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Short-term genetic selection for adolescent locomotor sensitivity to delta9-tetrahydrocannabinol (THC)

Chelsea R. Kasten[*,a], **Yanping Zhang**[a], **Ken Mackie**[b], and **Stephen L. Boehm II**[a,c]

[a]Department of Psychology, Indiana University – Purdue University – Indianapolis, Indianapolis, IN

[b]Department of Psychological and Brain Sciences and the Gill Center, Indiana University Bloomington, IN

[c]Indiana Alcohol Research Center, Indianapolis, IN

Abstract

Cannabis use is linked to positive and negative outcomes. Identifying genetic targets of susceptibility to the negative effects of cannabinoid use is of growing importance. The current study sought to complete short-term selective breeding for adolescent sensitivity and resistance to the locomotor effects of a single 10 mg/kg THC dose in the open field. Selection for THC-locomotor sensitivity was moderately heritable, with the greatest estimates of heritability seen in females from the F2 to S3 generations. Selection for locomotor sensitivity also resulted in increased anxiety-like activity in the open field. These results are the first to indicate that adolescent THC-locomotor sensitivity can be influenced via selective breeding. Development of lines with a genetic predisposition for THC-sensitivity or resistance to locomotor effects allow for investigation of risk factors, differences in consequences of THC use, identification of correlated behavioral responses, and detection of genetic targets that may contribute to heightened cannabinoid sensitivity.

Keywords

adolescent; cannabinoids; THC; locomotion; selection; genetic

Introduction

The Centers for Disease Control and Prevention (CDC) reported that, as of 2014, 7.4% of those aged 12–17 and 19.6% of those aged 18–25 had used marijuana in the past month. Although rates of use in adolescents and young adults have remained relatively stable since 2012, the percentage of individuals perceiving “no risk” of marijuana use has nearly doubled

[*]Corresponding author: Chelsea Kasten 402 N Blackford St, LD 124, Indianapolis, IN 46202, ckasten@iupui.edu.

Ethical Statement

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Author Contributions

All authors were responsible for the study concept, design, and writing and reviewing the manuscript. YZ was responsible for acquisition of behavioral data. CRK was responsible for colony management and data processing.

in that same time period to 17.6% of adolescents and 36.6% of young adults (Azofeifa et al. 2016). Preclinical studies have indicated that the cannabinoid system undergoes rapid development over the course of adolescence into adulthood. Although cannabinoid 1 receptor (CB1R) expression mimics the typical overexpression and cortical synaptic pruning pattern which occurs from adolescence to adulthood, CB1R binding in gray matter increases from adolescence to adulthood in regions including the hippocampus and cortex (Heng et al. 2011; Verdurand et al. 2011). This phenomenon may result from increased receptor functionality to compensate for losses in other systems, or may be indicative of CB1R's role in elongating axons from white matter to their final gray matter destinations (Romero et al. 1997; Keimpema et al. 2010; Verdurand et al. 2011).

In the USA, 28 states and the District of Columbia currently have laws permitting medical marijuana use, with some of those states also moving to permit recreational use and/or decriminalize the possession of small amounts of marijuana (Bestrashniy & Winters 2015; National Academies of Sciences 2017). In a recent review of existing policy and scientific research, the National Academies of Sciences (2017) reported that cannabis use is linked to both positive and negative outcomes. Modest effects exist for attenuating chemotherapy-induced nausea, chronic pain, and spasticity associated with multiple sclerosis. Conversely, increased cannabis use may result in development of cannabis and other drug abuse, with adolescent onset of use increasing such risks. Cannabis use is associated with impairing domains that are particularly important to normal development in adolescents, including cognitive decline as well as long-term impairments in attaining education, lower employment and income, and poorer social relationships. Although it is unclear whether medical marijuana laws contribute to the views and patterns of cannabis use in adolescents (Cerdá et al. 2017; Johnson et al. 2017) and how significantly adolescent use negatively impacts developmental trajectory (National Academies of Sciences, 2017), the potential unique susceptibility of adolescents to long-term consequences of cannabis use is an important consideration.

The psychoactive effects of the cannabis plant are attributed to THC (Pertwee, 2008). Behaviorally active cannabinoids produce a classic dose-dependent response in the tetrad assays: antinociception, hypothermia, catalepsy, and hypolocomotion (Martin et al., 1991; Wiley et al., 2014). Individuals that use cannabis often self-report subjective positive or negative changes in feelings of sedation (National Academies of Sciences, 2017), indicating that sensitivity or resistance to this effect of THC may influence future cannabinoid use in a positive or negative manner. Although sedation is often considered as a negative side effect of drug use, individual experiences of sedation are subjective. People that struggle with disorders characterized by hyperactivity or over-arousal may report feelings of sedation with positive terms, such as "relaxation," which is strongly linked to frequent adolescent cannabinoid use (Camera et al., 2012). Physical sedation may be examined preclinically by observing drug-induced changes in locomotor activity. Adolescent B6 mice are sensitive to THC-induced reductions in locomotion, whereas adolescent D2 mice do not demonstrate this same sensitivity (Kasten et al., 2017). Less than 1 in 5 adolescent marijuana users report feelings of sedation (Camera et al., 2012), indicating that sensitivity to the sedating effects of cannabis may inhibit excessive cannabis use. Limited use may protect individuals from the negative outcomes associated with adolescent use.

The goal of the current study was to determine whether sensitivity to the locomotor effects of Δ9-tetrahydrocannabinol (THC) could be influenced using a short-term selective breeding strategy to produce sensitive and resistant mice. Using a B6D2F2 founding population, adolescent mice were phenotyped for THC-induced reductions in basal locomotor activity in the open field following a 10 mg/kg injection of THC. Locomotor activity can be quickly measured during the adolescent time frame, testing can be reliably repeated across days to gather baseline and drug response within the same mice, and genotype-specific sensitivity to THC-induced locomotor reductions between male B6 and D2 adolescent mice (Kasten et al., 2017). The current study demonstrates the possibility to selectively breed for adolescent THC-induced activity reductions in a short-term line. Further, selection for overall activity reduction also produced a line difference in percent of distance spent in the center of the open field. This metric is often used as an indicator of anxiety-like behavior (Griebel & Holmes, 2013; Mohammad et al. 2016) and may be reflective of changes in anxiety levels self-reported by individuals that use cannabis (National Academies on Sciences, 2017).

2 Method

2.1 Animals

Sixty (30M, 30F) B6D2F1/J mice were purchased from Jackson Laboratory (Bar Harbor, ME). Mice arrived at 6 weeks of age and were housed five per cage within each sex on a 12:12 light cycle in facilities accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC). There was an acclimation period of 10 days before being paired into 30 breeder pairs of one male and one female. Resulting F2 offspring were phenotyped (see Section 2.2) at PND27–33. Following phenotyping, mice remained in their home cage until PND60+ when new breeders were paired. Offspring were housed 1–3 per cage with littermates of the same sex. Mice were distinguished by an ear punch (right, left, or no punch) which was done approximately a week before phenotyping. Food (LabDiet 5K20 for breeders, 5001 for all other rodents, St. Louis, MO) and water was available at all times apart from during behavioral tests. Breeder cages included nesting material (Ancare, Bellmore, NY) and paper domes (Shepherd, Watertown, TN) on Sani-Chips bedding (PJ Murphy Forest Products, Montville, NJ). All procedures were approved by the IUPUI School of Science Institutional Animal Care and Use Committee and conformed to the Guide for the Care and Use of Laboratory Animals (The National Academic Press, 2011).

2.2 Phenotyping

Phenotyping occurred over three days during PND27–33. Each day mice were moved into the behavioral testing room at the beginning of the dark cycle and were allowed an hour to acclimate. Following acclimation, mice were placed into Versamax activity monitors (Accuscan Instruments, Columbus, OH) for 20 minutes. A 20-minute test was chosen based on preliminary data within our lab indicating that this time point would reveal initial differences in sensitivity to the locomotor effects of THC (see **Section 3.1**). The Plexiglas activity boxes (40×40 cm, center of 20×20 cm) are housed in sound-attenuating chambers and record activity using photocell beams located 2 cm above the floor. The boxes are also equipped with a house light, which remained off. Day 1 served as a habituation day to

acclimate mice to the testing chamber (Phillips et al., 1995; Linsenbardt & Boehm, 2013). No injection was given on Day 1. On Day 2, a vehicle injection was administered to quantify baseline activity, and on Day 3, a 10 mg/kg THC injection was administered. Injections took place immediately prior to the activity session. It should be noted that females and males demonstrate different sensitivities to the locomotor effects of a range of THC doses (Britch et al. 2017), and therefore significant differences in sensitivity to a 10 mg/kg THC dose may be expected between the two sexes.

Overall response to THC was quantified as a mouse's change in activity from baseline (THC response-Baseline response), with more negative change scores indicating a larger reduction in activity following THC. Use of a change score allows consideration of baseline activity in quantifying the change induced by drug administration, thereby giving a less compromised indication of sensitivity or resistance to the locomotor effects of THC. Selection was determined based on an individual mouse's response to THC. Mice with strong negative or minimal THC-induced change score (THC total distance – vehicle total distance) were paired with one mouse of the opposite sex to complete a sensitive or resistant breeder pair, respectively. Breeder partners were determined by rank ordering the change in total distance traveled within sex and line. Up to 18 breeder partners of each line were determined by pseudorandomizing pairings accounting for family history. Within the sensitive line, the males and females demonstrating the largest reduction in baseline activity were paired. Within the resistant line, the males and females that demonstrated close to no change from baseline activity were paired. Although mice with an overt stimulant response to THC were not used as breeders, some resistant breeders did demonstrate a minimal stimulant response to THC, as demonstrated by parent averages in Figure 2A-B. Phenotyping and breeding was completed through S4.

2.3 Drugs

THC was obtained from the National Institutes of Health/National Institute on Drug Abuse (Bethesda, MD) at a concentration of 1 mg per 50 microliters of 95% ethanol. For a 10 mg/kg dose, 1 μl of THC concentrate was diluted into 0.1 ml of vehicle. Vehicle was comprised of an 18:1:1 ratio of 0.9% saline, Tween 80 (Sigma Aldrich, St. Louis, MO), and 200 proof ethanol (Pharmco, Inc., Brookefield, CT). Vehicle and THC were delivered via intraperitoneal injection in a volume of 0.1 ml per 10 g of body weight. The dose of ethanol contained in the vehicle is approximately equivalent to a dose of 0.3 g/kg.

2.4 Statistical Analysis

Cumulative estimated within-line heritability (h^2) was calculated as described in Linsenbardt & Boehm (2013) across F2 to S4 and F2 to S3 generations. Briefly, a response to selection score (R) was calculated as the mean THC-induced locomotor reduction score of a pair's individual offspring minus the mean of the parental generation for each line. A selection differential (S) was calculated as the mean THC-induced locomotor reduction score of the breeder pairs minus the average of parental generation for each line. The R of each generation was plotted against a cumulative S score. h^2 was calculated as the slope of a best-fit line of R/S using linear regression analysis for each line. R^2 values indicating the deviation of plot points against the line of best-fit were used as an indicator of additive

genetic variability. Within-line heritability for the sensitive and resistant lines was calculated for males and females independently as well as overall.

Apart from heritability estimates, F2 data were not included in analyses assessing line differences in behavior (e.g. Scibelli et al., 2011; Linsenbardt & Boehm, 2013). F2 data are shown on the graphs for reference. Total distance and percentage of distance traveled in the center of the open field were analyzed using line (sensitive or resistant) *generation (S1-S4) ANOVAs. Sexes were analyzed separately due to sex being a significant factor in vehicle and THC responses (see **Sections 3.4, 3.5**) and differences in within-line heritability (Table 1). Analyses were run on activity during the vehicle day, THC day, and the change score of activity (THC-vehicle). A more negative change score indicates a stronger response to THC, whereas a change score around 0 indicates no change in behavior between the two injection days. Percent of distance traveled in the center of the open field was calculated as [(center distance/total distance)*100]. Line*generation ANOVAS were also run to assess whether THC sensitivity changed from S1 to S4. Significance was set at $p < 0.05$ and corrected for all post hoc tests. All data are shown as the mean ± the standard error of the mean (SEM).

3 Results

3.1 F2 Variability and THC Time Course

B6D2F2 males and females demonstrated variability in their locomotor activity across days as well as their response to THC administration (Fig. 1A, B). A selection*sex*time repeated measures ANOVA was used to analyze the pattern of change of activity in the mice chosen as S1 breeders. Sex was determined not to be a significant factor in the initial F2 population; there was not a significant omnibus interaction, line*sex, sex*time, or main effect of sex (p's > .05). However, there were significant main effects of line and time, as well as a significant line*time effect (p's < .05). The effect of line at each time point was assessed using t-tests, with data collapsed across sex. Sensitive S1 breeders showed a significantly greater decrease in locomotion following THC administration at minutes 6–7 and 9–20 compared to resistant S1 breeders (p's < .05) (Fig. 1C).

3.2 Heritability of the Phenotype

Table I details h^2 estimates and R^2 values for adolescent THC-induced locomotor reduction selection. Selection scores were quantified as the change in activity following THC injection from the baseline activity. From the F2 to the S4 generation, the overall h^2 estimates for the sensitive and resistant lines were 0.0704 ± 0.05 and 0.2485 ± 0.35, respectively. It should be noted that in both cases the regression line indicated that response to selection became more negative over time, indicating that the resistant line also displayed increased sensitivity to THC-induced activity reduction over successive generations. R^2 values for the sensitive and resistant lines were 0.3750 and 0.1465, respectively, indicating that the sensitive line has greater remaining genetic variability to continue selection.

In males from the F2 to the S4 generation, the h^2 estimates for the sensitive and resistant lines were 0.0522 ± 0.06 and 0.2532 ± 0.40, respectively. Again, the direction of the regression line in both cases indicated that the response to selection became more negative

over time, even in the resistant line. R^2 values for the sensitive and resistant lines were 0.2092 and 0.1184, indicating limited remaining genetic variability.

In females from the F2 to the S4 generation, the h^2 estimates for the sensitive and resistant lines were 0.1115 ± 0.14 and 0.2462 ± 0.37, respectively. As for the overall and male calculations, the direction of the regression line indicated that both sensitive and resistant lines demonstrated an increase in THC-induced activity reduction across generations. R^2 values from the sensitive and resistant lines were 0.1853 and 0.1263, respectively, indicating minimal remaining genetic variability.

Phenotyping data revealed a large increase in sensitivity to THC in males and females of the 4^{th} generation (see Fig. 2A-B). Of particular concern is the major dip in sensitivity to THC in the resistant line. However, the 4^{th} generation breeders experienced an increase in health issues compared to previous generations of breeders. Six breeders (2 sensitive females, 2 sensitive males, 1 resistant female, 1 resistant male) died shortly following weaning of their first litters, while some females required wet food (2 sensitive, 3 resistant) to keep their weight up.

Due to concerns of possible developmental issues in S4 resulting from these health issues, we decided to also calculate the h^2 estimates for the F2 to S3 generations, leaving out S4. Overall h^2 estimates for the sensitive and resistant lines from the F2 to S3 generation were 0.1366 ± 0.07 and 0.0808 ± 0.58 with R^2 values of 0.6825 and 0.0096, respectively. For males, sensitive and resistant h^2 was estimated as 0.0042 ± 0.10 and 0.0172 ± 0.82 with R^2 values of 0.0009 and 0.0002, respectively. In females, sensitive and resistant h^2 estimates were 0.2592 ± 0.15 and 0.2081 ± 0.50 with R^2 values of 0.5847 and 0.0783, respectively. For overall and female estimates the direction of the regression line aligned with the direction of the selection; response to selection became more negative in the sensitive line and more positive or neutral in the resistant line. As evidenced by the R^2 calculations (Table 1), genetic variability was greater in the overall and female calculations of the sensitive line, indicating that continued breeding likely would have led to a stronger response to selection over successive generations. The small h^2 estimates and minimal additional genetic variance in the resistant line reflect the choice to select for minimal net change in activity score instead of a hypermobility response, thereby reducing genetic variability. Males maintained a neutral response to selection in both the sensitive and resistant lines with minimal remaining genetic variability, indicating that line differences would not continue to separate over successive generations.

3.3 Selection Phenotype Behavior

Selection for sensitivity or resistance to THC for S1-S4 was quantified as the change in behavior between the vehicle and THC day, with a more negative change indicating higher sensitivity. In males, a line*generation ANOVA analyzing the total distance change score revealed no significant interaction ($p > 0.05$). There was a main effect of selection generation; $F(3,175) = 5.30$, $p < 0.01$. There was also a main effect of line, with the resistant line demonstrating a smaller change in activity; $F(1,175) = 8.82$, $p < 0.01$ (Fig. 2A). In females, there was no interaction of line*generation on change score ($p > 0.05$). There was a main effect of selection generation; $F(3,189) = 5.35$, $p < 0.01$. There was also a main effect

of line, with the resistant line demonstrating a smaller change in activity; $F(1,189) = 14.43$, $p < 0.001$ (Fig. 2B).

To quantify line differences in anxiety-like activity a change score was calculated for percent of total distance traveled in the center of the open field. In males, a line*generation ANOVA analyzing the percent center distance change score revealed no significant interaction ($p > 0.05$). There was a main effect of selection generation; $F(3,175) = 3.71$, $p < 0.05$. There was also a main effect of line, with the sensitive line demonstrating a larger change in percent of distance spent in the center of the open field; $F(1,175) = 6.12$, $p < 0.05$. In females, there was an interaction of line*generation on change score; $F(3,189) = 5.93$, $p < 0.001$. There was a main effect of selection generation; $F(3,189) = 4.72$, $p < .01$. There was also a main effect of line; $F(1,189) = 10.69$, $p < 0.01$. Independent samples t-tests revealed that the sensitive line demonstrated a larger change in percent of distance spent in the center of the open field at S2, S3, and S4 (p's < 0.01) (Fig. 2D).

3.4 Vehicle and THC Total Distance

Because selection criterion was measured as the change in activity between two days, we also investigated whether line differences were present on the vehicle or THC day for both metrics across S1-S4 generations. Sex presented an overall significant factor, with males and females demonstrating a different pattern of response on the vehicle and THC days that drove the line changes in THC-induced locomotor sensitivity (p's < .01). On the vehicle day, a line*generation ANOVA revealed no significant interaction or main effect of line in males on total distance traveled (p's > 0.05). There was a significant main effect of selection generation; $F(3,181) = 6.23$, $p < 0.001$ (Fig. 3A). In females, there was no significant interaction of line*generation ($p > 0.05$). There was a main effect of selection generation; $F(3,189) = 5.45$, $p < 0.01$. There was also a main effect of line, with the sensitive line displaying more activity on the vehicle day than the resistant line; $F(1,189) = 7.17$, $p < 0.01$ (Fig. 3B).

On the THC day, a line*generation ANOVA revealed no significant interaction in males for total distance traveled ($p > 0.05$). There was a significant main effect of selection generation; $F(3,175) = 15.11$, $p < 0.001$. There was also a main effect of line, with the sensitive line traveling less than the resistant line; $F(1,175) = 12.21$, $p < 0.001$ (Fig. 3C). In females, there was no significant interaction of line*generation, or main effect of line (p's > 0.05). There was a significant main effect of selection generation; $F(3,189) = 11.08$, $p < 0.001$ (Fig. 3D).

3.5 Vehicle and THC Percent Center Distance

On the vehicle day, a line*generation ANOVA for S1-S4 revealed no significant interaction or main effect of line in males on percent of distance traveled in the center of the open field (p's > 0.05). There was a significant main effect of selection generation; $F(3,175) = 7.64$, $p < 0.001$ (Fig. 4A). In females, there was no significant interaction of line*generation ($p > 0.05$). There was a main effect of selection generation; $F(3,189) = 5.62$, $p < 0.01$. There was also a main effect of line, with the sensitive line spending a larger percent of the activity in the center of the field than the resistant line; $F(1,189) = 9.78$, $p < 0.01$ (Fig. 4B).

On the THC day, a line*generation ANOVA for S1-S4 revealed no significant interaction in males for percent of distance traveled in the center of the open field ($p > 0.05$). There was a significant main effect of selection generation; $F(3,175) = 14.52$, $p < 0.001$. There was also a main effect of line, with the sensitive line spending a smaller percentage of their traveled distance in the center of the open field; $F(1,175) = 5.18$, $p < 0.05$ (Fig. 4C). In females, there was a significant interaction of line*generation; $F(3,189) = 2.69$, $p < 0.05$. There was no main effect of line (p's > 0.05). There was a significant main effect of selection generation; $F(3,189) = 6.55$, $p < 0.001$, but the lines were not significantly different from each other at any selection generation (p's > .05) (Fig. 4D).

3.6 Difference in Change Scores between S1 and S4

In males, a line*generation ANOVA revealed no interaction on change in total distance (Fig. 5A) or percent center distance (Fig. 5C) between S1 and S4 mice (p's > 0.05). There was a main effect of line for total distance ($p < 0.05$), but not percent center distance ($p > 0.05$). There was a main effect of selection generation for total distance ($p < .001$) and percent center distance, with S4s being more sensitive ($p < 0.05$).

In females, a line*generation ANOVA revealed no interaction on change in total distance ($p > 0.05$) (Fig. 5B). There was an interaction of line*generation on percent center distance; $F(1,91) = 13.06$, $p < 0.001$. There was a main effect of line for total distance ($p < 0.05$), but not percent center distance ($p > 0.05$). There was a main effect of selection for total distance ($p < .001$) and percent center distance ($p < 0.05$). Independent-samples t-tests revealed that only the sensitive line demonstrated an increase in sensitivity to THC on change in percent center distance ($p < .001$) (Fig. 5D).

4 Discussion

The current study supports the notion that acute locomotor sensitivity to THC in adolescent mice is moderately heritable. These findings align with previous work indicating a role of genes in influencing a range of drug-related behaviors (e.g. Solecki et al. 2009; Meyer et al. 2010; Iancu et al. 2013; Moore et al. 2013; Hoffman et al. 2014; see Bühler et al. 2015 for human literature review). Females were more sensitive to selection than males, with the female lines showing significantly different locomotor activity changes at the terminal generation, whereas male differences were limited to a main effect of line (Figs. 2, 5). Selection for change in locomotor activity following 10 mg/kg THC also led to significant line differences in percent of distance traveled in the center of the open field in both males and females across S1 to S4 generations (Figs. 2, 5). This indicates that thigmotaxic activity following THC administration may be correlated response to selection for THC-induced activity reduction. This correlated trait may be expected due to the role of endocannabinoids and CB1Rs in anxiety-related behavior (Griebel & Holmes 2013). Interestingly, a nuanced examination of daily activity indicated that sex differences were present for activity on the vehicle and THC days. In females, the sensitive line demonstrated hyperlocomotion and travelled greater distance in the center of the open field when vehicle was administered (Figs. 3B, 4B). This line difference in behavior on the vehicle day (day 2) was attenuated by THC administration on day 3 (Figs. 3D, 4D). Conversely, the male lines displayed similar

activity on the vehicle day (Figs. 3A, 4A), with THC administration significantly altering the behavioral response between lines on day 3 (Figs. 3C, 4C).

Heritability estimates for THC-induced activity reduction were relatively low from the F2 to S4 generations. In the sensitive line, approximately 5% and 11% of the THC-induced activity reduction response was attributable to the genetic selection in males and females, respectively. Conversely, the heritability estimates in the resistant line were around 25% for both sexes. However, the direction of the estimates indicated that the resistant line also developed increased sensitivity to THC-induced activity reduction, which appears to be driven by heightened sensitivity in the S4 generation (Figs. 2, 5). As mentioned, the fourth generation experienced fecundity issues, which potentially contributed to the S4 behavior that was inconsistent with responses in generations S1-S3. Estimates of heritability from the F2 to S3 populations indicated that, in females, approximately 26% and 21% of the response was attributable to genetic selection for sensitivity and resistance to THC-induced activity reduction, respectively. Further, additive genetic variability was not exhausted in the sensitive females (R^2 = .5847). Heritability estimates in the males suggested minimal contribution of genetic selection to the behavioral response (< 2%), as well as exhaustion of variability to drive further selection in both lines (R^2's < .001).

The current phenotyping paradigm was chosen due to our previous findings that male adolescent B6 mice are sensitive to the locomotor effects of 10 mg/kg THC, whereas D2 mice are not, and therefore this behavior was expected to be heritable in males. Unexpectedly, males showed a minimal response to selection, whereas females demonstrated a moderate heritability that is on par with short-term selection for locomotor response to drugs of abuse (e.g. Linsenbardt & Boehm 2013). Recent work in the field has revealed strong sex-differences in both behavioral and neurobiological outcomes of THC administration, particularly during the adolescent time period. Female rodents exhibit increased nociception response, altered locomotor activity, a more robust discrimination profile, impaired object memory, and stronger short-term and long-term withdrawal profiles (Harte-Hargrove & Dow-Edwards 2012; Zamberletti et al. 2012; Craft et al. 2013; Llorent-Berzal et al. 2013; Wakley et al. 2015; Silva et al., 2016; Britch et al. 2017; Wiley et al. 2017). It should be noted that these sex-differences do not persist across every behavioral domain and may be strain-dependent (Keeley et al. 2015). Importantly, previous work has demonstrated that a females are often more sensitive to the behavioral effects of THC (Wakley et al. 2015; Britch et al. 2017; Wiley et al. 2017). Such sensitivity is potentially due to rapidly increased levels of the active metabolites 11-OH-THC and CBN in females, puberty status, and the role that receptors other than CB1R play in mediating behavioral effects of THC in females (Craft et al. 2013; Silva et al. 2016; Britch et al. 2017). The former studies indicate that the higher heritability estimates in females demonstrated herein may be dependent on a stronger behavioral response in females. At this age-point, the 10 mg/kg dose of THC may be more effective in producing a reduction in locomotor activity in females with a genetic background that is sensitive to THC. Use of sensitive and insensitive progenitor lines may confer a greater behavioral range in females, thereby contributing to greater response to selection. In males, ability to selectively breed for a reduction in locomotor activity may be heightened by using a higher dose of THC and/or performing phenotyping at a different age. Although the current study did not collect blood samples for

analysis of THC or metabolite levels, future studies may consider targeting separate doses in males and females that produce the same level of active THC and metabolites.

Further, epigenetic influences may result in transgenerational alterations in behavior under both naïve and drug-induced states. These transgenerational consequences have been demonstrated for multiple addictive drugs, including cannabinoids (for review, see Vassoler et al. 2014; Yohn et al. 2016; Szutorisz & Hurd 2016). Of these studies, Szutorisz et al. (2016) investigated whether transgenerational influences were mediated by the sex of the offspring. Parental mice received repeated adolescent exposure to THC or vehicle and naïve offspring were tested. Their results revealed sex-dependent changes in striatal mRNA expression levels of genes that encode receptors important for synaptic plasticity, including CB1Rs. Behaviorally, parental THC exposure resulted in a reduction in activity during adulthood in naïve female, but not male, offspring. Sex- and selection-dependent transgenerational effects may underlie the locomotor activity on the vehicle day (day 2) in the current study. Male offspring travel the same distance regardless of selection influence, whereas the female offspring of the sensitive line travel more than their resistant counterparts following vehicle injection (Figure 3). In females, the line-dependent changes in baseline activity occur during S3 and S4, potentially indicating a sex-dependent role of multigenerational epigenetic inheritance.

Using the criteria outlined by Crabbe et al. (1990), there are several limitations to interpretation of the current short-term selection data. We provide moderate evidence that selection pressure contributed to heritable behavioral differences in adolescent sensitivity to acute THC. Significant line differences are present for all behaviors in both males and females, with significant differences between the lines at a generational level in females. However, the lack of replication of the selective breeding project is a major limitation that restricts the ability to interpret how heritable the selection criteria truly are. A future experiment could replicate the short-term selective breeding experiment in an entirely new population of B6D2F2 mice. A second limitation is the use of only two mouse strains as the progenitor population. Using only two strains limits the initial presence of genetic variability, while the short-term selection may not drive relevant gene loci to homozygosity. Future work might initiate short-term selective breeding from a more diverse founding population that consists of alleles from more than 2 progenitor strains (see Hitzemann et al. 2014).

Furthermore, selective breeding is not the only strategy for estimating the extent to which adolescent THC-induced reductions in locomotor activity is heritable. Recombinant Inbred (RI) strains offer an alternative strategy. In particular, the advanced intercross BXD RI strains were also derived from a cross between B6 and D2 mice, but were extensively inbred forcing all gene loci to homozygosity. The BXD RI strains offer the added benefit of allowing for the easy genetic mapping of quantitative trait loci (QTLs), or chromosomal regions that might contain genes influencing the behavior, as all of the strains have been genotyped and sequenced (Plomin et al. 1990; Williams & Williams 2017). The BXD strains have been routinely employed to determine genetic associations with locomotor response following drug administration (e.g. Crabbe et al. 1983; Alexander et al. 1996; Jones et al. 1999; Palmer et al. 2006). Newer techniques in RNA-seq and ATAC-seq make it possible to

identify epigenetic changes in the transcriptome and accessible DNA regions following THC treatment that may be possible for use in technologies that further our understanding of behavioral differences (Scott-Boyer & Descehpper 2013; Goldowitz et al. 2014; Yeo et al. 2016; Crabbe 2016).

Several behavioral directions should also be pursued in either the short-term selected lines and/or the BXD RI strains. These include the phenotyping of adult animals to assess whether genetic susceptibility to adolescent THC-induced reductions in locomotor activity also extends into adulthood, how an adolescent or adult history of THC treatment alters other behaviors, and evaluating other behaviors that may by mediated by common genes and therefore represent correlated responses to selection. Based on a recent review of literature assessing the effects of cannabinoid use in human populations conducted by the National Academies of Sciences (2017), behaviors of interest may include cognitive performance, social interaction, assays associated with schizophrenic and depressive phenotypes, and sensitivity to other drugs of abuse. Behaviors should be assessed under naïve conditions as well as following drug exposure to assess whether changes in behavior are due to a genetic predisposition or altered sensitivity to THC exposure.

In conclusion, we provide evidence that adolescent sensitivity to THC's locomotor response is amenable to selection pressure in mice. These results are the first to indicate that an adolescent THC-induced reduction in locomotor activity is a moderately heritable phenotype that is associated with anxiety-like activity in the open field. Females show a greater divergence in selection than their male littermates. Lines should undergo at least one replication to confirm evidence of the heritable behavioral phenotype (Crabbe et al. 1990). One component of problematic drug use is negative reinforcement (Koob, 2013). Frequent adolescent marijuana users are more likely to report feeling of euphoria (20%) and relaxation (46%) than sedation (17%) and anxiety (11%) (Camera et al., 2012). This may indicate that individuals that are sensitive to the sedative and anxiety-provoking effects of cannabis may be less likely to use marijuana frequently because it induces negative consequences rather than relieves them. Thereby, these sensitive individuals would be less likely to use cannabinoids at a level that induces long-term negative effects. However, for individuals that suffer from disorders that lead to heightened states of activity and arousal, sedation may be a desirable outcome that aids in completion of day-to-day tasks, thereby leading to use that is more frequent. Activity in mice offers strong face validity for sedation activity in humans, which the National Academies of Sciences (2017) suggests should be tracked in all human studies investigating cannabis use. As such, lines with a genetic predisposition for THC-sensitivity or resistance to locomotor reduction and anxiety allow for investigation of risk factors, differences in consequences of THC use, identification of correlated behavioral responses, and detection of genetic targets that may contribute to the development of treatment and interventions for those predisposed to heightened cannabinoid sensitivity.

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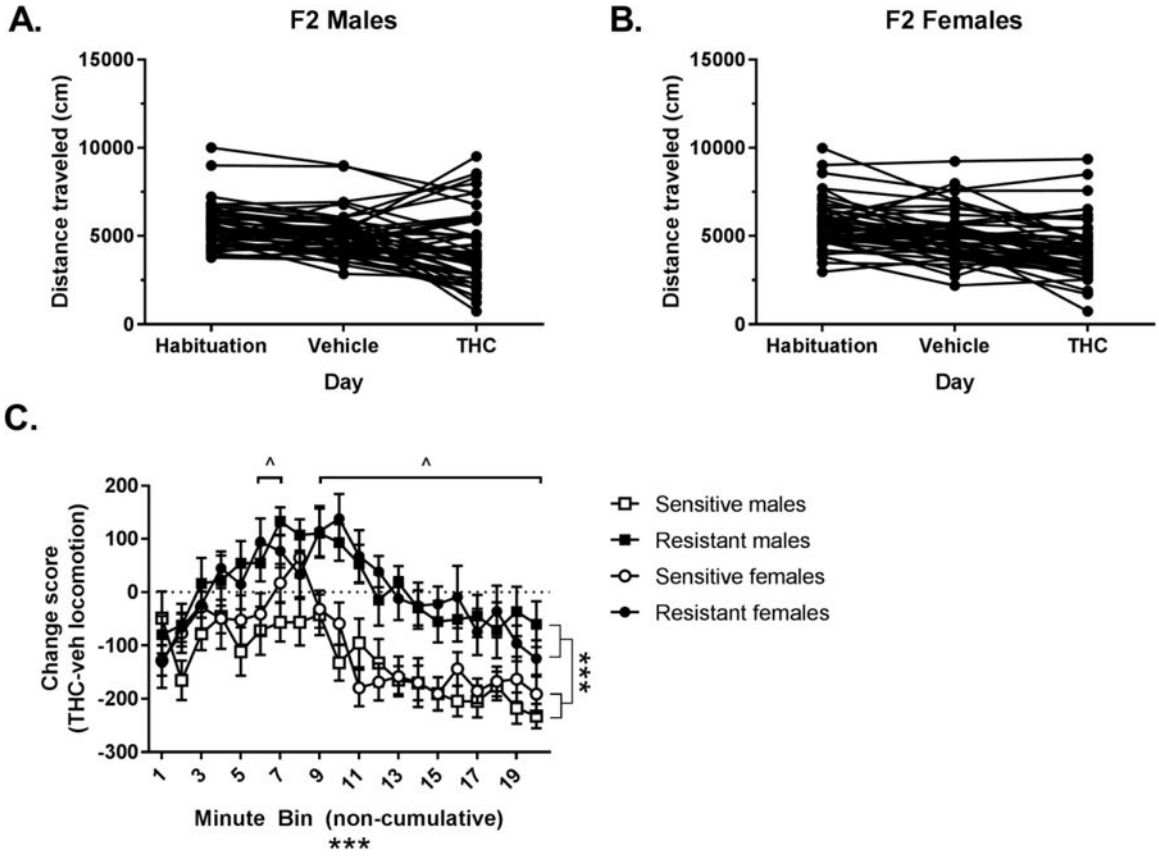

Figure 1.

demonstrates the variability in F2 response across days in males (A) and females (B). A 20-minute testing session is able to capture significantly different change scores following THC administration in the sensitive and resistant S1 breeder population (C). Asterisks (***) indicate a main effect of $p < .001$. Carrot (^) indicates a significant effect of selection at that time point at $p < .05$.

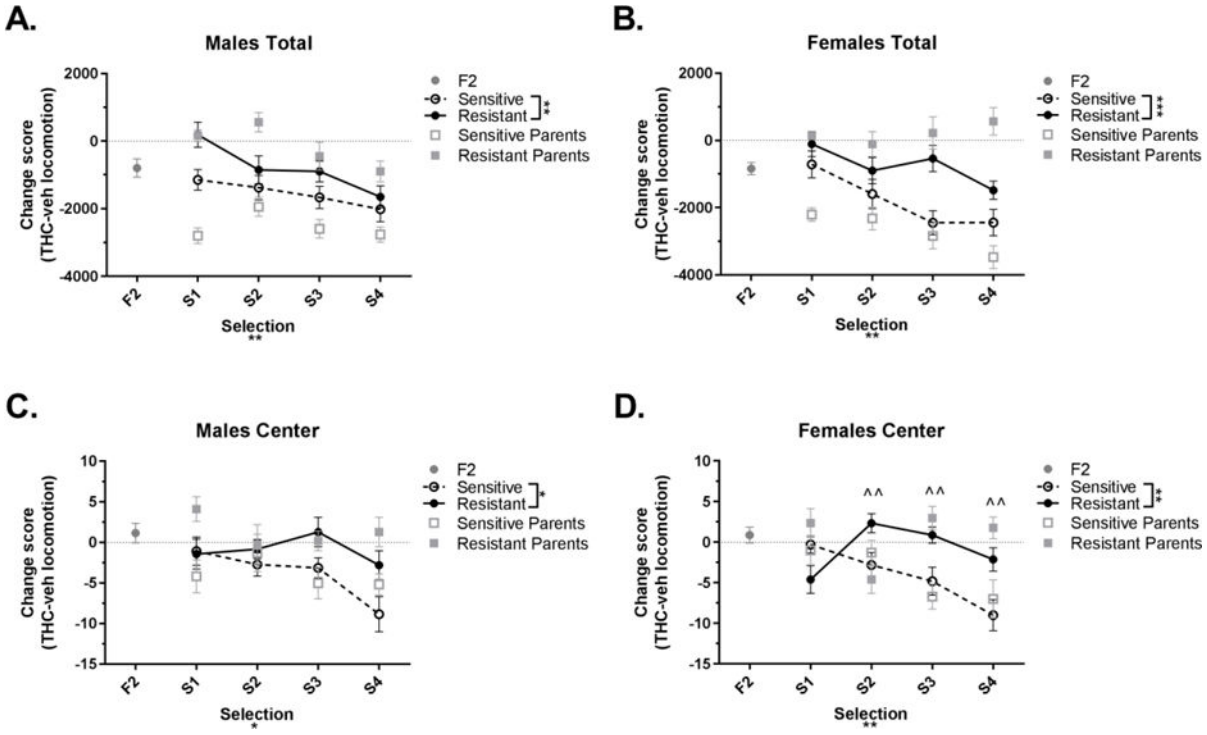

Figure 2.

depicts the change in individual locomotor scores for total distance traveled (A, B), and percent of distance traveled in the center of the open field (C, D). Data were analyzed for S1-S4 generations, F2 is shown for reference only. Change response of parents for each generation is shown for reference in gray. Parents were selected from the phenotyped offspring in the previous generation. The difference between gray and black data points within each generation represents the response to selection (R) within each generation. Asterisk indicates a main effect at $p < 0.05$ (*), $p < 0.01$ (**) and $p < 0.001$ (***). Carrot indicates that the lines are different from each other within that selection generation at $p < 0.01$ (^^).

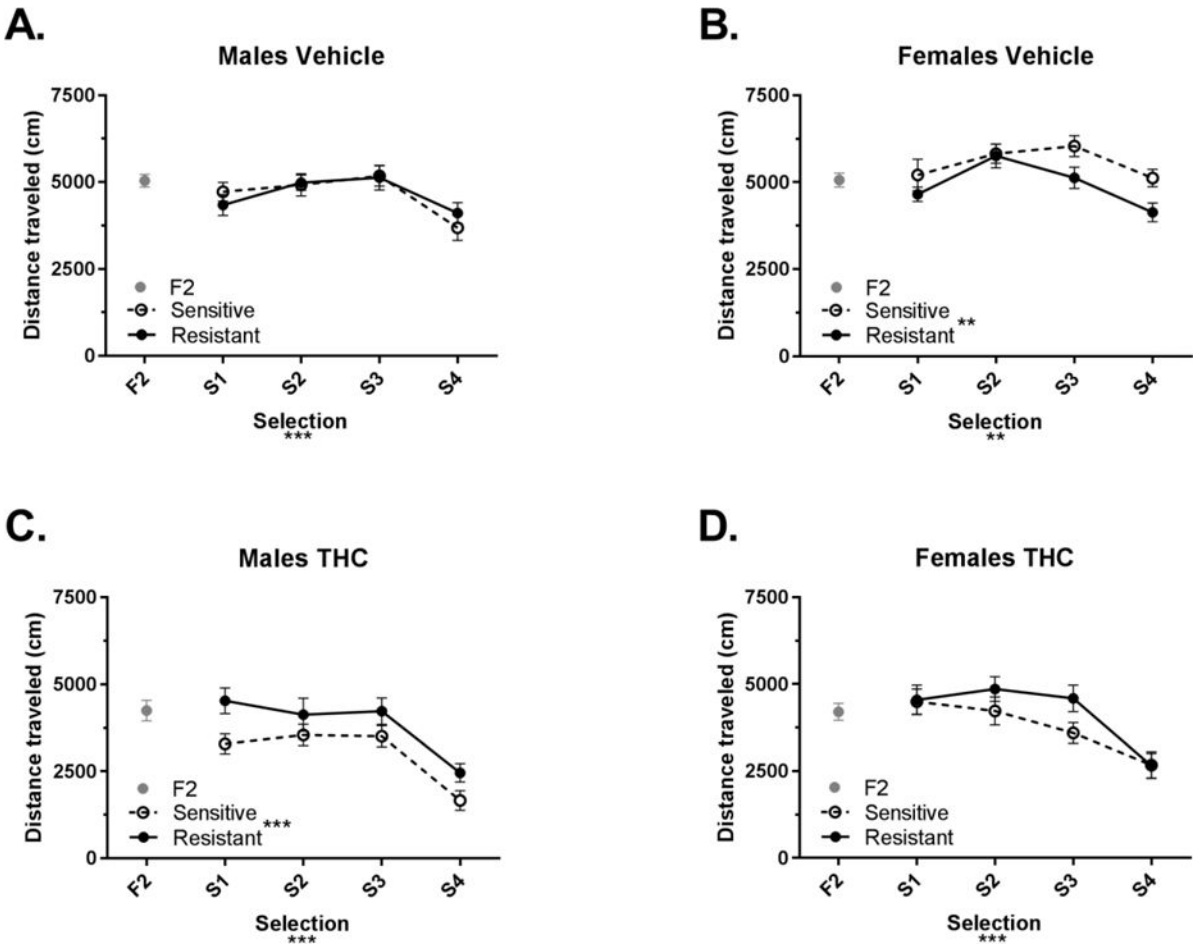

Figure 3.

depicts total distance traveled on vehicle (A, B) and THC (C, D) administration days. Data were analyzed for S1-S4 generations, F2 is shown for reference only. Asterisk indicates a main effect at $p < 0.01$ (**) and $p < 0.001$ (***).

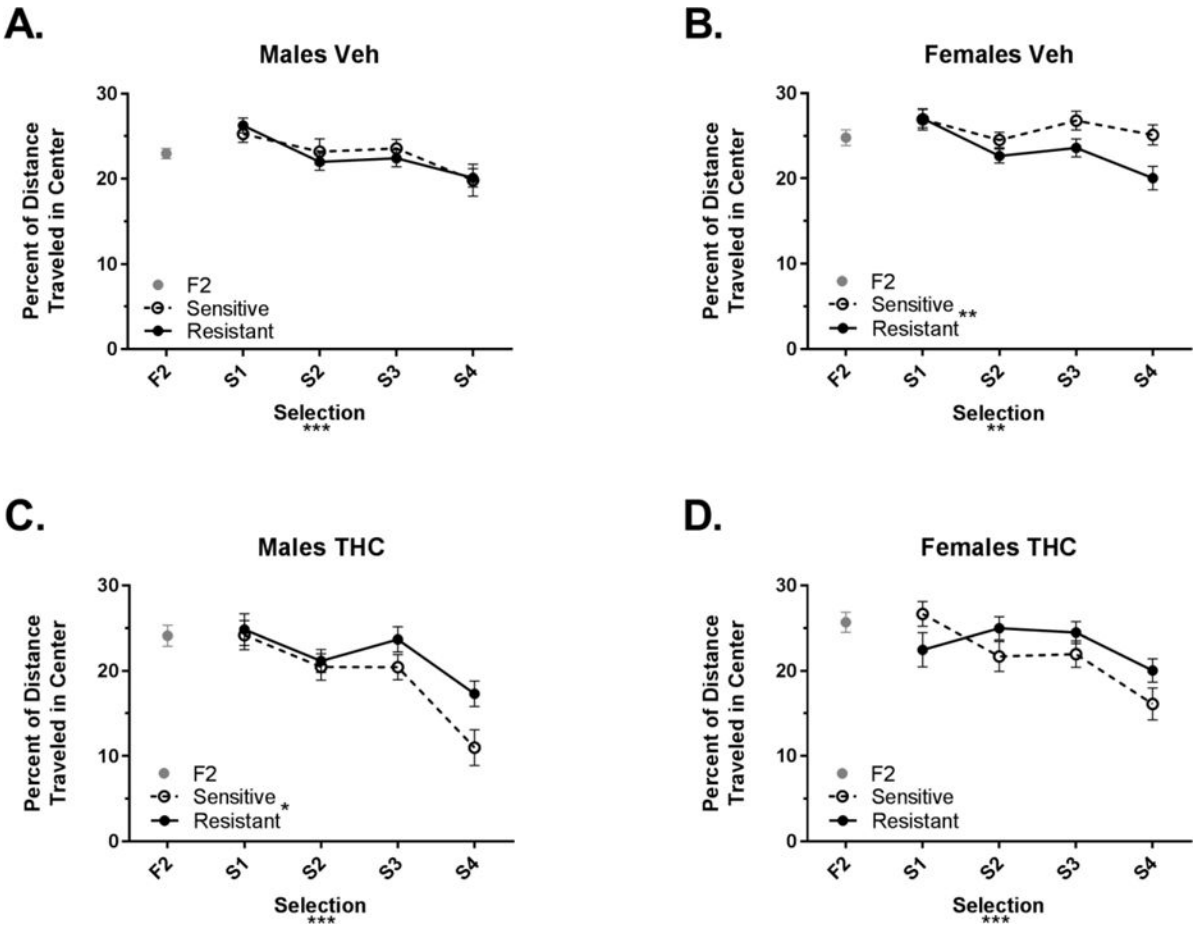

Figure 4.

depicts percent of distance traveled in the center of the open field on vehicle (A, B) and THC (C, D) administration days. Data were analyzed for S1-S4 generations. F2 is shown for reference only. Asterisk indicates a main effect at $p < 0.05$ (*), $p < 0.01$ (**), and $p < 0.001$ (***).

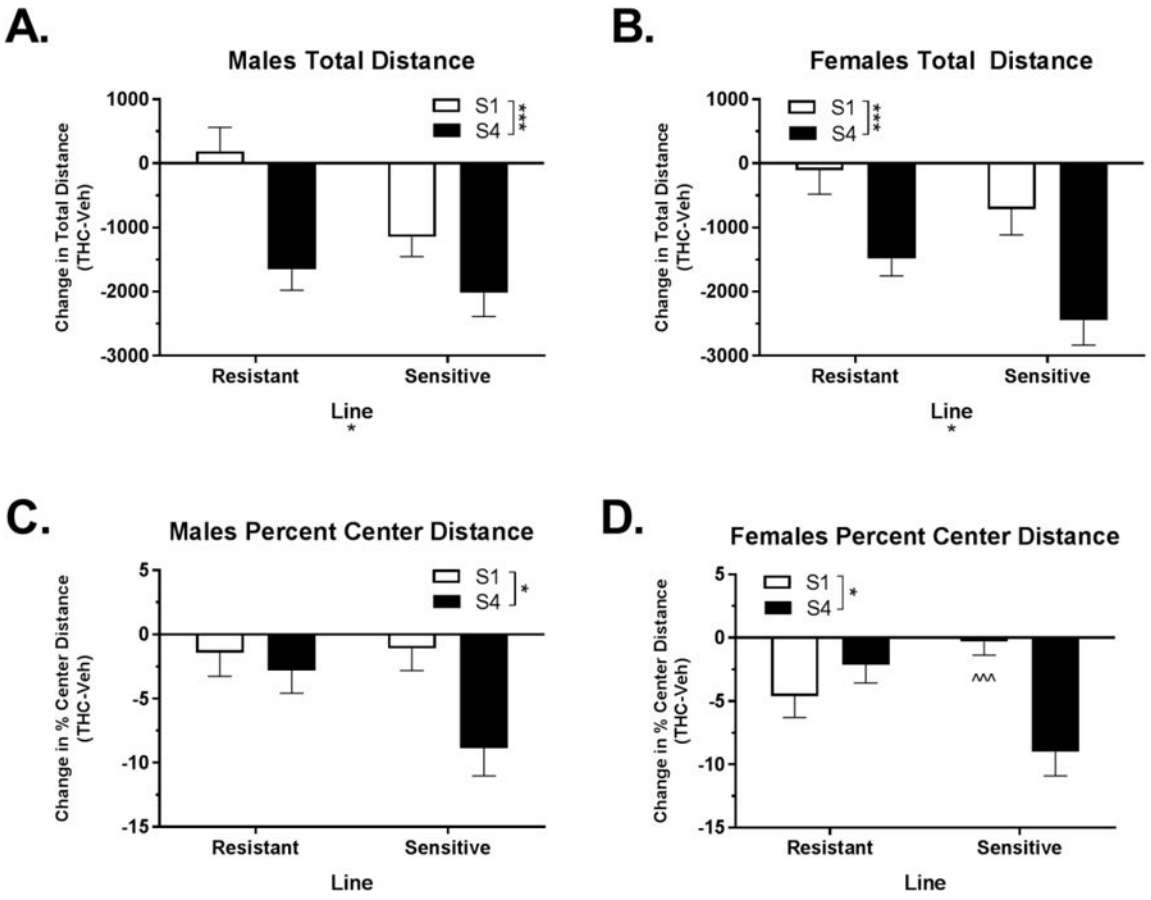

Figure 5.

depicts the shifts in THC sensitivity from S1 to S4 for the resistant and sensitive lines for change in total distance (A, B), and change in percent of distance spent in the center (C, D). Asterisk indicates a main effect at $p < 0.05$ (*) and $p < 0.001$ (***). Carrot indicates that the selection generations are different from each other within that line at $p < 0.001$ (^^^).

Table I

Table I includes the h^2 estimates and R^2 values (±SEM) for the sensitive and resistant lines from F2 to S3 and S4 generations. Asterisk (*) indicates a negative regression value, indicating that sensitivity to THC-induced sedation increased across generations of breeding.

	F2 through S4				F2 through S3			
	Sensitive		Resistant		Sensitive		Resistant	
	h^2	R^2	h^2	R^2	h^2	R^2	h^2	R^2
Overall	0.0704* ± 0.05	*0.3750*	0.2485* ± 0.35	*0.1465*	0.1366* ± 0.07	*0.6852*	0.0808 ± 0.58	*0.0096*
Males	0.0522* ± 0.06	*0.2092*	0.2532* ± 0.40	*0.1184*	0.0042* ± 0.10	*0.0009*	0.0172* ± 0.82	*0.0002*
Females	0.1115* ± 0.14	*0.1853*	0.2462* ± 0.37	*0.1263*	0.2592* ± 0.15	*0.5847*	0.2081 ± 0.50	*0.0783*