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Paternal THC exposure in rats causes long-lasting neurobehavioral effects in the offspring

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ABSTRACT

Developmental neurotoxicity of a wide variety of toxicants mediated via maternal exposure during gestation is very well established. In contrast, the impacts of paternal toxicant exposure on offspring neurobehavioral function are much less well studied. A vector for paternal toxicant exposure on development of his offspring has been identified. Sperm DNA can be imprinted by chemical exposures of the father. Most but not all of the epigenetic marks in sperm are reprogrammed after fertilization. The persisting epigenetic marks can lead to abnormal genetic expression in the offspring. We have found that paternal delta-9-tetrohydrocannabinol (THC) exposure in rats causes changes in methylation of sperm (Murphy et al., 2018). This is similar to cannabis-associated changes in sperm DNA methylation we found in human males who smoke cannabis (Murphy et al., 2018). In the current study we investigated the intergeneration effects of THC exposure of young adult male rats (0 or 2 mg/kg/day orally for 12 days) to the neurobehavioral development of their offspring. This paternal THC exposure was not found to significantly impact the clinical health of the offspring, including litter size, sex ratio, pup birth weight, survival and growth. However, it did cause a long-lasting significant impairment in attentional performance in the offspring relative to controls when they were tested in adulthood. There was also a significant increase in habituation of locomotor activity in the adult offspring of the males exposed to THC prior to mating. This study shows that premating paternal THC exposure even at a modest dose for a brief period can cause deleterious long-term behavioral effects in the offspring, notably significant impairment in an operant attention task. Further research should be conducted to determine the degree to which this type of risk is seen in humans and to investigate the mechanisms underlying these effects and possible treatments to ameliorate these long-term adverse behavioral consequences of paternal THC exposure.

1. Introduction

Cannabis is widely used in the US and with broadening legalization, its use is increasing. With this widespread use, the potential health risks of cannabis are of growing concern, including effects on reproduction and development. Considerable research has investigated risks associated with maternal exposure to cannabis and THC during gestation to assess the risk for persistent effects in the offspring (El Marroun et al., 2018). In contrast, very little has been done regarding paternal exposure to marijuana or THC prior to conception and its impacts on the development of offspring. With the discovery and characterization of epigenetic modifications of DNA of all cells, including sperm, new vectors are being investigated by which preconception paternal chemical exposure may affect the development of future offspring (Soubry et al., 2014).

We have recently shown that chronic preconception exposure of male rats to THC, the primary psychoactive compound of interest in cannabis, significantly alters genomic marking of the sperm (Murphy et al., 2018). These data were convergent with data from human males showing that cannabis smoking is associated with significant alterations in methylation of the human sperm genome (Murphy et al., 2018). Important overlaps were seen with the methylation changes in the sperm of rats and human male cannabis smokers.

As offspring inherit a portion of their DNA methylation pattern from paternal sperm (Tang et al., 2015), epigenetic data on males exposed to THC or cannabis suggest a mechanism by which paternal cannabis use

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may impact the development of the next generation. To date though, there is little data to suggest how epigenetic effects of paternal cannabis or THC exposure may impact function in the offspring, including neurological and behavioral functions. Similar work in rodent models suggests that paternal exposure to other drugs of abuse, such as alcohol, nicotine, stimulants and opiates, can alter the behavior of the offspring (Goldberg and Gould, 2018). These effects tend to be specific to certain behavioral outcomes, rather than general defects, but can impact functions that are relevant to neurobehavioral disorders. It is hypothesized that paternal THC exposure prior to conception will carry similar risks.

In the current study, we exposed young adult male rats to 0 or 2 mg/kg/day of THC for 12 days, the same exposure which was found to significantly alter methylation of sperm in our previous study (Murphy et al., 2018). Then we assessed the effects of that exposure on reproduction and neurobehavioral development of the offspring. Behavioral function was measured in a test battery including tests of locomotor activity, emotional function and cognition.

2. Methods

2.1. Design

The intergenerational effects of paternal THC exposure were investigated by dosing young adult Sprague-Dawley rats with 2 mg/kg/day of THC by gavage. Controls received vehicle. The rats were then bred with drug-naive females. One male and one female offspring from each litter were tested on a battery of behavioral tests to assess locomotion, emotional function and cognition. Subjects were maintained on a reversed 12/12 day-night cycle and had ad libitum access to food and water, unless stated otherwise. All testing occurred under low, ambient light conditions during the animal's dark phase (between 8:00–17:00). All study protocols were approved by the Institutional Animal Care and Use Committee at Duke University and conducted in accordance with federal guidelines.

2.2. Paternal THC exposure

Nine-week-old, sexually mature male Sprague Dawley rats were housed 2–3 per cage and were dosed daily for 12 days via oral gavage. There were two treatment groups: controls (N = 9) with 4 ml of vehicle only (10% ethanol, 1% Triton X-100 in saline) and THC-exposed rats (N = 8) receiving 2 mg/kg THC (Sigma-Aldrich St Louis, MO, USA) in 10% ethanol, 1% Triton X-100 in saline. This THC dose was selected because it modeled human moderate daily cannabis use (Harte and Dow-Edwards, 2010; Irimia et al., 2015; Rubino et al., 2009). The oral rout of exposure was chosen because many people consume cannabis by this route. More self-administer cannabis by smoking but this was not chosen as a route for the current study because chronic smoke exposure is stressful for rats and the dose administered is difficult to control. The males were group housed 2–3/cage with all animals housed with others of the same treatment group. The sequence of paternal THC exposure and offspring behavioral testing is displayed in Table 1.

2.3. Paternal behavioral testing

The THC-exposed and control males were tested on the 16-arm radial maze to determine if the dose of THC given affected cognitive function. The methods for 16-arm radial maze testing were the same as used for the offspring detailed below.

2.4. Mating

One day after the end of THC exposure the male rats were mated to drug naïve females. Each THC-exposed and control male was housed together with a drug-naive young adult female Sprague-Dawley rat for four days. The dams were housed singly with their litters. Weaning occurred on day 21 after birth. Then the offspring were housed in same-sexed groups.

2.5. Behavioral testing of the offspring

Behavioral assessment began during adolescence and continued into adulthood with a battery of tests to index long-term effects of paternal THC exposure on offspring locomotor activity, cognition and emotional response during the latter stages of development into adulthood. There were six control litters and seven litters with THC exposed fathers. One male and one female from each litter were tested on the following behavioral test battery. This test battery covers a variety of cognitive, motor and emotional functions. It has been found to be sensitive to the effects of a variety of developmental toxicant exposures (Levin et al., 2010; Roegge et al., 2008; Timofeeva et al., 2008a; Timofeeva et al., 2008b).

2.5.1. Week 4: elevated plus maze

The rats were tested on the elevated plus maze (Med Associates, St Albans, VT, USA) to assess their anxiety-like behavior vs. risk-taking behavior. The maze measured 142-cm x 104-cm x 76-cm high and consisted of two arms with 15-cm high, enclosed walls and two open arms with 2-cm railings. Each rat was assessed individually on the elevated plus maze for a single five-min session. The percentage of time the rat spent in the open vs. enclosed arms of the maze was calculated as an index of anxiety vs. risk taking. Also, the number of crossings across the center was counted as a measure of activity. The dependent measures were percent of time in the open arms to index anxiety-like behavior and the number of center crossings to measure locomotion in this five-min test.

2.5.2. Week 5: figure-8 locomotor activity test

Locomotor activity and its habituation were assessed in an enclosed maze in the shape of a figure-8 with two side alleys. The Figure-8 apparatus had a continuous alley measuring 10-cm x 10-cm, with the entire maze measuring 70-cm x 42-cm. Animals were permitted to freely explore the apparatus. Locomotor activity was indexed by the crossing of eight photo-beams located at approximately equal points throughout the alley. Photo-beam breaks were tallied in 5-min blocks across the one-hour test session. The mean number of photobeam breaks per five-min block within the session indexed locomotor activity.

Table 1
Paternal THC exposure and behavioral testing of offspring.

Paternal THC	Behavioral testing of offspring					
(2 mg/kg/day)	Postnatal weeks					
For 12 days Ending One day Before Mating	4 Elevated Plus Maze	5 Figure-8 Locomotor Activity Test	6 Novelty Suppressed Feeding	7 Novel Object Recognition	8–11 Radial Arm Maze	12–40 Operant Visual Attention Task

Table 2
Developmental health measurements.

	Maternal		Litter		Birth weight (g)		Weaning Weight (g)	
	%Pregnant	Weight gain (g)	Number	%Male	Male	Female	Male	Female
Control	66.7	62.5 ± 7.2	12.2 ± 0.8	46.3 ± 5.0	7.4 ± 0.3	7.0 ± 0.2	47.9 ± 1.7	46.3 ± 1.2
THC	77.8	62.1 ± 3.3	13.3 ± 0.7	57.8 ± 4.6	7.6 ± 0.2	7.3 ± 0.2	47.1 ± 2.8	45.3 ± 2.4

The linear trend of decreasing beam breaks over the twelve sequential time blocks within the session indexed the habituation of activity with experience in the apparatus over the one-hour session.

2.5.3. Week 6: novelty suppressed feeding

To assess fear responsivity, the offspring rats were tested for the suppression of feeding behavior in a novel environment. Each rat had food restricted for 24-h prior to the test session. The novel environment consisted of a plastic rectangular cage (different from the home cage) placed in the middle of a brightly lit testing room, with no cage top and no bedding in the cage. Twelve standard rat chow pellets were weighed before testing and were spread across the cage floor in 4 rows of 3 pellets each. The sessions lasted 10 min. Eating was defined as the act of chewing the food and not merely sniffing, holding, or carrying the food around in the mouth. The food pellets, which remained after the test session, were weighed to determine the amount of food eaten. The dependent measures were: amount of food eaten, latency to begin eating, the number of eating bouts and the duration of eating.

2.5.4. Week 7: novel object recognition

Recognition of a novel vs. familiar object was used to test attention and memory in a low-motivational state. Tests were conducted in opaque plastic enclosures measuring 70-cm x 41-cm x 33-cm. Objects consisted of plastic, glass, or ceramic material and were randomized for each animal. Animals were first habituated to the apparatus in two 10-min sessions over the course of two days. Testing began on day 3 with a 10-min familiarization session in which two identical objects (A/A) were placed in the cage for the animal to explore. The A/A session was then followed by a 1-h period spent in the animal's home cage. The animal was then placed back in the enclosure with one object from the A/A session and with another, dissimilar, "novel" object (A/B session). Between sessions, the objects were wiped clean in order to avoid odor recognition cues by the rats. The test session lasted for ten min. Analysis considered the preference in the first and second halves of the sessions. The behavior during the first five-min block within the session was with a more clearly differential novelty of the two objects compared with the second five min of the test session. The time in seconds spent actively exploring each object was recorded during each five-min block during the ten-min session and used for analysis.

2.5.5. Week 8–11: radial-arm maze

Spatial learning and memory were tested in the 16-arm radial maze. The maze was made of wood black painted with a central platform (50-cm diameter) and 16 radiating arms, each 10-cm wide x 60-cm in length. A food cup was positioned 2 cm from the end of each arm. Visual cues (cardboard shapes) were on the walls of the testing room to facilitate spatial orientation. The rats were habituated in the maze for two 10-min sessions in which they were placed on the central platform inside a large, black, round, opaque cylinder, with half-pieces of sugar coated cereal (Froot Loops®; Kellogg's Inc., Battle Creek, MI, USA). For the test sessions, twelve of the arms were baited at the beginning of each session to test working memory performance and the other four arms were always left un-baited to test reference memory (Hall et al., 2016). The baited arms of the maze for each rat remained constant throughout the entire series of testing sessions, but which arms were baited differed randomly between rats. Each trial began by placing the rat on the central platform inside the opaque cylinder for 10 s. Then the cylinder was lifted and the rat was allowed to roam the maze freely. Each session lasted 10 min or until the rat had entered all twelve baited arms, whichever occurred first. Each rat was assessed for working and reference memory errors over 18 sessions. Working memory errors were counted as repeat entries into baited arms, and reference memory errors were counted as entries into the arms that were never baited. Duration of responding was calculated as the total session time divided by the number of arm entries. There was one session run per day. The dependent measures were the number of working and reference memory errors as well as response duration (seconds per arm entry).

2.5.6. Weeks 12–40: operant visual attention task

The attention test was conducted as described in detail previously (Hall et al., 2016). There is a long period of training for it. All of the rats began at the same age. Each rat was placed in an operant chamber and trained to press one of two retractable levers in response to a visual cue light that was illuminated for a duration of 500 ms. If the cue-light became illuminated ("signal" trial), the animal needed to press the lever designated as the "signal" lever to receive a 20 mg food pellet reward. If the cue-light was not illuminated ("blank" trial), the animal needed to press the opposite lever in the chamber to receive the reward. The position (left, right) of "signal" and "blank" levers was randomized among the rats. If the rat made no response within 5 s of insertion of the response levers into the chamber, both levers retracted and a response "failure" was recorded. There were equal numbers of "signal" and "blank" trials in each test session with a total of 240 trials. "Hit" responses were correct choices on the signal trials while "correct rejection" responses were correct choices on blank trials. Percent correct hit and percent correct rejection per session were the dependent measures for response accuracy on this attention task. Analysis was conducted of the choice accuracy data including these factors as well as THC exposure and sex.

2.6. Data analysis

For each behavioral test, the data were evaluated by analysis of variance. Litter was the unit of variance. The between-litters factor was THC treatment. The within litter factor was sex. Within-subjects repeated factors were sessions and time blocks within session. Because each litter contributed one male and one female, sex was treated as a repeated measure within litter. Significance was assumed at the level of $p < 0.05$ (two-tailed). For interactions at $p < 0.10$, we also examined whether lower-order main effects were detectable after subdivision of the interactive variables (Snedecor and Cochran, 1967). The $p < 0.10$ criterion for interaction terms was not used to assign significance to the effects, but rather to identify interactive variables requiring subdivision for lower-order tests of the main effects of THC, the variable of chief interest. A cut-off of $p < 0.05$ (two-tailed) was used as the threshold for statistical significance.

3. Results

3.1. Paternal effects

There were seven litters from young adult Sprague-Dawley male rats

Table 3
Statistical analyses of behavioral tests of the offspring.

Elevated plus maze					
Percent open arm time	*p*-Value	Mean percent			
	Control	THC			
THC	0.49	27.4		32.0	
	Male			Female	
Sex	0.12	24.6		35.2	
THC × Sex	0.91	Male		Female	
Control		21.7		33.1	
THC		27.0		36.9	
Center Crosses	p-value	Mean number			
		Control		THC	
THC	0.55	2.25		2.79	
		Male		Female	
Sex	0.14	1.85		3.23	
THC × Sex	0.44	Male		Female	
Control		1.17		3.33	
THC		2.43		3.14	
Figure-8 Apparatus Activity	p-value	Mean beam breaks/5-min block			
		Control		THC	
THC	0.22	45.4		42.8	
		Adolescent		Adult	
Age	< 0.0005	37.8		50.2	
		Male		Female	
Sex	< 0.05	42.3		45.6	
THC × Sex	0.68	Male		Female	
Control		43.2		47.5	
THC		41.5		44.0	
5-min Block	< 0.0005				
THC × Age × Block	< 0.05	Mean linear trend			
Habituation Linear Trend		Control		THC	
Adolescent	0.99	1.28		1.27	
Adult	< 0.05	1.32		1.97	
Novelty Suppressed Feeding	p-value	Mean seconds			
		Control		THC	
THC	0.12	124.9		91.6	
		Male		Female	
Sex	0.90	107.8		106.2	
THC × Sex	0.54	Male		Female	
Control		132.5		117.3	
THC		86.6		96.7	
Novel Object Recognition	p-value	Mean seconds			
		Control		THC	
THC	0.42	30.0		27.7	
		Male		Female	
Sex	0.83	29.2		28.3	
THC × Sex	0.61	Male		Female	
Control		29.5		30.6	
THC		29.0		26.4	
Novel vs. Familiar	< 0.0005	Novel		Familiar	
		33.4		24.2	
5-Min Time Block	< 0.0005	Min 1–5		Min 6–10	
		34.5		23.1	
Radial-Arm Maze	p-value	Mean errors			
		Control		THC	
THC	0.61	8.63		8.34	
		Male		Female	
Sex	0.81	8.41		8.54	
THC × Sex	0.85	Male		Female	
Control		8.51		8.76	
THC		8.32		8.35	
Error type	< 0.0005	Working		Reference	
		10.42		6.53	
Session Block	< 0.0005	1–3	4–6	7–9	10–12
		9.49	9.92	7.64	6.85
Signal Detection Attention Test	p-value	Mean percent correct			
		Control		THC	
THC	< 0.05	87.4		84.1	
		Male		Female	
Sex	0.88	85.8		85.4	
THC × Sex	0.66	Male		Female	
Control		87.1		87.7	
THC		84.7		83.5	
		Hit		Correct rejection	
Trial Type	< 0.0005	82.0		89.2	
THC × Trial type	0.07	Control		THC	
Hit	< 0.05	84.9		79.6	
Correct Rejection	0.49	89.9		88.6	

that were orally dosed with 2 mg/kg/day of THC for 12 days and then mated with drug-free females. For comparison there were six litters from young adult Sprague-Dawley male rats that were orally dosed with the vehicle only for 12 days and then mated with drug-free females. No significant effects were seen clinical health and body weight of the THC exposed males. Nor were there any significant THC effects seen with radial-arm maze performance of the paternal rats exposed to THC vs. controls.

3.2. Clinical signs of health

No significant effects were seen with litter size, sex ratio, birthweight or subsequent growth (Table 2). This moderate dose of THC administered to male rats during 12 days did not significantly affect rates of conception and birth indices (Table 3).

3.3. Elevated plus maze test of anxiety-like behavior

There was no significant effect of paternal THC exposure on behavior in the elevated plus maze, either with center crosses (short-term locomotor activity during a five-min session) or percent open arm time (measure of anxiety, lower scores indicate greater anxiety). These results are shown in Table 2.

3.4. Figure-8 apparatus locomotor activity test

This test of locomotor activity and its habituation in a one-hour session was run twice, once during adolescence and again in young adulthood. Significant main effects were seen with testing age ($F(1,11) = 77.10$, $p < 0.0005$), sex ($F(1,11) = 5.79$, $p < 0.05$) and time block within session ($F(11,121) = 56.53$, $p < 0.0005$). The activity scores were greater for adults, the females were more active than males and activity scores declined over the course of the session. All of these effects were expected and demonstrated the validity and reproducibility of the test. The THC main effect was not significant. However, there was a significant paternal THC x age x session time block interaction ($F(11,121) = 2.02$, $p < 0.05$) indicating that the differential rates of habituation over the course of the test session between rats with control and THC-treated fathers were differentially expressed when the offspring were adolescents and adults. Follow-up tests of the linear trend effects of paternal THC exposure in adolescents and adults over the course of the twelve 5-min blocks within the 1-h session indicated that adult rats with THC-exposed fathers showed significantly ($F(1,11) = 5.62$, $p < 0.05$) more rapid habituation of locomotor activity than the offspring of controls (Fig. 1). This effect was not seen when the rats were adolescents.

3.5. Novelty suppressed feeding test of fear response

In this test of fear response, there was a trend toward an effect of THC decreasing the latency to begin eating in the novelty suppressed feeding test, but this was not significant ($p = 0.12$). The mean latency to begin eating for male control offspring was 132.5 ± 14.7 s, while male offspring of THC-treated fathers took 86.6 ± 14.3 s to begin eating. For females, control offspring took 117.3 ± 20.8 s, while the

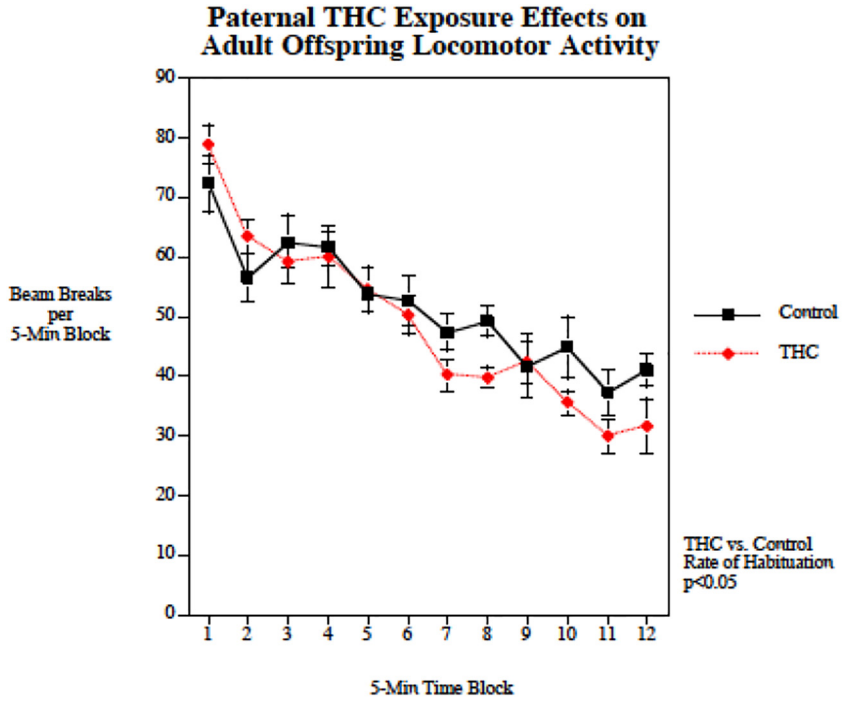

Fig. 1. Paternal THC exposure effects on locomotor activity of offspring in the figure-8 apparatus (mean ± sem). Adult offspring of THC-exposed fathers showed a significantly ($p < 0.05$) more rapid habituation of locomotor activity in the Figure-8 apparatus.

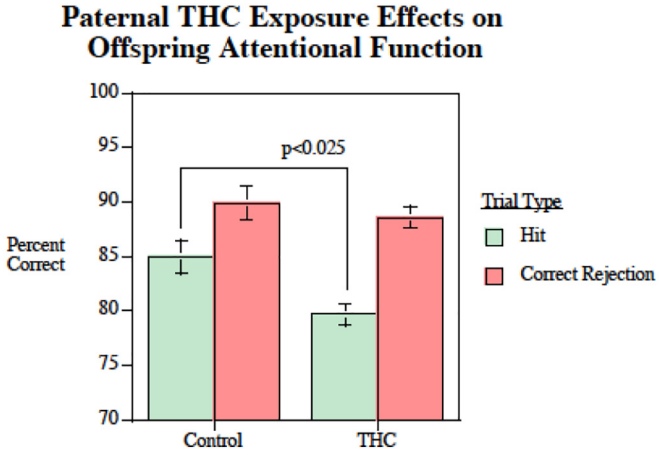

Fig. 2. Paternal THC exposure effects on attentional of offspring in the visual signal detection task (mean ± sem). Adult offspring of THC-exposed fathers showed a significant ($p < 0.025$) impairment in percent correct hit response on the signal detection attention operant test.

female offspring from THC-treated fathers took 96.7 ± 27.2 s to begin eating.

3.6. Novel object recognition test of non-spatial memory

In this test of non-spatial memory there was a significant ($F(1,11) = 25.04$, $p < 0.0005$) main effect of the rats investigating the novel object more than the familiar object. There was also a significant decrease in investigation as the test progressed and the objects became less novel ($F(1,11) = 24.65$, $p < 0.0005$). This demonstrates that the test was operating as intended. There were no significant paternal THC-related effects in the novel object recognition test.

3.7. 16-Arm radial maze test of spatial memory

In this test of spatial working and reference memory, the rats learned with significant ($F(3,66) = 7.43$, $p < 0.0005$) reduction of errors over the four successive 3-session blocks. No significant effects of paternal THC exposure were detected with error rates on this test. There was also a significant quickening in response with continued training ($F(3,66) = 72.25$, $p < 0.0005$), but again no significant paternal THC effect was detected.

3.8. Operant visual signal detection test of attention

In this operant visual signal detection test of attentional function the main effect of paternal THC treatment was significant ($F(1,11) = 4.91$, $p < 0.05$) with the offspring of fathers exposed to THC prior to mating having significantly lower percent correct (84.1 ± 0.7) than controls (87.4 ± 0.7). There was an interaction between paternal THC treatment and trial type ($F(1,11) = 4.15$, $p < 0.07$) that prompted further analysis of the simple main effects of paternal THC within each trial type (Percent hit and percent correct rejection). This further analysis showed that paternal THC treatment caused a significant ($F(1,11) = 8.81$, $p < 0.025$) decrease in percent hit performance (Control = 84.9 ± 1.5%; THC 79.6 ± 1.0%), while percent correct rejection was not significantly affected (Control = 89.9 ± 1.5%; THC = 88.6 ± 1.0%) (Fig. 2). There was also a three-way interaction of paternal THC exposure x sex of the offspring x session ($F(5,55) = 2.17$, $p < 0.08$) that prompted tests of the linear trend effects of paternal THC exposure over the six sessions for each sex. There were no significant differences in the linear trend of improvement. Fig. 3 shows the percent correct data broken down in detail by sex and session.

4. Discussion

Twelve consecutive days of THC exposure in young adult male rats at a dose that modeled moderate cannabis use (2 mg/kg/day, PO) produced significant behavioral effects in their offspring relative to the

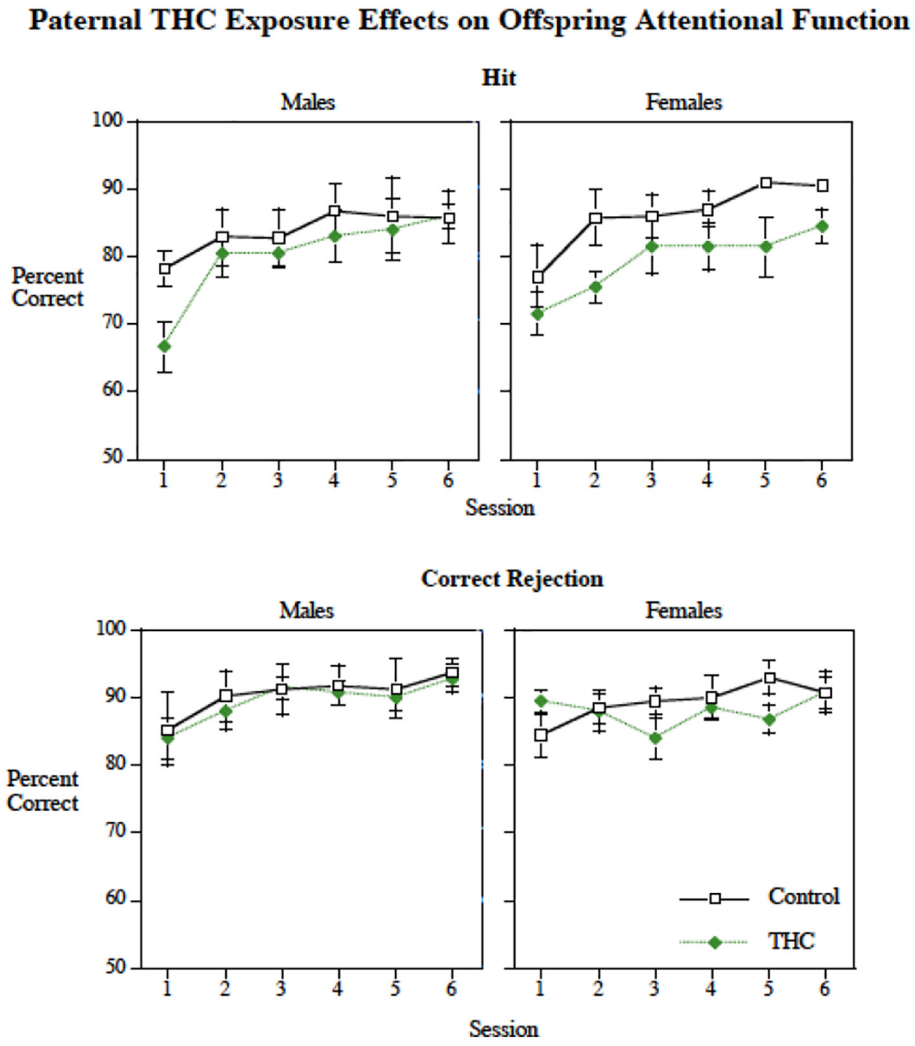

Fig. 3. Detailed breakdown of percent correct in the visual signal detection task (mean ± sem) divided by sex and session.

offspring of males exposed to the vehicle. This dose and duration of exposure did not produce overt health impairments in either the male rats or their offspring and it did not cause cognitive impairment in the male rats directly exposed. However, twelve days of 2 mg/kg/day of THC in young adult male rats did cause significant behavioral effects in the offspring of these rats and drug naïve females. These behavioral effects of paternal THC were still seen in the offspring when they became adults. The paternal THC neurobehavioral effects were fairly specific, as the bulk of tests in the behavioral battery detected no effects of paternal THC exposure. Interestingly, both of the significant effects of paternal THC exposure prior to conception were evident in adulthood. Paternal THC exposure led to a small but significant increase in the slope of locomotor habituation in the figure-8 maze in adulthood. There was also a clearly significant paternal THC effect on attentional accuracy, whereby subjects were significantly less accurate in their choice of lever when the brief cue was presented beforehand (hits). Accuracy on trials where no cue was presented (correct rejections) was unaffected. Poor signal detection of the light cue may indicate an impairment in sustained attention.

The vehicle for THC used in this study contained 10% ethanol. Blood ethanol levels were not taken in this study. From other studies in the literature it is estimated that the blood ethanol concentrations would have been below approximately 115 mg% (Walker and Ehlers, 2009). This ethanol dose is relatively low for causing physiological effects. The controls also received the same dose of ethanol in the vehicle. However, it is possible that there may have been a unique interaction of paternal ethanol and THC on behavioral function in the offspring.

The present findings are generally in agreement with the existing literature on paternal preconception exposure to drugs of abuse. As with other drugs of abuse (Goldberg and Gould, 2018), paternal THC exposure led to highly specific alterations in behavior which affected select assays while sparing general offspring health and unrelated behaviors. These data are also complementary to the few available studies that have investigated paternal cannabinoid effects on behavior. Andaloussi and colleagues (Andaloussi et al., 2019) found that paternal exposure to the synthetic cannabinoid WIN55,212–2 during adolescence failed to produce baseline changes in activity, avoidance of open spaces, novel object recognition and stress responsivity in the next generation. Effects on anxiety-like open space avoidance and stress responsivity were only evident following a chronic unpredictable stress regime. Szutorisz and colleagues (Szutorisz et al., 2014) also failed to show changes in locomotor activity in offspring where both parents had been exposed to THC prenatally. Parental THC effects were only evident when undergoing heroin self-administration, where these offspring showed greater lever pressing for heroin under an elevated fixed ratio and altered behavior following the end of the self-administration. In general, these studies and the present data suggest that offspring of THC exposed fathers are generally healthy and behaviorally indistinguishable from developmentally typical animals, but that underlying differences may still be expressed under specific conditions. In the present study, the operant attention task challenged the subjects sufficiently to

detect the paternal treatment effect.

Attentional deficits are characteristic symptoms of attention deficit hyperactivity disorder (ADHD), and as such, are highly comorbid with a wide range of psychiatric and behavioral disorders (e.g. (Solberg et al., 2018)). In general, attention deficits tend not to have known causes, with the exception of toxicant-induced deficits such as fetal alcohol or tobacco exposure (Kingdon et al., 2016; Pagani, 2014). The present rodent data suggest that paternal epigenetic effects may represent an additional candidate cause of attentional dysfunction, as preconception THC exposure led to an inattentive phenotype in the absence of other risk factors. Further, these data indicate that THC is a compound of particular interest in this area and requires further investigation.

The neurobehavioral effects seen in the offspring of male rats exposed to THC prior to mating may have been caused by abnormal DNA methylation patterns in the sperm (Murphy et al., 2018). In our previous article we reported on the character of this THC-induced abnormal methylation (Murphy et al., 2018). In that study, we evaluated associations between cannabis/THC exposure and altered DNA methylation in sperm from humans and rats. DNA methylation, measured by reduced representation bisulfite sequencing, differed in the sperm of human users from non-users by at least 10% at 3979 CpG sites. Pathway analyses indicated Hippo Signaling and Pathways in Cancer development as enriched with altered genes. These same two pathways were also enriched with genes having altered methylation in sperm from THC-exposed versus vehicle-exposed rats ($p < 0.01$). Data validity is supported by significant correlations between THC exposure levels in humans and methylation for 177 genes, and substantial overlap in THC target genes in rat sperm in this study and genes previously reported as having altered methylation in the brain of rat offspring born to parents both exposed to THC during adolescence (Szutorisz et al., 2014). In humans, cannabis use was also associated with significantly lower sperm concentration (Murphy et al., 2018). Most of the methylation marks are removed upon fertilization, although there are some which remain and are passed on to the offspring (Tang et al., 2015).

With the current study, we have shown that this relatively short-term (12 days) and modest dose of THC (2 mg/kg/day) causes significant behavioral changes in the offspring. This extends our previous finding that the same exposure causes significant methylation changes in the sperm (Murphy et al., 2018). It has long been known that maternal exposure after conception to a wide variety of environmental chemicals, therapeutic drugs and drugs of abuse including cannabis can cause neurobehavioral impairments in the offspring. This study and others are contributing to a quickly emerging literature that paternal chemical exposure before conception can produce behavioral alterations in the offspring that persist into adulthood.

Transparency document

The Transparency document associated with this article can be found, in online version.

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