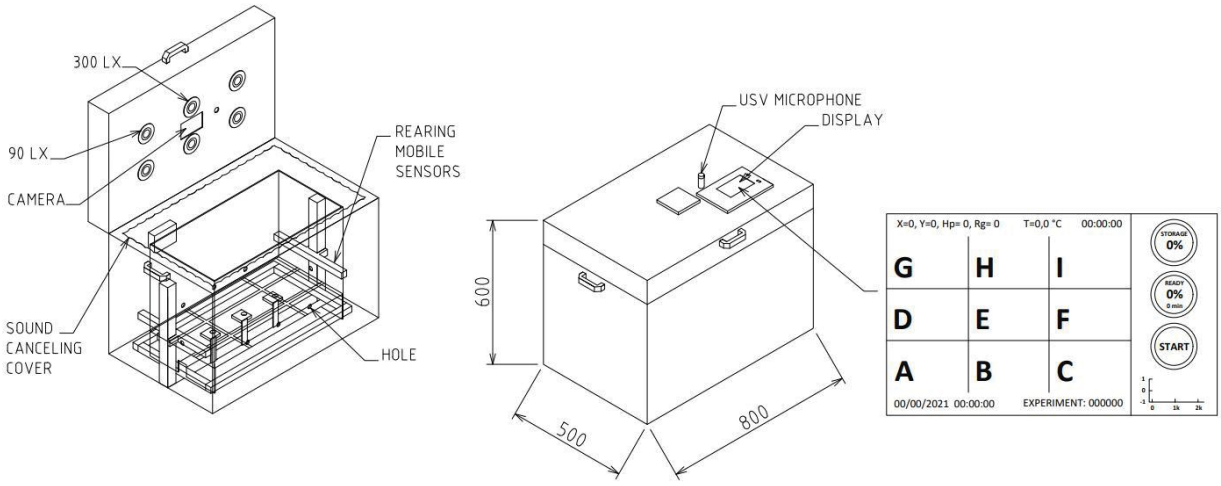

Figure 2. Second Version of Behavioral Pattern Monitor (BPM 2.0) for mice (Systel Information Technology/ UFPR, Curitiba, PR, Brazil). –(Patent Protocol BR 10 2022 020694 5- Attachment 2)

2. JUSTIFICATION

The development and validation of the BPM 2.0 for studies of mice models of CNS disorders can contribute to the realization of the simultaneous monitoring of several different behaviors as they occur in sequence and time in a single test. Numerous behavioral studies using mice can benefit from the development of the BPM 2.0. Moreover, as proposed for SmartCube® (ALEXANDROV *et al.,* 2015), the BPM profile could be behavioral signature for

different class of drugs, helping the researchers to screening new drugs for different neuropsychiatric disorders.

3. OBJECTIVES

The objective of this study was to develop and validate the BPM 2.0 (Systel Information Technology/ UFPR, Curitiba, PR, Brazil) for studies of mice models of CNS disorders.

3.1 SPECIFICS OBJECTIVES

3.1.1 Validation of the BPM 2.0 to assess the mania-like behavior induced by DAT inhibitors (MPH, GBR 12909 and Modafinil) in C57BL mice.

3.1.2 Assessment of the behavior profile in the BPM 2.0 of D1CT-7 mice, a proposed animal model of the Tourette Syndrome.

3.1.3 Assessment the behavioral alterations in the BPM 2.0 of D1CT-7 mice during acute stress induced by Space Confinement (SC).

4. METHODS

4.1 Experiment 1: Differential effects of dopamine transporter inhibitors in the mania-like behavior of the C57BL mice tested in the BPM 2.0.

4.1.1 Animals and environment

Ninety adult male C57BL mice (20-40 g/ approximately 4 months old) were used in this study. The mice were housed in groups of four in polycarbonate cages (29 cm × 9 cm × 12 cm) on a 12 h/12 h light/dark cycle (lights on at 7:00 AM) with controlled room temperature (21°C ± 1°C). Food and water were provided ad libitum. Animals were allowed to acclimate to these conditions for at least 7 days before the study. All of the experimental procedures were performed according to current Brazilian Law for Animal Experimental Ethics and Care (11.794/8, October 2008) and the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The institutional Ethical Board approved the experimental protocol (CEUA-UFPR, protocol no. 1241 – Attachment 3). All efforts were made to reduce the number of mice used and their suffering.

4.1.2 Drugs

MPH (Ritalina®, Novartis, São Paulo, Brazil) were dissolved in 0.9% saline and vaccuum filtered. GBR 12909 (Sigma MO, USA) was dissolved in 0.9% saline and vortexed for 30 s, after this, the solution was heated with heatgun for 2 minutes. Modafinil (Stavigile®, Libbs, Embu das Artes, SP, Brazil) were dissolved in 0.9% saline. All drugs were administered in a volume of 0.1 ml/10 g body weight. MPH (5 and 10

mg/kg), GBR 12909 (9 and 16 mg/kg), Modafinil (32, 64 and 100 mg/kg) and their respective vehicles were administered by intraperitoneal injection. Drug doses were based on previous studies demonstrating increased activity and exploration following MPH (KANAZAWA *et al.,* 2017; ASTH *et al.,* 2020); GBR 12909 (Young, et al., 2010b) and Modafinil (YOUNG, *et al.,* 2011).

4.1.3 Experimental Design

The mice were randomly distributed into experimental groups using Calculator Soup online calculator (https://www.calculatorsoup.com/index.php). Each test drug had 3 or 4 experimental groups (n=10 mice/group). MPH, GBR 12909, Modafinil , and their respective vehicles were administered by intraperitoneal injection immediately before the test. The experiments were performed during the light phase of the light/dark cycle.

4.1.4 Behavioral Tests

The session started by placing the mouse in the bottom left corner of the BPM 2.0 (described above). Locomotor activity was measured by the number of squares crossed (crossings); exploratory activity was scored by the number of holepokes and rearing; risk-taking behavior was calculated by the number of crossings and time (seconds) in the center area (E quadrant). Anxiety-like behavior was scored by the percentage of the time that the animal remains in the quadrants that receive more intensity of lights (300 lux- HEB quadrants) compared with those receive less intensity of lights (90 lux-GDAIFC quadrants) (see fig 4). Each session produced four files: Video, Audio, Report and Excel data (avi, wav, pdf and excel respectively). The video files was analyzed, when necessary to observe stereotyped behaviors. The audio files was not analyzed in this experiment.

The report file reported all scores in one test hour, and the excel file were used to score in specifics period of time (in the present study, each five minutes) of the test, which permit to evaluate changes in behaviors throughout the test in the BPM (detecting, for example, habituation).

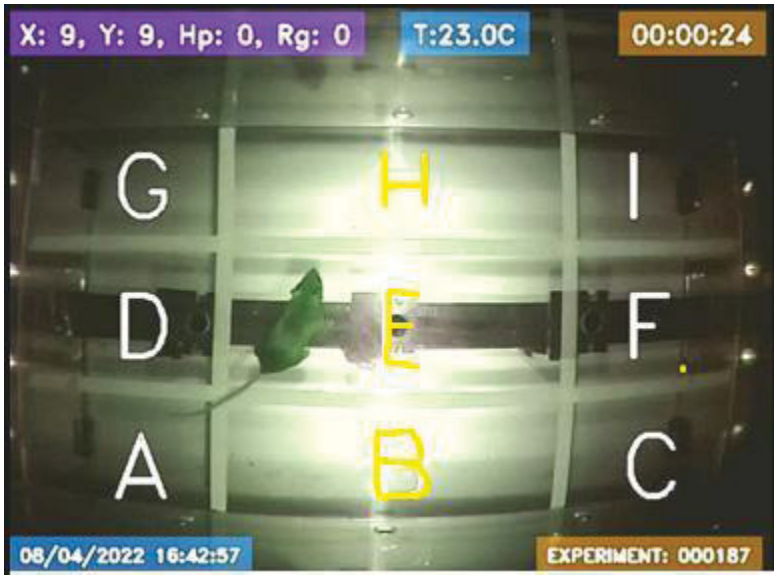

Figure 4. Different light intensities in the BPM 2.0 for mice (Systel Information Technology/ UFPR, Curitiba, PR, Brazil). HEB quadrants (300 Lux), GDAIFC quadrants (90 Lux)

4.1.5 Statistical analysis

The effect of DAT inhibitors was evaluated using one-way ANOVA, followed, if necessary, by Newman Keuls post-hoc test to compare treatment groups with appropriate control group. In all analysis, $p < 0.05$ was considered statistically significant.

4.2 Experiment 2: Evaluation of the behavioral profile of the animal model of TS(D1CT-7) mice in the BPM 2.0.

4.2.1 Animals and environment

Thirty-nine (19 D1CT-7 and 20 WT littermates) adult (3- to 4-month-old, weighing 20-30 g) males (20) and females (19), from University of Utah animal facilities were used. Mice experimentally naïve male and female Balb/c mice were purchased by Jackson Labs (Bar Harbor, ME, USA) and bred and genotyped as reported by Campbell (Campbell *et al.,* 1999). As the pattern of inheritance of D1CT-7 mice is autosomal dominant, WT females were breeded with heterozygous D1CT-7 males; this breeding scheme were selected to standardize maternal behavior. WT males and females, and D1CT-7 females were housed in group (2-4) cages with ad libitum access to food and water. D1CT-7 males were housed individually because the aggressive behavior when they are housed in group.

The room were maintained at 22°C, on a 12:12 hours light/dark cycle from 08:00 to 20:00 hours. Animals were tested during their light cycle between 12:00 and 16:00 hours to minimize any potential circadian effects. All experimental procedures were in compliance with the National Institute of Health Guidelines and approved by the Institutional Animal Use Committees of the University of Utah (IACUC protocol number 00001425).

4.2.2 Experimental Design

4.2.2.1 Experiment 2.1: **Evaluation of spontaneous locomotor and exploratory activities, risk taking behavior, anxiety-like behavior and USV.**

The same behavioral tests used in the item 4.1.4 were performed here. The video files were analyzed if necessary to observe stereotyped behaviors, grooming or tic-like behaviors during possible USV. The audio files were analyzed by the Avisoft SASLab Pro software (version 4.34; Avisoft Bioacoustics) and the number of calls were counted. The report file was used to report all scores in one test hour, and the excel file were be used to score in specifics period (each five minutes) of the test.

4.2.2.2 Experiment 2.2: Evaluation of repetitive behaviors (rearings and climbings) during acute stress caused by Space Confinement (SC) inside the BPM 2.0.

This experiment was realized to test if the BPM was able to detect the increase in repetitive behaviors (rearings and climbings) caused by acute stress (spatial confinement) inside the BPM 2.0 (GODAR et. al., 2016). Four weeks later, the same males used in experiment 4.2.1 were used here. The session started by confining the mouse inside a clear, bottomless plexiglas cylinder (10 cm in diameter × 30 cm in height) (see fig 5), that is placed in the H quadrant of the BPM. H quadrant was chosen because it does not have hole in the bottom to stimulate holepoke activity, and it receives 300 lux of lights (to be more stressful to the animal). Each session lasted 20 min (Godar, et. al., 2016). It was evaluated only the number of rearings and climbings and these results were compared with the number of rearing and climbings during the first 20 min of the experiment 2.1.

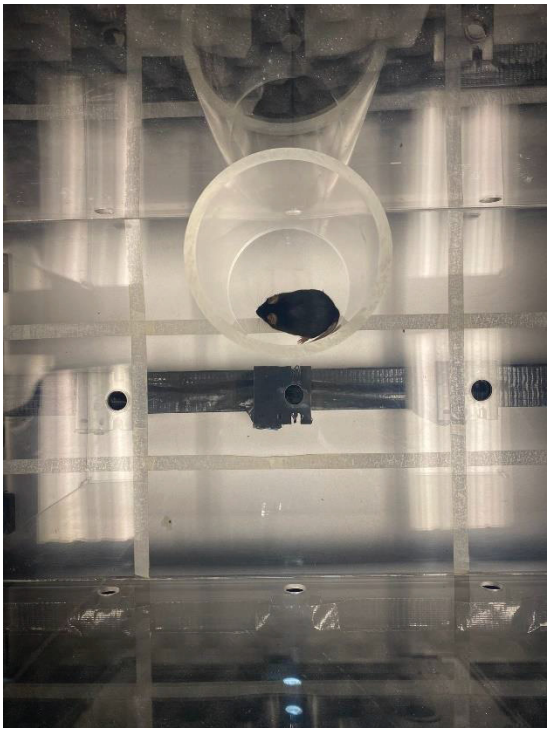

Figure 5. Space confinement BPM 2.0 for mice (Systel Information Technology/ UFPR, Curitiba, PR, Brazil).

4.2.3 Statistical analysis

The data were expressed as mean +/- SEM. Data from experiments 2.1 were analyzed using unpaired t test to compare D1CT-7 mice with their wild type. Males and females were analyzed together and separately. For USV measurement, the number of calls was analyzed using unpaired t test to compare D1CT-7 mice with WT. Data from experiments 2.2 (D1CT-7 mice during acute stress) were performed with two-way ANOVA (with genotype, and environmental condition as factors) followed by Tukey's test for post hoc comparisons. In all tests, values of $p<0,05$ were considered statistically significant.

5. RESULTS

5.1 Experiment 1: Differential effects of dopamine transporter inhibitors in the mania-like behavior in C57BL mice tested in the BPM 2.0.

5.1.1 Effects on acute GBR 12909 on BPM 2.0 behaviors

5.1.1.1 Locomotor Activity

The ANOVA revealed a significant difference among groups of GBR 12909 treatment on the number of total crossings at 15 min ($F_{2.,25}$= 3.75, p< 0.05), 20 min ($F_{2.25}$= 5.97, p<0.01), 25 min ($F_{2.25}$ =8.53, p<0.01), 30 min ($F_{2.25}$= 11.32, p<0.01) and 60 min ($F_{2.25}$= 20.90, p<0.001) of the experiment. GBR 12909 16 mg/kg increased locomotor activity at 15, 20, 25, 30 and 60 min (all p<0.05 compared to control group).The dose of 9mg/kg did not show difference on the number of crossings in any time (Fig. 8A). Therefore, only the highest dose of the GBR 12909 (16 mg/kg) increased the locomotor activity.

5.1.1.2 Anxiety-like Behavior

The ANOVA revealed a significant difference among groups of GBR 12909 treatment on the percentage of time spent in the 300 lux area (HEB squares) area at 25 min ($F_{2.25}$= 4.11, p< 0.05), 30 min ($F_{2.25}$= 4.96, p<0,05) and 60 min ($F_{2.25}$ =5.37, p< 0.05). GBR12909 16mg/kg increased the % time spent in bright area of BPM at 25, 30, and 60 min of the experiment (all p<0.05 compared with control group). The dose of 9mg/kg did not show difference on the percentage of time spent in the 300 lux area in any time (Fig. 8B). Therefore, only the highest dose of the GBR 12909 (16 mg/kg) decreased the anxiety-like behavior.

5.1.1.3 Exploratory Behavior

The ANOVA did not reveal significant difference among groups of GBR 12909 treatment in the number of holepokes (Fig. 8C) and rearings (Fig 8D) compared with control group in any time. Consequently, the GBR 12909 did not cause alterations in the exploratory behavior.

5.1.1.4 Risk-taking Behavior

The ANOVA revealed a significant difference among groups of GBR 12909 treatment on the center crossings (E square) at 20 min ($F_{2.25}$= 4.48, p<0.05), 25 min ($F_{2.25}$= 5.62, p<0.05) and 30 min ($F_{2.25}$ =3.67, p<0.03) and 60 min ($F_{2.25}$=6.78, p<0.01) . GBR 12909 16mg/kg increased center crossings at 20, 25, 30 and 60 min of the experiment (all p<0,05). The dose of 9mg/kg did not show difference on the center crossings in any time (Fig. 8E). Both doses of the GBR 12909 did not show difference

in the time in the center (E square) in any time (Fig. 8D). Therefore, only the highest dose of the GBR 12909 (16 mg/kg) increased center crossing and therefore increased the risk-taking behavior.

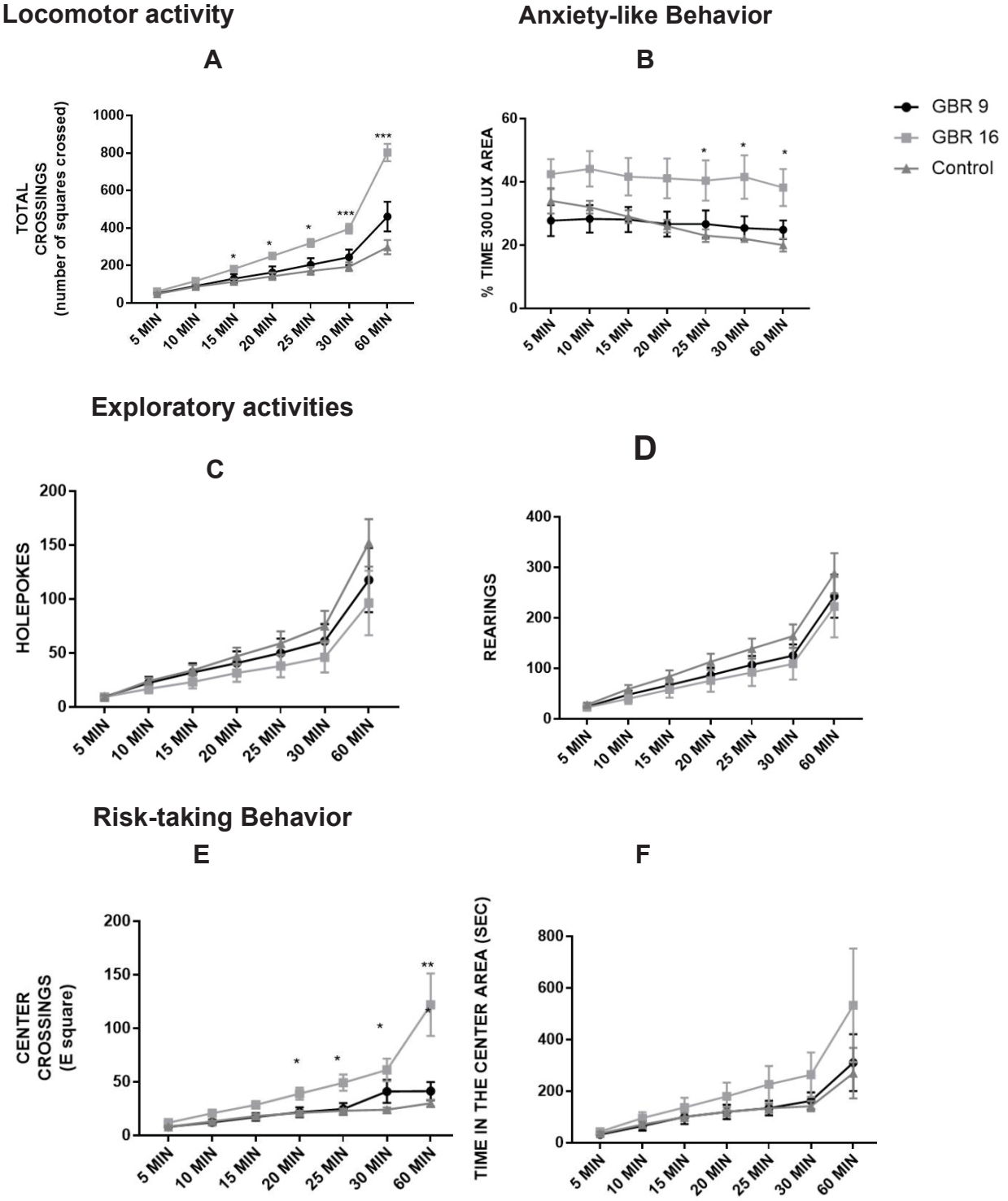

Fig. 8. Effects of acute administration of GBR 12909 (9 and 16 mg/kg) via i.p. in C57BL mice in the BPM 2.0. (A) number of total crossings (all squares). (B) % of time in the 300 lux area (HEB squares). (C) number of holepokes. (D) number of rearings. (E) number of crossings in the center area (E square). (F) Time in the center area (E square) in seconds. The data are expressed as mean +SD and represent cumulative numbers. N=10 mice/group. *p < 0,05, **p < 0,01, *** p < 0,001 comparing with control group.

5.1.2 Effects on acute Modafinil on BPM 2.0 behaviors

5.1.2.1 Locomotor Activity

The ANOVA revealed a significant difference among groups of Modafinil treatment on the number of crossings at 20 min ($F_{2.25}$= 3.57, p< 0.05), 25 min ($F_{3.26}$= 4.35, p<0.05), 30 min ($F_{2.25}$ =5.16, p<0.01) and 60 min ($F_{2.25}$= 7.80, p<0.001) of the experiment. Modafinil 32 mg/kg did not affect the number of crossings. However, Modafinil 64 mg/kg increased the number of crossing at 60 min, while modafinil 100 mg/kg increased at 20, 25, 30 and 60 min of the experiment (all p<0.05 compared with control group) (Fig. 9A). Therefore, Modafinil 64 and 100 mg/kg increased the locomotor activity.

5.1.2.2 Anxiety-like Behavior

The ANOVA revealed a significant difference among groups of Modafinil treatment on the percentage of time in the 300 lux area (HEB squares) at 20 min ($F_{2.25}$= 3.97, p< 0.05), 25 min ($F_{2.25}$= 4.81, p< 0.05), 30 min ($F_{2.25}$= 5.12, p< 0.01) and 60 min ($F_{2.25}$ = 8.29, p< 0.001) of the experiment. The dose of 32 mg/kg did not show difference on the percentage of time in the 300 lux area in any time. Modafinil 64 mg/kg increased the percentage of time in the 300 lux area only at 60 min of the experiment while 100 mg/kg increased at 20, 25, 30 and 60 min of the experiment (all p<0,05 compared with control group) (Fig. 9B). Therefore, Modafinil (64 and 100mg/kg) decreased the anxiety-like behavior.

5.1.2.3 Exploratory Behavior

The ANOVA did not show significant difference among groups of Modafinil treatment in the number of holepokes (Fig. 9C) and rearings (Fig 9D) compared with control group in any time. Consequently, Modafinil treatment did not cause alterations in the exploratory behaviors.

5.1.2.4 Risk-taking Behavior

The ANOVA revealed a significant difference among groups of Modafinil treatment on the center crossings (E square) at 30 min ($F_{2.25}$ =3.51, p<0.05) and 60 min ($F_{2.25}$=4.02, p<0.05) of the experiment. The doses of 32 and 64 mg/kg did not show difference on the center crossings in any time. However, Modafinil 100 mg/kg increased the center crossings at 30 and 60 min of the experiment (all p< 0.05 compared with control group) (Fig. 9E). Modafinil treatment did not show difference in the time in the center (E square) in any time (Fig. 9F). Therefore, only the highest dose of the Modafinil (100mg/kg) increased center crossing and consequently increased the risk-taking behavior.

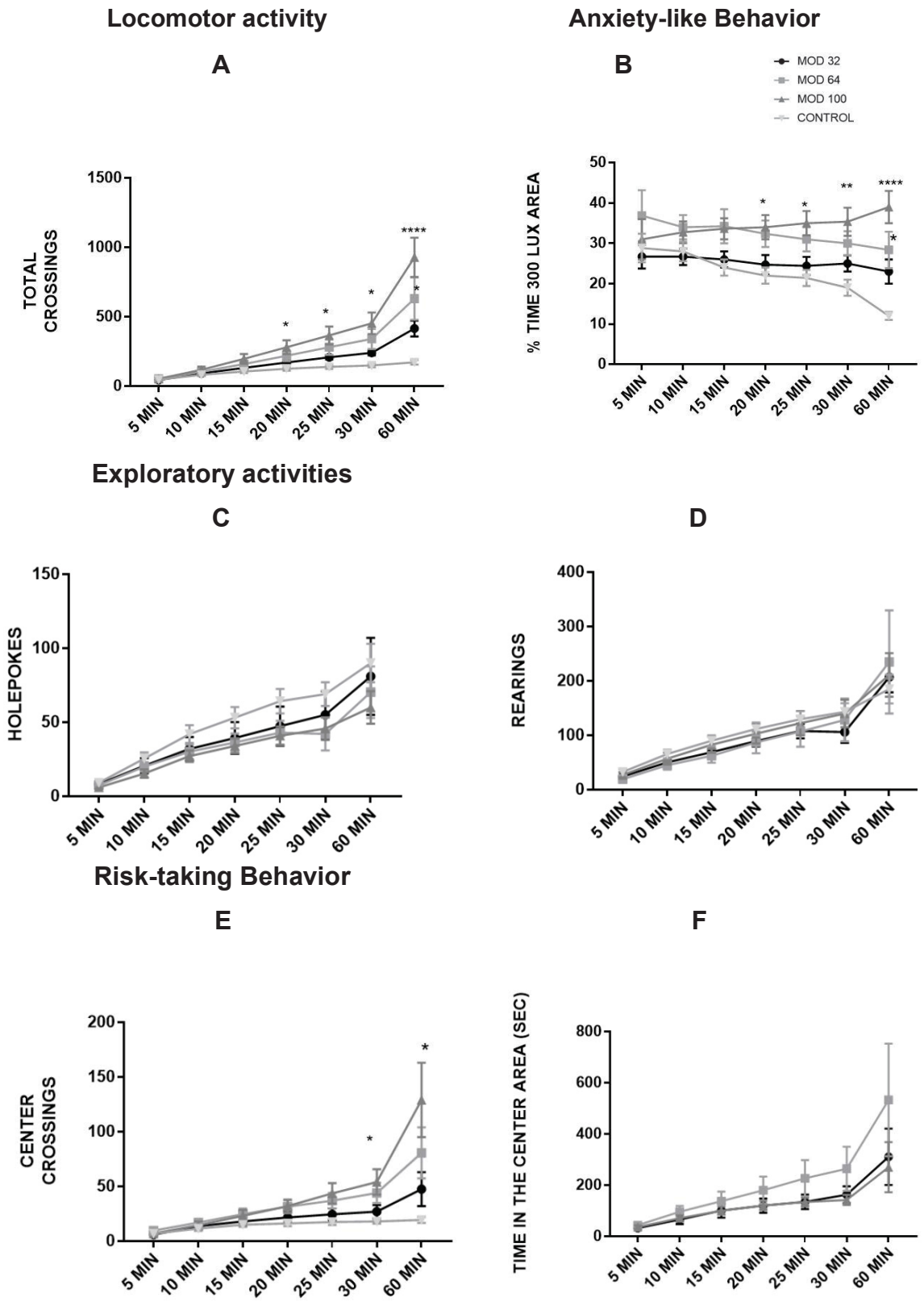

Fig. 9. Effects of acute administration of modafinil (32, 64 and 100 mg/kg) via i.p. in C57BL m the BPM 2.0. (A) number of total crossings (all squares). (B) % of time in the 300 lux area(HE squares). (C) number of holepokes. (D) number of rearings. (E) number of crossings in the c area (E square). (F) Time in the center area (E square) in seconds. The data are expressed mean +SD and represent cumulative numbers. N=10 mice/group. *p < 0.05, **p < 0.01, **** p 0.0001 comparing with control group.

5.1.3 Effects on acute MPH on BPM 2.0 behaviors

5.1.3.1 Locomotor Activity

The ANOVA revealed a significant difference among groups of MPH treatment on the number of total crossings at 5 min ($F_{2.26}$ = 4.1, p< 0.05), 10 min ($F_{2.26}$ = 6.75 , p< 0.01), 15 min ($F_{2.26}$ = 10.65, p< 0.01), 20 min ($F_{2.26}$ = 12.88 , p< 0,001), 25 min ($F_{2.26}$ = 14.62 , p< 0.0001), 30 min ($F_{2.26}$ = 15,18 , p< 0.0001) and 60 min ($F_{2.26}$ = 17.74 , p< 0.0001) of the experiment. MPH 5 and 10 mg/kg increased the number of crossing at 5, 10, 15, 20, 25, 30 and 60 min of the experiment (all p<0.05 compared with control group) (Fig 10A). Therefore, MPH 5 and 10 mg/kg increased the locomotor activity.

5.1.3.2 Anxiety-like Behavior

The ANOVA revealed a significant difference among groups of MPH treatment on the percentage of time in the 300 lux area (HEB squares) at 25 min ($F_{2.26}$= 4.43, p< 0.05), 30 min ($F_{2.26}$ = 6.51, p< 0.01) and 60 min ($F_{2.26}$ = 4.84, p< 0.05) of the experiment. The dose of 5 mg/kg did not show difference on the percentage of time in the 300 lux area in any time, but, the dose of 10mg/kg increased the percentage of time in the 300 lux area at 25, 30 and 60 min of the experiment (all p<0.05 compared with control group) (Fig. 10B). Therefore, MPH 10 mg/kg decreased the anxiety-like behavior.

5.1.3.3 Exploratory Behavior

The ANOVA revealed a significant difference among groups of MPH treatment on the holepokes at 10 min ($F_{2.26}$= 8.0, p< 0,001), 15 min ($F_{2.26}$ = 9.89 , p< 0,001), 20 ($F_{2.26}$ = 8.31 , p< 0,01), 25 min ($F_{2.26}$ = 7.66, p< 0,01), 30 min ($F_{2.26}$ = 7.52 , p< 0,01) and 60 min ($F_{2.26}$ = 7.87, p< 0,01) of the experiment. MPH 5 mg/kg decreased holepokes only at 60 min of the experiment, while MPH 10 mg/kg decreased holepokes at 10, 15, 20, 25, 30 and 60 min of the experiment (all p<0,05 compared with control group) (Fig 10C). Regarding to the rearings, the ANOVA revealed a significant difference among groups of MPH treatment on rearings at 10 min ($F_{2.26}$ = 4.41, p< 0,05), 15 min ($F_{2.26}$ = 3.75 , p< 0,01), 20 min ($F_{2.26}$ = 4.8 , p< 0,05), 25 min ($F_{2.26}$ = 4.91, p< 0,05) and 30 min ($F_{2.26}$ = 4.91, p< 0,05) of the experiment. MPH 5 mg/kg did not affected the rearings, however, MPH 10 mg/kg decreased rearings at 10, 15, 20, 25 and 30 min of the experiment (all p<0,05 compared with control group) (Fig 10D). These results showed that MPH treatment decreased the exploratory behavior in the first 30 min of the test.

5.1.3.4 Risk-taking Behavior

The ANOVA revealed a significant difference among groups of MPH treatment on the center crossings (E square) at 30 min ($F_{2.26} = 4.0$ $p < 0.05$) and 60 min ($F_{2,26} = 6,1$, $p < 0,01$) of the experiment. MPH 5 mg/kg increased center crossings at 60 min and MPH 10 mg/kg increased center crossings at 30 and 60 min of the experiment (all $p<0,05$ compared with control group) (Fig. 10E). MPH did not show difference in the time in the center (E square) in any time (Fig. 10F). Therefore, MPH 5 and 10 mg/kg increased center crossing and consequently increased the risk-taking behavior.

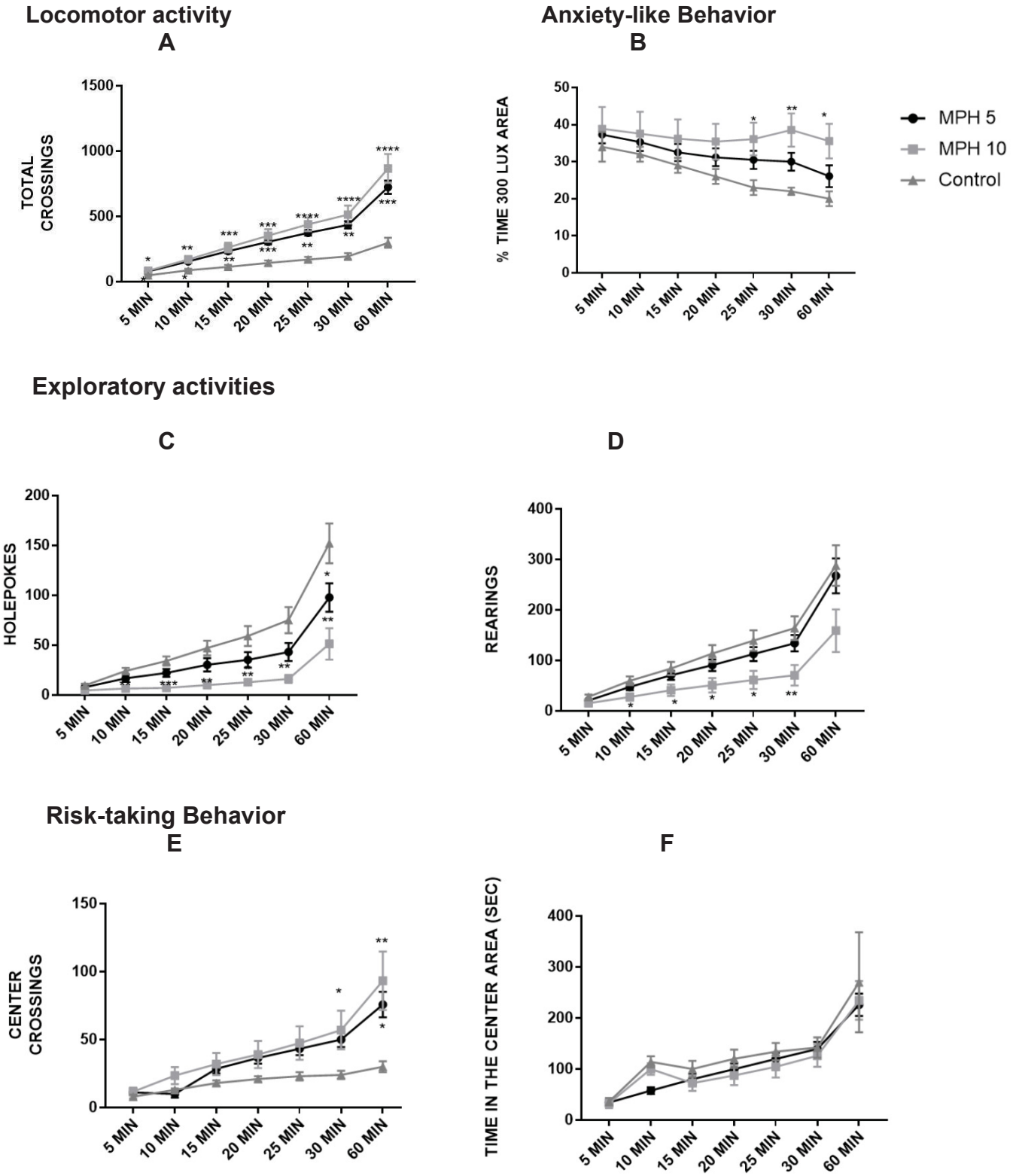

Fig. 10. Effects of acute administration of MPH (5 and 10 mg/kg) via i.p. in C57BL mice in the BPM 2.0. (A) number of total crossings (all squares). (B) % of time in the 300-lux area (HEB squares). (C) number of holepokes. (D) number of rearings. (E) number of crossings in the center area (E square). (F) Time in the center area (E square) in seconds. The data are expressed as mean +SD and represent cumulative numbers. N=10 mice/group. *p < 0.05, **p < 0.01, *** p < 0,001 and **** p < 0.0001 comparing with control group.

5.2. Experiment 2: Evaluation of the behavioral profile of animal model of the Tourete Syndrome (D1CT-7 mice) in the BPM 2.0.

5.2.1. Experiment 2.1 Evaluation of spontaneous locomotor and exploratory activities, risk taking behavior, anxiety-like behavior and USV.

5.2.1.1 Males

5.2.1.1.1 Locomotor Activity

Unpaired t test revealed that males D1CT-7 did more total crossings at 10 min (t_{18}=2.9, p<0,01), 15 min (t_{18}=3.1, p<0,01), 20 min (t_{18}=3.2, p<0,01), 25 min (t_{18}=3.3, p<0,01), 30 min (t_{18}=3.3, p<0,01) and 60 min (t_{18}=2.8, p<0,05), of the experiment compared with the wild type group (Fig. 11A). Therefore, D1CT-7 male mice had increased locomotor activity compared with wild type.

5.2.1.1.2 Anxiety-like Behavior

D1CT-7 males, did not show differences in the % of time in the 300 lux area in any time compared with the wild type group (Fig 11B). Therefore, D1CT-7 males did not show differences in the anxiety-like behavior compared with wild type group.

5.2.1.1.3 Exploratory Behavior

Unpaired t test revealed that males D1CT-7 did less holepokes at 5 min (t_{18}=5.3, p< 0,0001), 10 min ((t_{18}= 4.8, p< 0,001), 15 min (t_{18}=3.0, p<0,01), 20 min (t_{18}=3.1, p<0,01) and 25 min (t_{18}=2.2, p< 0, 05) of the experiment compared with the wild type group (Fig. 11C). In addition, unpaired t test revealed that males D1CT-7 did more rearings at 15 min (t_{18}=2.7, p< 0,05), 20 min (t_{18}=3.0, p<0,01), 25 min (t_{18}=2.9, p<0,01), 30 min (t= 2.9, p< 0,01) and at 60 min (t_{18}=2.9, p< 0, 05) of the test compared with the wild type males group (Fig. 11D). Therefore, D1CT-7 males had more exploratory behavior (rearings) compared with the wild type group, but they did less holepokes.

5.2.1.1.4 Risk-taking Behavior

D1CT-7 males, did not show differences in the number of center crossings (Fig 11E) or in time in center area (Fig 11D) in any time compared with the wild type group. Therefore, D1CT-7 males did not show differences in risk taking behavior compared with the wild type group.

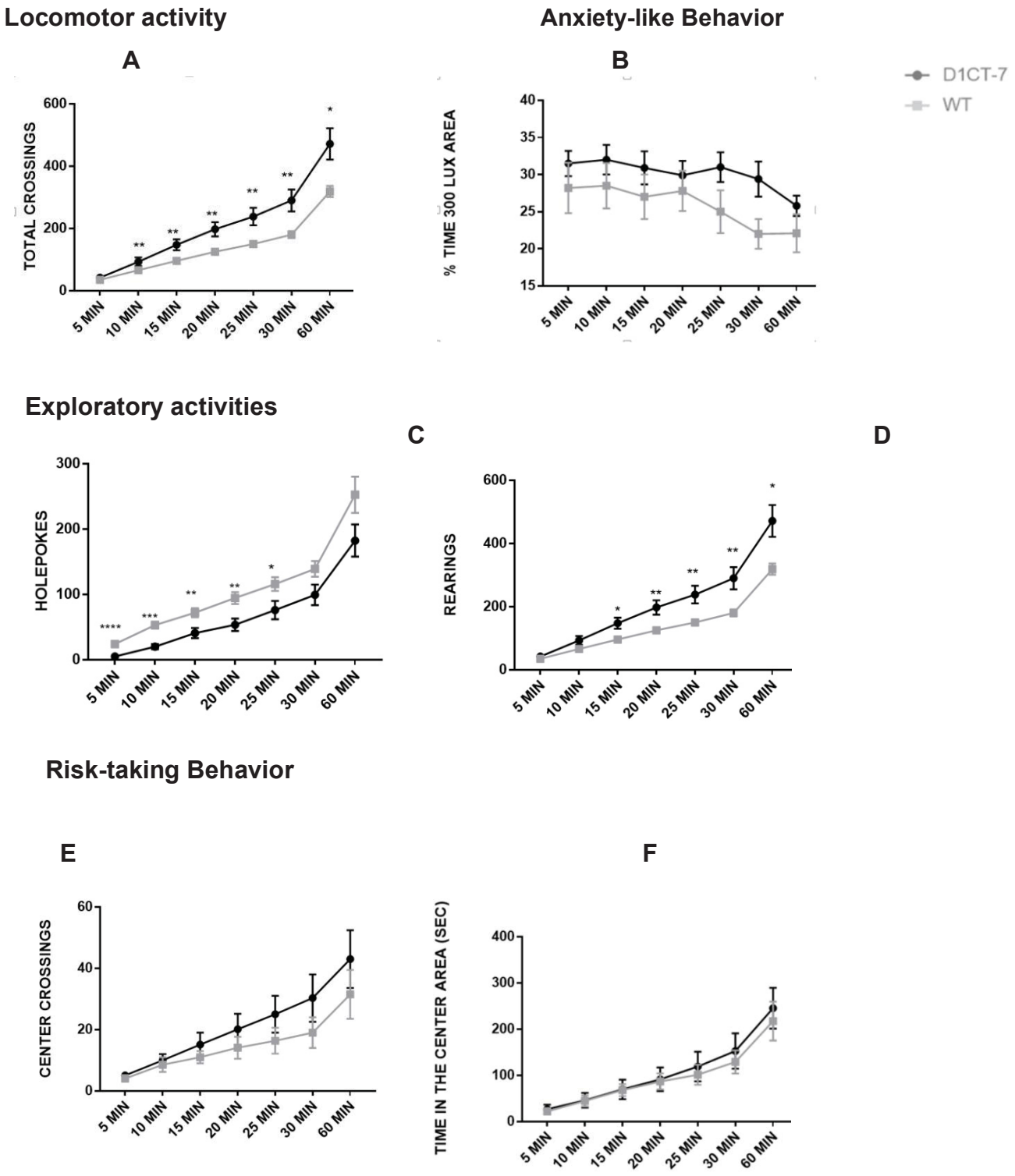

Fig. 11. Behavioral profile of the D1CT-7 male mice in the BPM. (A) number of total crossings (all squares). (B) % of time in the 300 lux area (HEB squares). (C) number of holepokes. (D) number of rearings. (E) number of crossings in the center area (E square). (F) Time in the center area (E square) in seconds. The data are expressed as mean ±SD and represent cumulative numbers. N=10 mice/group. *p < 0,05, **p < 0,01, *** p < 0,001, ****p< 0,0001, comparing with control group.

5.2.1.2 Females

5.2.1.2.1 Locomotor Activity

D1CT-7 females, did not show significant differences in the total crossings in any time compared with the wild type group (Fig. 12A). Therefore, D1CT-7 mice female did not have increased locomotor activity compared with the wild type group.

5.2.1.2.2 Anxiety-like Behavior

Unpaired t test revealed that D1CT-7 females showed differences in the % of time in the 300 lux area at 15 min (t_{17}=2.1, p<0,05) and 20 min (t_{17}=2.4, p<0,05) of the experiment compared with their wild type group (Fig 12B). Therefore, D1CT-7 females had less anxiety-like behavior in the middle of the test compared with the wild type group at 15 and 20 min of the experiment.

5.2.1.2.3 Exploratory Behavior

Unpaired t test revealed that females D1CT-7 did less holepokes at 5 min (t_{17}=5.1, p< 0, 0001), 10 min min (t_{17}=3.7, p< 0, 001) , 15 min min (t_{17}=3.5, p< 0, 001), 20 min min (t_{17}=3.3, p< 0,01), 25 min (t_{17}=4.0, p<0,001), 30 min (t_{17}=3.7, p< 0, 001)and 60 min(t_{17}=2.5, p< 0, 05) p< 0,05), compared with the wild type group (Fig. 12C). On the other hand, D1CT-7 females did not show significant differences in rearings in any time compared with wild type group (Fig. 12D). Therefore, D1CT-7 females had less exploratory behavior (holepokes) compared with the wild type group.

5.2.1.2.4 Risk-taking Behavior

D1CT-7 females did not show differences in the number of center crossings (Fig 12E) or in time in center area (Fig 12D) in any time compared with wild type group. Therefore, D1CT-7 females did not show differences in risk taking behavior compared with wild type group.

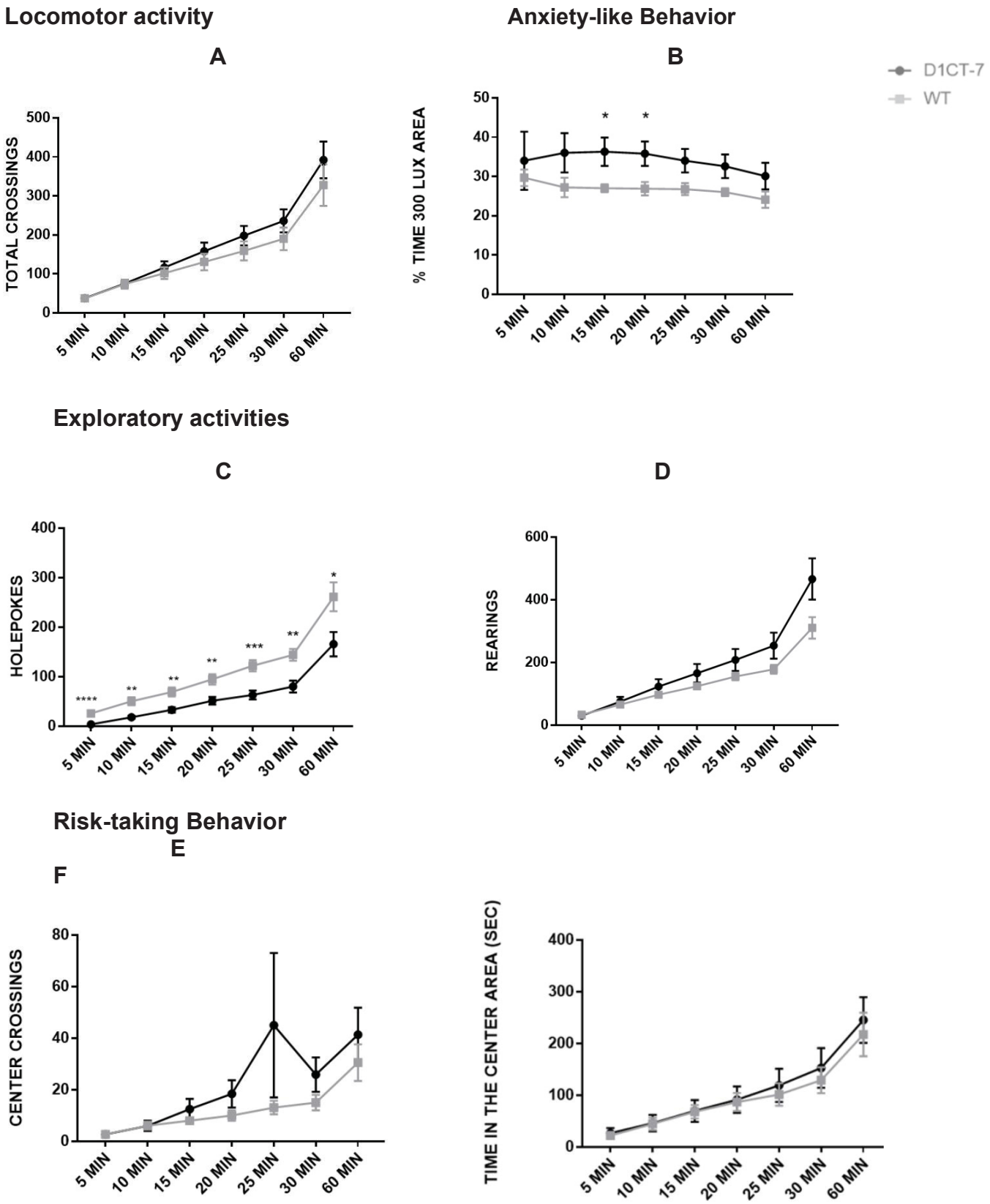

Fig. 12. Behavioral profile of the D1CT-7 female mice in the BPM. (A) number of total crossings (all squares). (B) % of time in the 300 lux area (HEB squares). (C) number of holepokes. (D) number of rearings. (E) number of crossings in the center area (E square). (F) Time in the center area (E square) in seconds. The data are expressed as mean +SD and represent cumulative numbers. N=10 mice/group. *p < 0,05, **p < 0,01, *** p < 0,001, ****p< 0,0001, comparing with control group

5.2.1.3 Males and Females

5.2.1.3.1 Locomotor Activity

Unpaired t test revealed that males and females D1CT-7 did more total crossings at 10 min (t_{37}=2.0, p< 0,05), 15 min (t_{37}=2.6, p <0.05), 20 min (t_{37}=2.8, p< 0,01), 25 min (t_{37}=3.1, p< 0,01), 30 min (t_{37}=3.0, p< 0,01) and 60 min (t_{37}=2.6, p<0.05) of the experiment compared with the wild type group (Fig. 13A). Therefore, D1CT-7 mice males and females have increased locomotor activity compared with the wild type group.

5.2.1.3.2 Anxiety-like Behavior

Unpaired t test revealed that D1CT-7 males and females showed differences in the % of time in the 300 lux area at 15 min (t_{37}=2.3, p< 0,05), 20 min (t_{37}=2.2, p< 0,05), 25 min (t_{37}=2.7, p< 0,05) and 30 min (t_{37}=2.8, p< 0,01) of the experiment compared with the wild type group (Fig 13B). Therefore, D1CT-7 males and females had less anxiety-like behavior compared with the wild type group in the first 30 min of the experiment.

5.2.1.3.3 Exploratory Behavior

Unpaired t test revealed that D1CT-7 males and females, did less holepokes at 5 min (t_{37}=7.5, p< 0,0001), 10 min (t_{37}=6.1, p< 0,0001), 15 min (t_{37}=4.7, p< 0,001), 20 min (t_{37}=4.7, p< 0,0001), 25 min (t_{37}=4.3, p< 0,001), 30 min (t_{37}=3.9, p< 0,001) and 60 min (t_{37}=3.2, p< 0,01) of the experiment compared with the wild type group (Fig. 13C). On the other hand, D1CT-7 males and females did more rearings at 15 min (t_{37}=2.5, p< 0,05), 20 min (t_{37}=2.8, p< 0,01), 25 min (t_{37}=2.9, p< 0,01), 30 min (t_{37}=3.2, p< 0,01) and 60 min (t_{37}=3.4, p< 0,01) of the experiment compared with the wild type group (Fig. 13D). Therefore, D1CT-7 males and females had more exploratory behavior (rearings), however, they did less holepokes compared with the wild type group.

5.2.1.3.4 Risk-taking Behavior

D1CT-7 males and females did not show differences in the number of center crossings (Fig 13E) or in time in center area (Fig 13D) in any time compared with the wild type group. Therefore, D1CT-7 males and females did not show differences in risk taking behavior compared with the wild type group.

5.2.1.4 Ultrasonic Vocalizations (USV) during

Low Frequence USV (below 40 Khz) was found in the first 30 min in D1CT-7 and WT male and females (Fig 14). No High Frequency USV (above 40) and long duration calls were found. An important limitation of the BPM 2.0 found, was that the USV microphone, that also recorded the noise from the BPM in the same frequency range of

the mice's USV, so que number of calls were not evaluated.

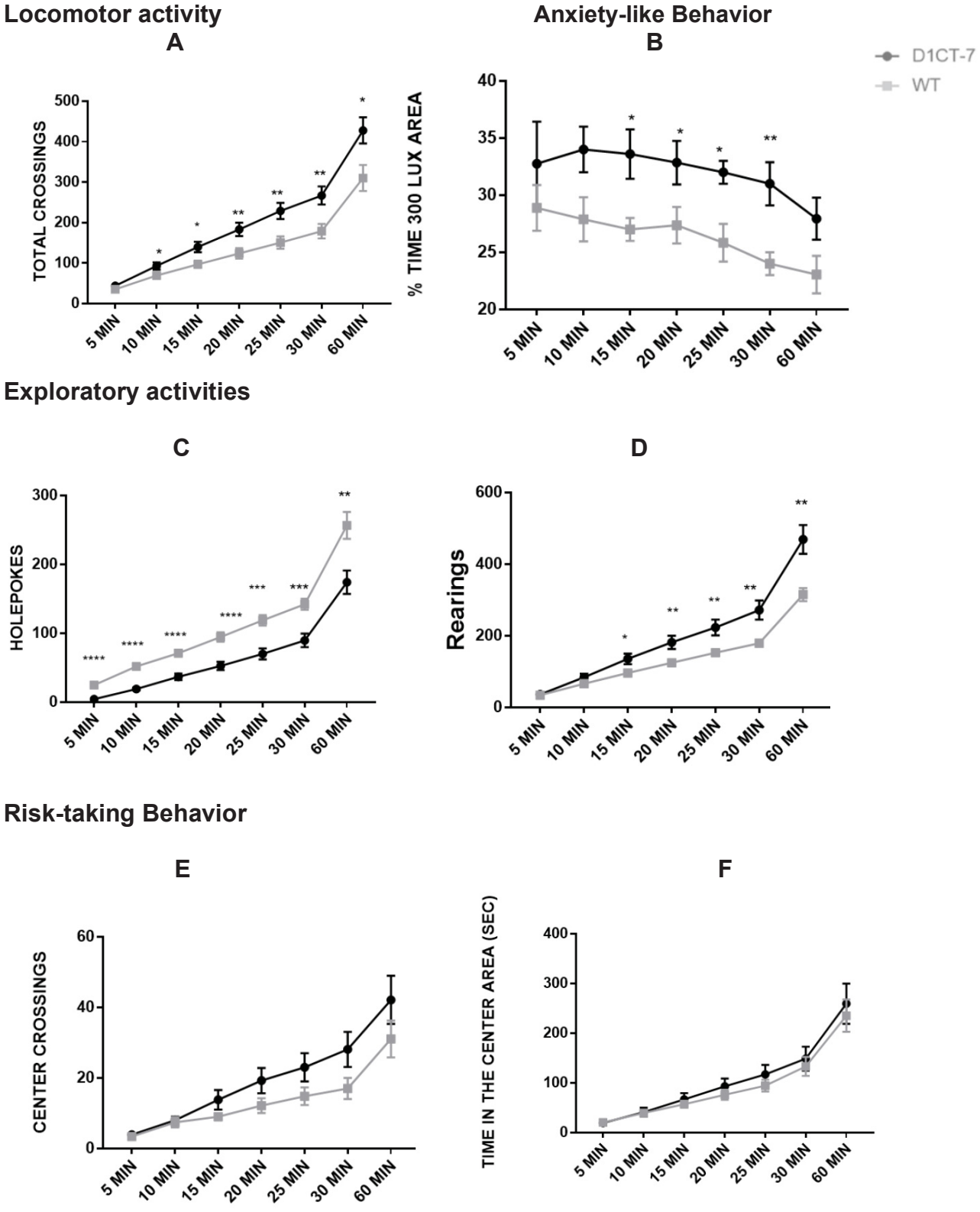

Fig. 13. Behavioral profile of the D1CT-7 males and females mice in the BPM. (A) number of total crossings (all squares). (B) % of time in the 300 lux area (HEB squares). (C) number of holepokes. (D) number of rearings. (E) number of crossings in the center area (E square). (F) Time in the center area (E square) in seconds. The data are expressed as mean +SD. N=10 mice/group. *p < 0,05, **p < 0,01, *** p < 0,001, ****p< 0,0001, comparing with control group

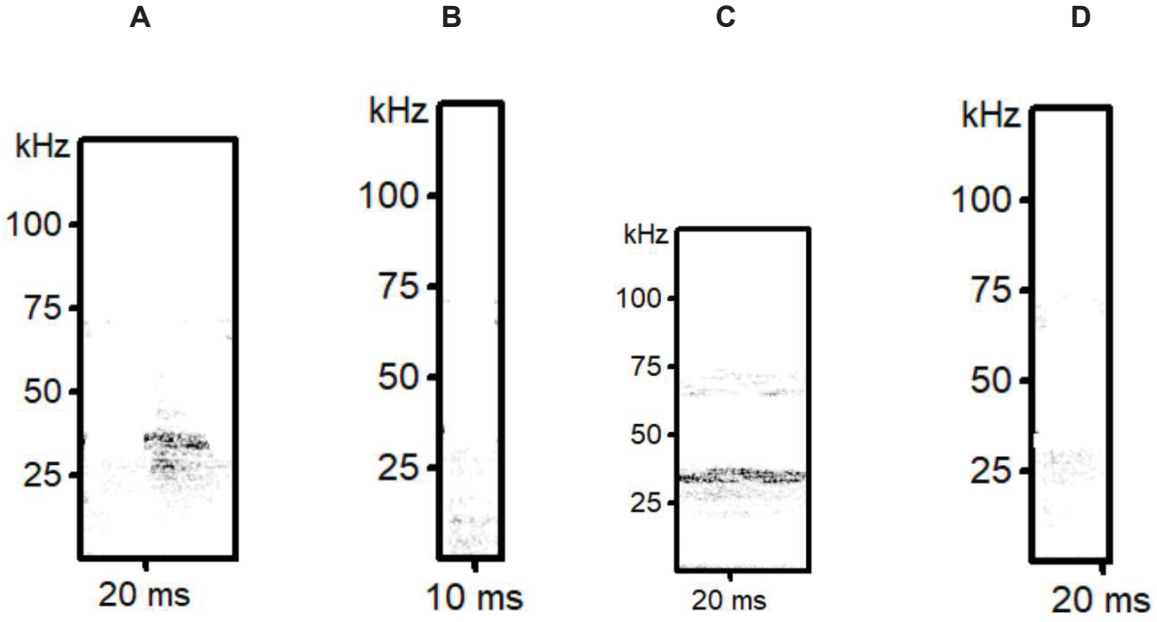

Fig. 14. D1CT-7 males's sound files analyzed using Avisoft Bioacoustics SAS lab software. Timestamps were added manually to spectrograms (Hamming window, FFT length = 512, frame size = 100%, overlap = 75%), and vocalizations were placed into the following categories: (A) Noise Vocalization. (B) Low frequence harmonic vocalization. (C) Noise from BPM 2.0. (D) Noise from de animal movements in the BPM 2.0.

5.2.2 Experiment 2.2: Evaluation of repetitive behaviors (rearings and climbings) during acute stress of D1CT-7 males caused by Space Confinement (SC) inside BPM 2.0

Two-way ANOVA revealed effect of the genotype ($F_{1,33=}$ 26.65, $p<0.01$) and environmental condition ($F_{1,33=}$ 29.25, $p<0.001$) during acute stress and spontaneous behavior of the D1CT-7 and WT males. A non-significant genotype × condition interaction was detected. Post hoc analyses revealed that D1CT-7 did more significant rearings and climbings during acute stress (SC) than during spontaneous behavior (SB) during 20 min of the experiment 2 ($p<0,0001$), no differences was observed in the WT group. Moreover, D1CT-7 males did more significant rearings and climbings during acute stress (SC) than the WT group ($p< 0,0003$) in the same environmental condition . These data suggest that the SC elicits a pronounced enhancement of repetitive moviments in D1CT-7, but did not significantly affect WT mice. (Fig15).

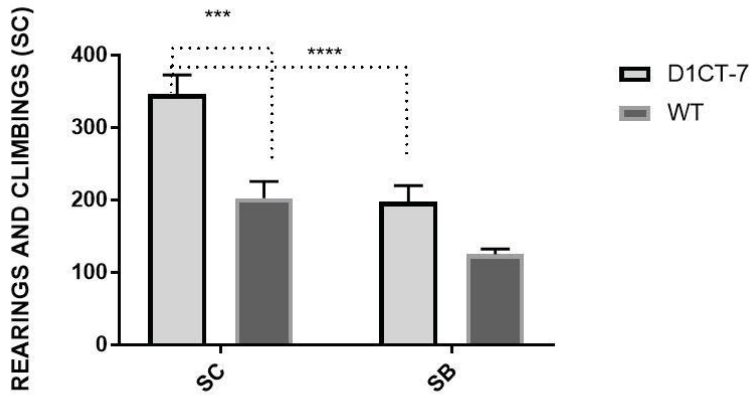

Fig. 15. Evaluation of rearings and climbings of the D1CT-7 males and their WT, during acute stress caused by Space Confinement (SC) and during spontaneous behavior (SB) inside BPM 2.0 during 20 minutes test. The data are expressed as mean +SD. N= 8-10 mice/group. ***p < 0,001, ****p< 0,0001 comparing with the WT group.

In summary, the experiment 1 showed that the BPM 2.0 was able to detect increase in locomotor activity and risk taking behaviors, and decrease in the anxiety-like behavior with all DAT inhibitors treatments. Moreover, the BPM 2.0, was able to detect a decrease in exploratory behaviors (holepokes and rearings) with MPH treatment (see table 1). In addition, the experiment 2, showed that the BPM 2.0 was able to detect increase in locomotor activity and rearings of the D1CT-7 males alone and males and females together. Additionally, the BPM 2.0 was able to detect a decrease in holepokes of the D1CT-7 males, females and males and females together compared with the control groups. Lastly, the BPM 2.0 detected a temporary decrease in the anxiety-like behavior of the D1CT-7 females alone and males and females together (see table 1).

Table1- Summary of results

		Locomotor Activity	Anxiety-Like	Exploratory Behaviors		Risk Taking Behaviors	
				Holepokes	Rearings	Center Cross	Time in the Center
Modafinil	64 mg/kg	↑	↓				
	100 mg/kg	↑	↓			↑	
GBR12909	9 mg/kg						
	16 mg/kg	↑	↓			↑	
MPH	5 mg/kg	↑		↓		↑	
	10 mg/kg	↑	↓	↓	↓	↑	
D1CT-7	Male	↑		↓	↑		
	Female		?	↓			
	Male + Female	↑	?	↓	↑		

6. DISCUSSION

The present study developed and validated the Brazilian Version of the Behavioral Patter Monitor for studies of mice models of CNS disorders.

First, the BPM 1.0 was developed, based on BPM for mice developed by Young and collaborators (YOUNG *et al.,* 2007; PERRY *et al.,* 2009; YOUNG *et al.,* 2011). The BPM 1.0 was technically valid to evaluate the manic-like behavior of Swiss mice treated with MPH (GONÇALVES, et. al., 2022). Moreover, it is able to discriminate between anticonvulsant drugs with (e.g., sodium valproate) and without (e.g., topiramate) clinical antimanic effects (GONÇALVES, et. al., 2022). Thus, it can be used for searching new antimanic-like drugs.

After this validation, the BPM 2.0 was developed according to description in the item 1.7. The BPM 2.0 was improved to solve some limitations of the BPM 1.0. In the BPM 2.0, the rearing sensors are mobile which permit to be adapted to any mouse size, a camera is integrated into the device, the new software used in this version never crashes and a new program was developed to analyze the results at any time-point of the test. Lastly, this version has an integrated USV microphone and thermometer.

In the experiment 1, it was evaluated the differential effects of different dopamine transporter inhibitors in the mania-like behavior of the C57BL mice tested in the BPM 2.0. GBR 12909 and modafinil were used to reproduce the results observed by Young and collaborators (YOUNG, *et. al*., 2010a; 2010b and 2011).

Regarding to locomotor activity, in the present study, C57BL mice treated with GBR 12909 (16 mg/kg) showed increased number of total crossings from 15 to 60 min of the test. These results are consistent with Young and collaborators (2010b), who showed that GBR 12909 (16 and 28 mg/kg) increased transitions and distance traveled of C57BL/6J mice from 20 to 60 min after GBR administration. This effect of GBR 12909 was also observed in Swiss mice submitted to open-field test (BASTOS *et al*., 2018).

Modafinil also increased the number of total crossings at dose of 64 and 100 mg/kg. The highest dose increased transitions from 20 to 60 min of the test, which is consistent with previous studies that showed increase in activity as measured by transitions at all three doses (32, 64 and 128 mg/kg) (YOUNG *et al.,* 2011). However,

the absence of effect with modafinil 32 mg/kg observed in the present study conflicts with the hyperlocomotion observed by Young *et al.*(2011) and Cope *et al.*(2017). This conflicting results can be due to lower sensitivity to modafinil of our C57BL mice supply, different methods used to prepare the drug solutions, differences between the BPM equipment, different period of testing in the light/ dark cycle, etc.

MPH increased the number of total crossings from 5 (only 10 mg/kg) to 60 min of the test in both doses (5 and 10 mg). Although MPH was tested only in the BPM 2.0 and 1.0, (present study; GONÇALVES *et al.*2022), these results are consistent with previous studies showing increased in locomotor activity in mice treated with MPH (BARBOSA *et al.,* 2011; SHANTHAKUMAR *et al.,* 2013; TONELLI *et al.,* 2013; SOUZA *et al.,* 2016; KANAZAWA *et al.,* 2017; ASTH *et al.,* 2020). In addition, as discussed above, Young and collaborators (2010b) showed that amphetamine (a DAT, NET, and SERT inhibitor and monoamine releaser) treatment also increased transitions in C57BL/6J mice in the BPM apparatus.

Thus, all dopamine transport inhibitors increased the number of total crossings (locomotor activity index) of the C57BL mice in the BPM 2.0. Although, amphetamines and MPH are considered the “gold standard” of animal model of mania, they are often used as models for different disorders, including, drug abuse, schizophrenia and tardive dyskinesia (YOUNG, et al., 2011), More selective DAT inhibitors, GBR 12909 and modafinil, on the contrary, may specifically reproduces the exploratory phenotype observed in patients with mania (PERRY *et al*., 2009; YOUNG *et al*., 2010b; YOUNG *et al*., 2011; BASTOS, *et al*., 2018, DE MIRANDA, *et al*., 2020).

Interestingly, all DAT inhibitors caused an increase in the number of total crossings in the first 20 minutes of the test, suggesting the assessment of locomotor activity just in the first 30 minutes of the test could be sufficient in many experiments, reducing time to perform a drug evaluation. It is a relevant issue for drug screening.

Regarding to exploratory behavior, GBR 12909 treatment did not increase holepokes or rearing at any dose or time. These results are inconsistent with Young and collaborators (2010b), who found that GBR 12909 (16 and 28.5 mg/kg) decreased holepoking at first 30 min. In a longer protocol (180 min of test) these authors also observed that GBR 12909 16 mg/kg increased holepoking from 150 to 180 min, while 9 mg/kg increased holepoking at 150 min. According to the Young and collaborators, the increased holepoking at final stages of the test would be result

of the effect of an active metabolite of GBR 12909 (Young, et al 2010b), which turns the 60 min test duration not sufficient to observe this effect. However, the 60 min protocol was used in most of the previous studies (RISBROUGH *et al.,* 2006; PERRY *et al.,* 2009; VAN ENKHUIZEN, *et al.,* 2013a, COPE, *et al.,* 2017, 2021). The conflicting result with 16 mg/ kg GBR 12909 can be due to the same factors listed above for locomotor activity. In addition, the results of these parameters in previous studies with DAT KD mice are less consistent than that observed with locomotor activity (KWIATKOWSKI *et al.,* 2019). Moreover, based on probability of typo I and type II error, it is expected some degree of non-reproducibility of positive results (HOWELS *et al.,* 2014). Thus, it could be expected some variability of positive results across studies.

Concerning to rearing, Young and collaborators (2010b) found that 16 mg/kg GBR 12909 treatment increased rearing from 90 to 180 min of the test, while 9 mg/ kg increased rearing at time periods of the 90, 150, and 180 min and 28.5 mg/kg increased rearing at 150 and 180 min only. In the same way, our experiment lasted only 60 min, an insufficient test length to observe this effect.

Modafinil treatment also did not change the number of holepokes and rearing in any dose or time in the experiment 1. Regarding to holepokes, these results are consistent with previous studies (YOUNG *et al.,* 2011; COPE *et al.,* 2017). Young and collaborators observed that modafinil did not change holepokes in C57BL/6J mice during 60min of BPM test. On the other hand, all three doses of modafinil (32, 64 and 128 mg/kg) significantly increased rearing from 20 to 60 min of the test (YOUNG *et al.,* 2011). Possible explanations are the same cited above, including that these behaviors may be not reproducible all the time. Another explanation may be related to the mechanism of action of the drug. Although modafinil exhibit significant binding to the DAT in the striatum, it also binds to the noradrenaline transporter (NET) in the thalamus (MADRAS *et al*, 2006). The reported effects of DAT and NET inhibitors on specific exploratory behavior have been contradictory (Young, et al., 2010b).

MPH treatment decreased holepokes at doses of 5 and 10 mg/kg, at 60 min and from 25 to 60 min of the test respectively. These results are consistent with Young and collaborators, where amphetamine (2.5, 5.0, and 10.0 mg/kg) significantly lowering holepoking behavior in C57BL/J6 mice in all time points (10 to 60 min) in the BPM (YOUNG et al 2010b). In the same way, MPH 10mg/kg

decreased rearings from 15 to 30 min of the test. These results are inconsistent with the observation that amphetamine in any dose or time caused alterations in rearings in the BPM (YOUNG *et al.,* 2010b). The serotonergic action of the amphetamine may account for their opposite effects on rearings in mice tested in the BPM.

The absence of MPH and amphetamine effects on increasing exploratory behaviors could be related to their additional effects on NET (already mentioned above), since NA have been demonstrated to affect exploratory behavior of rats in the hole board test (SARA *et al.,* 1995). On the other hand, Souza and collaborators (2016) showed that MPH 10 mg/kg increased holepokes in Swiss male mice on the hole board test. This conflicting results can be due to different mice strain and the differences between the BPM equipment and the hole board apparatus.

Thus, the BPM 2.0 was not able to reproduce some exploratory behaviors observed by Young and collaborators using the San Diego Instruments® apparatus (YOUNG *et al.,* 2010b, 2011; COPE *et al.,* 2017). These conflicting results can be explained by differences in the test length used, in drug source and preparations, methods (e.g., subjective day period testing), apparatus configuration, different animal supplier, etc. For example, different supplier can affect the manic-like behaviors of black Swiss mice (JUETTEN and EINAT, 2012). It is interesting to note that although 16 mg GBR 12909 did not affect holepokes in C57BL, it increased holepokes in 129/SvJ mice (YOUNG *et al.,* 2010b).

Another behavior analyzed in the experiment 1, was the risk-taking behavior, which was measured by the number of center crossings and the time (in seconds) in the center area (E quadrant). All DAT inhibitors increased center crossings, but no one increased the time in the center area.

GBR 12909 increased the center crossings at dose of 16 mg/kg from 20 to 60 min of the test. However, it did not increase the time in the center. In the same way, Young *et al.,* (2010b) found that C57BL/J6 mice treated with 16 mg/kg GBR 12909 displayed higher levels of center entries from 20 to 60 min of the test. They also found a nonsignificant trend effect of GBR 12909 for center duration (YOUNG *et al.*2010b).

Moreover, modafinil 100 mg/kg increased the center crossings from 30 to 60 min of the test, although it did not increase the time in the center at any dose or time. Unfortunately, there are no published studies evaluating the effects of modafinil in the risk-taking behavior in mice in the BPM. However, the selective DAT inhibitors

GBR12909 and modafinil modestly increased risk preference in the Iowa Gambling Task, while amphetamine induced a risk-averse preference (VAN ENKHUIZEN, et. al., 2013a). Selective DAT inhibitors also increased motivation and motor impulsivity (VAN ENKHUIZEN et. al., 2013b).

In addition, MPH 5 mg/kg (at 60 min of the test) and 10 mg/kg (from 30 to 60 min of the test), also increased center crossings, although it did not increase the time in the center in any dose or time. Young and collaborators found an opposite result with amphetamine (10 mg/kg) that significantly decreased center entries in the BPM (YOUNG *et. al.,* 2010b). On the other hand, Gonçalves and collaborators (2022) showed that MPH induced more center entries in Swiss mice tested in the BPM 1.0. As cited above, although amphetamine and MPH share DAT and NET inhibitory effects, amphetamine also has a serotonergic action, which may account for their opposite effects on risk taking behavior in mice tested in the BPM.

One new parameter assessed in BPM 2.0 was the percentage of the time that the animal remains in the quadrants that receive more intensity of lights (300 lux- HEB quadrants) relative to those quadrants that receive lower intensity of lights (90 lux- GDAIFC quadrants). This parameter is proposed to measure an anxiety-like behavior. All three DAT inhibitors in the highest doses (GBR 16mg/kg, Modafinil 64 and 100mg/kg and MPH 10mg/kg) increased the percentage of time in the HEB quadrants. These findings may lead to the conclusion that DAT inhibitors decrease anxiety-like behaviors in C57BL mice in the BPM. This is consistent with the fact that all of them increased risk-taking behavior (center crossings) but did not fit with the effects of psychostimulants in animal models of anxiety, such the light-dark test (BOURIN and HACOET *et al.,* 2003). However, previous study showed that DAT inhibitors (modafinil) decreased anxious-like behavior in sleep deprived rats (WADHWA, et. al., 2018). Studies using anxiolytic and anxiogenic drugs are need to evaluate the validity of this parameter as an anxiety-like behavior in the BPM.

In conclusion, the BPM 2.0 was capable and useful to differentiate the effect of DAT inhibitors on mania-like behaviors in C57BL mice, reinforcing its potential to give a behavioral signature for different drugs.

In order to study the usefulness of the BPM 2.0 to assess other CNS disorders models in mice, it was evaluated the behavioral profile of D1CT-7 mice, an animal model of TS(GODAR, *et al.,* 2016)). As expected, D1CT-7 male mice showed increased number of total crossings from 10 to 60 min of the test compared with WT.

These results are consistent with previous study that revealed that D1CT-7 male mice showed increased frequency and duration of walking in the open field test (SANTANGELO, *et al.,* 2018). On the other hand, D1CT-7 females mice did not show significant differences in the number of total crossings compared with WT. However, when males and females were analyzed together the number of total crossings remains significantly higher compared with WT. Hyperactivity is one the main features of the D1CT-7 mice (CAMPBELL, *et al.,* 1999, FOWLER, *et al.,* 2017; SANTANGELO, *et al.,* 2018). D1+ neurons in layers II–III of somatosensory cortical areas and the piriform layer II are glutamatergic neurons, that stimulate lateral cortical areas as well as deeper-layer corticostriatal glutamatergic neurotransmission and these alterations are accompanied by locomotor hyperactivity (CAMPBELL, *et al.,* 1999 and GODAR, *et al.,* 2016). Thus, the BPM 2.0 was able to detect the increased locomotor activity of the D1CT-7 mice.

Interestingly, D1CT-7 mice showed opposite results in the exploratory behaviors (rearing and holepokes). D1CT-7 male alone and male and female mice analyzed together showed a significant increase in the number of rearing (from 15 to 60 min of the test), while D1CT-7 males mice showed a decreased in holepokes (from 5 to 25 min of the test). Females alone and males and females analyzed together exhibited decreased holepokes in all times of the test. Females alone did not show differences in the number of rearing. These results are consistent with previous study that observed that D1CT-7 male mice showed increased frequency and duration of rearing and climbing in the open field test (SANTANGELO, *et al.,* 2018). As discussed above, these animals display tic-like manifestations that are accompanied by locomotor hyperactivity, perseverative allogrooming, digging, gnawing and leaping/rearing behaviors (GODAR, et. al., 2016 and SANTANGELO, *et al.,* 2018), thus the BPM 2.0 was able to detect the increased rearing behavior of the D1CT-7 mice, and because they perform a lot of rearings, the number of holepokes were significantly smaller when compared with their WT.

Regarding to risk taking behavior, D1CT-7 males and females alone and males and females analyzed together did not show significant difference in center crossings or in time in the center area. Unfortunately, there is no published study that analyzed risk-taking behavior of D1CT-7 mice. As discussed above, TS is often comorbid with several psychiatric conditions, including impulsivity that is related to the deficits in inhibitory control (MORAND-BEAULIEU, 2017) but, the BPM 2.0 did

not detect risk taking behaviors in the D1CT-7 mice.

On the other hand, D1CT-7 females showed increased the percentage of time in the HEB quadrants from 15 to 20 min of the test. When analyzed together D1CT-7 males and females mice also showed increased the percentage of time in the HEB quadrants from 15 to 30 min of the test. These results may suggest that D1CT-7 mice have less anxiety-like behavior in the BPM 2.0. at the first 30 min of the test. These results are inconsistent with TS characteristics in humans, since TS is often comorbid with several psychiatric conditions including anxiety disorders (HIRSCHTRITT, *et. al.,* 2015). However, there are not published studies that evaluated anxiety-like behaviors in D1CT-7 mice. Furthermore, as commented before, the interpretation of this parameter as an index of anxiety-like behavior needs further studies. Clearly, additional studies focusing anxiety-like behavior assessment in D1CT-7 mice are needed.

The BPM 2.0 was able to detect USV in D1CT-7 mice in both sex. No significant differences was found in the number of calls between D1CT-7 and WT. However, it was observed that the USV microphone recorded noise from the BPM 2.0 apparatus (noise from power cable and software), and these noises are in the same range frequency of the mice's USV. This may interfere in the USV analysis (e.g., some mice's USV may have been hidden by these noise records or the noise record may be interpreted as an USV). This is an important limitation of the BPM 2.0 and this technical problem needs to be solved.

Finally, we tested D1CT-7 males during 20 minutes of acute stress caused by SC inside BPM 2.0 to observe increase in the repetitive behaviors (GODAR, *et al*., 2016). It was analyzed rearings and climbings behaviors. D1CT-7 mice as expected, showed significant increase in the number of rearing (and climbing) compared with 20 minutes of spontaneous behavior in the whole BPM 2.0 apparatus. In addition, rearings and climbings in SC-exposed D1CT-7 mice were significantly greater than those observed in SC WT controls. These results are consistent with Godar and collaborators (2016), who observed increasing in other repetitive behaviors (tic-like responses and digging) of the SC-exposed D1CT-7 mice and they not observed significantly alter the behaviors of WT mice. Furthermore, tic-like and digging behaviors in SC-exposed D1CT-7 mice were significantly greater than those observed in SC WT controls (GODAR, *et. al*., 2016). These effects also are in line with previous reports documenting exacerbation of

leaping and climbing compulsions in D1CT-7 mice exposed to predator urine odor (MCGRATH *et al.,* 1999). Thus, these data suggest that the BPM 2.0 was able to detect TS-related phenotypes in D1CT-7 through the increase in the repetitive behaviors (rearings and climbings) during acute stress of D1CT-7 males caused by Space Confinement (SC).

After these experiments, another evaluation was made to detect the limitations of the *BPM 2.0*. As discussed above, the main limitation of the *BPM 2.0* found was the USV microphone. Due to the recorded noise from the *BPM 2.0* apparatus (noise from power cable and software) in all experiments, and as cited above, these noises were in the same range frequency of the mice's USV which can lead to a false result. This technical problem need to be solved in the next version of BPM 2.0. Furthermore, in this third version it will be developed a program to analyze the USV automatically.

7. CONCLUSION

In conclusion, the present results indicated that BPM 2.0 is a valid apparatus to test mice models of mania and useful to study the behavioral pattern of other CNS disorders (e.g., Tourette Syndrome). Further studies are being carried out to study the behavioral profile of mice submitted to other models (e.g., ouabain model of bipolar disorder).

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ATTACHMENT 1

Gonçalves, P. S., Pesquero, B. O., Valentin, D. T., Monteiro Pereira, V. C., & Andreatini, R. (2022). Effect of repeated sodium valproate and topiramate administration on mania-like behaviors induced by methylphenidate in mice. Acta neurobiologiae experimentalis, 82(4), 511–520. https://doi.org/10.55782/ane-2022-049 Attachment in the printed version

Link to the electronic version: https://pubmed.ncbi.nlm.nih.gov/36748974/

ATTACHMENT 2

INPI INSTITUTO NACIONAL DA PROPRIEDADE INDUSTRIAL

11/10/2022 870220094002 17:40

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Pedido nacional de Invenção, Modelo de Utilidade, Certificado de Adição de Invenção e entrada na fase nacional do PCT

Número do Processo: BR 10 2022 020694 5

Dados do Depositante (71)

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PETICIONAMENTO ELETRÔNICO Esta solicitação foi enviada pelo sistema Peticionamento Eletrônico em 11/10/2022 às 17:40, Petição 870220094002

Petição 870220094002, de 11/10/2022, pág. 1/21

PATENT PROTOCOL

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Dados do Pedido

Natureza Patente: 10 - Patente de Invenção (PI)
Título da Invenção ou Modelo de Utilidade (54): MONITOR DE PADRÃO DE COMPORTAMENTO PARA CAMUNDONGOS
Resumo: Descreve-se a presente patente de invenção como um monitor de padrão de comportamento para camundongos que, de acordo com as suas características gerais, propicia um monitor comportamental (1) em estrutura própria e específica baseada em um gabinete central (2), câmera de vídeo (3), microfone de vocalização ultrassônica (4), visor sensível ao toque (5), placas de circuitos com processadores integrados, rede de sensores infravermelhos de captura de movimentação, atividade exploratória e temperatura (6) e (7), e dispositivos armazenadores de dados, ambos os componentes integrados a uma suíte de programas de computador de controle de automação e geração e extração dos dados, com vistas a possibilitar completa otimização na análise do padrão comportamento de camundongos e similares dentro de um ambiente controlado objetivando a descobertas de novos fármacos para o tratamento de doenças do sistema nervoso central, aliando a análise por meio de algoritmos embarcados de vários comportamentos nos animais em um único teste.
Figura a publicar: 1

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PETICIONAMENTO ELETRÔNICO Esta solicitação foi enviada pelo sistema Peticionamento Eletrônico em 11/10/2022 às 17:40, sob o número BR1020220094302

Documentos anexados

Tipo Anexo	Nome
Relatório Descritivo	RD.pdf
Reivindicação	RV.pdf
Desenho	PRISCILA 01.pdf
Desenho	PRISCILA 02.pdf
Desenho	PRISCILA 03.pdf
Resumo	RS.pdf
Comprovante de pagamento de GRU 200	PED_PI_OS1690RA_P74P1COMP.pdf

Acesso ao Patrimônio Genético

☑ Declaração Negativa de Acesso - Declaro que o objeto do presente pedido de patente de invenção não foi obtido em decorrência de acesso à amostra de componente do Patrimônio Genético Brasileiro, o acesso foi realizado antes de 30 de junho de 2000, ou não se aplica.

Declaração de veracidade

☑ Declaro, sob as penas da lei, que todas as informações acima prestadas são completas e verdadeiras.

PETICIONAMENTO ELETRÔNICO Esta solicitação foi enviada pelo sistema Peticionamento Eletrônico em 11/10/2022 às 17:40, Petição 870220094012

ATTACHMENT 3: CEUA

MINISTÉRIO DA EDUCAÇÃO
UNIVERSIDADE FEDERAL DO PARANÁ
SETOR DE CIÊNCIAS BIOLÓGICAS
COMISSÃO DE ÉTICA NO USO DE ANIMAIS

UFPR

Nº 1241

CERTIFICADO

A Comissão de Ética no Uso de Animais do Setor de Ciências Biológicas da Universidade Federal do Paraná (CEUA/BIO – UFPR), instituída pela Resolução Nº 86/11 do Conselho de Ensino Pesquisa e Extensão (CEPE), de 22 de dezembro de 2011, **CERTIFICA** que os procedimentos utilizando animais no projeto de pesquisa abaixo especificado estão de acordo com a Diretriz Brasileira para o Cuidado e a Utilização de Animais para fins Científicos e Didáticos (DBCA) estabelecidas pelo Conselho Nacional de Controle de Experimentação Animal (CONCEA) e com as normas internacionais para a experimentação animal.

STATEMENT

The Ethics Committee for Animal Use from the Biological Sciences Section of the Federal University of Paraná (CEUA/BIO – UFPR), established by the Resolution Nº 86/11 of the Teaching Research and Extension Council (CEPE) on December 22nd 2011, **CERTIFIES** that the procedures using animals in the research project specified below are in agreement with the Brazilian Guidelines for Care and Use of Animals for Scientific and Teaching purposes established by the National Council for Control of Animal Experimentation (CONCEA) and with the international guidelines for animal experimentation.

PROCESSO/PROCESS: 23075.063525/2018-15

APROVADO/APPROVAL: 20/11/2018 – R.O. 10/2018

TÍTULO: O efeito antimaníaco de anticonvulsivantes bloqueadores de canais de sódio em diferentes modelos animais.

TITLE: The animanic-like effect of voltage-gate sodium channels blockers anticonvulsants on different animal models.

AUTORES/AUTHORS: Roberto Andreatini, Priscila Samaha Gonçalves, Luiz K Sales Kanazawa, Vivian Cristhiane Monteiro Pereira.

DEPARTAMENTO/DEPARTAMENT: Farmacologia

Profa. Dra. Katya Naliwaiko
Coordenadora da CEUA

Documento assinado eletronicamente por **ISELEN ABREU FLORENTINO IVANOSKI, MEDICO VETERINARIO**, em 07/02/2019, às 23:09, conforme art. 1º, III "b", da Lei 11.419/2006.

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