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Lack of Genotype Effect on D1, D2 Receptors and Dopamine Transporter Binding in Triple MOP-, DOP-, and KOP-Opioid Receptor Knockout Mice of Three Different Genetic Backgrounds

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Abstract

We investigated D1, D2 receptors and dopamine transporter (DAT) binding levels in mice lacking all three opioid receptors and wild-type (WT) mice on three different genetic backgrounds. Quantitative autoradiography was used to determine the level of radioligand binding to the D1 and D2 receptors and DAT labeled with [³H]SCH23390, [³H]raclopride, and [³H]mazindol, respectively in triple-opioid receptor knockout (KO) and WT maintained on C57BL/6 (B6) and 129/SvEvTac (129) as well as C57BL/6 × 129/SvPas (B6 × 129) strains. No significant genotype effect was observed in D1, D2 receptors and DAT binding in any regions analyzed in any of the strains studied, suggesting that a lack of all three opioid receptors does not influence D1, D2 receptors and DAT expression, irrespective of their genetic strain background. However, strain differences were observed in D1 binding between the three strains of mice studied. Lower levels of D1 binding were observed in the substantia nigra of B6 × 129 WT mice compared with the 129 WT mice and in the olfactory tubercle of B6 × 129 WT compared with B6 WT and 129 WT mice. Lower levels of D1 binding were observed in the caudate putamen of B6 × 129 KO mice compared with 129 KO mice. In contrast, no significant strain differences were observed in D2 and DAT binding between the three strains of mice in any regions analyzed. Overall, these results indicate a lack of modulation of the dopaminergic system by the deletion of all three opioid receptors regardless of different background strains.

Keywords

[³H]SCH23390; [³H]raclopride; [³H]mazindol; strain; inbred; knockout; triple; opioid

INTRODUCTION

A number of studies have shown the existence of interactions between opiodergic and dopaminergic systems with the use of genetically modified mice (Becker et al., 2001; Chefer et al., 2004; Lena et al., 2004; Spilewoy et al., 2000; Tien et al., 2003). A small but a significant upregulation of both D1 and D2 receptors (7.4 and 12.6%, respectively) have been shown in the brains of μ -opioid receptor (MOPr) knockout (KO) mice of a mixed C57BL/6 and 129 (B6 $\times$ 129) background versus wild-type (WT) controls (Lena et al., 2004). In addition, acute treatment with apomorphine, a mixed D1- and D2-receptor agonist, enhanced locomotor activity in MOPr KO mice of B6 $\times$ 129 genetic background compared with its WT control (Tien et al., 2003). Moreover, loss of D1 receptors in the *Drd1a*^{tm1Jae} strain or D2 receptors in B6 $\times$ 129 strain has been shown to lead to an increase in the analgesic effects of a MOPr agonist morphine suggesting an involvement of D1 and D2 receptors in the analgesic response to morphine as determined in both hot plate and tail flick tests (Becker et al., 2001; King et al., 2001). This behavioral and neurochemical evidence in KO mice suggests that there is a clear interaction between opiodergic and dopaminergic systems.

There is growing evidence from different strains of mutant mice with genetic ablation of the same gene(s) showing that the interactions of genetic background and/or with gene(s) can influence the behavior and neurochemistry of mutant animals (Boehme and Ciaranello, 1981; Cabib et al., 2002; D'Este et al., 2007). Although the utilization of transgenic or KO mice has become a useful tool to investigate the role of receptors and neurotransmitter systems, it remains possible that alterations in behavior or neurochemistry observed in mutant mice may reflect not only the lack of function of the gene(s) but also the effect of strain and its interaction with the gene deleted (Morice et al., 2004). For instance, MOPr KO mice of congenic B6 strain demonstrated a significantly higher level of baseline locomotor activity compared with its WT control, whereas MOPr KO mice bred on a mixed B6 $\times$ 129 background from successive heterozygote matings of the original KO strain displayed lower level of basal locomotion compared with its WT control (Hummel et al., 2004). Moreover, genetic background has been shown to influence individual threshold responses to both the locomotor and rewarding effects of cocaine in dopamine transporter (DAT) KO mice of three different strains (B6, DBA, and hybrid B6 $\times$ DBA) suggesting that the behavioral phenotypes of mice lacking the DAT is dependent on strain (Morice et al., 2004).

Strain differences in the dopaminergic system have been shown to exist between inbred strains of mice (He and Shippenberg, 2000; Janowsky et al., 2001; Puglisi-Allegra and Cabib, 1997). For instance, lower basal dopamine (DA) uptake was observed in 129 mice compared with B6, DBA, and Swiss-Webster mice (He and Shippenberg, 2000). Although acute amphetamine treatment increased DA efflux both in B6 and 129 strains of mice, lower levels of amphetamine-induced DA efflux were detected in the striatum of 129 mice compared with the B6 strain of mice suggesting the existence of strain differences in the dopaminergic system of these mice (Chen et al., 2007). Moreover, quantitative trait loci studies also found significant strain differences in the binding of [¹²⁵]RTI-55 to DAT in the neostriatum of B6, DBA and B6 $\times$ DBA recombinant inbred mouse strains; the DBA allele has shown to be associated with higher $B_{\max}$ values relative to the B6 allele (Janowsky et

al., 2001). Overall, the evidence points to profound strain differences in the dopaminergic system of mice, which could account for some of their distinct behavioral phenotypes.

Although mice lacking MOPr, δ -opioid receptor (DOPr), or μ -opioid receptor (KOPr) have been generated from different laboratories and used for a number of years to investigate the role of opioid receptors in the central nervous system (Chefer et al., 2005; Filliol et al., 2000; Loh et al., 1998; Matthes et al., 1996; Schuller et al., 1999; Simonin et al., 1998; Sora et al., 1997; Zhu et al., 1999), the influence of genetic background on behavioral and neurochemical phenotypes of the KO animals is not clear (Chefer et al., 2005; Hall et al., 2003; Hummel et al., 2004). Moreover, although the effect of MOPr, KOPr, and DOPr genes on the dopaminergic system has been extensively studied with the use of single MOPr, KOPr, or DOPr KO mice (Becker et al., 2002; Chefer and Shippenberg, 2006; Chefer et al., 2000, 2003, 2004, 2005; Job et al., 2007; Lan et al., 2007; Robledo et al., 2004), compensatory changes in the remaining other receptors has been observed in those KO mice, which could influence the dopaminergic system (Clarke et al., 2002; Lena et al., 2004; Yoo et al., 2004). To investigate the interaction of the opioid receptor system with the dopaminergic system, we carried out quantitative receptor autoradiography of D1, D2 receptors and DAT binding in the brains of triple-opioid receptor KO and WT mice. Moreover, to determine whether these possible alterations are strain dependent, quantitative receptor autoradiographic studies were carried out in mice lacking all three classical opioid receptors generated from three different genetic backgrounds; B6, 129, and B6 $\times$ 129 genetic background.

MATERIALS AND METHODS

Animals

Triple mutant mice (B6 $\times$ 129 background) lacking MOPr, DOPr, and KOPr genes were generated by interbreeding of single MOPr (Matthes et al., 1996), DOPr (Filliol et al., 2000), and KOPr (Simonin et al., 1998) KO mice as previously described (Simonin et al., 2001). In brief, double MOPr/DOPr KO mice (50% B6 and 50% 129) were mated with double MOPr/KOPr KO mice (50% B6 and 50% 129) to generate triple-opioid receptor KO mice maintained on a mixed background (50% B6 and 50% 129). Both the triple-opioid receptor KO and WT mice are on a mixed of B6 and 129 background. triple-opioid receptor KO and WT mice brains from congenic (backcrossed to at least F10) B6 and coisogenic 129 mice were generated as described previously (Ansonoff et al., 2006). All male animals used in this study were >9 weeks of age and were genotyped by PCR at weaning. After sacrificing the animals, the brains were immediately removed and were then quickly frozen by being placed into isopentane (-30°C) and subsequently stored at -80°C . All experiments were carried out in accordance with the European Communities Council Directive of November 24, 1986 (86/609/EEC) and the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978).

Materials

[^3H]SCH23390 (70.3 Ci/mmol), [^3H]raclopride (60.1 Ci/mmol), and [^3H]mazindol (20.6 Ci/mmol) were purchased from PerkinElmer Life Sciences (PerkinElmer House, Cambridge,

UK). Mianserin, cis-flupentixol, sulpiride, desipramine, and mazindol were purchased from Sigma-Aldrich (Sigma-Aldrich Company, Poole, UK).

DA D1, D2 receptors and DAT autoradiography

Frozen brains were placed into a Cryostat (Zeiss Microm HM505E, Jena, Germany), and were allowed to equilibrate at -20°C . Twenty micrometers of coronal sections were then cut (300- μm apart) and thaw-mounted onto gelatin coated ice-cold slides to assess levels of binding from fore- to hind-brain regions. Consecutive sections were taken for determination of total and nonspecific binding. Slides were then stored in plastic boxes containing anhydrous calcium chloride with the indicator cobalt chloride (Drierite, BDH, UK) at -20°C for subsequent autoradiographic binding.

Autoradiography binding was carried out for the DA D1 and D2 receptors and the DAT in accordance with the previously described methods (Bailey et al., 2007, 2008; Lena et al., 2004). For the D1 receptor binding, sections were prewashed for 20 min in 50 mM Tris-HCl buffer, pH 7.4, containing 120 mM NaCl, 5 mM KCl, 2 mM CaCl_2 , and 1 mM MgCl_2 at room temperature. The sections were then incubated in the same buffer at room temperature in the presence of 4 nM [^3H]SCH23390 and 1 mM mianserin, which blocks binding of [^3H]SCH23390 to 5-HT₂ and 5-HT_{1c} receptors for 90 min to label the D1 receptors. The D2 receptors were preincubated in the same Trisbuffer as the D1 receptors. Incubation was carried out in the presence of 4 nM [^3H]raclopride for 60 min. Nonspecific binding was determined in adjacent sections in the presence of 10 μM of cis-flupentixol for the D1 receptors or 10 μM of sulpiride for the D2 receptors. Both incubations were terminated by rapid rinses (6×1 min) in ice-cold 50 mM Tris-HCl buffer, pH 7.4, followed by a dip into ice-cold distilled water, before they were rapidly dried in a stream of cold air. For DAT binding, sections were preincubated for 5 min at 4°C in 50 mM Tris-HCl buffer, pH 7.9, containing 300 mM NaCl and 5 mM KCl. Sections were then incubated for 45 min at 4°C in the same buffer containing 4 nM [^3H]mazindol and 0.3 μM desipramine, which blocks potential binding to norepinephrine uptake sites. Nonspecific binding was determined in the presence of 10 μM mazindol for DAT binding. After incubation, sections were then rinsed twice for 1 min in ice-cold Tris-buffer, pH 7.9, dipped into ice-cold distilled water, and then rapidly dried in a stream of cold air. Dried tissue sections were apposed to Kodak BioMax MR films (Eastman Kodak Co., Rochester, NY, USA) in X-ray cassettes together with a set of tritium standards ([^3H]Microscale™, Amersham, UK) and developed after 4 (D₁ receptor and DAT) or 5 (D₂ receptor) weeks. Films were developed in the 50% of Kodak D19 developer solution. Sections from WT and KO animals from B6, 129, and B6 $\times$ 129 strains were processed together to ensure a paired protocol for binding, film apposition, and image analysis.

Autoradiographic image analysis

All films were analyzed by video-based computerized densitometry using an MCID image analyzer (Imaging Research, St. Catharines, ON, Canada) as previously described (Kitchen et al., 1997). Brain images were analyzed using left and right hemispheres to allow duplication of results. The cortical and olfactory tubercle structures were analyzed by sampling five to eight times with a box size $15 \times 15 \text{ mm}^2$ in a box tool. All other regions

were analyzed by freehand drawing tools. Structures were identified by reference to the mouse atlas of Franklin and Paxinos (2001). Specific binding was calculated by subtracting the level of nonspecific binding from the total binding level.

Data analysis

Three-way ANOVA (for the factors genotype, strain, and region) was used for comparison of quantitative measures of D1, D2 receptors and DAT binding in WT and triple-opioid KO mice of three different genetic background followed by a Bonferroni post-hoc test. A P -value of 0.05 was used as the cutoff for significance in all cases.

RESULTS

D1 binding in the brains of WT and triple-opioid receptor KO mice maintained on B6, 129, and B6 × 129 background

Three-way ANOVA showed a significant strain ($F_{(2,402)} = 36.21, P < 0.001$), region ($F_{(14,402)} = 537.60, P < 0.001$), strain × region interaction ($F_{(28,402)} = 3.44, P < 0.001$) and strain × genotype interaction ($F_{(2,402)} = 3.60, P < 0.05$) effects with no genotype or strain × genotype × region effects in the expression of the D1 receptor in WT and triple-opioid receptor KO mice of the three different genetic backgrounds. Post-hoc analysis showed B6 × 129 KO mice had significantly lower D1 receptor binding than 129 KO mice in the caudate putamen ($P < 0.05$) (Figs. 1A and 1B, Table I). Lower levels of D1 binding were observed in the olfactory tubercle ($P < 0.05$) and substantia nigra ($P < 0.001$) of B6 × 129 WT compared with 129 WT. Lower levels of D1 receptors were observed in the olfactory tubercle of B6 × 129 WT compared with B6 WT ($P < 0.001$). No significant genotype effect was detected in D1 binding in any of the three different genetic backgrounds in any of the region analyzed.

D2 binding in the brains of WT and triple-opioid receptor KO mice maintained on B6, 129, and B6 × 129 background

Three-way ANOVA showed significant region ($F_{(3,116)} = 19.42, P < 0.001$) effect with no strain, genotype, strain × genotype interaction, or strain × genotype × region interaction effects in levels of D2 receptor binding in WT and triple-opioid receptor KO mice of the three different genetic backgrounds (Table II). No significant difference in D2 receptor binding was observed in any of the regions of the WT and triple-opioid receptor KO mice of the three different genetic backgrounds.

DAT binding in the brains of WT and triple-opioid receptor KO mice maintained on B6, 129, and B6 × 129 background

Three-way ANOVA showed region ($F_{(6,182)} = 12.85, P < 0.001$) effect with no strain, genotype, strain × genotype interaction, or strain × genotype × region interaction effects in levels of DAT binding in WT and triple-opioid receptor KO mice of the three different genetic backgrounds (Table III). No significant difference in DAT binding was observed in any of the regions of WT and triple-opioid receptor KO mice of the three different genetic backgrounds.

DISCUSSION

Receptor interactions between opioidergic and dopaminergic systems were investigated by examining the effect of the deletion of all three opioid receptor (MOPr, DOPr, and KOPr) on D1, D2 receptors and DAT binding in triple-opioid receptor KO mice. Moreover, as genetic background has been shown to influence behavioral and neurochemical phenotypes of gene KO mice, the effect of deletion of all three opioid receptors on D1, D2 receptors and DAT binding was investigated in triple-opioid receptor KO generated on different genetic backgrounds (129, B6, and B6 × 129 background). The receptor binding results obtained in this study demonstrate that there is no significant genotype difference in D1, D2 receptors and DAT binding in mice lacking triple-opioid receptors of three different genetic backgrounds compared with WT controls in any regions analyzed. However, strain differences in D1 binding were detected within triple-opioid receptor WT and KO mice across the three different genetic backgrounds, but neither in D2 nor in DAT binding.

The absence of an effect of ablation of all three opioid receptors on D1 receptor binding irrespective of their genetic background is at odds with previous pharmacological studies showing that blockade of opioid receptors could modulate D1 receptors (Elwan and Soliman, 1995; Unterwald et al., 1997). For instance, repeated intermittent treatment with a nonselective opioid receptor antagonist naloxone was shown to decrease both $B_{\max}$ and K_d values of DA D1 ($[^3\text{H}]\text{SCH23390}$) receptors in rats (Elwan and Soliman, 1995). Moreover, acute administration of a nonselective opioid receptor antagonist nalmefene produced an increase in D1 binding ($[^{11}\text{C}]\text{SCH23390}$) potential in the striatum of rats as measured by positron emission tomography (PET) (Unterwald et al., 1997) suggesting that pharmacological blockade of opioid receptors could modulate DA D1 receptors. However, another study showed that naloxone-induced conditioned place aversion is maintained in D1 receptor KO mice suggesting that endogenous opiate-mediated hedonic homeostasis is not dependent on the presence of D1 receptors (Narayanan et al., 2004). Although blockade of opioid receptors by using nonselective antagonists can modulate D1 receptors, the long-term ablation of all three opioid receptors afforded by the triple KO mice strongly suggest that a tonic role of opioid systems in the modulation of D1 receptors is lacking and provide compelling evidence that modulation of D1 receptors is not dependent on the presence of all three opioid receptors. A similar phenotype is seen with respect to DAT/opioid interactions is that the lack of significant difference in DAT binding in the brains of triple-opioid receptor KO mice irrespective of their genetic background compared with WT mice is in contrast to previous pharmacological findings (Bhargava and Gudehithlu, 1996). Chronic blockade of opioid receptors by a non-selective opioid receptor antagonist naltrexone was shown to result in downregulation of DAT density but not K_d (Bhargava and Gudehithlu, 1996). We previously reported in abstract form that lower levels of DAT binding were observed in the medial part of caudate putamen of triple-opioid receptor KO mice compared with WT maintained on C57BL/6 × 129 mixed background (Yoo et al., 2006). This study was expanded to incorporate WT and KO mice of other backgrounds (B6 and 129) and the statistical analysis incorporating strain as a factor (three-way ANOVA) did not reveal a significant effect of genotype, strain, or genotype × strain interaction. Although it is possible, blockade of endogenous opioid tone by opioid antagonists may differ from genetic

ablation of all three opioid receptor genes in respect to modulation of endogenous opioid peptide levels, as with D1 binding the results from triple KO mice do not provide compelling evidence for a tonic interaction between DAT and opioid receptors. The lack of alteration of D2 receptors in mice lacking triple-opioid receptors irrespective of their genetic background in this study accords with a previous finding, which showed acute administration of a nonselective opioid receptor antagonist nalmefene produced no changes in D2 binding ($[^{11}\text{C}]\text{N-methylspiperone}$) potential as measured by PET (Unterwald et al., 1997) suggesting that both pharmacological blockade and genetic ablation of opioid receptors may not play a role in the modulation of D2 receptors. Another possibility for the lack of effect in the triple KO is that there are opposing interactions with the dopaminergic system of each individual opioid receptor. Previous studies using single opioid KO mice have shown that the mesoaccumbal DA neurotransmission is differentially modulated by different opioid subtypes. For instance, DA release were increased in the nucleus accumbens of KOPr KO compared with its WT control (Chefer et al., 2005), whereas DA release seems decreased in the nucleus accumbens of MOPr and DOPr KO mice (Chefer et al., 2003, 2004). In addition, basal DA uptake was shown to be decreased in the Acb of MOPr and DOPr KO mice (Chefer et al., 2003) and reuptake of DA in the Acb of MOPr KO mice was slower than WT mice (Mathon et al., 2006) suggesting that DOPr and MOPr play an important role in mediating DA release in Acb by regulating DAT. However, reuptake of DA was shown to be increased in the Acb of KOPr KO mice suggesting an inhibitory role of KOPr on mediating dopamine release possibly via DAT (Chefer et al., 2005). As a result, the lack of genotype differences observed in DAT binding could be due to the opposing influence of MOPr, DOPr, and KOPr on DAT regulation, which could counteract each other. Accordingly, deletion of MOPr and DOPr may counteract the effect of increasing DA neurotransmission by deletion of KOPr leading to balance of DA neurotransmission, which may result in no compensatory changes of D1, D2 receptors and DAT binding in triple-opioid receptor KO mice compared with its WT control.

The lack of significant difference in D1 binding between B6 WT and 129 WT strain is in accordance with Schlussman et al. (2003), who found no differences in D1 receptor binding between B6 and 129 strain (Schlussman et al., 2003). Other recent studies have also shown that there was no significant strain difference between B6 and 129 in baseline locomotor activity (Chen et al., 2007). In addition, responses in the prepulse inhibition test, which is believed to be modulated by DA and its receptor subtypes, by D1-like agonist SKF82958 administration showed similar trends in either B6 or 129 mice (Ralph-Williams et al., 2003). Together, these results show a lack of strain difference between B6 and 129 on the D1 receptor. Interestingly, however, we found WT of mixed B6 $\times$ 129 strain displayed lower levels of D1 receptors in olfactory tubercle and substantia nigra compared with 129 and/or in olfactory tubercle compared with B6 strain. The observed lower levels of D1 receptors in B6 $\times$ 129 mixed strain compared with 129 and B6 background alone suggests that genetic heterogeneity between inbred and hybrid strains contributes to the regulation of D1 receptor levels.

In this study, there were no significant strain differences in D2 and DAT binding in any brain regions of B6 $\times$ 129 background compared with 129 background as well as compared with B6 background, which accords with previous findings showed that administration of

the D2-like agonist quinpirole showed similar response in either B6 or 129 mice in the prepulse inhibition test (Ralph-Williams et al., 2003). Moreover, the lack of strain effect in DAT binding between B6 and 129 is also consistent with the finding that there was no significant difference in the surface expression of DAT in the striatum of B6 compared with 129 mice (Chen et al., 2007). However, interestingly B6 and 129 strains have been shown to differ in dopamine-related behaviors (Kalueff and Tuohimaa, 2004; Paulus et al., 1999). For instance, B6 strains exhibited higher levels of locomotor and grooming activity compared with 129 strains (Kalueff and Tuohimaa, 2004; Paulus et al., 1999). However, it is unlikely that strain differences in dopaminergic related activity between B6 and 19 are associated with strain differences in D2 or DAT expression. Nevertheless, our results reinforce the lack of strain effect observed between B6 and 129 on D2 receptor and DAT suggesting that genetic background does not influence D2 and DAT binding at least among B6, 129, and B6 × 129 backgrounds.

In summary, the lack of difference in D1, D2 receptors and DAT binding in the brain of triple-opioid receptor KO mice, irrespective of genetic background indicates a lack of modulation of dopaminergic system by the deletion of all three opioid receptor. This data implies that expression of endogenous opioid tone does not play a role in modulating D1, D2 receptors and DAT binding.

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Fig. 1.

A: Representative autoradiograms of $[^3\text{H}]\text{SCH23390}$ binding to D1 receptors in the brain sections of wild-type (WT) and triple-opioid receptor knockout (KO) mice from different genetic backgrounds; C57BL/6 (B6), 129 (129), and C57BL/6 $\times$ 129 (B6 $\times$ 129). The sections shown are from the level of the caudate putamen (bregma 1.10 mm) and substantia nigra (bregma -3.16 mm). The color bar represents pseudocolor interpretation of black and white film images in fmol/mg tissue. **B:** Quantitative autoradiography of $[^3\text{H}]\text{SCH23390}$ binding in the brains of wild-type (WT) and tripleopioid receptor knockout (KO) mice from different genetic background; C57BL/6 (B6), 129 (129), and C57BL/6 $\times$ 129 (B6 $\times$ 129). Values are expressed as means $\pm$ SEM of five to six mice. Three-way ANOVA showed there was a significant effect of strain ($F_{(2,402)} = 36.21$, $P < 0.001$), region ($F_{(14,402)} = 537.60$, $P < 0.001$), strain $\times$ region interactions ($F_{(28,402)} = 3.44$, $P < 0.001$), and strain $\times$ genotype interactions ($F_{(2,402)} = 3.60$, $P < 0.05$). * $P < 0.05$ compared with 129 KO; ### $P < 0.001$ compared with B6 WT; † $P < 0.05$ and ††† $P < 0.001$ compared with 129 WT. CPu, caudate putamen; Tu, olfactory tubercle; SN, substantia nigra.

Quantitative autoradiography of [³H]SCH23390 binding in the brains of wild-type and triple opioid receptor knockout mice from different genetic background

TABLE I

Region	[³ H]SCH23390-specific binding (fmol/mg tissue)					
	B6		129		B6 × 129	
	Wild-type	Triple KO	Wild-type	Triple KO	Wild-type	Triple KO
Caudate putamen	409.2 ± 12.4	403 ± 13.2	393.0 ± 34.4	447.2 ± 19.2	329.4 ± 13.9	361.6 ± 22.1 [*]
Nucleus accumbens core	248.8 ± 16.0	243 ± 19.8	236.0 ± 20.6	268.1 ± 15.0	211.3 ± 26.5	238.3 ± 32.3
Nucleus accumbens shell	226.6 ± 24.4	232 ± 20.0	235.6 ± 19.5	240.3 ± 16.9	196.3 ± 27.3	229.0 ± 27.1
Olfactory tubercle	386.0 ± 15.5	341 ± 17.3	330.5 ± 19.0	310.2 ± 16.6	247.4 ± 19.7 [‡]	261.1 ± 24.2
Clastrum	87.3 ± 5.7	84.4 ± 4.6	102.1 ± 3.6	105.3 ± 4.6	71.2 ± 6.5	77.9 ± 3.8
Dorsal endopiriform	93.4 ± 5.7	89.9 ± 4.5	95.9 ± 2.9	96.2 ± 3.2	71.3 ± 7.0	82.6 ± 4.3
Cingulate cortex	34.4 ± 2.9	34.8 ± 2.3	35.7 ± 2.2	32.5 ± 3.8	27.6 ± 2.5	32.8 ± 1.8
Motor cortex	22.8 ± 2.0	20.7 ± 1.5	24.1 ± 0.9	24.0 ± 1.6	22.7 ± 3.7	18.9 ± 0.7
Nigrostriatal bundle	91.3 ± 8.6	90.7 ± 6.1	122.1 ± 10.4	101.0 ± 6.7	69.6 ± 4.5	78.0 ± 7.9
Amygdala	48.2 ± 4.9	37.9 ± 2.5	49.2 ± 5.9	43.8 ± 3.9	30.2 ± 3.7	35.7 ± 4.8
Thalamus	20.7 ± 2.3	19.6 ± 1.4	24.3 ± 2.1	20.0 ± 1.0	17.9 ± 4.6	15.0 ± 1.8
Hypothalamus	23.5 ± 3.0	19.9 ± 2.7	21.3 ± 2.2	21.6 ± 3.9	12.8 ± 3.5	16.0 ± 3.6
Hippocampus	24.0 ± 3.6	21.9 ± 4.0	33.2 ± 2.2	31.6 ± 2.5	24.7 ± 5.3	25.4 ± 2.7
Substantia nigra	206.3 ± 33.2	200 ± 20.7	232.5 ± 27.7	209.0 ± 23.5	118.0 ± 10.2 [§]	164.6 ± 30.6
Ventral tegmental area	24.8 ± 7.9	22.6 ± 1.2	31.5 ± 3.1	33.0 ± 6.0	17.7 ± 3.8	17.5 ± 9.3

Quantitative autoradiography of [³H]SCH23390 binding in wild-type and triple-opioid receptor knockout (KO) mice from different genetic backgrounds; C57BL/6 (B6), 129 (129), and C57BL/6 × 129 (B6 × 129). Three-way ANOVA showed there was a significant effect of strain ($F(2,402) = 36.21$, $P < 0.001$), region ($F(14,402) = 537.60$, $P < 0.001$), strain × region interactions ($F(28,402) = 3.44$, $P < 0.001$), and strain × genotype interactions ($F(2,402) = 3.60$, $P < 0.05$).

^{*} $P < 0.05$ compared with 129 KO;

[†] $P < 0.001$ compared with B6 WT;

[‡] $P < 0.05$ and

[§] $P < 0.001$ compared with 129 WT.

Values are expressed as means ± SEM of five to six mice.

Quantitative autoradiography of [³H]raclopride binding in wild-type and triple opioid receptor knockout mice from different genetic backgrounds

TABLE II

Region	[³ H]raclopride-specific binding (fmol/mg tissue)					
	B6		129		B6 × 129	
	Wild-type	Triple KO	Wild-type	Triple KO	Wild-type	Triple KO
Caudate putamen	70.4 ± 13.0	75.6 ± 9.7	89.1 ± 13.4	86.8 ± 14.8	78.2 ± 16.3	78.5 ± 18.7
Olfactory tubercle	51.2 ± 9.1	58.2 ± 7.9	62.9 ± 8.9	59.8 ± 9.0	58.5 ± 12.8	57.3 ± 13.3
Nucleus accumbens core	40.6 ± 6.3	38.7 ± 3.3	48.9 ± 9.2	43.0 ± 6.0	39.8 ± 10.4	42.8 ± 11.1
Nucleus accumbens shell	30.7 ± 5.9	31.4 ± 2.2	43.5 ± 7.5	36.4 ± 5.7	36.8 ± 11.0	39.1 ± 12.3

Quantitative autoradiography of [³H]raclopride binding in wild-type and triple-opioid receptor knockout (KO) mice from different genetic backgrounds; C57BL/6 (B6), 129 (129), and C57BL/6 × 129 (B6 × 129). Values are expressed as means ± SEM of five to six mice. Three-way ANOVA showed significant region ($F(3,116) = 19.42, P < 0.001$) effect with no strain, genotype, strain × genotype interaction, or strain × genotype × region interaction effects.

Quantitative autoradiography of [³H]mazindol binding in brains of wild-type and triple-opioid receptor knockout (KO) mice from the different genetic backgrounds

TABLE III

Region	[³ H]mazindol-specific binding (fmol/mg tissue)							
	B6		129		B6 × 129		Wild-type	Triple KO
	Wild-type	Triple KO	Wild-type	Triple KO	Wild-type	Triple KO		
Nucleus accumbens core	73.1 ± 13.9	52.4 ± 9.5	68.2 ± 14.2	59.0 ± 11.2	60.8 ± 11.6	46.8 ± 16.9		
Nucleus accumbens shell	58.0 ± 12.3	37.9 ± 5.1	48.9 ± 8.1	44.6 ± 8.7	38.8 ± 12.5	38.2 ± 14.8		
Olfactory tubercle	67.9 ± 12.1	93.9 ± 15.0	87.1 ± 8.2	81.9 ± 6.8	91.1 ± 8.5	76.1 ± 13.1		
Caudate putamen	111.4 ± 19.8	88.7 ± 12.4	108.3 ± 17.1	87.0 ± 16.0	113.4 ± 22.2	77.9 ± 24.7		
Subthalamic area	52.2 ± 12.4	59.2 ± 7.3	41.9 ± 7.8	48.2 ± 10.4	54.6 ± 15.0	42.1 ± 16.7		
Substantia nigra	77.1 ± 13.8	79.0 ± 21.1	62.3 ± 15.2	49.8 ± 5.7	77.5 ± 10.0	84.3 ± 4.6		
Ventral tegmental area	75.7 ± 15.0	77.2 ± 14.8	63.0 ± 19.3	54.6 ± 9.6	114.5 ± 12.0	99.4 ± 4.7		

Quantitative autoradiography of [³H]mazindol binding in brains of wild-type and triple-opioid receptor knockout (KO) mice from different genetic background; C57BL/6 (B6), 129 (129), and C57BL/6 × 129 (B6 × 129). Values are expressed as means ± SEM of five to six mice. Three-way ANOVA showed there was a significant effect of region ($F(6,182) = 12.85, P < 0.001$) with no strain, genotype, strain × genotype interaction, or strain × genotype × region interaction effects.