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Female adolescent exposure to cannabinoids causes transgenerational effects on morphine sensitization in female offspring in the absence of in utero exposure

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Abstract

Female adolescent marijuana use is increasing, yet the effect on future offspring is unknown. Here, adolescent female Sprague Dawley rats (postnatal-day 30; PN30) were given subcutaneous (s.c.) injections with the cannabinoid agonist WIN-55,212 (WIN) or its vehicle (VEH) for three consecutive days using a twice-daily, increasing dosage regimen (1 mg/kg day 1; 2 mg/kg day 2; 4 mg/kg day 3). As adults (PN60), females were mated with drug-naïve males. Their adult female offspring (VEH-F1 or WIN-F1) were tested for behavioral sensitization by administering morphine (0 or 7.5 mg/kg s.c.) every other day for a total of five administrations. Following five days of abstinence, all animals received a morphine challenge (7.5 mg/kg s.c.) and locomotor activity was monitored. At completion of behavioral testing, mu opioid receptor (OPRM1), FosB, cFos, and dopamine receptor mRNA expression was measured in the nucleus accumbens as well as OPRM1 and corticotropin-releasing hormone mRNA in the paraventricular nucleus. In addition, plasma corticosterone levels were examined. On the day of challenge, morphine-pretreated WIN-F1 animals demonstrated a significantly enhanced response to morphine compared to morphine-pretreated VEH-F1 animals. Also following the morphine challenge, significantly higher levels of OPRM1 in the nucleus accumbens were observed in WIN-F1 animals. Together, these findings demonstrate transgenerational effects of adolescent exposure to cannabinoids in the absence of any in utero exposure.

Keywords

Cannabinoids; adolescence; morphine; sensitization; transgenerational

Introduction

Marijuana is currently the most commonly abused illicit drug in the United States and Europe. In 2009, 16.7 million Americans over the age of 12 years (Substance Abuse and

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Conflicts of interest

The authors declare no conflict of interest.

Mental Health Services Administration (SAMHSA), 2011) and 12 million Europeans over the age of 15 years (Thanki et al., 2012) reported recent marijuana use, with use often initiated during adolescence. While adolescent marijuana use is more prevalent among males, use among adolescent females is increasing, with recent surveys indicating that 6.4% of females aged 12–17 used marijuana regularly as compared to 8.3% of males (SAMHSA, 2011). Thus, female adolescent marijuana use is an area of growing public health concern.

Adolescence is a period of transition from childhood to adulthood (Spear, 2000), occurring predominately between 10–19 years of age (Deroche et al., 1992b). This period is associated with the maturation of the endocannabinoid system in brain regions that regulate reward, motivation, and cognition (Marco and Laviola, 2012). Thus, marijuana use by adolescents is of particular concern as developing systems may be more vulnerable to exogenous insults such as exposure to cannabinoids (Rice and Barone, 2000). Indeed, in humans, adolescent marijuana use has been associated with cognitive deficits, addiction, and psychiatric disorders during adulthood (Cohen et al., 2008; Egerton et al., 2006; Schneider, 2008). Similarly, in animal models, cannabinoid use during adolescence, which in the rat is defined as PN28–PN42 (Andersen, 2003), has also been shown to have long-term consequences on brain and behavior. For example, cannabinoid exposure during adolescence, but not adulthood, causes cross-sensitization to opioids and stimulants in dopaminergic neurons (Pistis et al., 2004) and increases levels of heroin self-administration in adulthood (Ellgren et al., 2007).

In recent years efforts have been made to understand sex differences in the endocannabinoid system that might lead to disparities in the long-term consequences of adolescent marijuana use (Craft et al., 2013; Krebs-Kraft et al., 2010; Viveros et al., 2011, 2012). In females, the endogenous cannabinoid system is an important modulator of reproductive processes, with CB1 receptors as well as endogenous cannabinoids (anandamide) expressed in the ovaries, uterine endometrium, and other peripheral endocrine tissue (Bari et al., 2011). As female adolescence is a period of significant development within the reproductive axis, cannabinoid exposure during this critical period could result in lasting modifications in female reproduction, some of which might impact future offspring.

We recently developed a rodent model to examine the effects of female adolescent cannabinoid exposure on subsequent generations. The CB1/CB2 receptor agonist WIN 55,212 (WIN) was administered to female rats on three consecutive days coinciding with their early adolescent period (PN30–PN32). These females were then drug-free until mating at PN60. When the male offspring of adolescent WIN-exposed females were examined, they demonstrated enhanced morphine-induced conditioned place preference (Byrnes et al., 2012). These results indicate that a three-day exposure to cannabinoids during adolescence can induce significant effects on male offspring.

In the current study, the effect of female adolescent cannabinoid exposure on future female offspring is examined. Based on our previous results (Byrnes et al., 2012), our working hypothesis was that adolescent cannabinoid exposure would enhance morphine-induced locomotor sensitization in first generation (F1) female offspring. In addition to examining behavior, the current study also measured changes in gene expression and blood

corticosterone levels in response to morphine. Increased expression of the immediate early gene transcription factor FosB in the nucleus accumbens has been shown to accompany locomotor sensitization. Therefore, we analyzed levels of FosB within the nucleus accumbens, as well as cFos, immediately following behavioral testing. Expression of mu opioid receptor (OPRM1) within the nucleus accumbens was examined given that this receptor is the primary target of morphine. In addition, dopamine D1 and D2 receptor mRNA expression within the nucleus accumbens were examined because dopamine signaling has been shown to be a key mediator in the expression of sensitization (Chen et al., 2009; Vanderschuren and Kalivas, 2000). Finally, plasma corticosterone and corticotropin-releasing hormone (CRH) mRNA from the paraventricular nucleus (PVN) of the hypothalamus were measured as a myriad of maternal effects (including prenatal stress, drug exposure and endocrine disruptor exposure), manifest as changes in the regulation of the HPA axis in the offspring (Bale, 2011; Bohacek and Mansuy, 2013; Guerrero-Bosagna and Skinner, 2012). Our results indicate that female offspring of dams exposed to cannabinoids during adolescence have alterations in physiological systems associated with reward and stress.

Materials and methods

Animals and housing

All animals were housed in standard plastic laboratory cages (40 cm×20 cm×18 cm) at Tufts University Cummings School for Veterinary Medicine (North Grafton, Massachusetts, USA). Animals were maintained on a 12-hour light/dark cycle with lights on at 07:00 and procedures were performed during the light phase. Food and water were available ad libitum. All procedures were approved by the Institutional Animal Care and Use Committee of Tufts University, and were carried out in accordance with the National Research Council (NRC) *Guide for the Care and Use of Laboratory Animals*, 1996. All efforts were made to minimize animal distress and reduce the number of animals used.

F0 (parental generation) animals and F1 animals

The animals used in this study are the female littermates from previously published work from our laboratory (Byrnes et al., 2012). As described previously (Byrnes et al., 2012), female Sprague-Dawley rats were purchased from Charles River Laboratories (Kingston, New York State, USA) at PN23. Animals were group housed and allowed to acclimate to the vivarium for seven days, at which point 16 females began a twice daily subcutaneous (s.c.) regimen of increasing doses of the CB1/CB2 receptor agonist WIN 55,212 (WIN; Sigma-Aldrich; St Louis, Missouri, USA) and 16 females received the 1.0% Tween-80 saline vehicle (VEH) for a total of three days (1 mg/kg on day 1, PN30; 2 mg/kg on day 2, PN31; 4 mg/kg on day 3, PN32). All injections occurred within the homecage. At PN60, all females (16 VEH and 16 WIN) were introduced to a colony male and allowed to mate (two females of the same treatment per one naïve male in one cage). Prior to parturition (i.e. 21 days after initial pairing with a male), females were separated and housed singly. At parturition, all litters were weighed and culled to a size of 10 pups, five male and five female. F1 female offspring only were used for the current studies. The offspring of females exposed to either vehicle or WIN 55,212 during adolescence (VEH-F1 and WIN-F1,

respectively) were weaned on PN21 and group-housed until testing (PN60). Female offspring were group housed with littermates, five per cage until they reached 250 g at which point they were switched to 2–3 per cage. Cages were not assigned to the same experimental group. There were at least six distinct litters represented within each group and a maximum of two pups from the same litter in any one group. As reported previously, WIN administration in adolescent females did not affect body weight or fertility in the dams, nor were there effects on litter size, gender composition, or pup body weight (Byrnes et al., 2012).

Morphine-induced locomotor sensitization

At 60 days of age, F1 female animals were tested for morphine-induced locomotor sensitization. VEH-F1 and WIN-F1 females received either saline (0.9% NaCl, 1 ml/kg, s.c.) or 7.5 mg/kg morphine sulfate (Butler Schein Animal Health, Dublin, Ohio, USA), and locomotor activity was monitored for 90 min using an automated 32-beam infrared photobeam frame which surrounded the open field (SmartFrame[®] Activity Cage Rack System; Hamilton-Kinder, Poway, California, USA). This procedure was repeated every other day for a total of five administrations to induce sensitization. Animals then remained in their home cages for a five-day period of abstinence followed by a challenge test for the expression of sensitization. On the day of challenge (day CH), all animals received 7.5 mg/kg morphine and were tested for locomotor activity for 90 min. Animals were sacrificed immediately after testing using a brief exposure to CO₂ (<30 s) followed by decapitation. Trunk blood was collected into heparinized tubes, centrifuged, and plasma stored at –20°C until assayed for corticosterone. Brains were rapidly removed, frozen in –20°C methylbutane and stored at –80°C until processed for quantitative Polymerase Chain Reaction (qPCR).

Corticosterone radioimmunoassay

Plasma corticosterone levels were measured by radioimmunoassay using commercially available kits (Diagnostics Products Corporation, Los Angeles, California, USA) according to the respective protocol. The detection limit of the assay was 20 ng/mL and all samples were run in duplicate in a single assay.

Gene expression analysis using qPCR

To collect samples for analysis of gene expression, frozen brains were mounted on a cryostat and bilateral micropunches were taken from the nucleus accumbens (1 mm; starting at: +2.5 mm A/P, ±1.5 mm M/L, –7 mm D/V, a region encompassing both core and shell) and PVN (1 mm; starting at: –1.4 mm A/P, ±0.5 mm M/L, –8 mm D/V) according to Paxinos and Watson (1997). Tissue punches were then homogenized in lysis buffer and total RNA extracted using the RNeasy kit from Qiagen (Valencia, California, USA) according to the manufacturer's protocol. Complementary DNA (cDNA) was synthesized using the RETROscript kit from Applied Biosystems (Carlsbad, California, USA). *Polymerase chain reaction* (PCR) was performed using an AB 7500 (Applied Biosystems) under standard amplification conditions: 2 min at 50°C, 10 min at 95°C, 40 cycles of 15 s at 95°C and 60 s at 60°C. All PCR primers were TaqMan[®] Gene Expression Assays purchased from Applied

Biosystems. The amplification efficiency of each of these assays has been validated by Applied Biosystems and averages 100% ($\pm 10\%$). Assay ID and accession numbers were as follows: S16: Rn01476520_g1, cFos: 00487426_g1, FosB: Rn00500401_m1, OPRM1: Rn00565144_m1, CRH: Rn01462137_m1, D1: Rn03062203_s1, D2: Rn01418275_m1

Quantification of gene transcription

Final quantification of mRNA was obtained using the comparative cycle threshold (CT) method (User Bulletin #2, Applied Biosystems). Data are reported as relative transcription or the n -fold difference relative to a calibrator cDNA. In brief, the housekeeping gene for the rat brain tissue, S16, was used as an internal control against which each target signal was normalized; this is referred to as the change in cycle threshold (target gene - housekeeping gene). Validation studies confirmed that the raw CT values of S16 did not vary by treatment group, confirming S16 as an appropriate housekeeping gene. The CT was then normalized against the calibrator (i.e. the mean gene transcription for the vehicle/saline group for the target gene in each brain region) and data are presented as calibrator – change in cycle threshold ($-CT$). As detailed above, all animals received an injection of morphine (7.5 mg/kg) prior to analysis.

Statistical analyses

The sensitization data were analyzed with a three-way repeated measures analysis of variance (ANOVA) test followed by Tukey's pairwise comparisons. The within-subject factor was day, and the between-subject factors were maternal adolescent exposure (VEH-F1 or WIN-F1) and pretreatment regimen (repeated saline or repeated 7.5 mg/kg morphine). Three statistical outliers (as determined by the extreme studentized deviate (ESD) outlier test, or Grubbs' test, with alpha set to 0.05) were removed from this and all subsequent analyses giving an n of 7–12 for each group. Both corticosterone and gene expression data were analyzed using a two-way ANOVA followed by Tukey's pairwise comparisons where appropriate. Between-subject factors were maternal adolescent exposure and pretreatment regimen. Significance was defined as $p < 0.05$.

Results

WIN-F1 female offspring demonstrate enhanced expression of morphine sensitization

Sensitization data was analyzed with a repeated measures three-way ANOVA, which revealed a significant day $\times$ maternal adolescent exposure interaction ($F(2,68)=4.60$; $p=0.013$), a day $\times$ pretreatment regimen interaction ($F(2,68)=50.84$; $p<0.001$), and a maternal adolescent exposure $\times$ pretreatment regimen interaction ($F(1,34)=4.54$; $p=0.04$). These data are shown in Figure 1(a). On day 1, there was a significant reduction in locomotor activity in those animals that received morphine compared to those that received saline ($p<0.05$). No differences between VEH-F1 and WIN-F1 females pretreated with saline were observed on any day. In contrast, WIN-F1, but not VEH-F1, females pretreated with repeated morphine demonstrated significant morphine-induced locomotor sensitization which was obvious even by the treatment day 5 ($p<0.05$ when compared to treatment day 1) On day CH, while both VEH-F1 and WIN-F1 animals pretreated with repeated morphine exhibited significantly enhanced locomotor activity when compared with pretreated saline animals ($p<0.05$), the

expression of sensitization as measured on day CH was significantly greater in WIN-F1 animals compared to VEH-F1 animals ($p<0.05$).

Data was also examined as a change from day 1 activity. Locomotor activity on day 1 was subtracted from activity on day 5 and day CH for each animal (see Figure 1(b)). This data was analyzed with a three-way repeated measures ANOVA, which revealed a significant day $\times$ maternal adolescent exposure interaction ($F(1,37)=7.22$; $p=0.011$); and a day $\times$ pretreatment regimen interaction ($F(1,37)=33.60$; $p<0.001$). Subsequent two-way ANOVAs were performed for each day. On day 5, there was a significant main effect of pretreatment regimen ($F(1,34)=16.78$; $p<0.001$). Tukey's test revealed that WIN animals that received repeated morphine had significantly greater locomotor activity than those that received saline ($p<0.05$). On day CH, there was a significant main effect of pretreatment regimen ($F(1,34)=73.20$; $p<0.001$) as well as a main effect of maternal exposure ($F(1,34)=5.54$; $p=0.025$). Tukey's post test revealed that in both the VEH- and WIN-F1 animals, repeated morphine significantly enhanced responding when compared to repeated saline when all animals were challenged with morphine ($p<0.05$). Moreover, WIN-F1 animals pretreated with repeated morphine had significantly greater locomotor activity on day CH than did their VEH-F1 counterparts ($p<0.05$). Thus, maternal adolescent exposure to WIN 55,212 resulted in enhanced morphine-induced locomotor sensitization in female offspring, an effect that does not appear to be due to any shift in the general response to injections or testing conditions.

Differential regulation of gene expression in WIN-F1 female offspring

Gene expression levels in the nucleus accumbens were examined in VEH-F1 and WIN-F1 animals 90 min after the morphine challenge (7.5 mg/kg, s.c.). While there were no significant changes in the immediate early gene Fos family, there was a trend towards an increase in the expression of the FosB gene in all animals pretreated with morphine regardless of maternal exposure (see Figure 2(a); main effect of pretreatment regimen ($F(1,28)=3.42$; $p=0.07$)). There was no significant effect of maternal adolescent exposure and no interaction. A similar trend towards increased expression of cFOS as a function of pretreatment with repeated morphine was also observed (Figure 2(b); ($F(1,31)=3.07$; $p=0.08$)). Once again, these were no significant effects of maternal adolescent exposure and no interaction. The number of animals in these groups varied from 6–11 because some analytes were undetectable. We also examined dopamine D1 and D2 receptor expression levels in the nucleus accumbens. No significant differences in either dopamine D1 or D2 receptor gene expression were observed. These data are shown in Table 1.

OPRM1 expression levels in the nucleus accumbens as well as the PVN were also examined 90 min following the morphine challenge. As shown in Figure 3(a), there was a significant main effect of maternal adolescent exposure ($F(1,27)=6.59$; $p=0.016$) but no main effect of pretreatment regimen nor an interaction. In contrast, no changes in OPRM1 mRNA expression levels were observed within the PVN (see Figure 3(b); all $ps>0.05$).

Plasma corticosterone following morphine treatment in F1 animals

On day CH, all animals received 7.5 mg/kg morphine and were tested for locomotor activity for 90 min. Plasma corticosterone levels were measured immediately following testing. Therefore, as with gene expression, corticosterone levels were measured 90 min after morphine administration and immediately after measurements of locomotor activity testing. While there were no statistically significant differences, there was a modest trend towards a main effect of maternal adolescent exposure ($F(1,34)=2.88$; $p=0.09$) as well as a main effect of pretreatment regimen morphine dose ($F(1,34)=3.38$; $p=0.07$). As shown in Figure 4(a), these trends appeared to be primarily driven by increased corticosterone levels in WIN-F1 females pretreated with saline. Indeed, when compared to VEH-F1 females pretreated with saline, WIN-F1 females demonstrated a significantly more robust corticosterone response on day CH ($t=2.27$, $p<0.05$). Examination of CRH mRNA in the PVN, however, did not reveal any significant difference between the groups (Figure 4(b)).

Discussion

The current findings demonstrate enhanced expression of morphine-induced locomotor sensitization in WIN-F1 females compared to VEH-F1 controls. While no changes in gene expression or corticosterone secretion correlated with these behavioral effects, WIN-F1 animals pretreated with saline and administered morphine for the first time on day CH, did demonstrate alterations in both OPRM1 expression in the nucleus accumbens, as well as augmented corticosterone response. The current findings indicate that significant transgenerational effects can arise in the female offspring of mother's exposed to cannabinoids during adolescent development. These data are similar to previous findings in male offspring following this same cannabinoid regimen (Byrnes et al., 2012). Taken together, these findings support the hypothesis that adolescent female cannabinoid exposure can induce effects in both male and female offspring in the absence of in utero exposure.

In the present study, we found enhanced expression of morphine-induced locomotor sensitization in WIN-F1 females. Sensitization is hypothesized to occur via plasticity in motor, motivational, and cognitive neural systems, and is thought to contribute to drug craving and relapse (Barb et al., 1992; Drevets et al., 2001; Kantak et al., 2009; Spano et al., 2007). Both VEH-F1 and WIN-F1 animals that received morphine had a reduction in locomotor activity on day 1 compared to those animals that received saline. This expected effect is due to the sedative properties of morphine. Additionally, both VEH-F1 and WIN-F1 animals demonstrated morphine-induced locomotor sensitization with significant increases in locomotor activity on day 5 compared to day 1 and day CH when compared to saline-pretreated controls. However, there was a significant enhancement in the magnitude of expression of sensitization on day CH between WIN-F1 animals and VEH-F1 controls. This suggests that WIN-F1 animals may have an increased vulnerability to processes involved in morphine-induced locomotor sensitization which emerge following a period of abstinence.

Drug sensitization can last for days, weeks or even months after cessation of drug exposure (Craft, 2008). The immediate early gene FosB is a member of the Fos family of transcription factors and is implicated in neural plasticity in addiction and sensitization within the nucleus accumbens (Anier et al., 2010). Therefore, we examined the levels of FosB in WIN-F1 and

VEH-F1 animals at the conclusion of testing on day CH. A large body of literature indicates that FosB and its truncated form (FosB) are important for drug sensitization. Recently, this has been shown true with morphine-induced sensitization as well (Kaplan et al., 2011). Consistent with this, we found a trend towards increased FosB expression in the nucleus accumbens following pretreatment with repeated morphine. It should be noted that the sensitization paradigm that we utilized was designed to produce only weak sensitization so as not to produce a ceiling effect. Animals were administered 7.5 mg/kg of morphine every other day for a total of five administrations, and challenged with the same dose five days later. Therefore, it is perhaps not surprising that the effect observed with the FosB expression was only a marginal effect. We also examined the expression of cFos following the sensitization challenge day. Another member of the Fos family of immediate early gene transcription factors, cFos, is a classical marker for neuronal activity. Morphine is known to increase the expression of cFos within the nucleus accumbens (Lajtha and Sershen, 2010). Consistent with this finding, we found a trend towards increased cFos in the nucleus accumbens in animals that received a sensitizing regimen of morphine. Likely, this effect did not reach significance because acute morphine administration can also increase cFos expression and all animals received a challenge injection of morphine. Nonetheless, the trends toward increased expression of Fos genes in the nucleus accumbens were observed regardless of maternal adolescent drug history. Therefore, differential effects on Fos genes as a function of maternal adolescent cannabinoid exposure is not a likely candidate for the underlying mechanism mediating the enhanced expression of morphine-induced locomotor sensitization observed in WIN-F1 females.

Opioid receptors are highly expressed by nucleus accumbens medium spiny neurons (Mansour et al., 1995), although their role in addiction remains unclear. Local opioid infusion into the nucleus accumbens modulates behavior in a biphasic manner. Morphine initially suppresses locomotor activity and subsequently causes hyper-locomotion (Cunningham and Kelley, 1992). Previously, mu opioid antagonists have been shown to play a role in the reinstatement of food-seeking behavior (Guy et al., 2011), but not heroin-seeking behavior (Stewart and Vezina, 1988). More recently, it has been suggested that persistent neuroadaptations in the nucleus accumbens mu opioid receptors can contribute to drug-seeking behaviors (Li et al., 2008). Consistent with this notion, endogenous opioid peptides such as B-endorphin, are released in the nucleus accumbens by cocaine (Roth-Deri et al., 2003) and stressful situations (Zangen and Shalev, 2003), both shown to reinstate drug-seeking behavior. Here, we report increases in expression of accumbal OPRM1 mRNA of WIN-F1 animals. While this effect on OPRM1 was a main effect of maternal adolescent exposure, it appears to be largely driven by WIN-F1 animals receiving their first exposure to morphine on day CH (i.e. saline-pretreated WIN-F1 animals). This suggests that the OPRM1 system of WIN-F1 females may respond differently to initial agonist exposure. It is unclear, however, whether the prior exposure to repeated injections of saline and behavioral testing in this group influenced the subsequent expression of OPRM1 mRNA in response to an acute morphine challenge. This will be important to determine in future studies as it may address the possible link between stress- and reward-related circuits in the observed effects in WIN offspring.

While changes in the OPRM1 transcription in the nucleus accumbens were observed, no effects on either the dopamine D1 or D2 receptor gene expression in the nucleus accumbens were detected. The lack of differences between the WIN-F1 and VEH-F1 females with regard to post-synaptic dopamine receptors is consistent with the similarities in Fos induction observed in these two groups. Indeed, previous findings indicate that Fos protein induction in the nucleus accumbens is dopamine dependent (Cousijn et al., 2012; Lajtha and Sershen, 2010). Together, these data suggest that modification in postsynaptic dopamine systems are unlikely to be critical mediators of the differential effects observed in WIN F1 females.

Repeated stress increases the propensity towards drug taking and augments psychomotor effects of opioids (Deroche et al., 1992a, 1992b). Corticosterone, the primary glucocorticoid in the rat, is secreted in response to stress as part of the hypothalamic-pituitary-adrenal (HPA) axis. WIN-F1 animals tended to have higher levels of circulating corticosterone in response to morphine than the VEH-F1 controls. However, while all of the animals received an injection of morphine, the trend towards an effect of maternal adolescent exposure on corticosterone secretion again appears to be primarily mediated by WIN-F1 animals experiencing morphine for the first time, similar to the OPRM1 findings. Thus, WIN-F1 animals show a much greater corticosterone response to an acute morphine challenge than VEH-F1 animals whereas both morphine-pretreated groups have similar increases in corticosterone levels on day CH. The administration of morphine causes a rapid rise in plasma levels of corticosterone in the rat followed by a decrease several hours later (Pechnick, 1993). Therefore, the alterations observed in morphine-induced corticosterone secretion in WIN-F1 females could be due to a difference in this biphasic response (i.e. corticosterone levels may stay elevated longer or increase more slowly as a function of maternal exposure). Future studies examining the time course of HPA reactivity following morphine would provide additional information regarding transgenerational effects on opioidergic modulation of the stress axis in the offspring of adolescent females exposed to cannabinoids. In that regard, repeated exposure to stressful stimuli have been shown to enhance the psychomotor effects of morphine (Deroche et al., 1993). Thus, there is a stress-induced enhancement of the pharmacological effects of morphine that is dependent on corticosterone and can be mimicked by repeated injections of corticosterone (Deroche et al., 1992b). It is possible that a similarly enhanced morphine-induced corticosterone response to the initial dose of morphine, may be observed in our WIN-F1 females that were then administered repeated morphine and thus, a shift in morphine-induced corticosterone during the early development phase of locomotor sensitization could be an underlying factor in the enhanced behavioral sensitization observed in WIN-F1 females.

We also examined the levels of CRH mRNA in the PVN. While we did not observe any significant effects, the pattern of expression was similar to the levels of circulating corticosterone. It is possible that the failure to detect statistically significant differences in CRH gene expression was due to large inter-animal variability in CRH gene expression. Given the critical role of CRH in the PVN in maintaining homeostasis, such individual variability following behavioral testing is not unexpected. Future studies should examine both basal levels of CRH mRNA as well as the effects of acute morphine when measured in the home cage of VEH-F1 and WIN-F1 animals. Nonetheless, in the context of the current

study, the similarity of the changes in both corticosterone secretion and the pattern of CRH gene expression in the PVN suggest that WIN-F1 animals may have an altered HPA response to OPRM1 activation. Future studies will be needed to elucidate the transgenerational effects of adolescent cannabinoid exposure on the interplay between the stress axis and reward pathways.

The mechanism of transmission of the observed phenotypes is unknown. One possibility is that the interaction between the dam and her offspring may be altered in such a way that WIN-F1 offspring do not receive the same level of maternal care as VEH-F1 offspring. This can be measured by examination of licking and grooming behavior and nursing postures (Champagne and Meaney, 2001). While these animals were not observed for maternal behavior, previous studies from this laboratory have shown that there are only very subtle differences in maternal care behavior of dams exposed to morphine during adolescence versus those exposed to saline (Johnson et al., 2011). Future studies are needed to test if that holds true for cannabinoid agonist exposure, as well. Another possible mechanism of transmission is that adolescent cannabinoid exposure alters the development of the reproductive axis and thus changes the prenatal environment. The endocannabinoid system is involved in the maturation of the reproductive axes (Bari et al., 2011) and exposure to exogenous cannabinoids may disrupt the development. Epigenetic changes within the gametes of the dam may also take place including methylation of DNA or posttranslational modifications to core histone protein (Sharma, 2012). Finally, differential imprinting of specific genes caused by epigenetic modifications may play a role in the mechanism of transmission (Keverne, 2013). Future studies will examine the F2 generation in order to determine effects that are transmitted via incorporation into the germline and effects that result from altered prenatal environment.

We previously found that male offspring of females exposed to cannabinoids during adolescence demonstrate enhanced conditioned place preference, particularly at lower doses. The data presented here was collected from female littermates from that work (Byrnes et al., 2012). While the same behavioral paradigms were not used in these two experiments, the results indicate a similar phenotype between the males and females. Enhanced conditioned place preference (CPP) as well as enhanced expression of locomotor sensitization both indicate phenotypes of increased sensitivity to the effects of morphine. Future studies will be needed to examine the sensitization behavior of the males and the CPP behavior of the females.

Overall, the current set of findings indicates that a relatively brief (three day) exposure to cannabinoid agonists in female adolescent rats can impact behavioral, endocrine, and gene transcription events in future offspring. These effects occur in the absence of any in utero exposure. The direction of the observed effects (i.e. enhanced expression of morphine-induced locomotor sensitization, and increased transcription of OPRM1 in the nucleus accumbens) lead to the hypothesis that female adolescent exposure to cannabinoids affects future offspring in a manner that may alter the nature of their responses to other drugs of abuse, such as opiates.

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Figure 1.

WIN-55,212 (WIN)-treated first generation (F1) females demonstrate enhanced expression of morphine locomotor sensitization: (a) total locomotor activity; (b) change from day 1. Female vehicle (VEH)- and WIN-treated F1 animals were administered either saline or morphine (7.5 mg/kg; s.c.) every other day for five days to induce locomotor sensitization. Following a five-day abstinence period, all animals were challenged with morphine (7.5 mg/kg; s.c; day of challenge (day CH)). (a) Data presented are mean ± standard error of the mean (SEM) of the total locomotor activity (ambulatory counts) on day 1, day 5 and day CH, (b) the difference from day 1 activity on day 5 and day CH is presented. * $p < 0.05$ WIN-F1 morphine-pretreated (F1 animals repeatedly administered morphine during sensitization testing) on day CH compared to VEH-F1 morphine-pretreated on day CH. + $p < 0.05$ WIN-F1 morphine-pretreated animals on day 5 or day CH compared to day 1. ++ $p < 0.05$ VEH-F1 morphine pretreated animals on day CH compared to day 1. # $p < 0.001$ morphine pretreated animals compared to the saline pretreated animals ($n = 7-12$).

Figure 2.

FosB levels in the nucleus accumbens tend to increase in response to morphine-induced locomotor sensitization in both vehicle (VEH)-first generation (F1) and WIN-55,212 (WIN) (F1) females. quantitative real-time Polymerase Chain Reaction (qPCR) was utilized to examine the expression levels of (a) FosB and (b) cFOS (parental generation) 90 min following the day of challenge (day CH). Data are expressed as mean $\pm$ standard error of the mean (SEM) of the $\Delta\Delta CT$ value. In both VEH- and WIN-F1 females, there was a non-significant trend towards an increase in FosB and cFOS expression levels within the nucleus accumbens following morphine subcutaneous (s.c.) pretreatment ($p=0.07$ and 0.08 respectively) ($n=6-11$).

Figure 3.

Levels of mu opioid receptor (OPRM1) mRNA expression. There is an increase in OPRM1 mRNA expression levels in the nucleus accumbens in WIN-55,212 (WIN)-first generation (F1) females. qrtPCR was used to examine the expression levels of OPRM1 mRNA (a) within the nucleus accumbens and (b) the paraventricular nucleus of the hypothalamus (PVN). Data are expressed as mean $\pm$ standard error of the mean (SEM) of the $\Delta\Delta Ct$ value. Within the nucleus accumbens, there was a main effect of maternal treatment with WIN-F1 animals demonstrating significantly increased levels of OPRM1 mRNA ($p < 0.05$, *main effect). There were no significant changes within the PVN ($n = 7-12$).

Figure 4.

Levels of corticosterone and corticosterone-releasing hormone (CRH). (a) WIN-55,212 (WIN)-first generation (F1) females tend to have increased levels of corticosterone. Plasma corticosterone levels were measured using a radioimmunoassay. Data are expressed as mean $\pm$ standard error of the mean (SEM) corticosterone (ng/mL). WIN-F1 females showed a non-significant trend towards increased blood corticosterone regardless of pretreatment ($p=0.09$). (b) CRH in the paraventricular nucleus (PVN) of the hypothalamus was also measured using qrtPCR. Data are expressed as mean $\pm$ SEM of the $\Delta\Delta CT$ value. There were no significant changes in CRH expression within the PVN ($n=7-12$).

Dopamine receptor mRNA expression levels in the nucleus accumbens do not change. Quantitative, real-time Polymerase Chain Reaction (qPCR) was used to assess the mRNA expression levels of dopamine D1 and D2 receptors in the nucleus accumbens. Data are expressed as mean (standard error of the mean (SEM)) of the CT (change from the change in cycle threshold, calibrator – change in cycle threshold) value. There were no significant changes.

Table 1

Brain region	F0 (Parental generation) condition ^a	F1 condition ^b	n	D1 expression	D2 expression
Nucleus accumbens	Vehicle	Saline	8	1.000 (0.552)	1.000 (0.480)
		Morphine	8	0.932 (0.697)	0.832 (0.677)
	WIN 55,212	Saline	10	1.229 (0.302)	1.186 (0.274)
		Morphine	10	1.398 (0.382)	1.046 (0.660)

^a Adolescent exposure in F0 females;
^b pretreatment regimen plus morphine challenge in first generation (F1) females.