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SELF ADMINISTRATION OF OXYCODONE BY ADOLESCENT AND ADULT MICE AFFECTS STRIATAL NEUROTRANSMITTER RECEPTOR GENE EXPRESSION

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Abstract

Illicit use of prescription opioid analgesics (e.g., oxycodone) in adolescence is a pressing public health issue. Our goal was to determine whether oxycodone self administration differentially affects striatal neurotransmitter receptor gene expression in the dorsal striatum of adolescent compared to adult C57BL/6J mice. Groups of adolescent mice (4 weeks old, $n = 12$) and of adult mice (11 weeks old, $n = 11$) underwent surgery during which a catheter was implanted into their jugular veins. After recovering from surgery, mice self administered oxycodone (0.25 mg/kg/infusion) 2 h/day for 14 consecutive days or served as yoked saline controls. Mice were sacrificed within 1 h after the last self-administration session and the dorsal striatum was isolated for mRNA analysis. Gene expression was analyzed with real time PCR using a commercially available neurotransmitter receptor PCR array containing 84 genes. We found that adolescent mice self administered less oxycodone than adult mice over the 14 days. Monoamine oxidase A (*Maoa*) and neuropeptide Y receptor 5 mRNA levels were lower in adolescent mice than in adult mice without oxycodone exposure. Oxycodone self administration increased *Maoa* mRNA levels compared to controls in both age groups. There was a positive correlation of the amount of oxycodone self administered in the last session or across 14 sessions with *Maoa* mRNA levels. Gastrin-releasing peptide receptor mRNA showed a significant Drug $\times$ Age interaction, with point-wise significance. More genes in the dorsal striatum of adolescents (19) changed in response to oxycodone self administration compared to controls than in adult (4) mice. Overall, this study demonstrates that repeated oxycodone self administration alters neurotransmitter receptors gene expression in the dorsal striatum of adolescent and adult mice.

Keywords

oxycodone; adolescent; adult; self administration; neurotransmitter receptor gene expression; dorsal striatum

INTRODUCTION

Non-medical use of prescription opioids such as oxycodone in adolescents and young adults has increased in recent years (Johnston et al., 2009, 2013). This is particularly worrisome

since the adolescent brain is undergoing maturation processes (Spear and Brake, 1983). Exposure to prescription opioids during adolescence may alter the normal developmental processes and increase the likelihood of neurobiological changes which may predispose adolescents to be more susceptible to develop addiction to drugs upon subsequent exposure.

There is accumulating evidence that adolescents show differential response to drugs of abuse compared to adults. For example, adolescent rats showed a significantly higher level of nicotine intake compared with both adult male (Levin et al., 2003) and female (Chen et al., 2007) rats. Periadolescent male but not female rats have higher motor activity in response to morphine than do adult rats (White et al., 2008). However, adolescent rats showed decreased cocaine self administration (SA) (Belluzzi et al., 2005) and cocaine-induced locomotor sensitization (Laviola et al., 1995; Collins and Izenwasser, 2002). Adolescent rats self administered less morphine than adults in 1-h self administration sessions. Also, cue-induced reinstatement was less robust in rats that began morphine self-administration during adolescence vs. adulthood (Doherty et al., 2009). Periadolescent (35-day-old) rats did not exhibit the morphine-induced place preferences found in the adult rats (Bolanos et al., 1996). Our earlier study found that adolescent mice self-administered less oxycodone than adult mice (Zhang et al., 2009).

The differential responses to drugs of abuse in adolescent animals may be due to differential neurobiological alterations induced by drugs of abuse in adolescents. Prescription opioids activate mu opioid receptors (MOP-r) located on the GABAergic interneurons in the ventral tegmental area and the substantia nigra, disinhibiting dopamine neurons and resulting in increases in dopamine release in the ventral and dorsal striata (Johnson and North, 1992). The increases of dopamine levels subsequently alter gene expression in the striatum (e.g., Spangler et al., 1993; Angulo and McEwen, 1994; Unterwald, 2001; Unterwald et al., 2001) which may be associated with changes in behavioral effects of MOP-r agonists in rodents, such as increased locomotor activity, development of conditioned place preference and self administration (Kruzich et al., 2003; Schlussman et al., 2008; Zhang et al., 2009; Picetti et al., 2012; Seip et al., 2012).

Studies found that gene expression altered in response to drugs of abuse in adult rodent brains (e.g., Wang et al., 1999; Nestler, 2001; Rodriguez Parkitna et al., 2004). However, there have been few studies on prescription opioid-induced alterations in gene expression in the adolescent brain (Ellgren et al., 2007). Thus, it is essential to determine how exposure to a prescription opioid such as oxycodone affects gene expression in the adolescent brain. In our earlier study, adolescent mice self administered significantly less oxycodone than adult mice (Zhang et al., 2009). We hypothesized that there were differential neurobiological alterations in brain regions associated with reward between adolescent and adult mice as a consequence of oxycodone self administration.

We tested this hypothesis by studying gene expression of neurotransmitter receptors in the dorsal striatum, a brain region closely involved in reward, locomotor regulation and habitual learning (Ito et al., 2002). Since changes in multiple neurotransmitter systems may be involved in the behavioral profile of oxycodone self administration, we chose to use the neurotransmitter receptor array which profiles the expression of 84 genes involved in modulating biological processes through neurotransmitter receptors and five housekeeping genes (Qiagen, Valencia, CA, USA).

To our knowledge, this is one of the few studies comparing the neurobiological changes induced by oxycodone between adolescent and adult subjects (see also Ellgren et al., 2007). The current study should initiate the elucidation of molecular mechanisms underlying the

actions of oxycodone, and could shed light on the mechanisms underlying prescription opioid addiction in adolescents versus adults.

Although it is impossible to develop an animal model that exactly matches all the behavioral and neurobiological alterations taking place in human adolescence, the mouse model we have chosen (4-week-old mice) provides a rodent model of a neurological developmental period that bears substantial analogy to human adolescence (Spear, 2000; Adriani et al., 2004). The self-administration paradigm used in this study aims to model drug self administration or abuse in human adolescents.

EXPERIMENTAL PROCEDURES

Subjects

Male adolescent and adult (4 or 11 weeks old on arrival) C57BL/6J mice (Jackson Laboratory, Bar Harbor, ME, USA) were housed in groups of up to five with free access to food and water in a light-(12:12-h light/dark cycle, light on at 7:00 pm and off at 7:00 am) and temperature-(25 °C) controlled room. The adolescent mice were weaned at 3 weeks of age at the Jackson Laboratory. Animal care and experimental procedures were conducted according to the *Guide for the Care and Use of Laboratory Animals* (Institute of Laboratory Animal Resources Commission on Life Sciences 1996). The experimental protocols used were approved by the Institutional Animal Care and Use Committee of The Rockefeller University.

Self-administration procedure

Catheter implantation—Following acclimation for 7 days, the mice were anesthetized with a combination of xylazine (8.0 mg/kg i.p.) and ketamine (80 mg/kg i.p.). After shaving and application of a 70% alcohol and iodine preparatory solution, incisions were made in the midscapular region and anteromedial to the foreleg. A catheter of approximately 6 cm in length (ID: 0.31 mm, OD: 0.64 mm) (Helix Medical, Inc., CA, USA) was passed subcutaneously from the dorsal to the ventral incision. After exposure of the right jugular vein, a 22-gauge needle was inserted into the vein to guide the catheter into the jugular vein. Once the catheter was inside the vein, the needle was removed and the catheter was inserted to the level of a silicone ball marker 1.1 cm from the end. The catheter was tied to the vein with surgical silk. Physiological saline was then flushed through the catheter to avoid clotting and the catheter then capped with a stopper. Antibiotic ointment was applied to the catheter exit wounds on the animal's back and forearm. Mice were individually housed after the surgery and were allowed 4 days of recovery (due to the limited period of adolescence in the mouse (Spear, 2000; Adriani et al., 2004) before being placed in operant test chambers for the self-administration procedure (Zhang et al., 2006). See Table 1 for details of age and experimental procedure.

Intravenous self-administration chamber—The self-administration chamber, ENV-307W (21.6 cm × 17.8 cm × 12.7 cm, Med. Associates, St. Albans, VT, USA), was located inside a larger sound attenuation chamber (Med. Associates). The front, back and top were constructed of 5.6 mm polycarbonate. Each chamber contained a wall with two small holes (0.9 cm diameter, 4.2 cm apart, 1.5 cm from the floor of the chamber). One hole was defined as active, the other was inactive. When the photocell in the active hole was triggered by a nose-poke, an infusion pump (Med. Associates) delivered an oxycodone infusion of 20 µl/3 s from a 5-ml syringe. The syringe was connected by a swivel via Tygon tubing. The infusion pump and syringe were outside the chamber. During infusion, a cue light above the active hole was illuminated. Each injection was followed by a 20-s “time-out” period during which poking responses were recorded but had no programmed

consequences. All responses at the inactive hole were also recorded. Mice were tested during the dark phase of the diurnal cycle (all experiments were performed between 8:00 am and 12:00 pm).

Oxycodone self administration—A 2-h self-administration session was conducted daily. Each day, mice were weighed and heparinized saline (0.02 ml of 30 IU/ml solution) was used to flush the catheter to maintain patency. During self-administration sessions, mice in the oxycodone (Sigma, St. Louis, MO, USA) groups were placed in the self-administration chamber and a nose-poke through the active hole led to an infusion of oxycodone (0.25 mg/kg/infusion) under an FR1 schedule for 14 days. Drug volume was controlled by a computer for individual animals to follow daily changes in body weight. Mice in the control groups received yoked saline infusions during all sessions (saline was infused in the control mouse whenever the oxycodone mouse self-administered oxycodone). At the end of the experiment, only data from mice that passed a catheter patency test (defined as loss of muscle tone within a few seconds after administration of a shortacting anesthetic) with injection of 30 μ l of ketamine (5 mg/ml) (Fort Dodge, IA, USA) were included in the analysis of data.

RNA extraction

Mice were sacrificed within 1 h after the last self-administration session; the dorsal striatum from each mouse was dissected from the brain and homogenized in Qiazol (Qiagen, Valencia, CA). Total RNA was isolated from homogenates of the dorsal striatum using the miRNeasy kit (Qiagen, Valencia, CA). The quality and quantity of RNA from each sample were determined spectrophotometrically. Genomic DNA was removed from the isolated RNA using RT² HT First Strand Kit (Qiagen, Valencia, CA). Complementary DNA was then synthesized from 500 ng of total RNA with the same kit.

Mouse neurotransmitter receptors RT²-Profiler PCR Array

The mouse neurotransmitter receptors RT² Profiler PCR Array (PAMM-060Z, Qiagen, Valencia, CA) profiles the expression of 84 genes involved in modulating biological processes through neurotransmitter receptors and five housekeeping genes (β -actin; glyceraldehyde 3-phosphate dehydrogenase, Gapdh; hypoxanthine guanine phosphoribosyl transferase 1, Hsp90ab1: Heat shock protein 90 alpha (cytosolic), class B member 1, Hprt-1; and β -glucuronidase, Gusb) by real-time PCR using the SYBR Green detection method. Total RNA (500 ng) was reverse transcribed using the First Strand cDNA Synthesis Kit (Qiagen, Valencia, CA) following manufacturer's instructions. The generated cDNA was diluted with an appropriate volume of instrument-specific 2 $\times$ SuperArray RT² Real-Time SYBR Green PCR Master Mix (PA-012) and ultra pure water, and 10 μ l of this reaction mix was added to each well of the PCR array. The real-time PCR reaction was performed in an ABI Prism 7900 HT Sequence Detection System (Applied Biosystems, Foster City, CA, USA). The cycling conditions were optimized by the manufacturer and were as follows: 2 min at 50 $^{\circ}$ C, 10 min at 95 $^{\circ}$ C and 40 cycles of 15 s at 95 $^{\circ}$ C and 1 min at 60 $^{\circ}$ C. The ABI Prism 7900 HT Sequence Detection System was used to calculate the Ct value for each well. Data were normalized to the mean of the four housekeeping genes and analyzed by the comparative Ct-method ($2^{-\Delta\text{CT}}$). All significant changes in gene expression levels are reported; the complete list of genes assayed on the array is shown in Table 4 and can also be found at the manufacturer's website (http://www.sabioscience.com/rt_pcr_product/HTML/PAMM-060ZE-4.html).

In order to minimize any systematic errors, sample preparation procedures were standardized across animals, animal ages and drug conditions and samples. All animals and tissue samples were handled identically. All animals were sacrificed at the identical time

point in the 14-day self-administration paradigm. All brains were dissected by the same experienced dissectors and homogenized by another experienced investigator. Total RNA was extracted from all samples at the same time by the same investigator. RNA quantity and quality were determined in all samples on the same day, and all cDNA libraries were constructed in a single assay in a single 96-well plate. A validation experiment using independent groups of animals from an independent self-administration experiment was also carried out several months after this study. The mean CT values for all targets from the validation experiment were similar to those observed in the current study (e.g., *Maoa* 25.13 from adult control mice of this experiment versus 25.78 from adult control mice of validation experiment). In addition, all gene expression data passed recommended quality control tests, including elimination of genomic DNA, reverse transcription and the PCR procedure.

Statistical analysis

Differences in self administration measured as number of nose pokes in the active hole in each self-administration session across the 14 sessions were assessed by a two-way analysis of variance (ANOVA), Age Group $\times$ Drug Condition. Differences in expression of genes were also analyzed by two-way ANOVA, Age Group $\times$ Drug Condition. Regression analyses were conducted to determine whether the level of gene expression was related to the amount of oxycodone self administered.

Differences in expression of genes between the two groups in adolescents or adults were analyzed by *t*-tests and allowance for testing multiple genes was achieved by the Bonferroni correction. To evaluate whether a group of small *p*-values occurred more often than expected by chance, a recently developed partition test was applied to the *t*-tests (Ott et al., 2012). For a given threshold, *r*, ranging between 0 and a suitable upper limit like 0.10, the observed number of *p*-values smaller than *r* are contrasted by a chi-square statistic with the expected number, *nr*, where *n*=number of tests carried out. The largest chi-square statistic over the range of *r* represents the test statistic whose associated (experiment-wise) significance level is obtained in randomized samples (labels case and controls are randomly permuted).

RESULTS

Oxycodone self administration in adolescent and adult mice

Self-administration behavior of adolescent and adult C57BL/6 mice is shown in Fig. 1A. Three-way ANOVA, Age $\times$ Drug Condition $\times$ Session, showed that there was a significant main effect of Age, $F(1, 20)=23.95$, $p<0.0001$. There was also a significant Drug Condition, $F(1, 20)=40.95$, $p<0.000005$ with a significant Age $\times$ Drug Condition Interaction, $F(1, 20)=33.22$, $p<0.00002$. Adolescents self administered fewer oxycodone infusions than adults over the course of 14 consecutive daily sessions (Fig. 1A). The number of nose pokes at the active hole during the 20-s time out period is shown in Fig. 1B. Three-way ANOVA, Age $\times$ Drug Condition $\times$ Session, showed that there was a significant main effect of Drug Condition, $F(1, 20)=51.89$, $p<0.000002$, with neither a significant main effect of Age nor Age $\times$ Drug Condition Interaction. The number of nose pokes at the active hole during the time out period was higher in the oxycodone groups compared to that of yoked saline control groups in both age groups.

Effect of oxycodone self administration on neurotransmitter receptor gene expression in the dorsal striatum

The effect of oxycodone self administration on the expression of 84 neurotransmitter and receptor genes in adolescent and adult mice was analyzed with a two-way ANOVA, Age $\times$

Condition (see Table 5 for complete list). Of the 84 genes on the array, five genes (*Chrna6*, *Gabra6*, *Chrnd*, *Gabrp*, *Npy6r*) were disregarded because of low abundance (CT value > 35, a threshold suggested by the manufacture). Thus, the remaining 79 genes were analyzed. Multiple genes were found to show point-wise statistical significance ($p \leq 0.05$), and two genes, *Maoa* and *Npy5r*, exhibited an experimentwise significant age effect, $p_B = 0.000211$, using the Bonferroni correction for $3 \times 79 = 237$ tests (see Table 5). We focused our main analysis on genes showing significant effects with an experiment-wise p value ($p < 0.0002$ as mentioned above); other genes with p value greater than 0.0002 are presented in Table 5.

Genes differing between the oxycodone group and yoked saline controls

Two-way ANOVA, Age $\times$ Drug Condition, found that there were seven genes (*Anxa9*, *Chrm3*, *Chrna1*, *Chrb4*, *Maoa*, *Mc2r*, *Prokr1*) showing a point-wise significant main effect of Drug Condition, see Table 2A.

Genes differing between adolescent and adult mice

Two-way ANOVA found that five genes (*Comt*, *Gabra3*, *Gabrg1*, *Npy5r*, *Maoa*) showed a significant main effect of Age, see Table 2B. Only the two genes showing an experiment-wise significance are detailed below.

Monoamine oxidase A (*Maoa*)

The effect of oxycodone self administration and Age on *Maoa* mRNA levels in the dorsal striatum following 14-day oxycodone self administration or yoked-saline in the adolescent and adult mice is shown in Fig. 2. Two-way ANOVA, Age $\times$ Drug Condition, showed a significant main effect of Age, $F(1, 18) = 22.15$, $p < 0.0002$, and of Drug Condition, $F(1, 18) = 9.19$, $p < 0.01$, with no significant interaction ($p = 0.72$). Thus, there were lower *Maoa* mRNA levels in the dorsal striatum of adolescent than adult mice (with or without oxycodone). Levels of *Maoa* mRNA were higher in mice that had self-administered oxycodone compared with age-appropriate yoked saline controls.

Neuropeptide Y receptor 5 (*Npy5r*)

The effect of Age on *Npy5r* mRNA expression in the dorsal striatum is shown in Fig. 3A. The levels of *Npy5r* mRNA in the adolescent mice were lower than those of the adult mice, $F(1, 18) = 34.86$, $p < 0.00002$.

Genes showing significant interaction

Two-way ANOVA showed that there was a significant interaction of Age $\times$ Drug Condition of gastrin-releasing peptide receptor (*Grpr*) mRNA levels, with no significant main effect of Age or of Drug Condition. The interaction effect, $F(1, 19) = 11.07$, $p < 0.005$, was seen as a decrease in means from adolescent to adult mice in controls, and an increase in means from adolescent to adult for Drug Condition, shown in Fig. 3B.

In addition, two other genes (*Npffr1*, *Ntsr1*) also showed a point-wise significant interaction effect (Table 2C).

Genes differing between oxycodone and yoked saline control in the adolescent group

Using a newly developed partition test (Ott et al., 2012) to further examine gene expression between mice that had self administered oxycodone and yoked saline controls in each age group, we found 19 genes with small p -values showing a difference between the two groups in the adolescent mice, with an experiment-wise significance level of $p = 0.103$, indicating “suggestive” significance, see Table 3A. Thus, it is unlikely that these 19 genes had

relatively low p values by chance alone. There were only four genes showing a difference between the two groups in the adults, see Table 3B.

Correlation between the amount of oxycodone self administered and *Mao* mRNA levels

To further quantify the relationship between the amounts of oxycodone self-administration and subsequent oxycodone-induced alteration in *Mao* mRNA levels across the two age groups, we examined the correlation between the increases in *Mao* mRNA levels in response to the amount of oxycodone self administered across all sessions, or during the last session, by each mouse. This analysis revealed a significant positive correlation between the total amounts of oxycodone that had been self-administered and *Mao* mRNA levels, $r=0.855$, $p<0.0005$ across all mice in the two age groups (Fig. 4A). Interestingly, a significant positive correlation between the total amounts of oxycodone and *Mao* mRNA levels was found, $r=0.826$, $p<0.05$ in adolescent mice (Fig. 4B) but not in adult mice. Further, there is also a significant positive correlation between the amount of oxycodone self administered in the last session and *Mao* mRNA levels across all mice, $r=0.745$, $p<0.01$ (Fig. 4C). The greater the amount of oxycodone self-administered, the higher the *Mao* mRNA levels.

DISCUSSION

Prescription opioid abuse (with oxycodone as a major example) is currently a massive public health problem, and adolescents tend to be especially sensitive to this health impact. In the present study, we have presented the first systematic comparison of striatal neurotransmitter receptor gene expression between adolescent and adult mice that had acquired and established oxycodone self administration in 14-day sessions.

Consistent with our earlier report (Zhang et al., 2009), we found that adolescent mice in the current study self administered less oxycodone than adult mice. Since multiple neurotransmitter systems are involved in the behavioral profile resulting from oxycodone self administration, we have chosen to examine transcription profiles of neurotransmitter receptors in the striatum, a brain region that is closely involved in reward, locomotor regulation and habitual learning (e.g., Ito et al., 2002; Porrino et al., 2007; Belin and Everitt, 2008).

Main gene targets differentially affected as a factor of age (adolescence versus adulthood) or oxycodone self administration

Maoa mRNA showed experiment-wise significant differences in expression between adolescents and adults. Adolescent mice were found to have lower *Maoa* mRNA levels in the dorsal striatum compared to adults. Monoamine oxidases are enzymes that metabolize monoamines including dopamine, norepinephrine and serotonin. A number of psychiatric disorders such as alcoholism, drug abuse and impulsive and risk-taking behaviors were found to be associated with abnormalities in monoamine oxidase levels (Gottfries et al., 1975; Wiberg et al., 1977; Schooler et al., 1978; Shekim et al., 1989; Devor et al., 1993). A mutation that resulted in inactivation of the monoamine oxidase A gene was found in males with a history of aggression, and sexual assault behavior (Brunner et al., 1993). A study in rats found that selective monoamine oxidase inhibitors decrease morphine-reinforced behavior (He and Grasing, 2006). The findings of lower *Maoa* mRNA levels in adolescents compared to adults are interesting because adolescents are characterized by increases in novelty seeking and risk taking (Spear, 2000). Decreases in *Maoa* mRNA levels suggest less *Maoa* activity, thus resulting in the accumulation of dopamine and other monoamines in the synaptic cleft in the adolescent brain. The slower metabolism of dopamine and monoamines by *Maoa* found in the dorsal striatum of the adolescent may be associated with characteristic behaviors exhibited in adolescence. This finding is consistent with a microdialysis study

showing that the basal dopamine levels in the nucleus accumbens of adolescent rat brain were higher than those of the adult (Badanich et al., 2006). Also, Walker and Kuhn found that cocaine increases stimulated dopamine release more in periadolescent than adult rats (Walker and Kuhn, 2008), even though no age-related difference was observed in basal or cocaine-stimulated nucleus accumbens dopamine in both periadolescent and adult rats in another study (Frantz et al., 2007). In addition, the mRNA level of another metabolic monoamine enzyme, *Comt*, also showed a decrease in the dorsal striatum in adolescent mice compared to that of the adult, further suggesting that the metabolism of monoamines in the adolescent brain may be slower than in the adult brain

Furthermore, mice that self administered oxycodone showed higher *Maoa* mRNA levels compared to yoked saline controls in both age groups tested. The positive correlation between the amount of oxycodone self administered and *Mao* mRNA levels found in the present study suggests that oxycodone self administration may increase *Maoa* mRNA levels in the dorsal striatum. Such alteration may be mechanistically related to increases in striatal dopamine levels as a result of oxycodone exposure (Zhang et al., 2009). These are among the first studies on *Maoa* mRNA in adolescent subjects in response to self administration of a prescription opioid

The gene encoding *Npy5r* also showed an experiment-wise significant difference in expression between adolescents and adults. This result is interesting since neuropeptide Y is distributed throughout the brain and gastrointestinal tissues and is centrally involved in many physiological functions such as the regulation of food intake, locomotion and psychomotor activities through its receptor. An earlier study found that neuropeptide Y causes a powerful attenuation of morphine withdrawal via Y5-like receptors, suggesting that NPY and Y5-like receptors deserve attention with regard to basic mechanisms and possible therapeutic potential in opioid dependence and withdrawal (Woldbye et al., 1998).

Gene expression of gastrin-releasing peptide (GRP) receptor (*Grpr*) was increased in mice that had self-administered oxycodone during adolescence but decreased in adults. This finding is intriguing, since accumulating evidence indicates that GRP and its receptor are involved in the neurobiological alterations associated with psychiatric and neurological disorders (Roesler et al., 2006a; Hodges et al., 2009). A translocation in the *Grpr* gene has been associated with autism (Ishikawa-Brush et al., 1997). Studies in rodents have found that Grprs in brain areas such as hippocampus and amygdala play an important role in regulating synaptic plasticity and aspects of behavior that might be altered in disorders such as anxiety, depression and memory dysfunction (e.g., Roesler et al., 2006b; Chaperon et al., 2012). Thus, increases in *Grpr* mRNA levels in adolescent mice or decreases in adult mice that had self administered oxycodone may be closely associated with the oxycodone self-administration behavior. These are also among the first findings on *Grpr* in responses to MOP-r agonist in adolescents.

It is important to note that even though adolescent and adult mice were both shipped from the Jackson Laboratory, adolescent mice might experience a different degree of stress than adult mice during the shipping process, given the fact that the adolescent mice were separated from their mother approximately 1 week before shipping. However, using the same ages of C57BL/6 mice housed in our animal facility, we found a similar behavior of oxycodone self administration difference between adolescent and adult mice.

Overall gene response to oxycodone self administration in adolescent versus adults

Using the newly developed partition test, we found that 19 genes show significant difference between mice in the oxycodone and yoked saline control in adolescent groups, whereas only four genes were found to differ in the adult groups. This illustrates that adolescent dorsal

striatum gene responses to oxycodone self administration likely involve a more complex and potentially more far-reaching set of adaptations. During the normal adolescent period, many brain neuronal systems undergo developmental alterations (for review, see Spear, 2000). Thus, the greater number of genes altered in response to oxycodone self administration in the adolescent group indicates that adolescent brains are more sensitive to oxycodone and may be more vulnerable to oxycodone exposure, especially considering that the amount of oxycodone taken by adolescent mice was significantly less than by adult mice (Zhang et al., 2009). This is of specific concern given the fact that prescription opioid abuse has increased among adolescents in recent years (Johnston et al., 2009, 2013).

In contrast to earlier reports showing that adolescent rats expressed higher dopamine receptor mRNA levels than adults in the striatum (Teicher et al., 1995; Tarazi et al., 2000), no significant differences were found in dopamine receptors (D1–D5) mRNA levels between adolescent and adult in the yoked saline controls. This finding may be due to the fact that the adolescent mice in this study were sacrificed at an age near to the young adult stage. Another interesting discovery in the current study was that striatal mRNA levels in the adolescent mice were lower than those of the adult mice for all the genes found to be either experiment-wise (*Maoa* and *Npy5r*) or point-wise (*Comt*, *Gabra3* and *Gabrg1*) significant between the adolescent and adult groups. Earlier studies found that there is a massive loss of synapses in neocortical brain regions during adolescence in monkeys (e.g., Bourgeois et al., 1994). A similar pruning process had been found in humans (e.g., Huttenlocher, 1984) and rodents (e.g., Cressman et al., 2010; Liston and Gan, 2010). The magnitude of synapse elimination was enormous and may likely be accompanied by massive loss of neurons during the adolescent period, which could be a reason for the detection of lower mRNA levels in the adolescent found in this study.

The change of *Maoa* function may in part contribute to increase in oxycodone self administered over the 14-day sessions as dorsal striatum is thought to be involved in mediating habitual learning and compulsive-like behaviors (e.g., Ito et al., 2002; Porrino et al., 2007; Belin and Everitt, 2008). This hypothesis should be tested in future studies, to determine whether protein levels of the *Maoa* are increased under such conditions. In addition, several other important neurotransmitter and neuropeptide receptor genes including glutamatergic, noradrenergic, serotonergic and opioid receptors genes are not included in the array used in the current study. Future studies are needed to examine changes in these receptors by oxycodone self administration between adolescent and adult mice.

In summary, the current study demonstrated that adolescents and adults differed in specific gene expression in the dorsal striatum, including *Maoa*, in the absence of oxycodone exposure. Of interest, oxycodone self administration led to increases in *Maoa* gene expression in both adolescent and adult mice. The qualitatively different transcription profiles in striatal neurotransmitter receptors between adolescents and adults provide initial avenues for later system-wide functional analyses. Further studies focused on the gene products of the targets identified herein, and their differences in adolescents versus adults, would also be important for mechanistic analysis. The genes identified in this study also constitute appropriate candidates for further study, testing the mechanistic impact of these targets for pharmacotherapy, and their relationship to the developmental stage of prescription opioid self exposure.

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Abbreviations

GRP	gastrin-releasing peptide
Grpr	gastrin-releasing peptide receptor
ANOVA	analysis of variance
Maoa	monoamine oxidase A
MOP-r	mu opioid receptors
Npy5r	neuropeptide Y receptor 5.

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Figure 1.

The number of self-administered infusions of oxycodone (0.25 mg/kg per infusion) by both adolescent and adult mice is shown in A. Adults self administered more infusions of oxycodone than adolescents. The number of nose pokes at the active hole during 20-s time out period is shown in B.

Figure 2.

Alteration in monoamine oxidase A (*Maoa*) mRNA levels in the dorsal striatum following 14-day oxycodone or yoked-saline self administration in the adolescent and adult mice. There were lower levels of *Maoa* mRNA in the adolescent than in the adult mice. The levels of *Maoa* mRNA were higher in mice that had self administered oxycodone compared with those of the yoked saline controls.

Figure 3.

(A) The effect of Age on neuropeptide Y receptor Y5 (*Npy5r*) mRNA expression in the dorsal striatum. The levels of *Npy5r* mRNA in the adolescent mice were significantly lower than those of the adult mice. (B) A significant Age × Drug Condition Interaction in the expression of gastrin-releasing peptide receptor (*Grpr*) mRNA levels. The interaction effect was seen as a decrease in means from adolescent to adult mice in yoked saline control mice and increase in means from adolescent to adult in mice that had self-administered oxycodone.

Figure 4.

The correlation between the amount of oxycodone self administered with oxycodone-induced increases in *Mao* mRNA level. There is a significant positive correlation between the total amount (mg) of oxycodone self-administered across the 14 sessions with the levels of *Mao* mRNA, $r = 0.855, p < 0.0005$ across all mice in the two age groups (A). A significant positive correlation between the total amounts (mg) of oxycodone and *Mao* mRNA levels was also found, $r = 0.826, p < 0.05$ in adolescent mice (B). There was also a significant positive correlation between the amount (mg) of oxycodone self administered in the last session and *Mao* mRNA levels across all mice, $r = 0.745, p < 0.0054$ (C).

Table 1

The age (postnatal-day) of mice in each experimental procedure

Age and experimental procedure	On arrival	Surgery	Oxycodone self administration	Sacrifice and isolation of mRNA
Adolescent (Postnatal Day)	28	35	39–52	52
Adult (Postnatal Day)	77	84	88–101	101

Table 2

The genes showing significant alterations as a result of oxycodone self administration in both age groups are listed in A. The genes showing significant difference between the two age groups are listed in B. The genes showing significant interaction of age and oxycodone self administration are listed in C.

Gene	Name	P-value
(A)		
<i>MaoA</i>	Monoamine oxidase A	0.001
<i>Anxa9</i>	Annexin A9	0.029
<i>Chrm3</i>	Cholinergic receptor, muscarinic 3, cardiac	0.047
<i>Chrna1</i>	Cholinergic receptor, nicotinic, alpha polypeptide 1 (muscle)	0.013
<i>Chrn4</i>	Cholinergic receptor, nicotinic, beta polypeptide 4	0.013
<i>Prokr1</i>	Prokineticin receptor 1	0.015
<i>Mc2r</i>	Melanocortin 2 receptor	0.014
(B)		
<i>Npy5r</i>	Neuropeptide Y receptor 5	0.00001
<i>MaoA</i>	Monoamine oxidase A	0.00017
<i>Gabrg1</i>	Gamma-aminobutyric acid (GABA) A receptor, subunit gamma 1	0.001
<i>Comt</i>	Catechol-O-methyltransferase	0.003
<i>Gabra3</i>	Gamma-aminobutyric acid (GABA) A receptor, subunit alpha 3	0.002
(C)		
<i>Grpr</i>	Gastrin releasing peptide receptor	0.005
<i>Npffr1</i>	Neuropeptide FF receptor 1	0.023
<i>Ntsr1</i>	Neurotensin receptor 1	0.032

Table 3

The genes showing difference between oxycodone and yoked-saline using the newly developed partition test are listed in A for adolescent and B for adult

Gene	Name	P value	Direction
(A)			
<i>Grpr</i>	Gastrin-releasing peptide receptor	0.0076	↓
<i>Mc2r</i>	Melanocortin 2 receptor	0.0077	↑
<i>Sstr1</i>	Somatostatin receptor 1	0.0153	↑
<i>Npffr1</i>	Neuropeptide FF receptor 1	0.0153	↓
<i>Sstr3</i>	Somatostatin receptor 3	0.0156	↑
<i>Cckbr</i>	Cholecystokinin B receptor	0.0319	↑
<i>Chrm4</i>	Cholinergic receptor, nicotinic, beta polypeptide 4	0.0375	↓
<i>Gabrg2</i>	Gamma-aminobutyric acid (GABA) A receptor, subunit gamma 2	0.0419	↑
<i>Chrm3</i>	Cholinergic receptor, muscarinic 3, cardiac	0.0425	↑
<i>Gabra5</i>	Gamma-aminobutyric acid (GABA) A receptor, subunit alpha 5	0.0471	↑
<i>Npy1r</i>	Neuropeptide Y receptor Y1	0.0483	↑
<i>Gla1</i>	Glycine receptor, alpha 1 subunit	0.0533	↑
<i>Gabra2</i>	Gamma-aminobutyric acid (GABA) A receptor, subunit alpha 2	0.0533	↑
<i>Ntsr1</i>	Neurotensin receptor 1	0.0538	↑
<i>Chrna1</i>	Cholinergic receptor, nicotinic, alpha polypeptide 1 (muscle)	0.0614	↑
<i>Chrm3</i>	Cholinergic receptor, nicotinic, beta polypeptide 3	0.0634	↑
<i>Npffr2</i>	Neuropeptide FF receptor 2	0.0696	↑
<i>Chrne</i>	Cholinergic receptor, nicotinic, epsilon polypeptide	0.0724	↑
<i>Chat</i>	Choline acetyltransferase	0.0764	↑
(B)			
<i>Maoa</i>	Annexin A9	0.012072	↑
<i>Comt</i>	Catechol-O-methyltransferase	0.017574	↓
<i>Anxa9</i>	Monoamine oxidase A	0.026001	↑
<i>Prokr1</i>	Prokineticin receptor 1	0.026207	↑

Arrows indicate increases or decreases of the target gene mRNA levels of mice that had self administered oxycodone compared to those of the saline controls.

Table 4

The symbol and descriptions of the 84 neurotransmitter receptor genes in the array used in the current study

84 genes	Symbol	Description
1	Ache	Acetylcholinesterase
2	Anxa9	Annexin A9
3	Brs3	Bombesin-like receptor 3
4	Cckar	Cholecystokinin A receptor
5	Cckbr	Cholecystokinin B receptor
6	Chat	Choline acetyltransferase
7	Chrm1	Cholinergic receptor, muscarinic 1, CNS
8	Chrm2	Cholinergic receptor, muscarinic 2, cardiac
9	Chrm3	Cholinergic receptor, muscarinic 3, cardiac
10	Chrm4	Cholinergic receptor, muscarinic 4
11	Chrm5	Cholinergic receptor, muscarinic 5
12	Chma1	Cholinergic receptor, nicotinic, alpha polypeptide 1 (muscle)
13	Chma2	Cholinergic receptor, nicotinic, alpha polypeptide 2 (neuronal)
14	Chma3	Cholinergic receptor, nicotinic, alpha polypeptide 3
15	Chma4	Cholinergic receptor, nicotinic, alpha polypeptide 4
16	Chma5	Cholinergic receptor, nicotinic, alpha polypeptide 5
17	Chma6	Cholinergic receptor, nicotinic, alpha polypeptide 6
18	Chma7	Cholinergic receptor, nicotinic, alpha polypeptide 7
19	Chmb1	Cholinergic receptor, nicotinic, beta polypeptide 1 (muscle)
20	Chmb2	Cholinergic receptor, nicotinic, beta polypeptide 2 (neuronal)
21	Chmb3	Cholinergic receptor, nicotinic, beta polypeptide 3
22	Chmb4	Cholinergic receptor, nicotinic, beta polypeptide 4
23	Chmd	Cholinergic receptor, nicotinic, delta polypeptide
24	Chme	Cholinergic receptor, nicotinic, epsilon polypeptide
25	Chmg	Cholinergic receptor, nicotinic, gamma polypeptide
26	Comt	Catechol-O-methyltransferase
27	Drd1a	Dopamine receptor D1A
28	Drd2	Dopamine receptor D2
29	Drd3	Dopamine receptor D3
30	Drd4	Dopamine receptor D4
31	Drd5	Dopamine receptor D5
32	Gabra1	Gamma-aminobutyric acid (GABA) A receptor, subunit alpha 1
33	Gabra2	Gamma-aminobutyric acid (GABA) A receptor, subunit alpha 2
34	Gabra3	Gamma-aminobutyric acid (GABA) A receptor, subunit alpha 3
35	Gabra4	Gamma-aminobutyric acid (GABA) A receptor, subunit alpha 4
36	Gabra5	Gamma-aminobutyric acid (GABA) A receptor, subunit alpha 5
37	Gabra6	Gamma-aminobutyric acid (GABA) A receptor, subunit alpha 6
38	Gabrb2	Gamma-aminobutyric acid (GABA) A receptor, subunit beta 2
39	Gabrb3	Gamma-aminobutyric acid (GABA) A receptor, subunit beta 3

84 genes	Symbol	Description
40	Gabrd	Gamma-aminobutyric acid (GABA) A receptor, subunit delta
41	Gabrg1	Gamma-aminobutyric acid (GABA) A receptor, subunit gamma 1
42	Gabrg2	Gamma-aminobutyric acid (GABA) A receptor, subunit gamma 2
43	Gabrp	Gamma-aminobutyric acid (GABA) A receptor, pi
44	Gabrq	Gamma-aminobutyric acid (GABA) A receptor, subunit theta
45	Gabbr1	Gamma-aminobutyric acid (GABA) C receptor, subunit rho 1
46	Gabbr2	Gamma-aminobutyric acid (GABA) C receptor, subunit rho 2
47	Gad1	Glutamic acid decarboxylase 1
48	Galr1	Galanin receptor 1
49	Galr2	Galanin receptor 2
50	Galr3	Galanin receptor 3
51	Gla1	Glycine receptor, alpha 1 subunit
52	Gla2	Glycine receptor, alpha 2 subunit
53	Gla3	Glycine receptor, alpha 3 subunit
54	Gla4	Glycine receptor, alpha 4 subunit
55	Glr1b	Glycine receptor, beta subunit
56	Qrfpr	Pyroglutamylated RFamide peptide receptor
57	Npffr1	Neuropeptide FF receptor 1
58	Prokr1	Prokineticin receptor 1
59	Prokr2	Prokineticin receptor 2
60	Npffr2	Neuropeptide FF receptor 2
61	Gpr83	G protein-coupled receptor 83
62	Grpr	Gastrin-releasing peptide receptor
63	Htr3a	5-hydroxytryptamine (serotonin) receptor 3A
64	Maoa	Monoamine oxidase A
65	Mc2r	Melanocortin 2 receptor
66	Nmur1	Neuromedin U receptor 1
67	Nmur2	Neuromedin U receptor 2
68	Npy1r	Neuropeptide Y receptor Y1
69	Npy2r	Neuropeptide Y receptor Y2
70	Npy5r	Neuropeptide Y receptor Y5
71	Npy6r	Neuropeptide Y receptor Y6
72	Ntsr1	Neurotensin receptor 1
73	Pgr15l	G protein-coupled receptor 15-like
74	Ppyr1	Pancreatic polypeptide receptor 1
75	Prhr	Prolactin-releasing hormone receptor
76	Slc5a7	Solute carrier family 5 (choline transporter), member 7
77	Sstr1	Somatostatin receptor 1
78	Sstr2	Somatostatin receptor 2
79	Sstr3	Somatostatin receptor 3
80	Sstr4	Somatostatin receptor 4
81	Sstr5	Somatostatin receptor 5

84 genes	Symbol	Description
82	Tacr1	Tachykinin receptor 1
83	Tacr2	Tachykinin receptor 2
84	Tacr3	Tachykinin receptor 3

Table 5

The results of two-way ANOVAs on condition (oxycodone or saline) $\times$ age (adolescent or adult) and the interaction

Gene	Condition	Age	Con $\times$ Age	Gene	Condition	Age	Con $\times$ Age
Ache	0.438	0.177	0.358	Gabrq	0.366	0.331	0.693
Anxa9	0.029	0.674	0.383	Gabbr1	0.658	0.134	0.405
Brs3	0.725	0.611	0.152	Gabbr2	0.378	0.329	0.341
Cckar	0.063	0.285	0.388	Gad1	0.293	0.226	0.614
Cckbr	0.057	0.309	0.350	Gallr1	0.793	0.296	0.833
Chat	0.284	0.525	0.367	Gall2	0.225	0.354	0.945
Chrm1	0.705	0.800	0.208	Gira3	0.445	0.622	0.510
Chrm2	0.555	0.558	0.753	Gira1	0.458	0.259	0.149
Chrm3	0.047	0.126	0.743	Gira2	0.191	0.323	0.685
Chrm4	0.886	0.379	0.498	Gira3	0.940	0.838	0.748
Chrm5	0.358	0.021	0.916	Gira4	0.354	0.326	0.372
Chna1	0.013	0.052	0.951	Girb	0.136	0.088	0.660
Chna2	0.406	0.863	0.684	Qrfpr	0.325	0.052	0.866
Chna3	0.627	0.694	0.801	Npfr1	0.264	0.272	0.023
Chna4	0.415	0.670	0.971	Prokr1	0.015	0.092	0.149
Chna5	0.108	0.726	0.956	Prokr2	0.718	0.600	0.921
Chna7	0.833	0.144	0.693	Npfr2	0.349	0.324	0.223
Chnb1	0.204	0.359	0.510	Gpr83	0.624	0.355	0.835
Chnb2	0.863	0.691	0.517	Grpr	0.591	0.337	0.004
Chnb3	0.314	0.125	0.745	Htr3a	0.483	0.295	0.175
Chnb4	0.013	0.270	0.812	Maoa	0.007	0.00017	0.724
Chme	0.437	0.510	0.333	Mc2r	0.014	0.246	0.097
Chng	0.646	0.605	0.640	Nmur1	0.977	0.152	0.905
Comt	0.343	0.003	0.081	Nmur2	0.736	0.091	0.487
Drd1a	0.695	0.022	0.881	Npy1r	0.118	0.106	0.374
Drd2	0.994	0.869	0.360	Npy2r	0.231	0.017	0.235
Drd3	0.617	0.503	0.597	Npy5r	0.633	0.00001	0.423
Drd4	0.848	0.581	0.579	Ntsr1	0.626	0.919	0.032
Drd5	0.372	0.356	0.351	Pgr15l	0.525	0.507	0.276

Gene	Condition	Age	Con × Age	Gene	Condition	Age	Con × Age
Gabra1	0.594	0.113	0.808	Ppyr1	0.588	0.426	0.483
Gabra2	0.070	0.237	0.872	Prhr	0.335	0.282	0.272
Gabra3	0.812	0.002	0.341	Slc5a7	0.339	0.126	0.913
Gabra4	0.698	0.062	0.800	Sstr1	0.069	0.766	0.253
Gabra5	0.739	0.195	0.299	Sstr2	0.193	0.175	0.738
Gabrb2	0.374	0.026	0.955	Sstr3	0.060	0.472	0.374
Gabrb3	0.682	0.114	0.816	Sstr4	0.622	0.661	0.706
Gabrd	0.521	0.761	0.781	Sstr5	0.323	0.205	0.245
Gabrg1	0.558	0.001	0.263	Tacr1	0.130	0.190	0.652
Gabrg2	0.079	0.012	0.994	Tacr2	0.377	0.261	0.280
				Tacr3	0.406	0.049	0.249