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Early life stress alters opioid receptor mRNA levels within the nucleus accumbens in a sex-dependent manner

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Abstract

Early life stress (ELS) strongly impacts mental health, but little is known about its interaction with biological sex and postnatal development to influence risk and resilience to psychopathologies. A number of psychiatric disorders, such as social anhedonia and drug addiction, involve dysfunctional opioid signaling; moreover, there is evidence for differential central opioid function in males vs. females. The present study examined opioid receptor gene expression in the nucleus accumbens (NAc) and amygdala of male and female rats subjected to a neonatal predator odor exposure (POE) paradigm to model ELS. Brain tissue was collected at two developmental time points: neonatal and juvenile. Results showed that, following the neonatal POE experience, opioid receptor mRNA levels in the NAc were differentially regulated at the neonatal and juvenile time points. POE down-regulated neonatal mu- and kappa-opioid receptor mRNA levels in neonatal females, but upregulated mu- and delta-opioid receptor mRNA levels in juvenile females. Intriguingly, POE had no significant effect on NAc opioid receptor mRNA levels in males at either time point, indicating that the impact of POE on opioid system development is sex-dependent. Finally, POE failed to alter amygdalar opioid receptor gene expression in either sex at either time-point. The spatiotemporally- and sex-specific impact of ELS within the developing brain may confer differential risk or resilience for males and females to develop atypical opioid-regulated behaviors associated with conditions such as depression and addiction.

Keywords

Opioids; Nucleus accumbens; Amygdala; Early life stress; Development; Sex differences

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Conflicts of interest

The authors report no conflicts of interest.

1. Introduction

Environmental perturbations, such as early life stress (ELS), occurring during important developmental time periods are known to impact mental health risk (Cohen et al., 2013; Pechtel and Pizzagalli, 2011). For example, clinical studies have revealed that individuals who experience child abuse and/or neglect display heightened susceptibility for developing affective disorders, e.g. depression and anxiety later in life (Gorka et al., 2014; Heim and Nemeroff, 2001; Bale et al., 2010). Importantly, biological sex is another contributing factor that influences mental health risk, and sex differences are observed in diagnoses, onset, and manifestation of various psychopathologies, including depression, anxiety disorders, eating disorders, and schizophrenia (Aleman et al., 2003; Kessler et al., 1993; Kessler et al., 1994; Hudson et al., 2007; Striegel-Moore et al., 2008). Therefore, it is important to understand how ELS interacts with biological sex to influence brain development.

Animal models of ELS have indicated that the neonatal environment has long-term effects on behavior and neurobiological systems (Bale et al., 2010; Eiland and McEwen, 2010; Roth et al., 2009). Early life stress also appears to result in pervasive alterations in behavioral processes related to opioid system functioning that manifest during the juvenile period, such as social anhedonia and higher risk for developing drug addiction (Arnold and Sivi, 2002; Enoch, 2011; Laviola et al., 2003; Ward and Stehm, 1991), suggesting that ELS impacts central opioid systems. Very little is known about sex differences in opioid receptor mRNA expression within the developing brain, and how it may be modulated by early life stress. The study aims to fill this gap by examining effects of ELS and biological sex on the nucleus accumbens (NAc) and the amygdala, two brain regions associated with high levels of opioid receptor expression (Auger and Olesen, 2009; Koob, 1992; Lutz and Kieffer, 2013; Trezza et al., 2010).

The goal of the present study is to characterize the developmental impact of early life stress on opioid receptor expression. Opioid receptor mRNA expression levels were measured in males and females in response to a neonatal predator odor exposure (POE) paradigm to assess changes in the development of the opioid system by ELS. This behavioral model was designed to examine the ecologically relevant impact of psychological “fear of harm” on the developing brain (Kigar et al., 2017). Sex-dependent changes in mRNA levels of the opioid receptors in the nucleus accumbens and amygdala were examined at both neonatal and juvenile time points to determine immediate as well as long-lasting effects of early life stress.

2. Results

2.1. Neonatal POE decreases MOR and KOR mRNA levels in female NAc at P3

Mu-, kappa-, and delta-opioid receptor (MOR, KOR, DOR, respectively) mRNA levels were assayed in postnatal day (P3) nucleus accumbens (NAc) and amygdala samples to determine whether the POE experience immediately altered the expression of those receptor genes. A treatment $\times$ sex interaction ($F_{(1,21)} = 7.886$, $p = 0.012$) was found in NAc MOR mRNA levels, whereas both a main treatment effect ($F_{(1,20)} = 12.906$, $p = 0.002$) as well as a treatment $\times$ sex interaction ($F_{(1,20)} = 9.23$, $p = 0.007$) were seen for NAc KOR mRNA levels

(Fig. 1A). There were no effects on mRNA levels of DOR. Post-hoc analyses using Tukey's Honest Significant Difference (HSD) test revealed that females exposed to POE exhibited diminished levels of MOR ($p = 0.01$) and KOR ($p < 0.001$) mRNA relative to non-stressed female controls or males, whereas no effect was seen for NAc DOR mRNA levels. Opioid receptor mRNA levels were not altered in stressed males. These findings demonstrate that POE selectively downregulates MOR and KOR mRNA levels in neonatal female NAc.

In contrast to the NAc-specific effects described above, no effects due to the POE experience were observed in the amygdala at P3 (Fig. 1B).

2.2. Neonatal POE increases MOR and DOR mRNA levels in female NAc at P33

The mRNA levels of opioid receptors in the juvenile NAc and amygdala were analyzed at P33 to investigate whether the POE experience had lasting effects on the opioid system. In the NAc, neonatal POE elevated MOR mRNA levels (main effect of treatment: $F_{(1,37)} = 4.24$, $p = 0.047$) (Fig. 2A). Furthermore, in contrast to the P3 time point at which no effects on DOR mRNA were observed, there were main effects of sex ($F_{(1,37)} = 5.629$, $p = 0.023$) and a treatment $\times$ sex interaction ($F_{(1,37)} = 5.866$, $p = 0.021$) for DOR mRNA levels at the P33 time point. Post-hoc analyses with Tukey HSD confirmed that POE significantly increased MOR ($p = 0.026$) and DOR ($p = 0.009$) mRNA levels specifically in stressed females compared to control females at P33. In contrast to the P3 time point, there were no statistically significant effects of POE or sex on mRNA levels of KOR at P33 in the NAc. No effects were seen in males for any of the opioid receptor mRNA levels at P33.

Similar to the neonatal time point, no effects due to the POE experience were observed in the amygdala at P33 (Fig. 2B).

3. Discussion

The data in this experiment indicate that early life stress (ELS) alters opioid receptor mRNA expression in a sex-, time- and region-dependent manner. Specifically, experiencing the predator odor exposure (POE) caused decreases in MOR and KOR mRNA levels in the NAc of stressed females early in development at the neonatal time point (P3) (Fig. 1A). At the juvenile time point (P33), neonatally POE-exposed female rats displayed elevated MOR and DOR mRNA levels within the NAc (Fig. 2A). Therefore, the impact of POE on opioid receptor mRNA levels varied across developmental time-points. Considering that these changes were observed only in females, biological sex appears to play an important role in how ELS alters opioid receptor mRNA levels within the developing brain. Interestingly, there were no effects due to POE at either time point in the amygdala for any of the opioid receptors (Figs. 1B and 2B), indicating that the impact of ELS on the opioid system is partially region-specific.

3.1. Neonatal POE as a model of ELS

Neonatal predator odor exposure has been used previously and validated as a potent rodent stressor during the neonatal period (Conely and Bell, 1976; Lyons and Banks, 1982). However, previous experiments typically expose each group of animals to only one type of predator odor, either as a singular occurrence, or across multiple sessions. The present POE

paradigm introduces the novel aspect of variable odors - cat (Adamec and Shallow, 1993), novel adult male rat (Conely and Bell, 1976; Lyons and Banks, 1982; Tanapat et al., 1998), and ferret (Baisley et al., 2011; Bakshi et al., 2012; Rajbhandari et al., 2015) - on different days to prevent habituation of the pups to a specific odor. Using POE as a model of early life stress mimics a naturalistic “fear of harm” without depriving pups of maternal care and warmth, food or drink, or other resources for extended periods of time. Importantly, neonatal POE induces a surge of cortisol immediately following exposure (Tanapat et al., 1998), leading to multiple molecular and behavioral effects, including a decrease in cell proliferation in the dentate gyrus (Tanapat et al., 1998), as well as alterations in pup vocalizations (Ayers et al., 2015; Lyons and Banks, 1982), and freezing behavior in juvenile animals (Ayers et al., 2015). The present study extends these observations by examining long-term outcomes of neonatal POE on opioid receptor gene expression. These long-lasting effects may be critical to understanding the mechanisms and potential treatment of abnormal development and atypical opioid-regulated behaviors later in life in response to this form of ELS.

It is important to note that various types of early life stressors, including different types of predator odors, can yield different molecular and behavioral outcomes. For instance, maternal separation and lipopolysaccharide (LPS) injections are two distinct stressors that, when administered perinatally, result in opposing outcomes of juvenile social play and vasopressin expression. While maternal separation leads to increased vasopressin mRNA expression in the bed nucleus of the stria terminalis (BNST) and increased levels of juvenile social play (Veenema and Neumann, 2009), LPS injections result in decreased vasopressin mRNA expression in the BNST and lower levels of juvenile social play (Taylor et al., 2012). Other studies have shown that specific predator odors can also yield contrasting outcomes. For example, tri-methylthiazoline (TMT; fox odor) has been shown to induce Fos activation in different brain regions than cat odor (McGregor et al., 2002; Staples et al., 2008). Additionally, these studies found that rats demonstrate retreat or escape behavior when exposed to cat but not fox odor. Habituation can also occur after repeated exposure to the same predator odor (Dielenberg and McGregor, 1999; Staples, 2010). Furthermore, research from the Bakshi lab has compared directly the effects of predator (ferret) exposure with standard lab stressors (foot-shock, restraint stress) and found that predator exposure causes far greater activation in key regions associated with defensive behaviors (Baisley et al., 2011). Additionally, exposure to the same stressor at different time points during development, such as adolescence versus adulthood, can have differential effects on neuroendocrine and gene expression (Bakshi et al., 2008). Therefore, the findings in the current study are likely to be unique to the variable POE paradigm used.

3.2. ELS impacts opioid receptor mRNA levels in the NAc, not the amygdala

In the present experiment, opioid receptor mRNA levels were altered in the NAc in response to POE while there were no detectable changes within the amygdala. These findings suggest the possibility that opioid receptor mRNA expression within the NAc may be more responsive than the amygdala to POE. However, this does not rule out the possibility that other systems, such as dopamine, may be changing within the amygdala to influence development. Furthermore, opioid receptor changes in the NAc could influence amygdala-

mediated processes indirectly by modulating the sensitivity of the NAc to amygdala input (Kelley et al., 1982; Brog et al., 1993; Wright et al., 1996). Finally, the amygdala is a heterogeneous grouping of nuclei, e.g., central, medial, basolateral, each with distinct afferent inputs from other brain regions (Kim et al., 2016). At this time, we cannot rule out the possibility that opioid receptor expression changes may differ between these subregions of the amygdala—an effect that would be masked by our method of tissue collection in the current experiment. This will be an important avenue to explore in future studies.

While the opioid system is implicated in a wide variety of behaviors, the nucleus accumbens and amygdala are involved in regulating different types of opioid-regulated behaviors. The amygdala has been highly implicated in social reward/anhedonia (Auger and Olesen, 2009), while opioid signaling in the NAc is involved in drug addiction as well as feeding behavior (for reviews, see: Kelley et al., 2005; Baldo and Kelley, 2007). Additionally, opioid receptors in these two regions have been found to be involved in distinguishable aspects of appetitively motivated behavior, such as hedonic food reward (Pecina and Berridge, 2005) versus incentive salience attribution (DiFeliceantonio and Berridge, 2012). Specifically, MORs in the amygdala mediate the incentive properties and motivation to seek out reward, while the activation of MORs in the NAc increases the hedonic perception of reward during/after consumption (Pecina and Berridge, 2005; Barbano and Cador, 2007; Mahler and Berridge, 2009; Der-Avakian and Markou, 2012; DiFeliceantonio and Berridge, 2012; Mahler and Berridge, 2012; Baldo et al., 2013). It is intriguing that neonatal POE altered opioid receptor levels within the NAc and not the amygdala. This could suggest a level of anatomical specificity to MOR disruption by neonatal POE.

Interestingly, we also found altered KOR and DOR mRNA expression levels in the nucleus accumbens but not the amygdala. Delta-opioid receptors have been found to interact with MORs to affect dopamine release and can influence feeding and drug-seeking behaviors (Pentney and Gratton, 1991; Acquas et al., 1993; Yoshida et al., 1999; Ragnauth et al., 2000; Simmons and Self, 2009). Kappa-opioid receptors, on the other hand, have opposing effects to MOR and DOR on dopamine release in the NAc (Di Chiara and Imperato, 1967). In this brain region, they have been shown to inhibit dopamine, glutamate, and GABA release, and have been associated with modulation of depressive-like behavior (Hjehmstad and Fields, 2001; Thompson et al., 2000; Hjehmstad and Fields, 2003; Ebner et al., 2010). Intriguingly, the co-regulation of the opioid receptor subtypes observed in this study may also be compensatory. For example, MOR knockout mice demonstrate decreased anxiety- and depressive-like behaviors, while DOR knockout mice have been shown to display increased levels of anxiety- and depressive-like behaviors (Filliol et al., 2000; Yoo et al., 2004; Lutz and Kieffer, 2013). Therefore, it is important for future studies to determine whether changes in levels of receptor expression may be maladaptive or adaptive in this model. All together, these data demonstrate that opioid receptors in the NAc are impacted by neonatal POE, which could underlie differences in juvenile and later adult behavior. Further studies including behavioral outcome measures are necessary to elucidate whether ELS has differential effects on different types of opioid-sensitive behaviors.

3.3. Effect of ELS on opioid receptor mRNA levels differs across development

Although the functional consequences of the changes observed in opioid receptors in response to POE within the NAc are yet to be determined, the differences between time points may be particularly important in elucidating the impact of ELS across neuronal development. In this experiment, two developmental time points were included, one during the neonatal period (P3) and one during the juvenile period (P33). These two time points are critical to the development of neurological disorders and particularly susceptible to disturbances from the environment (Bale et al., 2010; Holder and Blaustein, 2014; Rice and Barone, 2000; Roth and Sweatt, 2010; Schneider, 2013; Sisk and Foster, 2004). Changes in opioid receptor mRNA levels at P3 were observed to examine the immediate effects of POE at a time when the brain is highly plastic and connections and synapses are forming rapidly. Therefore, disruptions during this time point may have substantial and permanent effects on brain development. It will also be essential to determine the functional role of the lasting differences in MOR and DOR mRNA levels in females during the juvenile period. It is considered by many that the juvenile period may underlie a second period of vulnerability to social and environmental perturbations. The increase in MOR and DOR could suggest either increased risk or resilience to further stressors during the juvenile period. Dysregulation of opioid receptors during the juvenile period may alter juvenile social interactions and thereby further influence brain development. Therefore, further experimentation is needed to elucidate the direct impact of early life stress versus those due to altered juvenile social interactions on adult opioid receptor expression.

Importantly, we observed that the impact of neonatal POE on specific opioid receptor mRNA varied across development. It has been shown that, in the typically-developing neonatal rat brain, higher levels of MOR and KOR mRNA expression occur during early stages of development, whereas higher DOR mRNA levels appear to be expressed later in development (Kornblum et al., 1987; Zhu et al., 1998). These differential expression patterns may indicate varied receptor function; however, the functional role of opioid receptors during the neonatal period is still relatively unclear and will require future mechanistic studies. Nevertheless, the present results suggest that POE may impact the basic neurobiological mechanisms that underlie heterogeneity among MOR, KOR, and DOR gene expression patterns across development.

3.4. ELS alters NAc opioid receptor mRNA levels in females, not males

Alterations in OR mRNA levels were observed only in the females in the present experiment. These sex-specific findings suggest that biological sex plays a role in how ELS alters the opioid system at the molecular level. The mechanisms underlying sex-specific effects on opioid system regulation are not fully understood; however, it is likely that gonadal hormones are involved. For example, research shows that levels of estrogen can stimulate release of endogenous opioids, which in turn regulates concentration (Piva et al., 1995) and internalization (Eckersell et al., 1998) of opioid receptors. Estrogen can also facilitate the formation of opioid receptor dimers, which can result in sex differences in opioid receptor action (Lee and Ho, 2013). Interestingly, gonadectomies in male mice do not alter opioid receptor protein expression or ligand affinity, but do influence the potency of opioid agonists (Candido et al., 1992). Therefore, it is important to note that while no

changes in opioid receptor mRNA levels were observed in males, it is possible that males could exhibit differences in other parameters of opioid receptor function within the NAc and amygdala, such as ligand binding dynamics. Taken together, these observations suggest that differences in levels of sex steroid hormones in males and females at both timepoints may modify how stress regulates opioid receptor mRNA expression in a sex-specific manner. They also suggest that neonatal stress can exacerbate differences in opioid action within the female versus male brain, as POE induces dramatic differences in opioid receptor expression in females but not males.

3.5. Concluding summary

Our results demonstrate that early life stress (ELS) in the form of POE impacts the developing brain in a region-, time-, and sex-dependent manner. The early changes that occur in the neonatal period could re-shape neuronal connections (particularly within the NAc) in a sex-dependent manner, causing a cascade of events that re-program the opioid system, possibly resulting in the opioid receptor mRNA changes seen at the juvenile time point in the present study. This chain of events could engender enduring perturbations in behaviors that are regulated by central opioid signaling, such as social anhedonia and drug addiction later in life. Accordingly, the opioid system plays a role in a variety of processes such as reward, neurogenesis, stress, anxiety, analgesia, and addiction (Drolet et al., 2001; Kieffer and Gaveriaux-Ruff, 2002; Le Merrer et al., 2009; Lutz and Kieffer, 2013; Trigo et al., 2010). The present study is an initial step to address developmental consequences of ELS on the opioid system, and more studies are necessary to explore the effect of ELS on various behaviors and systems through changes in the opioid system.

Finally, the present findings indicate that the impact of ELS on the opioid system in the NAc is dependent upon biological sex. It remains to be elucidated whether POE-induced ELS and the lasting consequences on opioid receptor regulation in females builds risk or resilience to various mental health disorders. Nevertheless, this sex difference in the response of the opioid system could offer an explanation for sex differences in social anhedonia, addiction, and susceptibility to psychopathologies with atypical opioid-sensitive behaviors.

4. Experimental procedure

4.1. Animals and weaning environment

Untimed-pregnant Sprague Dawley female rats (~ 15 d pregnant) purchased from Charles River Laboratories (Wilmington, MA) were kept in an animal facility and allowed to deliver normally. Cages were checked regularly to determine the day of birth, which was considered postnatal day 0 (P0). On P0, litters were culled to 10 and composed of 5 males and 5 females to standardize litter size and sex composition. Pups found to be particularly small or appearing unhealthy were euthanized by rapid decapitation. Animals raised to the juvenile period were left with dams undisturbed following the predator odor exposure until weaning. Juveniles were weaned at P21 and then housed in cages of 5 with mixed-sex littermates. Animals were housed under standard laboratory conditions: reverse light/dark cycle of 12 h/12 h, food and water *ad libitum*. All procedures were approved by the University of Wisconsin-Madison Animal Care and Use Committee.

4.2. Predator odor exposure (POE) paradigm

As we have previously reported (Kigar et al., 2017), neonatal predator odor exposure (POE) was used to model early life stress (ELS), discussed in detail in Section 3.1. This paradigm was adapted to neonates from a well-established paradigm of predator stress for adult Sprague-Dawley rats (Baisley et al., 2011; Bakshi et al., 2012; Rajbhandari et al., 2015) in order to model the lasting effects of early life adversity in the adolescent brain. The experimental groups consisted of non-stressed control males (CM) and females (CF), and stressed males (SM) and females (SF). A total of 12 litters were used, with half the litters randomly assigned to the experimental group and the other half assigned to the control group. Pups in both groups were taken out of their home cages and placed on a perforated platform, raised approximately 2 in. above the floor of a testing chamber (Kigar et al., 2017). Those in the experimental group were exposed to cat, novel adult male rat, and ferret odor on postnatal days 1–3, respectively, for 5 min each. Those in the control group were exposed to clean bedding. Litters were kept together throughout the exposure to either testing condition. All animals were returned to their respective dams within 10 min. A subset of 6 litters ($n = 5–7$ per group) was sacrificed 30 min after the last odor exposure on P3 and brain tissue collected to inspect mRNA levels of the opioid receptors in the nucleus accumbens (NAc) and amygdala. The remaining 6 litters ($n = 15$ per group) were reared normally until the juvenile period, just before the onset of puberty. Vaginal opening was monitored daily to ensure females were pre-pubertal. One female displayed vaginal opening by postnatal day 33. However, this animal was not a statistical outlier in our analyses, and therefore remained in the study. Animals were sacrificed and brain tissue collected from this group to measure opioid receptor mRNA levels in the same brain regions at P33 (Fig. 3). A subset of $n = 8–10$ per group from 4 of these litters was sacrificed for mRNA expression analysis by real-time quantitative polymerase chain reaction (RT-PCR), enabling all samples to be run in duplicate with controls and standards within the same plate.

4.3. Tissue collection

Both neonatal and juvenile rats were sacrificed via rapid decapitation. The nucleus accumbens and amygdala were microdissected and immediately snap frozen in isopentane (Fisher; catalog #03551–4) on dry ice. Nucleus accumbens samples were isolated using the anterior commissure fibers to guide tissue corer placement; central and medial amygdalae were microdissected from both lateral hemispheres. Tissue samples were homogenized and total RNA and DNA collected using an AllPrep DNA/RNA Mini Kit (Qiagen; catalog #80204).

4.4. Quantification of mRNA

RNA concentrations were determined using the NanoDrop 2000 (ThermoScientific; catalog #ND-2000LAPTOP) and converted to cDNA with the Promega ImProm-II™ Reverse Transcriptase System (Promega; catalog #A3800). RT-PCR was conducted using a Stratagene Mx3000P™ real time PCR system in duplicate using GoTaq Colorless Master Mix (Promega; catalog #M7132), SYBR green (Invitrogen; catalog #S33102), and ROX (Invitrogen; catalog #12223–012) as a reference dye. The amplification included an initial denaturing step at 95 °C for 2 min, followed by 40 cycles of 95 °C denaturing step for 30 s,

60 °C annealing step for 30 s, and 72 °C elongation step for 30 s. Specifically, primers designed to amplify mu-, kappa-, and delta-opioid receptor mRNA (OPRM1, OPRK1, OPRD1, respectively) (Table 1) were run on all cDNA samples, PCR controls were template and no template, standard curves were run to check efficiencies (90%–110%) in each assay, and dissociation melt curves were examined for a single peak. PCR products were run on DNA gels to ensure purity by confirmation of a single band. Relative cDNA levels were calculated using the comparative C_T method normalized to the average of three reference genes (Ywhaz, Hprt and Rpl13a) that were not statistically significant (Table 1).

4.5. Statistical analysis

All molecular tests were conducted and analyzed by researchers blind to group classification. PCR data were analyzed with two-way ANOVAs for each gene, each brain region, at each time point. Statistical analyses were performed using SigmaPlot 11.0. Potential outliers were screened using the Grubbs test for outliers (<http://graphpad.com/quickcalcs/Grubbs1.cfm>). All reported measures are listed as mean $\pm$ SEM. Significance is defined as a p value of < 0.05 .

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Abbreviations:

ELS	Early life stress
POE	predator odor exposure
MOR, KOR, DOR	mu-, kappa-, delta-opioid receptor
NAc	nucleus accumbens

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HIGHLIGHTS

- Neonatal predator odor exposure alters opioid receptor mRNA levels.
- Changes in expression are observed in the neonatal and juvenile nucleus accumbens.
- These changes in expression are sex-specific, restricted to females.
- Changes in expression do not occur in the neonatal nor juvenile amygdala.
- These changes could lead to sex-specific responses to opioids.

Fig. 1.

RT-PCR analysis of opioid receptors in nucleus accumbens and amygdala at postnatal day 3.

A. POE significantly decreases mu- and kappa-opioid receptor mRNA levels in the neonatal female nucleus accumbens. B. No changes in opioid receptor mRNA levels are observed in the neonatal amygdala. Control females, CF; stressed females, SF; control males, CM; stressed males, SM; mu-opioid receptor, MOR; kappa-opioid receptor, KOR; delta-opioid receptor, DOR. Values shown as mean $\pm$ SEM. ** indicates $p < 0.01$, *** indicates $p < 0.001$.

Fig. 2.

RT-PCR analysis of opioid receptors in nucleus accumbens and amygdala at postnatal day 33. A. POE significantly increases mu and delta-opioid receptor mRNA levels in the juvenile female nucleus accumbens. B. No changes in opioid receptor mRNA levels are observed in the juvenile amygdala. Control females, CF; stressed females, SF; control males, CM; stressed males, SM; μ -opioid receptor, MOR; kappa-opioid receptor, KOR; delta-opioid receptor, DOR. Values shown as mean $\pm$ SEM. * indicates $p < 0.05$, ** indicates $p < 0.01$.

Fig. 3.

Experimental timeline. Newborn rats were exposed to bedding mixed with odors from cat, novel adult male rat, and ferret odor on postnatal days 1–3 (P1–3), respectively, for 5 min in each. Control groups were exposed to clean bedding. Brains collected on P3 or P33.

Table 1

mRNA primer sequences and PubMed accession numbers for RT-qPCR.

Primer	Accession #	Forward	Reverse
Ywhaz	NM_013011	TTGAGCAGAAAGACGGAAGGT	GAAGCATTGGGGATCAAGAA
Hprt	NM_012583	GCAGACTTTTGCTTTTCCTTGG	CCGCTGTCTTTTAGGCTTTG
Rpl13a	NM_173340	AGCAGCTCTTGAGGGCTAAGG	GGGTTCACACCAAGAGTCCA
OPRM1	NM_013071	CATTACCATCATGGCCCTCTAC	TGTTGGTGGCAGTC TTCATT
OPRK1	NM_017167	GGATGTGGGTGGGATGAATAAG	TGCAGTGGTAATCTGGGTTAAA
OPRD1	NM_012617	CGTCTCGGACACCTTCCCTA	AGTGTAACCGACGATTCCAA