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IMPACT OF TETRAHYDROCANNABINOL ON TEMPORAL PERCEPTION AND EXPLORATORY BEHAVIOR IN A MOUSE MODEL OF BIPOLAR MANIA

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ABSTRACT

Bipolar disorder (BD) is a prevalent psychiatric disorder affecting about 1% of the world's population. However, there is still a lack of adequate treatment for BD. Mania state, the defining feature of BD, manifests insomnia, aggression, and motor hyperactivity, possibly due to dysregulation of the dopaminergic system. In BD lower dopamine transporter (DAT) expression and function are observed which leads to decreased dopamine metabolism which may cause elevated dopamine transmission during mania state.

Cannabis is used in higher rates in people with BD than in the general population. THC, a psychoactive compound present in cannabis, decreases dopamine levels over time, and therefore, with the elevated dopamine levels observed in bipolar mania, may be able to treat the mania-like symptoms of BD.

DAT knockdown (KD) mice reconstruct BD-related behaviors such as hyperexploratory and risky decision-making behavior in the behavioral pattern monitor (BPM) and may be useful to develop newer treatment strategies for BD mania. We investigated if hyperexploratory behavior of DAT KD mice would decrease after chronically administering THC (14 days) in the behavioral pattern monitor (BPM). 45 mice (51% DAT KD, 22% female) were intraperitoneal injected of either THC (3mg/kg) or saline for 14 consecutive days and then tested in the BPM.

Another cohort of DAT KD mice in the BPM, other cohort DAT KD mice were tested in the temporal discrimination task (TDT). The TDT measures temporal perception which altered in BD and by THC. Therefore, we used the TDT to investigate the effect of THC on temporal perception of these subjects. The data we provide is data from during the training phase. 45 mice (54% DAT KD, 56% female) were trained in the TDT task, with each session duration being 30 minutes.

The 19 sessions were insufficient for all mice to attain criterion in the TDT (> 85% correct), although current training revealed no sex or genotype differences in training to-date. Chronic THC treatment attenuated exploratory behavior in DAT KD mouse model of bipolar mania, with a trend effect ($p = 0.055$) in rearing, and a similar effect in holepoking.

Chronic THC treatment on DAT KD genotype might attenuate exploratory behavior in DAT KD model of bipolar mania. Moreover, when mice perform the TDT task consistently investigation on the effect of chronic THC treatment on temporal perception will be performed.

INTRODUCTION

Bipolar disorder (BD) affects about 1% of the world's population regardless of ethnic group, nationality, or socioeconomic status [1] [2]. BD includes changes in mania, depression, and euthymic state [3]. Mania manifests behaviorally as insomnia and besides that aggression, motor hyperactivity and overload of energy and speech [4]. The typical onset of BD is approximately during the adolescent phase of life [5]. A more serious disease course is present in early illness onset [2]. BD is classified into subtypes (BD I and BD II) depending on specific symptoms. BD I is described as alternations between manic episodes with depressive episodes, while BD II expresses in one depressive episode and more than one hypomanic state [5].

Currently there is a lack of a specific treatment for BD [3][6]. However, several countries are increasingly allowing cannabinoids to be made accessible for medicinal purposes, including treatment of mental disorders [7]. Legalization of cannabis use is expanded throughout the United States in both adults and adolescents [8], and rates of recreational cannabis use are particularly high among people with BD [9]. The cannabis plant contains hundreds of constituents also known as phytocannabinoids [10], with tetrahydrocannabinol (THC), being the main psychoactive ingredient [10][11]. During the past decades clinical and preclinical research has been done to identify potential therapeutic effects of cannabis on behavior and physiology [8].

Cannabinoids primarily use the endogenous cannabinoid (ECB) system to exert their effect. Cannabinoid 1 receptors (CB1Rs) and cannabinoid 2 receptors (CB2Rs) are the two main receptors in the ECB system, both operating as G-protein coupled receptors [10]. While CB1Rs are mainly located in the central and peripheral nervous system, CB2Rs are predominantly located in immune cells. THC is a partial agonist of both receptors [12]. THC primarily causes psychoactive effects via activating CB1Rs [7][12], but its effects on specific cognitive domains relevant to BD remain unclear. One such cognitive domain is that of temporal perception. Targeting this domain could interfere many of the mania-related behaviors described above.

THC administration slows down temporal perception [13]. Therefore, THC interferes with timing ability that could lead in changes in motor impulsivity [13]. There is an overestimation of time in humans when administered THC [14]. Temporal perceptions take place over multiple time periods that ranges from milliseconds to hours. It includes functions as sensory-motor timing [15]. Rodents are effectively able to measure time, and cannabis also slows their temporal perception [13]. Thus, the impact of cannabinoids on BD-relevant behaviors could be gleaned by testing their impact on a rodent model of BD performing a temporal perception task.

Genetic linkage studies implicate that dopamine transporter (DAT) polymorphisms are associated with BD [16][17]. It is also reported that patients with BD have lower DAT levels and reduced DAT expression [17][18]. Dysfunctional dopamine (DA) neurotransmission lead to inaccurate dopamine metabolism [19] that can cause neurological and psychiatric disorders, such as BD [19][20]. Homeostasis of dopamine is maintained by DAT by uptake of extracellular dopamine [20][21] from synapses into presynaptic terminals [16]. Dysfunction in DAT expression results in BD-relevant changes in behavior [21]. For example, in mania state elevated dopaminergic transmission is seen [6]. DAT functioning levels can be genetically or pharmacologically manipulated in mice [17]. DAT KD mice have 10% of normal DAT levels [22][23] that results in mania-like behaviors, such as increased risk-taking behavior and abnormal exploration [24].

The influence of THC on temporal perception in DAT KD mice is an interesting field to investigate in. Acute and long-term THC administration cause variation in effects on the dopaminergic system [12]. This link between THC and dopamine signaling has been broadly investigated. Dopaminergic cell firing and dopamine synthesis/release are increased by acute THC administration, while long-term administration of THC causes dulling of the dopamine release and neural activity [12].

In animals, the Temporal Discrimination Task (TDT) can be used to examine the effects of THC on temporal perception of a subject [13]. TDT is a retrospective timing schedule [15] where subjects learned to respond to a certain image depending on an interval that passed by. Manic BD patients often perceive time extremely accelerated [25] – this perception is able to be measured by the TDT task. The TDT task is also able to determine changes in temporal perception due to THC because THC slows down temporal perception [13]. Discrete choice response rates are used to be able to test drug effects on temporal discrimination with equal baseline response rates [14]. That means that after responding correctly to a stimulus the mouse will be rewarded (e.g. release of strawberry milk) and after an incorrect response there will be a timeout where there no rewards can be earned.

Another task is the behavioral pattern monitor (BPM) that can be used to understand the effects of THC on BP-relevant behaviors. It Is used to measure exploratory behavior in mania model. It is important to understand this aspect because motor patterns are disturbed in BD [20] and influenced by THC [26]. The BPM task is designed to track exploratory behavior, including total motor activity, patterns of motor activity, and reactions to novel stimuli [17][24]. Exploratory behavior is the act of minimizing stimulation and excitation, making the unknown recognized [27].

When DAT KD mice are tested in the mouse BPM, their profiles are consistent with manic BD patients in the human BPM (hBPM) [17][24]. DAT KD mice are used to model mania behavior of BD in patients [24] [28], with predictive validity seen where valproate treatment drove similar changes in exploration in people with BD mania and the DAT KD mice [18][22]. It is unclear if cannabis worsens or improves symptoms of bipolar disorders in users, such as motor activity and impulsive behavior [18] – a question that is difficult to answer in humans because of heterogeneity of symptoms in BD [24]. Therefore, utilizing animal models may be a solution to investigate if cannabis may be a candidate treatment for BD.

In this study we hypothesize that THC will slow down time perception of DAT KD mice and therefore minimize motoric impulsivity. We used BPM and TDT strategies to investigate the interaction of THC with BD effects. Use of TDT is novel for this DAT KD animal model of BD.

The purpose of this study was to examine the impact of THC on behaviors relevant to BD using, DAT KD mice. Effects were compared to their DAT wildtype (WT) littermates and vehicle treated mice. Hence, we tested whether cannabis constituents like THC affected the behavior of mice in the TDT and BPM.

METHODOLOGY

Animal subjects

C57 mice were kept in a temperature-controlled vivarium (21 ± 1°C). They lived under reversed day-night cycle circumstances. That means that lights were turned on at 7:00 PM and turned off at 7:00 AM. All mice were group-housed (maximum 4 mice per cage). TDT mice were trained in the mouse TDT (mTDT). 30 minutes before training all TDT mice were brought in the testing area and trained between 8.30 AM and 1 PM. Therefore, the training was done during the dark phase timeframe of the reversed day-night cycle. The TDT cohort was food-restricted at 85% of their baseline weight during training. The BPM cohort had *ad libitum* access to food. Both TDT and BPM cohort mice had unlimited access to water.

Animal subjects – TDT

In total this cohort consisted of 45 mice. DAT KD female mice (n= 11) and DAT KD male mice (n=13). DAT WT female mice (n=10) and DAT WT male mice (n=11) represented the control group. These mice were trained in the mTDT.

Animal subjects – BPM

Another cohort mice were used in the BPM. DAT KD female mice (n= 19) and DAT KD male mice (n=4). Also, DAT WT female mice (n=16) and DAT WT male mice (n=6) represented the control group. In total the cohort consisted of 45 mice.

Habituation

Initially, mice had to get used to getting strawberry milk. A small plastic cup with strawberry milk was put into the mice' cage for 20 minutes. Habituation was considered finished when each cage consumed all strawberry milk within the period they were exposed to strawberry milk. All cages completed habituation within one session.

FR1 Touch training

TDT mice were trained to use the touchscreen with an FR1 schedule. They were first daily trained (Monday – Friday) with the FR1 touch training until all mice accomplished > 60 magazine entries in 30 minutes on 2 consecutive days. These mice were then put on the TDT after when they reached this threshold. The FR1 touch training included a single image stimulus which showed up after 6 seconds, on either the left or right side, after a mouse initiated a trial by nose poking the award magazine. The mouse needed to touch the image to receive the reward. A new trial started again after a mouse nose poked the reward magazine.

Temporal Discrimination Task (TDT)

The TDT was carried out after the FR1 touch training. Each training session included 120 trials and took 30 minutes. The TDT started with the magazine light turned on and the house light turned off. The moment the mouse entered the magazine the trial was initiated (Fig. 1). This resulted in the magazine light being extinguished [13]. The mice needed to learn to respond to one of the two images appearing on the touchscreen. The touchscreen was divided by two fields. The two images appeared on either field. One image was a marble image while the other one was a fan image. These images were not specifically the same as the images used in FR1 touch training. After either 2 seconds (2 s) or 10 seconds (10 s) both images appeared on the touchscreen (Fig. 2). The mouse had to touch either the marble image or fan image. Each image was specifically correlated with a short (2 s) or long (10 s) trial.

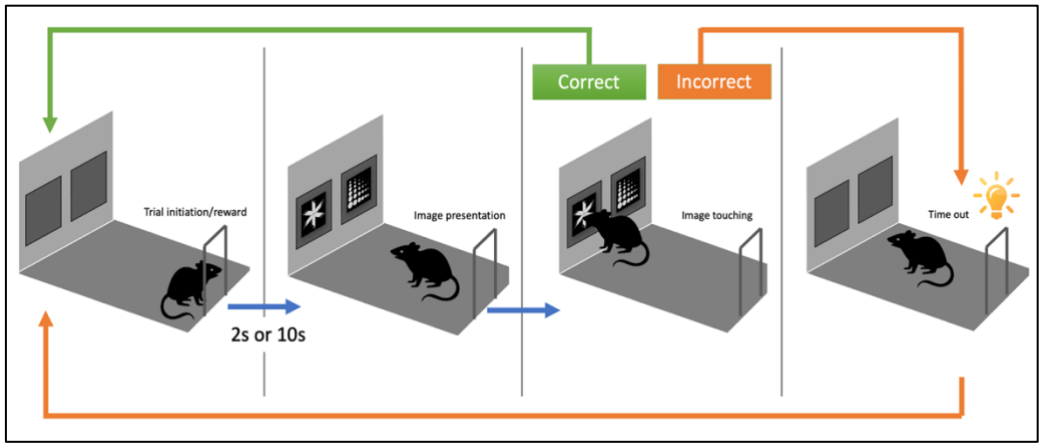

Fig. 1: A schematic presentation of the temporal discrimination task (TDT). The TDT is a chamber containing a touchscreen (with two fields) on the left and the magazine on the right. In the magazine the reward (strawberry milk) is released. The different stages are shown, starting with trial initiation, following image presentation (2 or 10 s later), and image touched by a mouse. After a correct trial the mouse can collect the reward in the magazine, while after an incorrect trial house light illuminates as a time out.

Two versions of TDT were used and randomized over the cohort. In version A the marble image was associated with 2 s and the fan image was associated with 10 s. Version B was the other way around, that means that the marble image had to be touched after 10 s and the fan image after 2 s of image pop-up. These two versions were used to control for any side bias the mice might have.

Incorrect trials were defined as a mouse touching the image that is not related to the short/long trial. The left or right field was not relevant to the timing. Correct trials were the ones when a mouse touched the images that is connected to the short/long trial. For example, when a mouse learned version A that means that failure to respond to the correct image caused a time-out punishment where house light was

illuminated. On top of that, all aperture was not responsive for 4s. When the mouse entered the magazine and left the magazine again, the following trial was initiated [13].

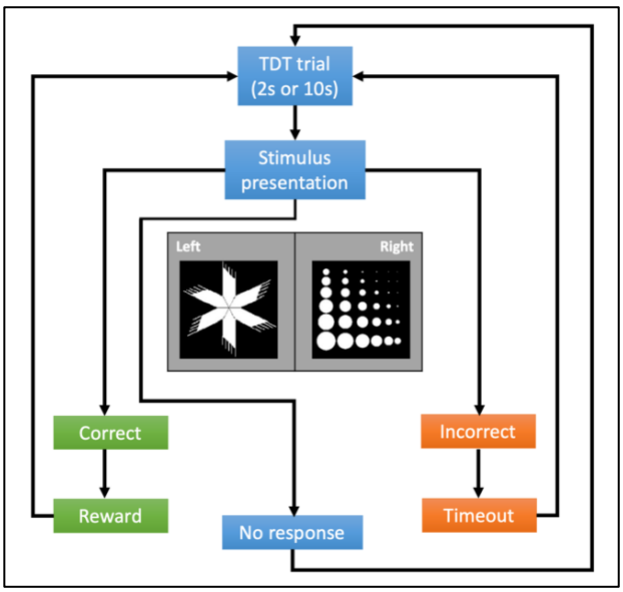

Fig. 2: A flowchart of a single trial in the TDT task. After trial initiation, 2 or 10 s later a fan image is shown on the left touchscreen field. A marble image is shown on the right touchscreen field. There are three outcomes after stimulus presentation: correct trial, incorrect trial, and no response.

This training was to be continued until all mice had a correct score of > 85% [15]. The score was calculated by %correct [correct/(correct + incorrect)*100] [13]. These scores had to be significantly above chance (score > 85%, $p < 0.05$) when a ttest was done for the number of trials to criterion from the last 4 days before the mouse was considered to have learned the TDT task. We kept track of baseline performance, including reward latency, touch latency and percent correct.

Behavioral Pattern Monitor (BPM)

BPM was used to track exploratory behavior in a novel circumstance [17]. This made it possible to record structural pattern of motor activity and exploration behavior of a rodent [3]. The BPM tracked 3 dimensions of exploratory behavior as mentioned earlier [20]. BPM was carried out on the first day and on the fourteenth day of drug treatment in BPM mice cohort. THC was administered for 14 consecutive days in this BPM experiment. Day 1 administration was associated with acute THC administration while after 14 consecutive days administration was considered long-term THC administration Exploratory behavior was monitored for 45 minutes. The mouse BPM (mBPM) chamber consisted of 3 holes on the ground and 8 holes on the walls. The holes function as objects that would be investigated by mice [20]. Infrared beams were built-in each hole. It functioned to keep track rearing, frequency of hole pokes, and the location of the animal [3]. One mouse was put in the chamber. During the 45 minutes run the location of the mouse was tracked every 55 milliseconds by infrared photobeams. Hole poking was also monitored by infrared photobeams that were located in each hole [20].

Drug treatment

There were two research arms in this mBPM study. Group A received saline administered while group B received THC. To prepare THC injections, the drug had to be dissolved in 7.5% Tween, 7.5% propylene glycol (PG) and saline. Drug treatment was carried out for the BPM mice. All of these mice were intraperitoneally (IP) injected with either saline or THC for 14 consecutive days, 0.3 mg/kg per mouse, with a volume of 5mL/kg, 30 minutes prior to testing. On days 2 till 13 the mice were IP injected between 12pm and 1pm to be consistent in timing of administering THC dose during the 14 consecutive days.

Statistical analysis

Two-way ANOVA for pairwise comparisons between subjects was used for TDT data as well as BPM data. Statistical analysis was conducted by using SPSS (v 26) or Graphpad Prism (version 9). The statistical probability for significance was fixed at 0.05.

TDT experiment

The statistical analysis for the number of trials to criterion was done over data about 4 consecutive sessions of $p < 0.05$. For the baseline performance in late TDT training stages analysis was conducted using sessions 15 to 19 for each mouse, as 19 was the lowest number of sessions completed by a single mouse. Progression over the five sessions leading up to session 19 was used in statistical analysis.

RESULTS

TDT experiment

Training

Using the TDT, we trained the DAT KD mice for 30 minutes to collect data during the training phase. The end task for TDT was not conducted since not all mice were performing the TDT task at criterion, not even all were above chance (score > 50%, $p < 0.05$). After 25 days of collecting TDT training data we saw that 17 out of 45 mice knew how to perform the TDT task significantly above chance. 9 out of 17 mice were DAT KD mice. Also, 9 out of 17 mice were female mice.

Ttests were conducted to compare acquisition and performance data in late TDT training stages. It was seen that acquisition of TDT was not affected by sex or by genotype. We checked for task learning by keeping track of the amount of trials mice completed 4 days in a row for those significantly above chance (Fig. 3A). Thus, looking at sex or genotype, these main factors did not affect the pace where the mice learned the TDT task (Fig. 3B-C). Only reward latency during the 10 s trials was significant ($p = 0.0253$) by sex, seen one day between female DAT KD mice and male DAT KD mice.

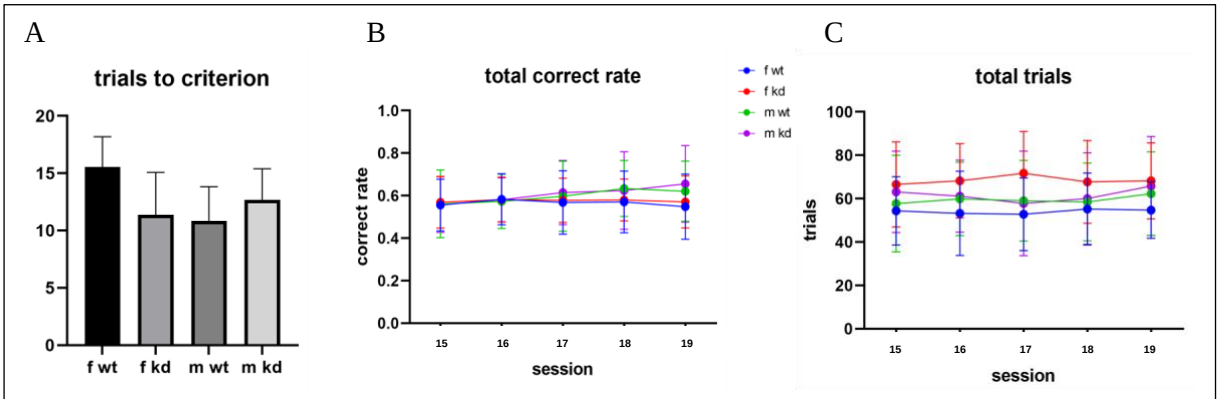

Fig. 3: A) Number of trials mice completed 4 days in a row significantly above chance. B) Number of total trials (sum of 2 s trials and 10 s trials) completed did not differ at this point in training between wildtype (WT) mice, dopamine transporter knockdown (DAT KD) mice, male (m) mice, or female (f) mice. C) Number of total trials (sum of 2 s trials and 10 s trials) responded to correctly similarly did not differ based on genotype or sex.

TDT performance from session 15 to 19

The performance in training stage was not affected in the 2 s, 10 s or combined trials. That means that in the late TDT training stages the performance was not affected by sex or genotype (Table 1A and 1B).

	2 s correct	2 s trials	2 s touch latency	2 s reward latency	10 s correct	10 s trials	10 s touch latency	10 s reward latency
p value	0.7622	0.3302	0.3451	0.2887	0.1695	0.1165	0.149	0.0253

Table 1A: The p values of outcome measures (2 s and 10 s trials) of the TDT from training days 15-19.

	Total correct trials	Total trials	Trials to task learning
p value	0.8441	0.215	0.6601

Table 1B: The p values of outcome measures (total trials) of the TDT from training days 15-19.

Impact of acute THC (day1) on hyperexploration of DAT KD mice in the BPM

The BPM was conducted on day 1 and 14. The impact of acute THC (day 1) on hyperexploration of DAT KD mice are shown below:

Significant effects on behavior were seen in genotype and drug, but not in genotype by drug factor. Genotype as a factor did not significantly affect pokes and rears but affected on other behavior measured. These measures were significantly affected by genotype, with spatial d ($F_{(1,37)} = 7.723$, $p = 0.009$), counts ($F_{(1,37)} = 6.806$, $p = 0.013$), transition ($F_{(1,37)} = 6.376$, $p = 0.016$), and distance traveled ($F_{(1,37)} = 6{,}480$, $p = 0.015$) (Table 2, genotype column). The other factor that had significant effects on behavior was drug. All measures were affected by drug, except for spatial d. Significant effects were seen in pokes ($F_{(1,37)} = 7.594$, $p= 0.009$), rears ($F_{(1,37)} = 19.150$, $p < 0.001$), counts ($F_{(1,37)} = 15.174$, $p < 0.001$), transitions ($F_{(1,37)} = 5.194$, $p = 0.029$), and distance traveled ($F_{(1,37)} = 6.459$, $p = 0.015$) (Table 2, drug column). No genotype*drug interaction was observed for any measures (Table 2, genotype*drug column).

The BPM was conducted on day 1 and 14. Based on *a priori* hypothesis, despite a lack of genotype*drug interaction, we examined the effect of THC within each genotype. In the DAT KD mice, we saw that THC significantly decreased poking ($F_{(1,37)} = 5.219$, $p = 0.028$), transitions ($F_{(1,37)} = 4.491$, $p = 0.041$), counts ($F_{(1,37)} = 13.066$, $p < 0.001$), distance traveled ($F_{(1,37)} = 5.854$, $p = 0.021$), and a trend decrease of spatial d ($F_{(1,37)} = 3.829$, $p = 0.058$). However, poking, transitions, counts, distance traveled, and spatial d were not significantly affected by acute THC treatment in WT mice. THC significantly decreased rears in DAT KD mice ($F_{(1,37)} = 11.241$, $p = 0.002$) as well as in WT mice ($F_{(1,37)} = 7.916$, $p = 0.008$) after acute THC treatment (Fig. 5). These data indicate a greater sensitivity of DAT KD mice to the effect of THC, relative to their WT littermates

BPM measures	Genotype	Drug	Genotype*Drug
Pokes	F(1,37) = 0.140, p = 0.710	F(1,37) = 7.594, p = 0.009	F(1,37) = 0.081, p = 0.496
Rears	F(1,37) = 2.258), p = 0.141	F(1,37) = 19.150, p < 0.001	F(1,37) = 0.580, p = 0.502
Spatial d	F(1,37) = 7.723, p = 0.009	F(1,37) = 1.925), p = 0.174	F(1,37) = 1.464, p = 0.127
Counts	F(1,37) = 6.806, p = 0.013	F(1,37) = 15.174, p < 0.001	F(1,37) = 0.132, p = 0.129
Transitions	F(1,37) = 6.376, p = 0.016	F(1,37) = 5.194, p = 0.029	F(1,37) = 0.839, p = 0.366
Distance traveled	F(1,37) = 6,480, p = 0.015	F(1,37) = 6.459, p = 0.015	F(1,37) = 0.219, p = 0.276

Table 2: The p values of factors genotype on behavior, drug on behavior and genotype by drug on behavior on day 1.

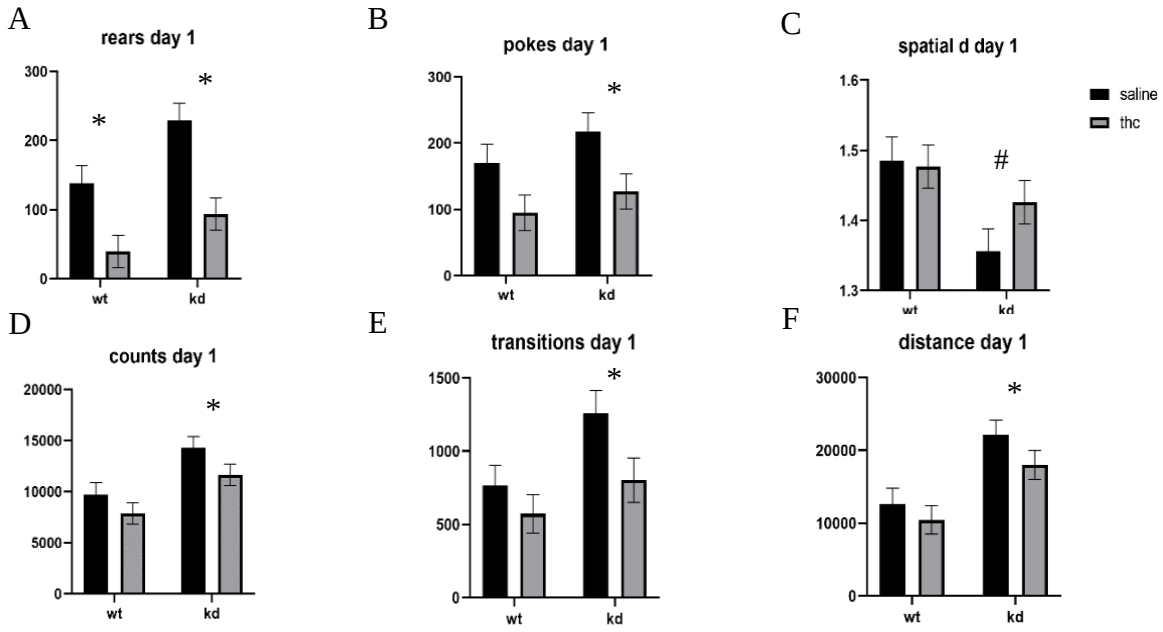

Fig. 5: The impact of acute (day 1) THC treatment (3 mg/kg), on behavior monitored in the behavioral pattern monitor (BPM). A) THC reduced the rearing of DAT KD mice ($p = 0.002$) as well as wildtype (WT) littermate mice ($p = 0.008$). B) A similar effect of THC was observed on holepoking ($p = 0.028$), with THC-induced reduced levels in DAT KD mice with no effect in WT littermates. C) Acute THC treatment showed a trend significance ($p = 0.058$) on spatial d in DAT KD mice, without affecting WT littermates. D) Interestingly, acute THC treatment significantly affected behavior measures, such as counts ($p < 0.001$), transitions ($p = 0.041$) and distance traveled ($p = 0.021$) in DAT KD mice but not significantly in WT littermates. Data presented as mean $\pm$ SEM. #= $p < 0.1$ vs. saline control.

Impact of chronic (14 days) THC on hyperexploration of DAT KD mice in the BPM

We focused on chronic THC treatment because chronic THC administration decreases dopamine release and neural activity [12]. This may encounter with mania state in bipolar where increased dopaminergic transmission is seen [6]. Outcome data were also analyzed by the main factors, drug and genotype.

After 14 days of THC treatment in mice, outcome data were analyzed by the main factors, drug and genotype. Significant effect on behavior was only seen in genotype. Drug did not affect behavior measures significantly as a main effect. There was a significant effect seen of sex on behavior. However, the cohort consisted only 22% males. Thus, the effect of sex on behavior would be difficult to confirm and cannot be determined until an additional cohort is included.

Genotype affected all behaviors significantly, except for poking. A trend effect ($F_{(1,37)} = 3.501$, $p = 0.069$) was seen between WT mice and DAT KD mice in poking. The other measures were affected significantly by genotype, with rears ($F_{(1,37)} = 4.103$, $p = 0.050$), spatial d ($F_{(1,37)} = 12.038$, $p < 0.001$), counts ($F_{(1,37)} = 42.886$, $p < 0.001$), transition ($F_{(1,37)} = 32.716$) ($p < 0.001$) and distance traveled ($F_{(1,37)} = 35.144$, $p < 0.001$) (Table 3).

Drug did not affect BPM performance significantly ($p > 0.05$) (Table 3). No genotype*drug interactions were observed either, but based on our *a priori* hypothesis was however, that chronic THC would affect DAT KD mice and not WT mice, these two groups were analyzed separately.

DAT KD mice performed fewer pokes after 14 days of THC administration compared to DAT KD mice who received saline for 14 consecutive days. However, this effect was not significant. Although, a trend effect of THC on rearing in DAT KD mice was seen after chronic THC treatment ($F_{(1,37)}$ = 3.927, p = 0.055). No significant effect of THC on other behavior measures in either WT mice or DAT KD mice was seen after chronic THC treatment (Fig. 6).

BPM measures	Genotype	Drug	Genotype*Drug
Pokes	F(1,37)= 3.501, p = 0.069	F(1,37) = 0.605, p = 0.442	F(1,37) = 0. 737, p = 0.399
Rears	F(1,37) = 4.103, p = 0.050	F(1,37) = 2.500, p = 0.122	F(1,37) = 1.978, p = 0.168
Spatial d	F(1,37) = 12.038, p < 0.001	F(1,37) = 0.133, p = 0.717	F(1,37) = 1.381, p = 0.247
Counts	F(1,37) = 42.886, p < 0.001	F(1,37) = 1.049, p = 0.312	F(1,37) = 0.045, p = 0.834
Transitions	F(1,37) = 32.716, p < 0.001	F(1,37) = 0.949, p = 0.336	F(1,37) = 0.009, p = 0.924
Distance traveled	F(1,37) = 35.144, p < 0.001	F(1,37) = 0.507, p = 0.481	F(1,37) = 0.000, p = 0.988

Table 3: The p values of factors genotype on behavior, drug on behavior and genotype by drug on behavior on day 14.

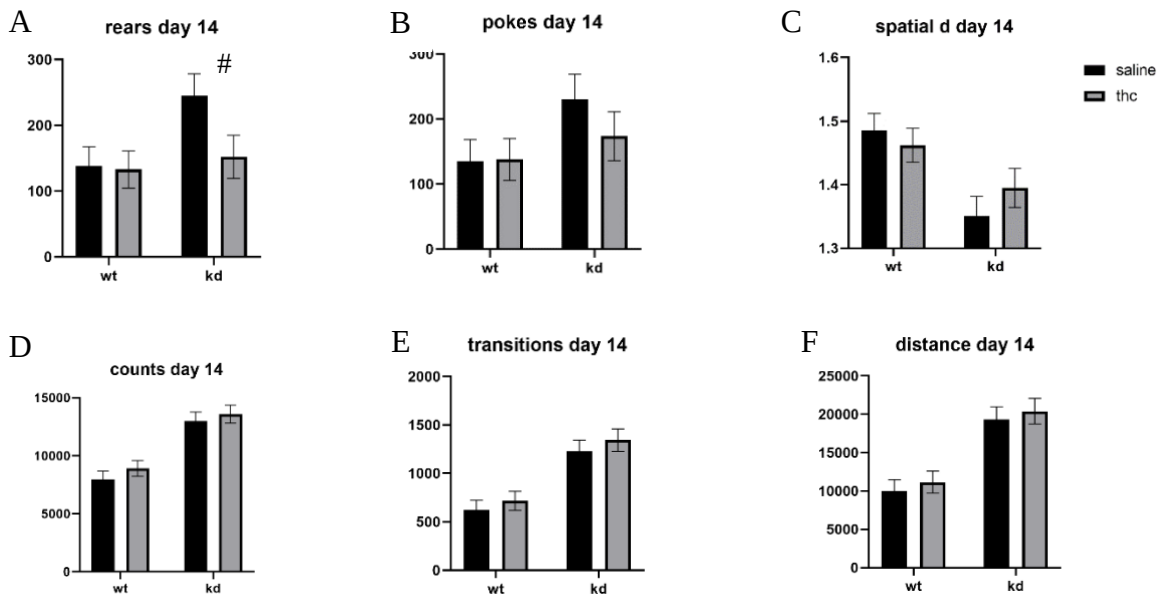

Fig. 6: The impact of chronic (14 day) THC treatment (3 mg/kg), on behavior in the behavioral pattern monitor (BPM). A) THC reduced the rearing of DAT KD mice with a trend significance (p=0.055), without affecting wildtype (WT) littermate mice. B) A similar effect of THC was observed on holepoking, with THC-induced reduced levels in DAT KD mice with no effect in WT littermates. C) Interestingly, THC also began to normalize spatial d in DAT KD mice without affecting WT littermates, although this effect was not significant. THC exerted no effect on activity measures however in either genotype, as measured by D) counts, E) transitions, F) distance traveled. Data presented as mean ±SEM. #= p < 0.1 vs. saline control.

DISCUSSION

Mice began learning the TDT task but required more training than there was time unfortunately. So far there was no significant effect of genotype or sex on acquisition of the task. In the BPM, we observed that acute THC exerted a greater effect on DAT KD mice than their WT littermates, attenuating several hyperexploratory behaviors, such as holepoking, transitions, counts, and distance travelled, and a trend decrease of spatial d. Interestingly, chronic THC had no effect on WT mice, but attenuated the hyper-specific exploration of DAT KD mice (as measured by rearing - one of the exploratory behavior measured) in the BPM, although no other effects reached significance or a trend.

Mice learned the TDT slowly. All mice had at least been trained on the TDT for 19 sessions but none reached the criterion (> 85% correct responding) to-date [15]. Genotype did not have a significant effect on current acquisition of TDT task. Unfortunately, we cannot tell if drug affected TDT performance because the mice did not reach the criterion yet to conduct any drug treatments. TDT studies done earlier showed that acquisition of the TDT task by individual mice took an average number of 16.6 ± 1.1 sessions to perform the task to criterion (> 85% correct responding), with a range of 6 to 42 sessions [15]. This explains why 19 sessions of training was insufficient for all mice to reach the criterion.
It was difficult to conclude if acquisition of the TDT task was faster in male mice compared to female mice because not all male mice reached the total correct criterion. If the study period was extended it would be more likely to be able to confirm if there was a difference in acquisition of TDT by sex.

Only reward latency during the 10 s trials was significant by sex in the TDT study. However, the difference was only significant on day 17 and only between female DAT KD mice and male DAT KD mice, with female DAT KD mice showing slower reward collection. No other measure was significantly affected by sex or by genotype on day 17 or any other day. Minor effect on day 17 might have been an artifact of statistical analysis, given the number of comparisons made. Thus, while training in the TDT takes some time, initial results indicate no effect of genotype in overall learning.

In the BPM study we saw that acute THC treatment decreased exploratory behaviors on WT mice as well as DAT KD mice on exploratory behaviors. Interestingly, this acute effect was consistently greater in the DAT KD mice vs. WT mice, with THC affecting holepoking, transitions, counts, and distance travelled, and a trend decrease of spatial d. However, we were mainly interested in the effect of chronic THC treatment on exploratory behaviors. THC attenuated hyperrearing in DAT KD mice (albeit at a trend). This effect should be clarified by adding further cohorts to this study given that similar directions of effects were seen for poking and spatial d in DAT KD mice.

Through cyclic voltammetry experiments in DAT KD mice striatal slices, elevated dopamine was observed [19]. The rate whereby dopamine is cleared remains unchanged by inhibitors of norepinephrine and serotonin transporters, by monoamine oxidase (MAO) (an enzyme that selectively inhibits dopamine degradation), or catechol-O-methyl transferase (COMT) [19]. Thus, other techniques affecting this dopamine clearance would be required, if indeed such elevated levels are seen in people with BD mania.

The catecholaminergic-cholinergic balance hypothesis indicates that elevated catecholamines levels occur during manic states and increased cholinergic functioning in depression state [3]. Dopamine, a catecholamine, is observed as the most fundamental factor in the pathophysiology of BD [3]. However, dysregulation in dopaminergic neurotransmission is not the only underlying factor in BD. Other neurotransmitters are also relevant in BD [6]. The brains of the mice from the BPM cohort were collected after BPM testing and will be assessed for potential changes in dopamine receptor expression, indicative of potential consequences of chronic THC treatment.

Difficulties in psychiatry studies occur when trying to use reproducible basic models, due to two main reasons: 1) Symptoms in psychiatric disorders are based on diagnostic categories (BD I and BD II) that are mainly subjective and heterogenous. That means that recreating the alternating episodes of mania and depression remain difficult [2][24]. 2) Knowledge about etiology and pathophysiology of

psychiatric disorders is still limited [24]. It is also challenging to model psychiatric disorders due to their complexity and these models include reproducibility issues [3][24]. Genetic variability and a variety of mania and depression symptoms make it difficult to model BD in rodents [29]. To understand the neurobiological substrates of bipolar circumstances it is remarkable useful to utilize animal models [3][20]. While these animal models can replicate a limited part of the disease. They may be beneficial to use to understand the pathophysiology of BD, and these models may also be useful to develop newer treatment strategies [4]. Currently, animal models are mainly focused on reproducing mania-like symptoms in rodents because mania is the core feature of BD [24]. That means that animal models like this lack reproducibility of depression-like symptoms.

Valproate is a standard treatment for BD. Valproate attenuates hyperactivity in animal models. However, valproate does not treat every facet of BD, and did not normalize DAT KD behaviors, such as increased specific exploration and more continuous movements [22]. In this study it was seen that there were trend effects on rearing, pokes, and spatial d (all of which are measures of exploratory behaviors) when administering chronic THC. Therefore, THC may be able to address aspects of BD that valproate or other BD treatments cannot address.

In conclusion, we were able to see that chronic THC on DAT KD genotype might attenuate exploratory behavior in DAT KD model of bipolar mania. However, we were not able to investigate the effect of chronic THC on temporal perception because the mice learned the TDT more slowly than anticipated.

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APPENDIX

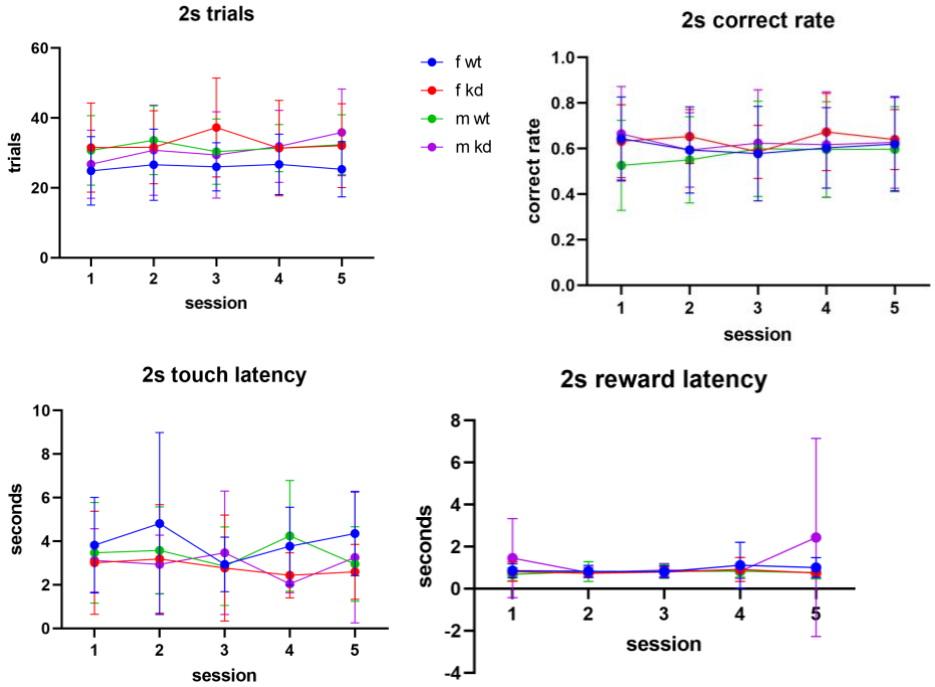

Fig. 7: A) Amount of 2s trials completed did not differ at this point in training between WT mice, DAT KD mice, male mice, or female mice (p = 0.3302). B) Amount of 2s trials responded to correctly similarly did not differ based on genotype or sex (p = 0.7622). C) Amount of 2s touch latency did not differ between DAT KD mice as well as WT mice. (p = 0.3451)D) Amount of 2s reward latency was similar in all mice. (p = 0.2887)

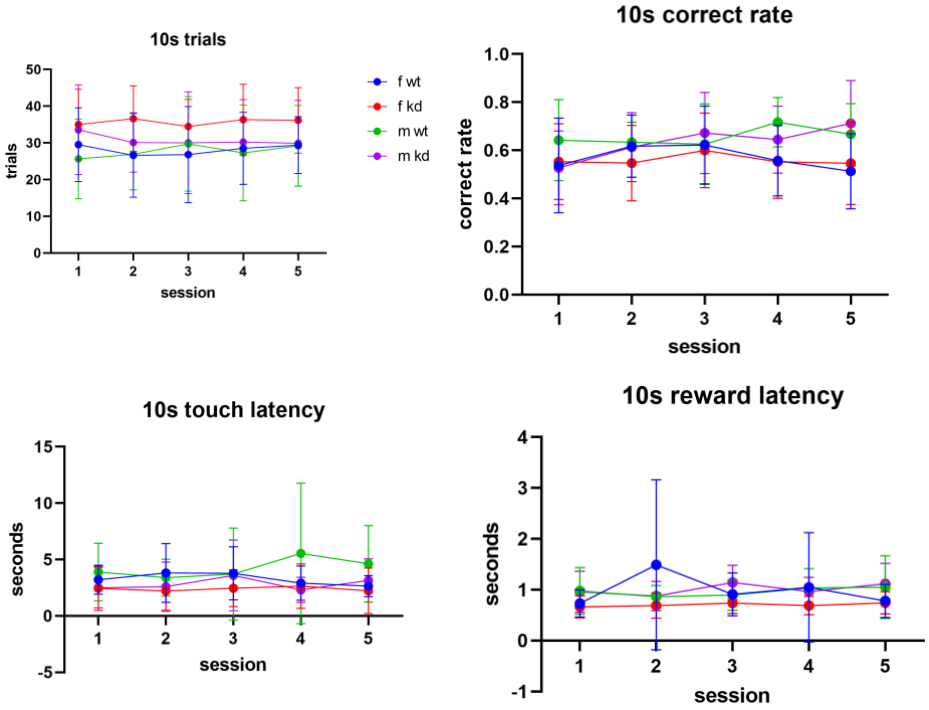

Fig. 8: A) Amount of 10s trials completed did not differ at this point in training between WT mice, DAT KD mice, male mice, or female mice ($p = 0.1695$)B) Amount of 10s trials responded to correctly similarly did not differ based on genotype or sex ($p = 0.1165$) C) Amount of 10s touch latency did not differ between DAT KD mice as well as WT mice ($p = 0.149$). D) Amount of 10s reward latency was similar in all mice. ($p = 0.0253$)