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Cross-generational THC exposure alters the developmental sensitivity of ventral and dorsal striatal gene expression in male and female offspring

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Abstract

Cannabis (*Cannabis sativa*, *Cannabis indica*) is the illicit drug most frequently abused by young men and women. The growing use of the drug has raised attention not only on the impact of direct exposure on the developing brain and behavior later in life, but also on potential cross-generational consequences. Our previous work demonstrated that adolescent exposure to ⁹-tetrahydrocannabinol (THC), the main psychoactive component of cannabis, affects reward-related behavior and striatal gene expression in male offspring that were unexposed to the drug during their own lifespan. The significant sex differences documented for most addiction and psychiatric disorders suggest that understanding the perturbation of the brain in the two sexes due to cannabis could provide insights about neuronal systems underpinning vulnerability to psychiatric illnesses. In the current study, we expanded our previous observations in males by analyzing the female brain for specific aberrations associated with cross-generational THC exposure. Based on the impact of adolescent development on subsequent adult behavioral pathology, we examined molecular patterns during both adolescence and adulthood. The results revealed a switch from the ventral striatum during adolescence to the dorsal striatum in adulthood in alterations of gene expression related to synaptic plasticity in both sexes. Females, however, exhibited stronger correlation patterns between genes and also showed locomotor disturbances not evident in males. Overall, the findings demonstrate cross-generational consequences of parental THC exposure in both male and female offspring.

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Conflict of interest

The authors declare no conflicts of interest.

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Keywords

marijuana; cross-generational transmission; development; striatum; gender; synaptic plasticity

1. Introduction

Over the past decade there has been a significant shift in society regarding marijuana (*Cannabis sativa*, *Cannabis indica*) such that the current perceived lack of health risk of this drug has led to more teens today smoking cannabis than cigarettes (Johnston et al., 2012). The growing acceptance in the Western world for the consumption of marijuana has important relevance particularly for women with far reaching implications. Firstly, more young women have increased their use of marijuana and this also extends to periods of pregnancy (SAMSHA, 2014). Secondly, the use of marijuana by women could possibly impact not only their immediate health, but also the health of their offspring, and such potential long-term consequences are still not known. It is clear that the biological differences between males and females influence the development of neuropsychiatric phenotypes (Figueira and Ouakinin, 2010; Savic and Engel, 2014; Shen et al., 2015), but the ways in which marijuana use causes long-term problems that affect the two sexes remain largely unexplored. As such, the inclusion of females in studies of cannabis is essential to decipher the neurobiological systems vulnerable in the different sexes to also ultimately guide targeted treatments in a sex-specific manner.

For decades researchers have debated whether the biological consequences of an individual's life experiences are reprogrammed from parent to offspring. While some studies report no remarkable effects of such experiences, a growing body of literature has challenged these findings and demonstrated significant aberrations that influence disease risk through the germline from parent to child. (Bohacek and Mansuy, 2013; Szyf, 2015). By now, several cases of cross-generational (parent-child) transmission regarding drugs of abuse have been published, describing behavioral phenotypes and molecular disturbances in the offspring of parents that were exposed to drugs before mating, including cannabinoids (reviewed in (Szutorisz and Hurd, 2015; Vassoler and Sadri-Vakili, 2014; Yohn et al., 2015)). However, most of these studies did not address the impact of gender in the context of naturally occurring cannabinoids such as Δ^9 -tetrahydrocannabinol (THC), the main psychoactive component of marijuana (Byrnes et al., 2012; Vassoler et al., 2013).

Recently, observations from our previous studies have shown that exposure of adolescent rats to THC before mating ("germline exposure") leads to cross-generational behavioral and gene regulation abnormalities in the striatum in the subsequent generation ("F1 offspring"), most likely achieved via impaired epigenetic regulatory processes (Szutorisz et al., 2014; Watson et al., 2015). Based on the observed striatal mRNA disturbances in F1 male progeny, along with the behavioral abnormalities identified in males, we set out to explore whether males and females are uniquely affected in this model. In the current study, we investigate potential sex-specific effects of cross-generational THC exposure on gene expression that could provide biological insights into neuropsychiatric vulnerability that frequently show sex differences in their incidence and course of the disorders.

2. Materials and Methods

2.1. Drugs

⁹-THC (50 mg/ml in ethanol solution) was evaporated under nitrogen gas, dissolved in 0.9% NaCl (saline) containing 0.3% Tween 80 to a concentration of 0.75 mg/ml (Dinieri and Hurd, 2012). Control “vehicle” (VEH) solution was saline containing 0.3% Tween 80.

2.2. Animals and cross-generational THC paradigm

The cross-generational THC animal model has been described in detail previously (Szutorisz et al., 2014; Watson et al., 2015) and is illustrated in Fig. 1. Briefly, for the F0 breeding parents, 21-day old male and female Long-Evans rats were purchased from Charles River Laboratories, Inc. (Wilmington, MA) and housed in same sex groups. After one week of acclimation in the facility, the rats were administered THC (1.5 mg/kg intraperitoneally, every 3rd day from postnatal day 28-49) or VEH. After birth at ~PND2, mixed litters were established combining an approximately equal number (12-14) of pups from THC- and VEH-exposed parents with a balanced proportion of males and females in each litter. The litters were cross-fostered to drug-naïve surrogates, which were used as nursing mothers. F1 offspring were weaned at ~PND24 and groups of 3-4 animals were maintained without any drug treatment or testing on normal 12-h light/dark cycle with *ad libitum* access to food and water until adolescence (~PND35) or adulthood (~PND62). Animal care and handling were performed by technicians unfamiliar with parental treatment history. Animals were anaesthetized with CO₂, decapitated, brains were frozen in isopentane, and stored at -80°C until subsequent experiments.

2.3. Quantitative reverse transcription PCR (qRT-PCR) analyses

Striatal brain regions (dorsal and ventral striatum) were dissected from frozen adolescent and adult brains of rats with a 15-gauge sample punch on a cold plate based on landmarks according to commonly used atlases (Paxinos and Watson, 2007). RNA was prepared from bilateral tissue punches using the RNAqueous-Micro Kit (Thermo Fisher Scientific, Waltham, MA) and cDNA was obtained with a first-strand synthesis kit (Quanta Biosciences, Gaithersburg, MD). Quantitative real-time PCR was performed using the LightCycler480 Probes Master reagent (Roche, Basel, Switzerland) and the TaqMan PCR program in a LightCycler 480 instrument (Roche).

The following Taqman-based assays (Applied Biosystems, Foster City, CA) were used in triplicate PCR reactions: *Cnr1*, Rn00562880_m1; *Grin1*, Rn01436038_m1; *Grin2A*, Rn00561341_m1; *Grin2B*, Rn00680474_m1; *Gria1*, Rn00709588_m1; *Gria2*, Rn00568514_m1; *Dlg4*, Rn00571479_m1; *Dlgap3*, Rn00597609_m1; *beta-2-microglobulin*, Rn00560865_m1. Each gene of interest was run in duplex with a reference gene (*beta-2-microglobulin*), data were normalized via the $\Delta\Delta$ CT method (Livak and Schmittgen, 2001).

To directly compare qRT-PCR results in male and female rats from the same litter, results described for male subjects were re-analyzed from previously published data (Szutorisz et al., 2014) and combined with that from female littermates that derived from the same breeding cohort. Processing and qPCR analysis of all male and female brain samples were

done simultaneously. Notably, the expression level of the reference gene was not different in males and females. In the adolescent male and female sample (N=5/treatment/sex), all subjects were derived from a different birth litter; in the adult sample (N=7-8/ treatment/sex) no more than two littermates per group were included in the experiment. No litter effects were found in the statistical analyses. None of the animals used for gene expression analyses underwent any behavioral testing or treatment, only handling.

2.4. Open field locomotor behavior testing

Rats were tested during the dark phase of the light/dark cycle in a standard squared plexiglass arena (40.6 cm × 40.6 cm; MED Associates Inc., St. Albans, VT), equipped with the Versamax activity monitor system (AccuScan Instruments, Inc., Columbus, OH). Locomotor activity was recorded for 60 minutes and the number of beam breaks were analyzed in 5 min time intervals. Test subjects were derived from the same breeding as the animals used for qRT-PCR experiments, each test group (N=10-12/treatment/sex) included animals from 5-7 birth litters and no litter effects were detected in the statistical analyses.

2.5. Statistical analyses

Outliers in the gene expression data were detected using Grubbs test. Treatment by gender interactions for single genes were tested using two-way analysis of variance models (ANOVAs). Comparisons of mRNA expression levels within each sex to assess direction of change in males and females were performed by Welch two-sample t-tests. Pearson correlations were calculated to assess and visualize the relationship between expression levels of genes using multivariate analyses in the JMP 12 software (SAS Institute, Cary, NC). Open field locomotor activity experiments were analyzed using ANOVAs with repeated measures using a multivariate model followed by post-hoc pairwise t-test comparisons when appropriate to address the effect of THC at specific time points.

3. Results

3.1. Cross-generational THC exposure leads to developmental gene expression abnormalities in the striatum of male and female offspring

Based on our initial findings (Szutorisz et al., 2014; Watson et al., 2015) and considering the central role of striatal circuitry in behaviors related to reward processing, motivation, emotion and motor activity (Everitt and Robbins, 2005; Girault, 2012; Koob and Volkow, 2010), we focused on studying gene expression profiles of the dorsal and ventral striatum (nucleus accumbens) in F1 subjects of parents with a prior history of adolescent THC or vehicle exposure. Different lines of evidence in our earlier work (Szutorisz et al., 2014; Watson et al., 2015) had already demonstrated alterations in the striatum as a consequence of germline THC exposure and the observed striatal changes pointed especially in the direction of components of the glutamatergic system and molecules relevant to synaptic plasticity.

To expand on the above work and address the impact of sex, here we characterized relevant gene expression profiles in the ventral and dorsal striatum of F1 male and female offspring (Table 1 and 2). We focused on genes encoding subunits of the CB1 receptor (*Cnr1*), the NMDA receptor (*Grin1*, *Grin2A*, *Grin2B*), and the AMPA receptor (*Gria 1*, *Gria2*). We also

included two other important regulators of synaptic function: *Dlg4* that encodes for postsynaptic density protein 95 (Psd-95) and *Dlgap3* that generates the Sapap3 protein, which interacts with Psd-95. Both genes have been broadly implicated in neuropsychiatric phenotypes in humans and animals (de Bartolomeis et al., 2014; Hall et al., 2015; Rauch and Carlezon, 2013; Wan et al., 2014; Wang et al., 2014; Zuchner et al., 2009). mRNA levels were measured by quantitative reverse transcription PCR at two developmental time points, in adolescent (PND35) and young adult (PND62) F1 animals.

The data provided several interesting observations and are shown in Tables 1 and 2. In adolescence, analysis of the combined ventral striatal male and female datasets using two-way ANOVAs revealed interactions between treatment and sex for *Cnr1* ($F(3,16)=2.07$, $p=0.04$), *Grin1* ($F(3,16)=4.28$, $p=0.012$) and *Grin2B* ($F(3,16)=15.81$, $p=0.01$), as well as a main effect of sex for *Grin2A* ($p<0.0001$), *Grin2B* ($p<0.0001$) and *Dlg4* ($p<0.0001$) and *Dlgap3* ($p<0.0001$), indicating sex-specific effects of parental THC exposure on these genes (Table 1). These effects were primarily related to the fact that females tended to have higher expression levels than males (see Female/Male ratio in Table 1) that was reduced by THC (Table 3). Some genes show remarkable sex-specific differences in their expression levels (e.g. *Dlg4* and *Dlgap3*). In contrast to the ventral striatum, the pattern of expression in the adolescent dorsal striatum showed no significant treatment and sex interactions, but there was a main effect of drug for *Grin2B* and sex for *Grin2A*, *Grin2B*, *Gria1*, *GriaB*, *Dlg4* and *Dlgap3* (Table 2).

In the adult ventral striatum, a significant main effect of THC was detected for *Grin2A* ($p=0.02$), with parental THC exposure leading to decreased expression (Table 1). A main sex effect was also evident for *Grin1*, *Grin2A*, *Grin2B*, *Gria1*, *Gria2*, *Dlg4* but no treatment and sex interactions were identified. A prominent feature of the adult dorsal striatal impairments noted for the genes studied was the impact of parental THC exposure (Table 2). Altered gene expression (reduced by THC) included *Cnr1* ($p=0.04$), *Grin1* ($p=0.003$), *Grin2A* ($p=0.001$), *Grin2B* ($p=0.014$), *Gria1* ($p=0.008$), *Gria2* ($p=0.001$), *Dlg4* ($p=0.02$), and *Dlgap3* ($p=0.004$). No significant interactions were detected between THC and sex, but the analysis indicated main sex effects for multiple genes [*Cnr1* ($p=0.0008$), *Grin2A* ($p<0.0001$), *Dlg4* ($p<0.0001$), and *Dlgap3* ($p<0.0001$)] (Table 2). Females expressed either lower (*Grin2A*, *Dlg4*) or higher (*Dlgap3*) levels than males.

Considering the significant contributions of sex-related alterations, Table 3 was created to provide an overview regarding the directionality of THC-related change. The data clearly emphasizes an intriguing developmental shift from ventral to dorsal striatal abnormalities in males and females between adolescence and adulthood. Adolescent females already showed an “adult-like” pattern with less ventral striatal abnormalities detectable and a tendency for THC-related down-regulation compared with their male littermates. This was interesting considering that at the adolescent developmental time point of our analysis (PND35), female rats are typically more advanced in puberty than males (Schneider, 2008).

3.2 Sex-related profiles in the relationships between the mRNA levels of genes relevant to synaptic plasticity

The observed mRNA level abnormalities raised questions about potential functional associations between the various genes in the different striatal region of each sex. As such, we explored co-expression patterns between genes using pairwise correlation analyses in the combined dataset of THC and vehicle animals. We focused on adulthood given the fact that our adult cohort had a larger sample size (N=8 in each treatment group and sex) and that most of our previous work focused on identifying molecular and behavioral alterations in adult offspring with parental THC exposure (Szutorisz et al., 2014; Watson et al., 2015).

Figures 2A and B show that the strength and pattern of ventral striatal correlations were quite similar between the male and female offspring. In contrast, the analyses revealed a different profile between the sexes in the dorsal striatum (Fig. 2C and D), with a number of the associations being stronger in females. In particular, glutamate receptor subunit genes in the dorsal striatum, especially *Grin2A*, had greater positive correlations with other NMDA and AMPA receptor subunit genes in females than in males. In males the correlations of *Grin2A* with other genes ranged from $r=0.15$ ($p=0.603$) to 0.48 ($p=0.477$) whereas in females the correlations were stronger, ranging from 0.69 ($p=0.005$) to 0.78 ($p=0.001$). Intriguingly, relationships between the mRNA levels of *Cnr1* and other genes were also highly significant in females (*Cnr1-Grin1*: $r=0.61$, $p=0.016$; *Cnr1-Grin2B*, $r=0.64$, $p=0.01$; *Cnr1-Gria1*, $r=0.66$, $p=0.007$; *Cnr1-Gria2*, $r=0.67$, $p=0.006$; *Cnr1-Dlg4*, $r=0.73$, $p=0.002$; *Cnr1-Dlgap3*, $r=0.59$, $p=0.021$) but not or less so in males.

3.3. Parental THC exposure alters the relationships between the mRNA levels of synaptic regulators in the dorsal striatum

In order to better assess the relationship of gene expression relevant to parental germline THC in each sex, we explored pairwise dorsal striatal mRNA level associations in adult offspring separately for the treatment groups (Fig. 3). Males failed to show a strong THC-related alteration in correlation patterns except for a few associations with the *Dlg4* gene (for example, in animals with VEH history: *Dlg4-Gria1*: $r=0.46$, $p=0.25$; *Dlg4-Gria2*: $r=0.24$, $p=0.57$; and in animals with THC history: *Dlg4-Gria1*: $r=0.82$, $p=0.023$; *Dlg4-Gria2*: $r=0.79$, $p=0.034$). In females, a larger number of genes showed strong relationships in association with parental germline THC exposure, particularly for *Dlgap3* and *Cnr1* genes (*Cnr1-Grin2A*: $r=0.87$, $p=0.039$; *Cnr1-Grin2B*, $r=0.72$, $p=0.009$; *Cnr1-Gria1*, $r=0.69$, $p=0.007$; *Cnr1-Gria2*, $r=0.74$, $p=0.006$; *Cnr1-Dlg4*, $r=0.79$, $p=0.002$; *Cnr1-Dlgap3*, $r=0.66$, $p=0.003$). Altogether, these results suggest that cross-generational THC exposure enhances the association between the expression of genes related to synaptic plasticity to a greater extent in females than males.

3.4. Parental germline THC exposure leads to decreased locomotor activity in adult F1 females

The observation that the effects of cross-generational THC exposure were most prevalent in the dorsal striatum, together with our previously published data pointing to behavioral impairments (e.g. increased “compulsive-like” drug seeking and stereotypy, (Szutorisz et al., 2014)) are interesting given the strong role of the dorsal striatum in regulating motor

behaviors. We therefore monitored the locomotor activity of adult male and female offspring in an open field test (Fig. 4). ANOVA analysis with repeated measures revealed a significant effect of parental THC history ($F(1,18)=6.12$, $p=0.02$). *Post hoc* pairwise t-test comparisons indicated decreased locomotor movements during the first 30 minutes (generally reflective of novelty exploration) of the test session ($p<0.05$) in females. No difference in locomotor activity was observed in males. In conclusion, the abnormal locomotor behavior associated with parental THC exposure in F1 females is consistent with the hypothesis that striatal molecular abnormalities in components of the glutamatergic system might relate to a motor behavioral functional outcome in adulthood.

4. Discussion

The results of our current study demonstrate that germline exposure to THC leads to significant gene expression abnormalities in both male and female F1 offspring, with different profiles noted in the magnitude of the disturbances between the sexes. Parental THC exposure was associated with altered mRNA expression of genes functionally implicated in the regulation of synaptic plasticity in the striatum, in line with the results of our previous work that only investigated male offspring (Szutorisz et al., 2014) or did not address sex-specific differences (Watson et al., 2015). Here, a key finding was the identification of alterations in the mRNA levels of a number of genes encoding regulators of glutamatergic synaptic plasticity in females and the relationships between different genes depending on sex. Moreover, the observed THC effects on the genes studied were more prevalent in the dorsal striatal subregion in adulthood in contrast to adolescence during which alterations were more evident in the ventral striatum.

A number of mRNA expression abnormalities seem to develop later in life (by early adulthood) in the dorsal striatum of both male and female offspring. This is interesting given that a number of neuropsychiatric disorders, including obsessive-compulsive disorder (OCD), also show a developmental progression and symptoms appear gradually over time, with clinical penetrance often evident in adulthood (Casey et al., 2015; Lenze and Wetherell, 2011; Schmitt et al., 2014). Indeed, a number of the genes currently investigated are relevant to some of these disorders. For example, genetic polymorphisms in the *Dlgap3* gene, which encodes the Sapap3 protein that is a component of the postsynaptic density and interacts with Psd-95 physically and functionally, have been associated with a higher incidence of phenotypes within the OCD spectrum in humans (Bienvenu et al., 2009; Crane et al., 2011; Wu et al., 2013). Similarly, genetic deletion of the *Dlgap3* gene in mice has been shown to result in a variety of OCD-like abnormalities such as extensive grooming, abnormal motor behavior, and anxiety (Welch et al., 2007). As a neurobiological consequence, loss of *Dlgap3* expression has been reported to cause dorsal striatal impairments of glutamate receptor regulation and neurophysiological function (Wan et al., 2014; Wan et al., 2011). *Dlg4*/Psd-95 disturbances have also been linked with a variety of neuropsychiatric phenotypes such as schizophrenia, bipolar disorder and increased morphine sensitivity (de Bartolomeis et al., 2014; Dean et al., 2015; Hall et al., 2015; Kristiansen and Meador-Woodruff, 2005; Wang et al., 2014).

Intriguingly, our previous genome-wide study exploring epigenetic alterations (DNA methylation) in the striatum of F1 offspring with parental germline THC exposure identified *Dlg4* as the most significant hub within a functional network (Watson et al., 2015). The genes identified in that network, including several glutamate receptor subunits and other molecules involved in the regulation of synaptic plasticity (e.g. *Grin2A* and *Dlgap3*), contained differentially methylated regions on the DNA level, emphasizing the long-term epigenetic disruptions in gene regulation due to parental THC exposure. Interestingly, the dynamic control of glutamate receptors (e.g. receptor trafficking) has been demonstrated to be regulated by DNA methylation (Sweatt, 2016).

The current study revealed several novel findings, one of which highlights that parental THC-related alterations are linked to sex-related changes in both the mRNA expression levels of a few individual genes and in co-expression patterns. The co-expression profiles shown in Figures 2 and 3 are particularly intriguing as co-regulation networks in recent years have been closely related to tissue- and cell type-specific biological functions and disturbances (Kopp et al., 2015; Monaco et al., 2015; Richiardi et al., 2015; Rotival and Petretto, 2014). Co-expression patterns in the brain are particularly of interest given the developmental concept of complex neuropsychiatric disorders, where it has been historically difficult to identify single causal molecular relationships (Gaiteri et al., 2014; Grennan et al., 2014). While the exact nature of the correlations between different genes and how germline THC exposure affects these relationships in subsequent progeny remains to be explored, our observations emphasize the importance of studying functional networks of genes in relation to cannabis exposure in the future. Moreover, although the current analysis focused on glutamatergic molecular abnormalities, future studies using genome-wide approaches are expected to provide greater insight about other neurotransmitter systems and neuronal components potentially impacted by parental germline THC exposure.

The finding that more significant abnormalities were identified in the dorsal striatum in adulthood is consistent with our previous report of impairment in dorsal (but not ventral) striatal long-term depression (LTD) as a functional neurophysiological change in synaptic plasticity in adult male offspring with parental THC exposure (Szutorisz et al., 2014). The mechanistic nature of the observed increased LTD remains to be explored in detail neurophysiologically. For example, which subtype of glutamate receptors are responsible for the observed effects and whether the synaptic plasticity is mediated by the endocannabinoid system needs to be investigated. The fact that parental THC exposure caused a highly significant strengthening of mRNA expression patterns between the *Cnr1* gene (which encodes the CB1 receptor) and various glutamate receptor subunit genes (especially *Grin2A*), as well as *Dlg4* and *Dlgap3*, is intriguing and suggests indeed an endocannabinoid link mechanistically that will be examined in subsequent neurophysiological studies.

It is unknown what particular behaviors may be causally linked to disturbances of striatal neurons expressing the molecular abnormalities currently detected in the adult dorsal striatum. It is clear that the parental history of THC did not induce a general gross impairment of motor behavior in either sex, but was quite specific to novelty reactivity in females. Within the dorsal striatum, the medial subregion is a crucial component of the prefrontal cortex-striatal circuit known to modulate novelty reactivity and integrate cognition

and motor behaviors (Rinaldi et al., 2010). As such, specifically investigating the dorsomedial subregion of the striatum may provide some insight into the sex-specific differences observed in novelty seeking behavior.

The observations summarized above are in line with the concept of epigenetically inherited phenotypes. In-depth investigations will be needed to provide insights about gene regulatory mechanisms underlying the transmission of cannabis effects through the germline and how they relate to sex. The endocannabinoid system plays important roles not only in the development of the cells and physiological systems of the brain, but also in reproduction. It is known that both male and female reproductive tissues express CB receptors and endocannabinoid ligands, and that in males THC can disrupt gonadal functions (Banerjee et al., 2011; Bari et al., 2011). Studies on the impact of cannabinoids on epigenetic changes in male fertility have been conducted in *Cnr1* null mutant mice that displayed abnormal histone retention in germ cells compared to wild type mice (Chioccarelli et al., 2010). In that study, *Cnr1* expression was demonstrated to be necessary for spermiogenesis by controlling chromatin condensation, resulting in poor sperm quality. Adverse effects of cannabis exposure on the ovary of females have also been found to present a higher risk of infertility due to anovulation (Klonoff-Cohen et al., 2006). The effects of cannabis on the sperm and oocyte epigenome that could potentially lead to multigenerational transmission remain to be explored in different generations to demonstrate true transgenerational effects. Specifically, subsequent studies are required to assess how possible epigenetic processes (e.g. DNA methylation) are involved in the transmission of cannabinoid effects from parent to offspring. Overall, our findings demonstrate that germline THC exposure can impact offspring phenotype and neurobiology in both sexes and could possibly confer enhanced risk for the development of neuropsychiatric disorders that may manifest differentially in the two sexes.

5. Conclusions

Providing concrete insights about specific neurobiological disturbances in female and male offspring with parental THC exposure will have far-reaching impact for the neuroscience field given the critical role of the endocannabinoid system in early brain development, a time period now acknowledged to drive the course of psychiatric disorders expressed in adulthood. The results also emphasize the switch of ventral to dorsal striatal disturbances from adolescence to adulthood that may also have significant relevance to the age-dependent vulnerability seen with different neuropsychiatric disorders. Overall, our research highlights the important consideration that should be given for examining sex-specific effects not only related to events that occur during one's own lifetime but even across generations.

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Highlights

- Parental THC exposure causes striatal dysregulation in the expression of genes functionally related to synaptic plasticity in both male and female offspring.
- Gene expression changes show a developmental shift between adolescence and adulthood.
- Female offspring have stronger alterations in the mRNA co-expression patterns of various synaptic plasticity-related genes in the dorsal striatum.
- Females with parental germline THC exposure exhibit decreased novelty seeking behavior, which is not observed in males.

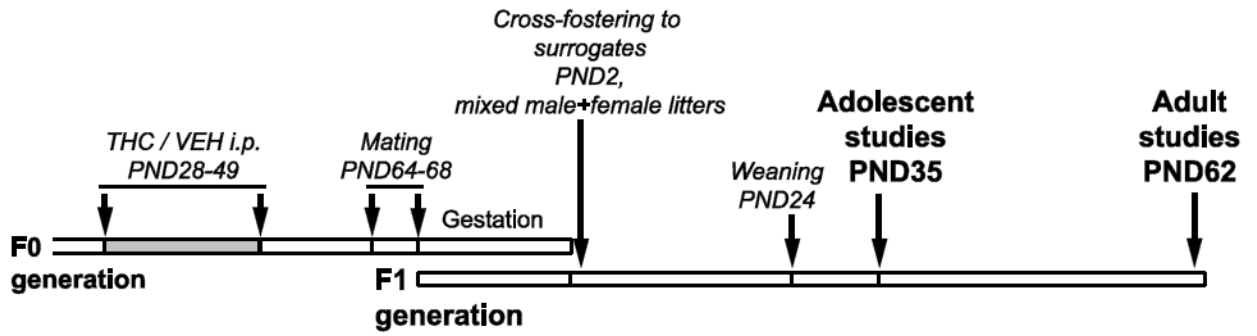


Figure 1.
Schematic overview of the experimental approach used to study the cross-generational effects of parental germline THC exposure in F1 offspring. For details, see Materials and Methods.

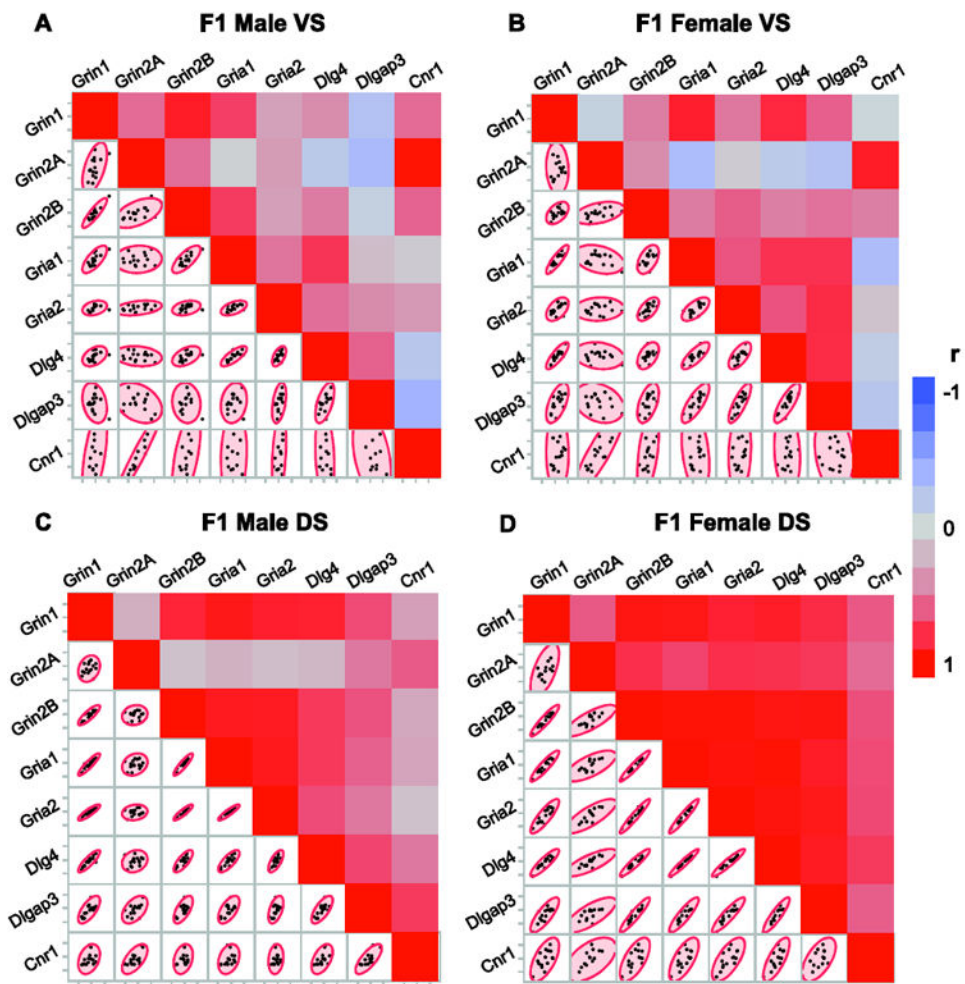


Figure 2.

Relationships between the mRNA levels of synaptic regulators within striatal subregions and sexes. Correlation analysis of the mRNA levels measured by qRT-PCR in samples combining offspring with parental THC or VEH exposure. (A, C) Adult F1 males. (B, D) Adult F1 females. Colors in heat maps represent Pearson correlation coefficient (r) values. Scatter plots show the distribution of mRNA levels between animal subjects. N= 16 (8 males +8 females)/treatment group. VS, ventral striatum; DS, dorsal striatum.

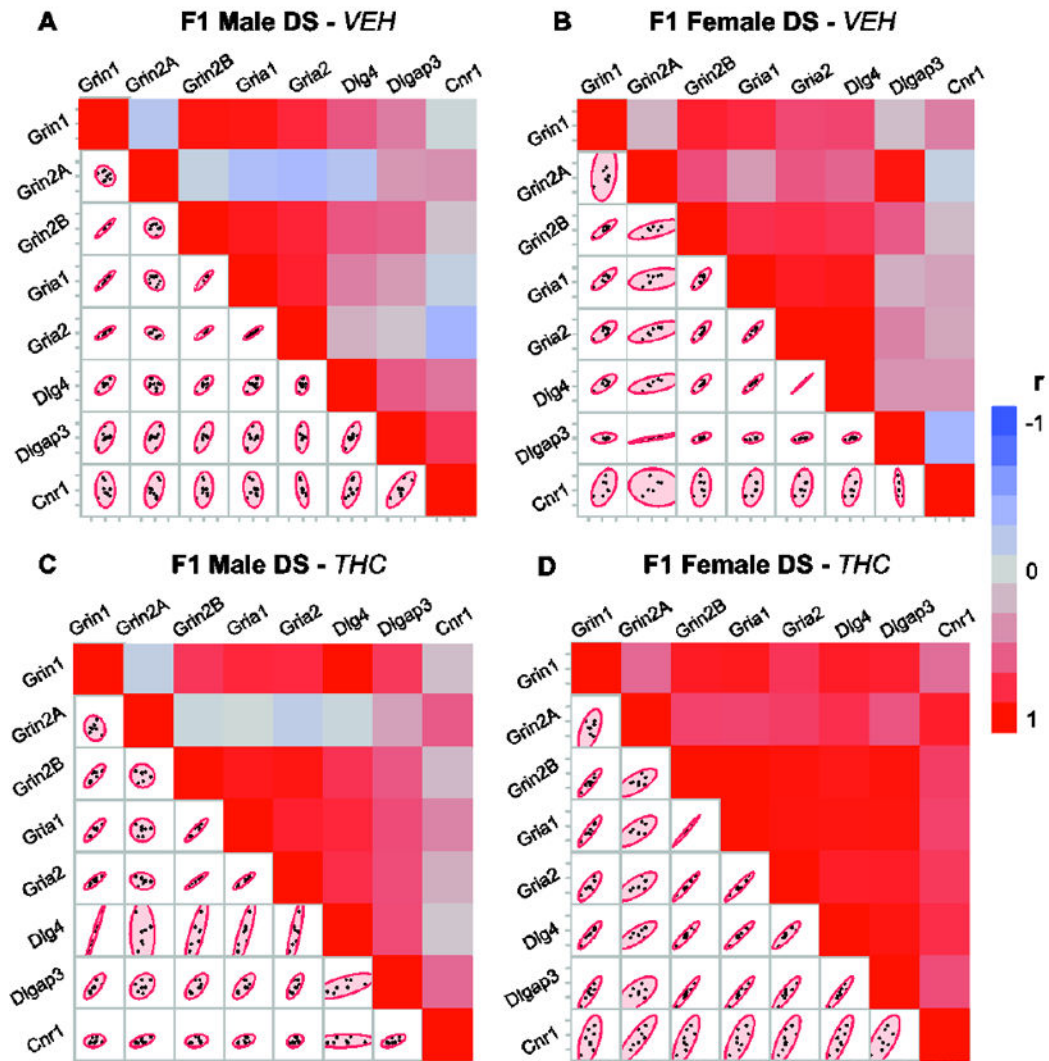


Figure 3.

Parental THC exposure alters the relationships between the mRNA levels of synaptic regulators in the dorsal striatum. Correlation analysis of the mRNA levels measured by qRT-PCR. (A, C) Germline VEH- and THC-exposed adult F1 males, respectively. (B, D) Germline VEH- and THC-exposed adult F1 females, respectively. Colors in heat maps represent Pearson correlation coefficient (r) values. Scatter plots show the distribution of mRNA levels between animal subjects. $N=8/$ treatment/sex. DS, dorsal striatum.

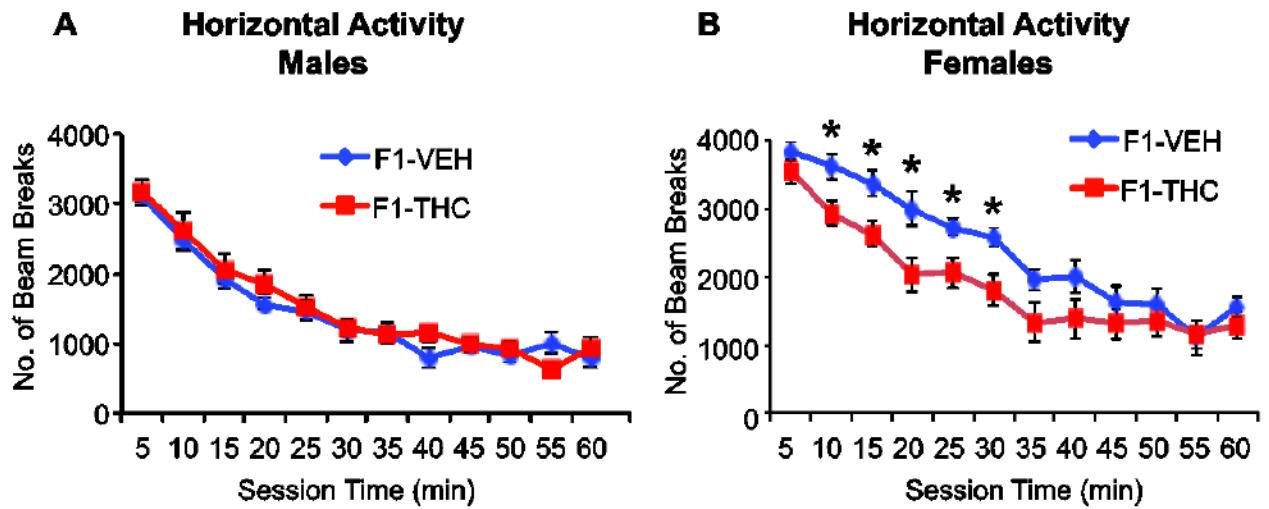


Fig. 4.

Parental germline THC exposure leads to decreased novelty-related locomotor activity in adult F1 females. Open field horizontal activity in adult F1 male (A) and female (B) offspring. N=10-12 animals/group. Values are expressed as a mean \pm SEM. * indicates $p < 0.05$ vs control (F1-VEH) subjects.

Table 1

Alterations of ventral striatal mRNA levels in F1 offspring analyzed by ANOVAs in combined male and female data sets.

Gene	Ventral Striatum - Adolescence					
	% THC/VEH ± SEM	% Female/Male ± SEM	F ratio	Effect (p-value)		
				Treatment *Sex	Treatment	Sex
<i>Cnr1</i>	110 ± 8.1	107 ± 8.2	2.07	0.040	0.391	0.515
<i>Grin1</i>	91 ± 4.2	122 ± 7.1	4.28	0.012	0.376	0.065
<i>Grin2A</i>	108 ± 8.4	166 ± 6.4	14.22	0.062	0.357	<0.0001
<i>Grin2B</i>	89 ± 4.0	129 ± 3.0	15.81	0.012	0.021	<0.0001
<i>Gria1</i>	90 ± 5.3	109 ± 5.2	2.41	0.069	0.175	0.255
<i>Gria2</i>	111 ± 5.9	105 ± 7.4	1.26	0.140	0.313	0.598
<i>Dlg4</i>	99 ± 9.8	185 ± 4.5	34.06	0.099	0.887	<0.0001
<i>Dlgap3</i>	105 ± 29.3	440 ± 10.6	51.67	0.312	0.609	<0.0001
Gene	Ventral Striatum - Adulthood					
	% THC/VEH ± SEM	% Female/Male ± SEM	F ratio	Effect (p-value)		
				Treatment *Sex	Treatment	Sex
<i>Cnr1</i>	82 ± 8.0	97 ± 2.9	0.95	0.713	0.157	0.402
<i>Grin1</i>	103 ± 4.5	107 ± 3.3	9.47	0.129	0.632	<0.0001
<i>Grin2A</i>	80 ± 7.9	151 ± 3.5	14.88	0.586	0.022	<0.0001
<i>Grin2B</i>	99 ± 3.4	132 ± 3.0	2.70	0.589	0.786	0.009
<i>Gria1</i>	108 ± 4.6	112 ± 3.5	10.59	0.679	0.175	<0.0001
<i>Gria2</i>	103 ± 3.5	112 ± 3.4	12.01	0.816	0.482	<0.0001
<i>Dlg4</i>	103 ± 7.5	197 ± 2.3	58.92	0.864	0.248	<0.0001
<i>Dlgap3</i>	109 ± 10.2	436 ± 5.4	27.31	0.793	0.305	<0.0001

N=10/treatment/sex in adolescent group; N=15-16/treatment/sex in adult group

Table 2

Alterations of dorsal striatal mRNA levels in F1 offspring analyzed by ANOVAs in combined male and female data sets.

Gene	Dorsal Striatum - Adolescence					
	% THC/VEH ± SEM	% Female/Male ± SEM	F ratio	Effect (p-value)		
				Treatment *Sex	Treatment	Sex
<i>Cnr1</i>	93 ± 3.0	113 ± 10.1	1.11	0.910	0.127	0.408
<i>Grin1</i>	98 ± 3.6	123 ± 3.0	1.30	0.277	0.632	0.142
<i>Grin2A</i>	102 ± 7.5	59 ± 6.0	24.05	0.738	0.684	<0.0001
<i>Grin2B</i>	86 ± 4.4	114 ± 3.4	17.43	0.165	0.004	<0.0001
<i>Gria1</i>	93 ± 3.1	128 ± 3.5	2.45	0.408	0.175	0.047
<i>Gria2</i>	97 ± 3.7	123 ± 2.6	2.72	0.157	0.549	0.031
<i>Dlg4</i>	96 ± 12.2	60 ± 2.9	144.40	0.177	0.258	<0.0001
<i>Dlgap3</i>	92 ± 25.8	186 ± 5.4	133.94	0.892	0.223	<0.0001
Gene	Dorsal Striatum - Adulthood					
	% THC/VEH ± SEM	% Female/Male ± SEM	F ratio	Effect (p-value)		
				Treatment *Sex	Treatment	Sex
<i>Cnr1</i>	87 ± 4.9	127 ± 4.2	6.29	0.909	0.039	0.001
<i>Grin1</i>	88 ± 2.3	106 ± 2.7	4.32	0.701	0.003	0.192
<i>Grin2A</i>	82 ± 7.2	57 ± 3.8	35.39	0.570	0.001	<0.0001
<i>Grin2B</i>	89 ± 2.8	99 ± 3.0	2.71	0.292	0.014	0.746
<i>Gria1</i>	88 ± 2.8	105 ± 3.0	3.17	0.840	0.008	0.290
<i>Gria2</i>	86 ± 2.6	100 ± 2.5	5.62	0.134	0.001	0.922
<i>Dlg4</i>	91 ± 8.8	51 ± 2.7	103.33	0.677	0.016	<0.0001
<i>Dlgap3</i>	85 ± 5.0	147 ± 3.5	22.51	0.080	0.004	<0.0001

N=10/treatment/sex in adolescent group; N=15-16/treatment/sex in adult group.

Table 3

Direction of striatal mRNA expression changes in male and female offspring with parental THC exposure.

Gene	Males				Females			
	Adolescence		Adulthood		Adolescence		Adulthood	
	VS	DS	VS	DS	VS	DS	VS	DS
<i>Cnr1</i>	↑	ns	ns	↓	ns	ns	ns	ns
<i>Grin1</i>	↑	ns	ns	↓	ns	ns	ns	↓
<i>Grin2A</i>	↑↑	ns	↓	↓↓	ns	ns	↓	↓
<i>Grin2B</i>	ns	ns	ns	ns	↓	↓↓	ns	↓↓
<i>Gria1</i>	ns	ns	ns	↓	↓	ns	ns	↓
<i>Gria2</i>	↑	ns	ns	↓	ns	ns	ns	↑↑
<i>Dlg4</i>	ns	ns	ns	↓	ns	ns	ns	↓
<i>Dlgap3</i>	↑↑	↑↑	ns	ns	ns	ns	ns	↑↑

Arrowheads indicate direction of THC-related change (up- or down-regulated). One red arrow: p<0.05, two red arrows: p<0.01, black arrow: 0.05 p 0.1, ns: non-significant. N =5/treatment/sex in adolescent group; N=7-8/treatment/sex in adult group. VS: ventral striatum, DS: dorsal striatum.