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Regional mRNA expression of the endogenous opioid and dopaminergic systems in brains of C57BL/6J and 129P3/J mice: Strain and heroin effects

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ABSTRACT

We have previously shown strain and dose differences in heroin-induced behavior, reward and regional expression of somatostatin receptor mRNAs in C57BL/6J and 129P3/J mice. Using Real Time PCR we examined the effects of five doses of heroin on the levels of the transcripts of endogenous opioid peptides and their receptors and dopaminergic receptors in the mesocorticolimbic and nigrostriatal pathways in these same mice. Compared to C57BL/6J animals, 129P3/J mice had higher mRNA levels of *Oprk1* in the nucleus accumbens and of *Oprd1* in the nucleus accumbens and a region containing both the substantia nigra and ventral tegmental area (SN/VTA). In the cortex of 129P3/J mice, lower levels of both *Oprk1* and *Oprd1* mRNAs were observed. *Pdyn* mRNA was also lower in the caudate putamen of 129P3/J mice. Strain differences were not found in the levels of *Oprm1*, *Penk* or *Pomc* mRNAs in any region examined. Within strains, complex patterns of heroin dose-dependent changes in the levels of *Oprm1*, *Oprk1* and *Oprd1* mRNAs were observed in the SN/VTA. Additionally, *Oprd1* mRNA was dose-dependently elevated in the hypothalamus. Also in the hypothalamus, we found higher levels of *Drd1a* mRNA in C57BL/6J mice than in 129P3/J animals and higher levels of DAT (*Slc6a3*) mRNA in the caudate putamen of C57BL/6J animals than in 129P3/J counterparts. Heroin had dose-related effects on *Drd1a* mRNA in the hypothalamus and on *Drd2* mRNA in the caudate putamen.

1. Introduction

The endogenous opioid system consists of the mu, kappa and delta opioid receptors and their ligands (β-endorphin, dynorphins and enkephalins respectively). The endogenous opioid system is widely dispersed throughout the CNS and the periphery (e.g. Boublik et al., 1983; DePaoli et al., 1994; Dumont and Lemaire, 1984; Jamensky and Gianoulakis, 1997; Konturek, 1980; Mansour et al., 1988, 1994; Minami et al., 1993; Pert et al., 1975; Sanchez-Blazquez and Garzon, 1985; Sharif and Hughes, 1989). In the CNS, opioid receptors and their ligands are found throughout the mesocorticolimbic and nigrostriatal dopaminergic systems, although the relative levels of each receptor and ligand show significant regional specificity (for review see e.g. Mansour et al., 1988).

The mesocorticolimbic and nigrostriatal dopaminergic projections originate from cell bodies in the ventral tegmental area and the substantia nigra respectively, and project throughout the limbic system and to the striatum. A third dopaminergic system, the tuberoinfundibular system, originates from cell bodies in the hypothalamus and projects to the pituitary and the median eminence. Within these dopaminergic systems there are five distinct dopamine receptors (D1-5).

Dopamine receptors are known to colocalize with specific endogenous opioid peptides. The medium spiny neurons of the striatonigral projection contain the endogenous opioid peptide dynorphin and the dopamine D1 receptor, whereas those of the striatopallidal projection contain enkephalin and the dopamine D2 receptor (e.g. Afifi, 1994; Gerfen et al., 1990; Le Moine and Bloch, 1995; McGinty, 2007).

Morphine, the biologically active metabolite of heroin (e.g. Inturrisi et al., 1983; Selley et al., 2001), binds to the mu opioid receptor on GABAergic interneurons in the substantia nigra and ventral tegmental area, which release GABAergic inhibition of dopamine release (e.g. Johnson and North, 1992). This disinhibition results in elevated levels of dopamine in the projection fields (e.g. Di Chiara and Imperato, 1988a,b; Spanagel et al., 1990) where it activates pre-and post-synaptic dopaminergic receptors.

In vivo, endogenous opioid peptides and their receptors modulate extracellular dopamine levels in nucleus accumbens and caudate putamen. Activation of the mu opioid receptor increases extracellular dopamine levels (Di Chiara and Imperato, 1988a; Spanagel et al., 1990, 1992). Conversely, acute stimulation of *Oprk1* lowers dopamine levels (Di Chiara and Imperato, 1988a; Spanagel et al., 1990, 1992; Zhang et al., 2003). Furthermore, administration of the selective *Oprk1* antagonist nor-BNI into the nucleus accumbens increased extracellular dopamine levels in the same region, demonstrating an ongoing inhibitory tone at the kappa opioid receptor (Spanagel et al., 1992).

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Consistent with this, we found that locally administered dynorphin A (1–17) decreased dopamine dialysate levels in the caudate putamen in mice, and blocked cocaine-induced conditioned place preference (Zhang et al., 2004a). This further implicates the kappa opioid receptor system in the dorsal striatum (i.e., caudate putamen) as an important mediator of the acquisition of reward-related behaviors of cocaine (Everitt and Robbins, 2005; Fagergren et al., 2003; Porrino et al., 2004). Thus, the mu and kappa opioid peptides and ligands countermodulate dopaminergic tone.

Opiates affect mRNA levels within the striatum, including those of the endogenous opioid system (e.g. Buzas et al., 1996; Garcia de Yebenes and Pelletier, 1993; Georges et al., 1999; Romualdi et al., 1990, 1991; Trujillo et al., 1995; Yukhananov et al., 1993) and the dopaminergic system (Spangler et al., 2003). In the nucleus accumbens and caudate putamen of rats, *Penk* mRNA levels were decreased by chronic administration of morphine (Basheer and Tempel, 1993; Georges et al., 1999; Uhl et al., 1988). We have shown that acute intermittent morphine administration to rats produced elevations in *Pdyn* and *Oprm1* mRNA in the whole brain, with no effect on *Penk* mRNA levels (Wang et al., 1999). Acute morphine withdrawal increased *Penk* mRNA expression in the hypothalamus and striatum (Fukunaga et al., 1996; Gudehithlu and Bhargava, 1995; Harbuz et al., 1991; Lightman and Young, 1987).

There is a large body of data demonstrating considerable individual differences in the vulnerability to develop addictive disease. One way to study these individual, and perhaps genetic, roots of vulnerability is to examine inbred strains of animals which are known to differ in their response to drugs of abuse (e.g. Belknap et al., 1989; Kosten et al., 1994). Numerous neurochemical and drug-induced behavioral differences have been described in C57BL/6J and DBA2 strains of mice (e.g. Castellano et al., 1976; Crabbe et al., 1980; De Waele et al., 1992; Jamensky and Gianoulakis, 1997; Phillips et al., 1994). The C57BL6 and 129 strains of mice are frequently used in the generation of knockout animals (e.g. Crawley et al., 1997); however these strains of mice have been studied much less extensively (for review see Crawley et al., 1997). Mice of various 129 sub-strains have consistently been shown to be less responsive to the locomotor stimulating effects of cocaine (e.g. Kuzmin and Johansson, 2000; Kuzmin et al., 2000; Miner, 1997; Schlussman et al., 1998, 2003a) or rewarding effects of heroin (e.g. Schlussman et al., 2008; Szumlinski et al., 2005) or cocaine (Miner, 1997). Szumlinski et al. showed that C57BL/6J mice developed conditioned place preference to heroin at a dose of 100 μg/kg, while 129/sVJ mice developed conditioned place aversion to the same dose (Szumlinski et al., 2005). We have shown significant differences in the development of heroin-induced conditioned place preference, in the same mice described in the study presented here, with C57BL/6J mice developing preference to relatively low doses of heroin and 129P3/J mice developing preference only to higher doses of heroin (Schlussman et al., 2008). We suggested that this might represent a decreased sensitivity to the rewarding effects of heroin in 129P3/J mice relative to C57BL/6J animals.

Here we extend our behavioral studies by examining the relative expression of mRNAs of the endogenous opioid and dopaminergic systems in the same mice we found to differ in their behavioral response to heroin.

2. Materials and methods

A total of 125 age-matched male mice (6 weeks old on arrival; Jackson Laboratory, Bar Harbor, ME), 55 C57BL/6J and 70 129P3/J, were studied. All animals were individually housed in an environmentally controlled room dedicated to this study. Food and water were available *ad lib* and animals were allowed two weeks to acclimate prior to the start of the experiments. Mice of each strain were randomly assigned to one of six groups, each administered a specific dose (0, 1.25, 2.5, 5, 10 or 20 mg/kg) of heroin (diacetylmorphine HCl, obtained from NIH-NIDA). This study was approved by the Rockefeller University Institutional Animal Care and Use Committee and included provisions to minimize pain and discomfort.

Mice whose tissue was studied here were from a study of heroin-induced conditioned place preference which has been reported elsewhere (Schlussman et al., 2008). Animals in the 0 mg/kg group received i.p. injections of isotonic saline on all days of the study. Animals in the other groups received i.p. injections of heroin or saline on alternate days for a total of 8 days (for details see Schlussman et al., 2008). Animals were sacrificed immediately following the testing session (24.5 h following the last conditioning session) by decapitation following brief CO_2 exposure (<20 s), their brains rapidly removed, and slices were cut with a rodent brain matrix (ASI Instruments, Warren, MI). The hypothalamus, cortex (an area containing the cingulate cortex area 1, the primary and secondary motor cortices and the prelimbic cortex (Franklin and Paxinos, 1997)), nucleus accumbens, the caudate putamen and a region containing both the substantia nigra and the ventral tegmental area (SN/VTA) were dissected and homogenized in guanidium thiocyanate as previously described (Branch et al., 1992). RNA was isolated from homogenates of the hypothalamus, cortex and caudate putamen with the RNAqueous system (Ambion [ABI], Austin TX) according to manufacturer's instructions. RNA from the nucleus accumbens and SN/VTA was isolated using an acid phenolic extraction (Chomczynski and Sacchi, 1987). Following RNA isolation, all samples were treated with DNase (Turbo DNA-free™, Ambion [ABI], Austin, TX). The quantity and quality of RNA in each extract was determined with the Agilent Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA).

cDNA was synthesized from each sample using the Super Script™ III first strand synthesis kit (Invitrogen, Carlsbad, CA). Five hundred ng of RNA from the hypothalamus, cortex and caudate putamen was used for reverse transcription. The entire nucleus accumbens and SN/VTA were utilized for generation of cDNAs. cDNAs were diluted 1:10 for real time PCR analysis.

Real time PCR analysis of the relative mRNA expression levels of *Oprm1*, *Oprk1*, *Oprd1*, *Penk*, proopiomelanocortin (*Pomc*; hypothalamus, caudate putamen only), *Pdyn*, tyrosine hydroxylase (*Th*; hypothalamus and SN/VTA only), DAT (*Slc6a3*; caudate putamen, nucleus accumbens and cortex) dopamine D1 receptor (*Drd1a*; hypothalamus, nucleus accumbens and caudate putamen), dopamine D2 receptor (*Drd2*; hypothalamus, nucleus accumbens and caudate putamen) and dopamine D3 receptor (*Drd3*; nucleus accumbens and caudate putamen) was conducted using commercially available primers and master mix (RT^2qPCR™ primer assays and RT^2 Real Time™ SYBR® Green PCR Master Mix; Qiagen, Valencia, CA) according to manufacturer's directions in an ABI Prism 7900 HT Sequence Detection System (Applied Biosystems, Foster City, CA). Water controls for each primer set were included in every assay. Any sample with a cycle threshold (Ct) greater than that of the water control or a Ct greater than or equal to 35 was not included in the analysis. All data were normalized to *Gapdh* and reported as $2^{-\Delta Ct}$ where ΔCt is the cycle threshold of the mRNA of interest minus the cycle threshold of *Gapdh*.

Data for each mRNA of interest was analyzed by two-way ANOVA, Strain X Dose, followed by Newman-Keuls *post hoc* analysis where appropriate. Due to missing data points in certain C57BL/6J mice, resulting in an incomplete ANOVA design, significant main effects of Strain, in the cortex and nucleus accumbens were also analyzed in the saline treated animals only, using two-tailed t or Mann-Whitney U tests as appropriate. Any sample that was ≥2.5 standard deviations from the strain mean was considered an outlier and dropped from the analysis.

3. Results

We only present data which reached or neared statistical significance. All our findings are shown in supplemental Figs. 1–11.

3.1. Endogenous opioid strain effects

We observed significant region-specific strain differences in the mRNA levels of several components of the endogenous opioid system.

3.1.1. SN/VTA

In the region containing the substantia nigra and ventral tegmental area, the relative levels of *Oprd1* mRNA were significantly higher in 129P3/J mice compared to C57BL/6J counterparts ($F_{(1,87)} = 7.32$, $p<0.01$; Fig. 1A).

3.1.2. Caudate putamen

In the caudate putamen, 129P3/J mice had lower levels of *Pdyn* mRNA than did C57BL/6J mice ($F_{(1,109)} = 7.67$, $p<0.01$; Fig. 1B).

3.1.3. Nucleus accumbens

Several strain differences in the relative mRNA expression levels were observed in the nucleus accumbens.

Levels of *Oprm1* mRNA were greater in the nucleus accumbens of 129P3/J mice, relative to C57BL/6J mice, although this just missed statistical significance ($F_{(1,58)} = 3.96$, $p = 0.051$). However, when *Oprm1* mRNA levels in saline controls were compared, this effect was significant ($U = 1.00$, $P<0.005$; Fig. 1C).

There were apparently higher levels of *Oprk1* mRNA in the nucleus accumbens of 129P3/J mice compared to C57BL/6J animals, however, this did not reach statistical significance ($F_{(1,57)} = 2.87$ $p = 0.096$; Fig. 1D). Such a trend was also observed in saline controls ($t = 1.74$ $p = 0.096$).

In the nucleus accumbens, *Oprd1* mRNA levels were significantly higher in 129P3/J mice than in C57BL/6J animals ($F_{(1,38)} = 4.61$, $p<0.05$; Fig. 1E). This significant difference was also observed between saline controls ($U = 6.00$, $p<0.05$).

A significant main effect of Strain on *Penk* mRNA levels was not observed, however there was a trend for higher levels of expression in 129P3/J mice when saline controls were examined ($t = 1.91$ $p = 0.072$; Fig. 1F).

3.1.4. Cortex and hypothalamus

Strain differences in the relative levels of mRNA for any component of the endogenous system were not found in either region.

3.2. Dopaminergic system strain effects

We observed regional strain differences in the relative expression levels of mRNAs of the dopaminergic system.

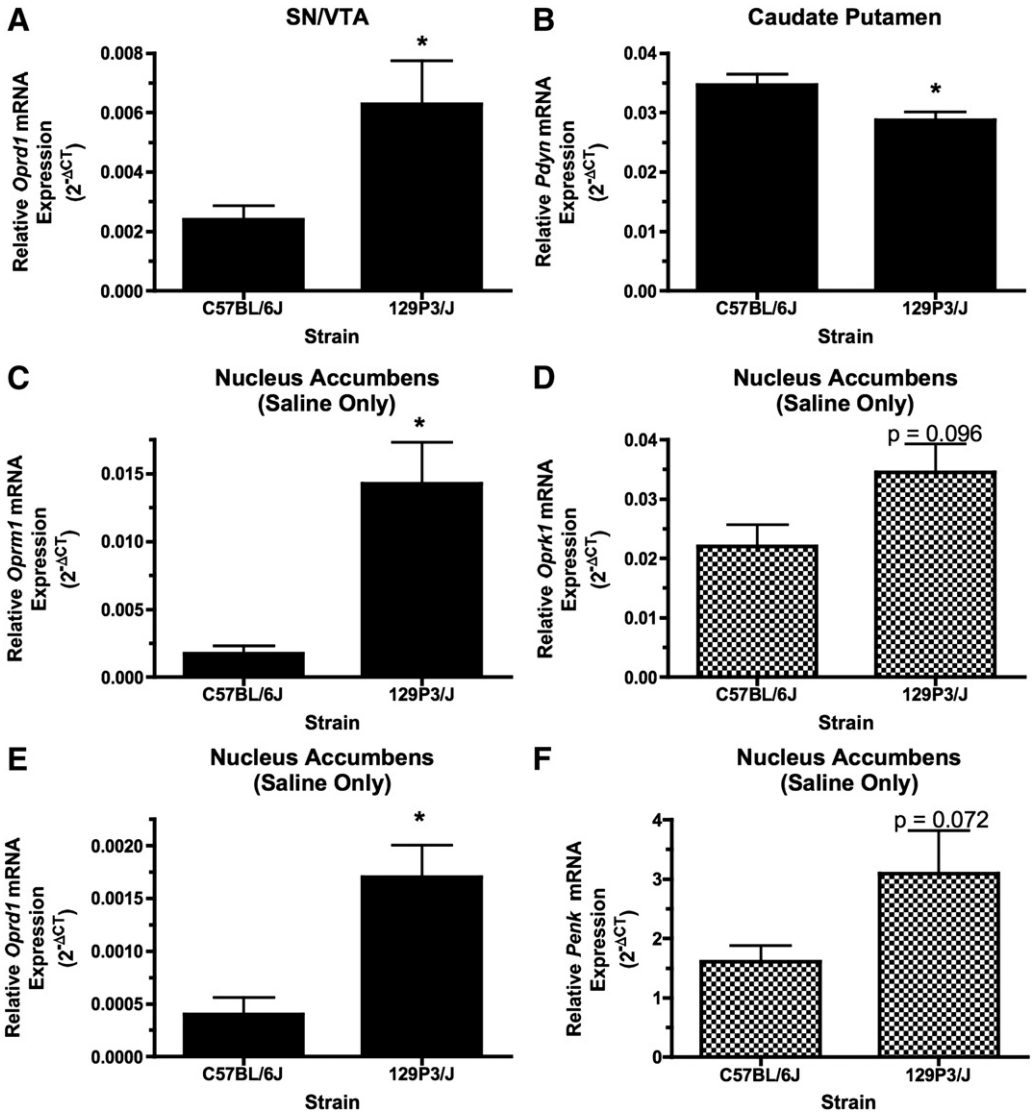

Fig. 1. Endogenous Opioid System; Strain Effects. Regionally specific strain differences in the relative expression levels of mRNA for components of the endogenous opioid system were found. Solid bars represent statistically significant differences; stippled bars represent strain differences which did not reach the 0.05 level of statistical significance.

3.2.1. SN/VTA

In the SN/VTA there was a trend toward higher levels of *Th* mRNA expression in C57BL/6J mice relative to 129P3/J animals ($F_{(1,99)}=3.11$, $p=0.08$; Fig. 2A).

3.2.2. Caudate putamen

In the caudate putamen there were lower levels of *Slc6a3* mRNA in 129P3/J mice but this difference just missed statistical significance ($F_{(1,106)}=3.90$, $p=0.051$; Fig. 2B).

3.2.3. Nucleus accumbens

In the nucleus accumbens of 129P3/J mice, we found a trend toward lower levels of *Slc6a3* mRNA compared to C57BL/6J mice, ($F_{(1,65)}=3.07$, $p=0.084$) In the saline controls, this difference was statistically significant ($U=15.00$, $p<0.05$; Fig. 2C).

3.2.4. Cortex

Strain differences in the relative levels of mRNA of the dopaminergic system were not found.

3.2.5. Hypothalamus

In the hypothalamus, 129P3/J mice had lower levels of *Drd1a* mRNA compared to C57BL/6J mice ($F_{(1,95)}=5.49$, $p<0.05$; Fig. 2D). In the hypothalamus, *Drd2* mRNA levels appeared higher in 129P3/J mice than in C57BL/6J mice, although this difference did not reach statistical significance ($F_{(1,79)}=3.35$, $p=0.071$; Fig. 2E).

3.3. Endogenous opioid system; heroin dose effects

Within strains, heroin had a complex pattern of effects on expression of several mRNAs. Interestingly, most of these were observed in the SN/VTA.

3.3.1. SN/VTA

A significant heroin Dose effect was observed, in this region, on the relative expression levels of mRNAs for all three endogenous opioid receptor genes (*Oprm1*, $F_{(5,101)}=5.31$, $p<0.0005$; *Oprk1* $F_{(5,97)}=3.59$, $p<0.01$; *Oprd1*, $F_{(5,87)}=2.95$, $p<0.05$). *Oprm1* mRNA levels were significantly reduced by heroin at doses of 5 and 10 mg/kg ($p<0.05$;

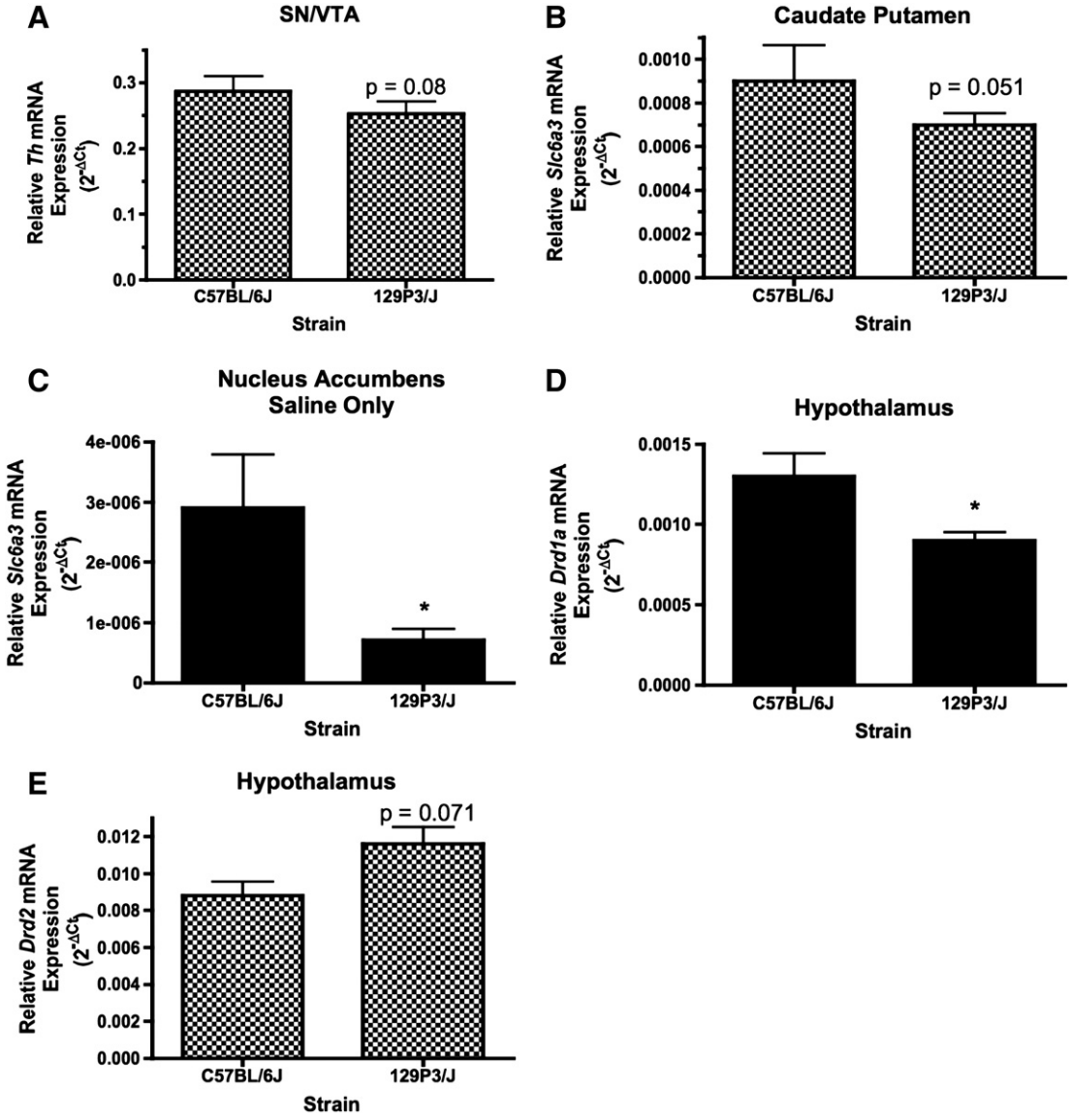

Fig. 2. Dopaminergic System; Strain Effects. Regionally specific strain differences in the relative expression levels of mRNA for components of the dopaminergic systems were found. Solid bars represent statistically significant differences; stippled bars represent strain differences that did not reach the 0.05 level of statistical significance.

Fig. 3A). *Oprm1* mRNA levels were also lower following 20 mg/kg of heroin, although this did not reach significance ($p = 0.08$). Lower doses of heroin (1.25 and 2.5 mg/kg) had no effect on levels of *Oprm1* mRNA. *Oprk1* mRNA levels were significantly increased by 2.5 mg/kg of heroin ($p < 0.05$; Fig. 3B). *Oprk1* mRNA levels appeared to increase following 1.25 mg/kg, but this did not reach statistical significance ($p = 0.07$). The higher doses of heroin had no effect on *Oprk1* mRNA. *Oprd1* mRNA levels in the SN/VTA were significantly elevated by the lowest dose of heroin ($p < 0.05$; Fig. 3D). We also observed a Strain by Dose interaction in the level of *Oprd1* mRNA in the SN/VTA ($F_{(5,87)} = 3.44$, $p < 0.01$). Newman-Keuls *post hoc* tests showed that the effect of the 1.25 mg/kg dose on expression levels of *Oprd1* mRNA was due to an elevation of *Oprd1* mRNA levels only in 129P3/J mice (data not shown). A significant main effect of heroin Dose on *Penk* mRNA expression was found in the SN/VTA ($F_{(5,104)} = 3.25$, $p < 0.01$; Fig. 3D). Newman-Kuels *post hoc* tests indicated an apparent lower level of *Penk* expression following the 10 mg/kg dose of heroin ($p = 0.08$).

3.3.2. Hypothalamus

In the hypothalamus, we observed a significant main effect of heroin Dose on relative levels of *Oprd1* mRNA ($F_{(5,78)} = 6.27$, $p < 0.0001$; Fig. 3E). Newman-Kuels *post hoc* tests showed that *Oprd1* mRNA levels were significantly elevated by the 10 mg/kg dose ($p < 0.05$). A significant Strain by Dose interaction was also observed ($F_{(1,78)} = 2.62$, $p < 0.05$). Newman-Kuels *post hoc* tests indicated that the main effect of Dose was due to an elevation of *Oprd1* mRNA by 10 mg/kg of heroin in C57BL/6J mice, but not in 129P3/J animals (data not shown).

3.4. Dopaminergic system; heroin dose effects

A significant effect of heroin Dose on mRNA levels of the dopaminergic system was found in the caudate putamen and the hypothalamus.

Caudate putamen: In the caudate putamen, there was a significant main effect of Dose on the relative levels of *Drd2* mRNA ($F_{(5,111)} = 4.07$, $p < 0.005$; Fig. 4A). Newman-Kuels *post hoc* tests showed that heroin significantly elevated *Drd2* mRNA levels at the 2.5 mg/kg dose.

Hypothalamus: We observed a significant main effect of heroin Dose on *Drd1a* mRNA ($F_{(5,95)} = 2.76$, $p < 0.05$ l; Fig. 4B). Newman-Kuels *post hoc* tests showed that heroin significantly elevated *Drd1a* mRNA levels at the 10 mg/kg dose.

Heroin dose effects on mRNAs of the dopaminergic system were not observed in any other region examined.

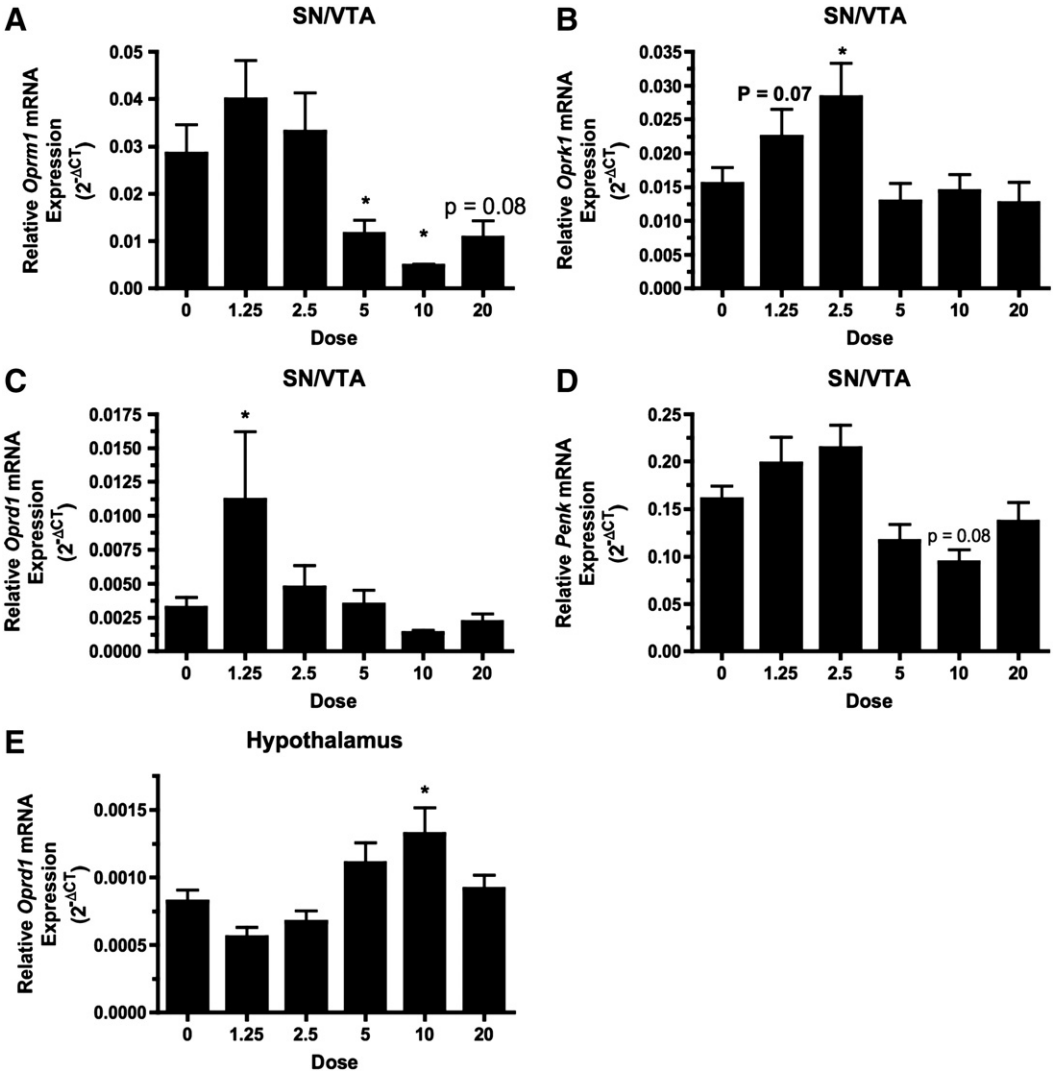

Fig. 3. Endogenous Opioid System. Dose Effects within strains, a statistically significant main effect of Heroin Dose on relative mRNA levels of components of the endogenous opioid system was found in several brain regions. * represents Newman-Kuels *post-hoc* test $p < 0.05$.

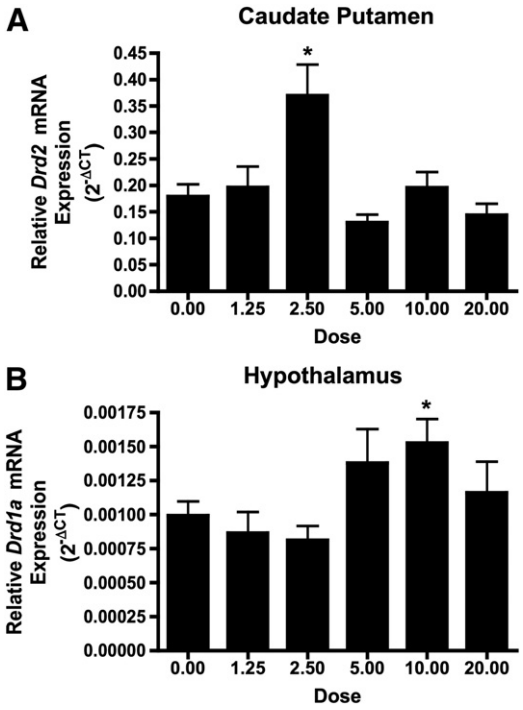

Fig. 4. Dopaminergic System; Dose Effects. Within strains, a statistically significant main effect of Heroin Dose was found for the relative expression of *Drd2* mRNA in the caudate putamen and on *Drd1a* mRNA in the hypothalamus. * Represents Newman-Kuels *post-hoc* test $p<0.05$.

4. Discussion

We identified region-specific strain differences in the relative expression levels of mRNA for important components of the endogenous opioid and the dopaminergic systems. The C57BL/6J and 129 strains of mice are known to differ in their behavioral response to cocaine (e.g. Miner, 1997; Schlussman et al., 1998, 2003a; Szumlinski et al., 2005; Unterwald and Cuntapay, 2000) and to heroin (e.g. Schlussman et al., 2008; Szumlinski et al., 2005). C57BL/6J mice have been shown to be more sensitive to thermal stimulation than 129/J (now 129P3/J) mice but less sensitive to morphine-induced antinociception (Mogil and Wilson, 1997). 129/J mice have also been reported to be relatively resistant to developing dependence to morphine (Kest et al., 2002). Strains of 129 mice are consistently hyporesponsive to the behaviorally stimulating effects of cocaine, showing little or no horizontal activity in response to cocaine (e.g. Kuzmin et al., 2000; Miner, 1997; Schlussman et al., 1998, 2003a). Additionally, 129Ola/Hsd mice failed to acquire cocaine self-administration while C57BL/6J and DBA mice did (Kuzmin and Johansson, 2000). We have previously shown, in the same animals studied in the present report, that C57BL/6J and 129P3/J mice differ in the locomotor-stimulating effect of heroin and in the establishment of heroin-induced conditioned place preference (Schlussman et al., 2008). Contrary to findings with cocaine, 129P3/J mice had a robust, dose-dependent locomotor response to heroin. The 129P3/J mice also developed conditioned place preference to heroin, although only to relatively high doses, to which C57BL/6J mice did not form a preference (Schlussman et al., 2008). Another substrain of 129 mice, 129×1/sVJ developed conditioned place aversion to a lower dose of heroin than we used (Szumlinski et al., 2005). Interestingly, this same sub-strain of mice was shown to require both contextual and drug cues in order to express conditioned place preference to morphine and it was suggested that higher relative levels of anxiety in the 129 strain of mice may explain these differences (Dockstader and van der Kooy, 2001). We have also reported that these same mice whose tissues were used in the present study show significant differences in the expression of specific somatostatin receptor mRNAs within discrete regions of the central nervous system (Schlussman et al., 2010).

In the region containing the substantia nigra and ventral tegmental area, and in the nucleus accumbens, we observed significantly higher levels of *Oprd1* mRNA in 129P3/J mice, and in the nucleus accumbens we report a trend toward higher levels of *Penk* mRNA in 129P3/J mice. Delta opioid receptors have been implicated in mediating anxiety-like behaviors. For instance, mice with life-long deletion of *Oprd1* show increased levels of anxiety (Filliol et al., 2000). Interestingly, 129/J mice have been shown to exhibit lower levels of anxiety, as measured in the open field, when compared to C57BL/6J mice (Montkowski et al., 1997). This may be related to a higher relative expression of *Oprd1* mRNA. We have reported that in the mice whose tissue was studied here, 129P3/J animals have higher levels of somatostatin receptor-2 mRNA in the caudate putamen (Schlussman et al., 2010). Somatostatin receptor-2 knockout mice have increased anxiety-like behavior (Viollet et al., 2000) which suggests that higher levels of Somatostatin receptor *2* mRNA might be associated with decreased levels of anxiety. However, although we did not examine *sstr-2* mRNA levels in brain regions other than the caudate putamen. However, another group has suggested that 129/sVJ mice have high levels of anxiety during acute opiate withdrawal, in a conditioned place preference paradigm (Dockstader and van der Kooy, 2001).

In addition to *Oprd1* mRNA, we also observed significantly higher levels of *Oprm1* mRNA, and a trend to higher levels of *Oprk1* mRNA in the nucleus accumbens of 129P3/J mice compared to that of C57BL/6J animals. The nucleus accumbens is an integral part of the reward pathway and is a major target of the mesocorticolimbic dopaminergic projection. We also found a small but statistically significant negative correlation between *Oprk1* mRNA levels in the nucleus accumbens of 129P3/J, but not in C57BL/6J, mice. The mu and kappa opioid receptors are thought to act in a countermodulatory manner to mediate dopaminergic tone (e.g. Zhang et al., 2004a,b, 2009). Therefore it is somewhat counterintuitive to find elevated levels of mRNA for both of these receptors in this important dopaminergic terminal field, although it must be remembered that mRNA levels may not directly reflect protein levels, and *Oprm1* and *Oprk1* densities have not yet been compared in these two strains. A recent study has shown that DBA/2J mice are more sensitive to the rewarding effects of heroin (as measured by conditioned place preference) than are C57BL/6J animals (Bailey et al., 2010). These authors did not report basal differences in MOP-r binding densities in the nucleus accumbens between these two strains but they did report that MOP-r density was decreased by chronic heroin administration in many brain regions of the C57BL/6J animals, including the nucleus accumbens shell, while MOP-r density was not altered in any region of the DBA/2J brain (Bailey et al., 2010). Interestingly, significant strain differences in the DAMGO stimulated [^{35}S] GTPγ were reported with higher levels of binding in the nucleus accumbens (core and shell) of C57BL/6J mice than in DBA/2J animals (Bailey et al., 2010).

In the caudate putamen we found a significantly lower level of *Pdyn* mRNA in 129P3/J mice than in C57BL/6J counterparts. This was unexpected. In another study, using solution hybridization / RNase protection, we did not find a difference in *Pdyn* mRNA in the caudate putamen of the two strains (Schlussman et al., 2003b). Additionally, dynorphin peptide lowers dopaminergic tone through its action on the kappa opioid receptor (e.g. Zhang et al., 2004a) and we did not observe differences in the basal levels of dopamine in the caudate putamen of these two strains (Zhang et al., 2001). Dynorphin, and other kappa opioid receptor agonists block the formation of cocaine-induced conditioned place preference (e.g. Zhang et al., 2004a,b, 2005). However we have reported that these same 129P3/J mice develop conditioned place preference to heroin, but only at relatively high doses (Schlussman et al., 2008). Previous studies have shown differences in the rewarding effects of opiates in C57BL/6J and DBA/2J

mice, with often contradictory results (e.g. see Bailey et al., 2010; Cunningham et al., 1992; Orsini et al., 2005; Semenova et al., 1995). Similar to the DBA mice, another strain, SWR, has also been shown to be less sensitive to the rewarding effects of opiates than C57BL/6J animals (Solecki et al., 2009). A recent study reported lower levels of *Pyn* and *Penk* mRNA in the dorsolateral striatum of SWR/J relative to either C57BL/6J or DBA/2J animals (Gieryk et al., 2010). In the nucleus accumbens we showed a trend toward higher levels of *Penk* mRNA levels in 129P3/J mice than in C57BL/6J animals. While we found that 129P3/J mice only develop conditioned place preference to relatively high doses of heroin (Schlussman et al., 2008), in the present study we did not find a statistically significant effect of heroin dose on *Penk* mRNA levels in this region. In an earlier report, Gieryk et al. reported that C57BL/6J mice had higher levels of *Penk* mRNA and lower levels of *Pdyn* mRNA in the nucleus accumbens than did either DBA/2J or SWR/J mice (Gieryk et al., 2010). Interestingly, this groups had previously shown that C57BL/6J have a high sensitivity to morphine reward (as measured by conditioned place preference) while both DBA/2Jand SWR/J mice showed lower levels of morphine reward (Solecki et al., 2009) which led them to suggest that sensitivity to morphine reward may be related to basal levels of nucleus accumbal *Penk* and *Pdyn* mRNA (Gieryk et al., 2010). In the present study, we did not observe differences in levels of either *Penk* or *Pdyn* mRNA in the nucleus accumbens, despite having shown strain differences in the sensitivity to heroin-induced conditioned place preference (Schlussman et al., 2008). In the absence of a direct comparison of C57BL/6J, DBA/2J, SWR/J and 129P3/J, it is difficult to interpret these data.

In the present study we found lower levels of mRNA for *Th*, the rate limiting biosynthetic enzyme for dopamine, in the region containing the substantia nigra and ventral tegmental area, which may suggest reduced dopamine biosynthesis. We also observed lower levels of mRNA for the dopamine transporter (*Slc6a3*) in both the caudate putamen and the nucleus accumbens of 129P3/J mice compared to C57BL/6J animals. Interestingly, rats that are more susceptible to acquisition of psychostimulant self-administration show lower levels of DAT binding sites in these regions (e.g. Flores et al., 1998) which led us to expect lower DAT mRNA levels in striatal regions of C57BL/6J mice. We did not observe an effect of heroin on S*Slc6a3* mRNA levels. A previous report demonstrated higher levels of DAT binding in the nucleus accumbens, caudate putamen and olfactory tubercle of DBA/2J mice, compared to C57BL/6J animals, following chronic heroin administration (Bailey et al., 2010). These authors suggested that strain differences in heroin-induced levels of DAT binding might be partially responsible for the strain differences in heroin-induced locomotor activity in the strains (Bailey et al., 2010). However, contrary to the findings with DBA/2J mice, we reported that both the 129P3/J and C57BL/6J mice utilized in the present study showed a robust locomotor response to heroin (Schlussman et al., 2008). Nonetheless, it would be of interest to examine DAT binding and mRNA levels in C57BL/6J and 129P3/J mice following chronic administration of heroin.

In the hypothalamus, we reported opposite strain differences on levels of mRNA for the *Drd1a* and *Drd2*. 129P3/J mice had lower levels of *Drd1a* mRNA, and trended toward higher levels of *Drd2* mRNA in this region. Activation of the stress responsive hypothalamic-pituitary-adrenal axis is, at least partially, regulated by dopaminergic *Drd1a* and *Drd2* (e.g. Borowsky and Kuhn, 1992; Eaton et al., 1996) and we have shown that *Drd2* exerts tonic inhibition on hypothalamic *Pomc* mRNA expression (Zhou et al., 2004). We have not yet examined HPA function, in depth, in these two strains of mice, but in the present report we did not find strain differences in hypothalamic *Pomc* mRNA levels.

We did not observe a strain difference on the expression level of mRNA for *Drd1a*, *Drd2* or *Drd3* in either the nucleus accumbens or the caudate putamen. These findings for *Drd1a* mRNA support our earlier finding that, within the entire nucleus accumbens or caudate putamen there was no difference in the dopamine D1 receptor binding density in these strains (Schlussman et al., 2003a). Inbred strains of rats which have been reported to be more susceptible to acquisition of psychostimulant self-administration have fewer *Drd2* binding sites in the caudate putamen or nucleus accumbens (e.g. Hooks et al., 1994) and lower levels of *Drd2* mRNA in the nucleus accumbens (e.g. Dalley et al., 2007; Hooks et al., 1994). Similarly, inbred strains of rats (e.g. F344 and Lewis) which are known to differ in their acquisition of drug self-administration (e.g. Guitart et al., 1992; Kosten et al., 2007) show differences in the density of *Drd2* and *Drd3*. Specifically, Lewis rats, which have a higher propensity to self administer drugs of abuse, had lower levels of *Drd2* binding in the nucleus accumbens and caudate putamen than did F344 rats. Lewis rats also have lower levels of dopamine D3 receptor binding in the nucleus accumbens (Flores et al., 1998). Based on our previous behavioral studies in these two strains of mice, we had hypothesized that C57BL/6J mice would have lower levels of *Drd2* and *Drd3* mRNA in the caudate putamen and nucleus accumbens than do 129P3/J mice.

In the region containing the substantia nigra and ventral tegmental area, heroin had a dose-dependent effect on the relative mRNA levels of all three endogenous opioid receptors. *Oprm1* mRNA was lowered by the three highest doses of heroin (5, 10 and 20 mg/kg). Heroin doses of 1.25 or 2.5 mg/kg did not alter *Oprm1* mRNA levels. In contrast, *Oprk1* mRNA levels were increased by the 1.25 and 2.5 mg/kg dose, while the higher doses had no effect. This may be reflective of the countermodulation of dopamine by *Oprm1* and *Oprk1*.

Few other heroin effects were observed in the present study. However, it must be noted that in the context of the original conditioned place preference study, these animals were sacrificed either 24 or 48 h following the last of four single injections of heroin. It is likely that additional effects would be observed following longer exposure to heroin. Additionally, real time PCR, like all PCR techniques, is based on an exponential function and, as a result, can only detect relatively large changes in mRNA levels. It is likely that additional heroin-induced effects on the mRNAs examined in the present report would be uncovered using techniques such as *in situ* hybridization or solution hybridization / RNase protection. Also, while we did not analyze the efficiency of amplification, it is possible that some of the strain effects that we report here may be due to strain differences in the efficiency of amplification. However, since the direction of strain-related differences in relative mRNA levels was gene-specific, we consider this possibility remote.

In summary, this report describes significant strain differences in the relative expression levels of important components of the endogenous opioid and dopaminergic systems. The previously reported behavioral differences between these two strains may be due, in part, to these strain differences in the endogenous opioid and dopaminergic systems.

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