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REDUCED EXPRESSION OF THE MU OPIOID RECEPTOR IN SOME, BUT NOT ALL, BRAIN REGIONS IN MICE WITH *Oprm1* A112G

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Abstract

OPRM1 A118G is a common single nucleotide polymorphism (SNP) in the coding region of the human mu opioid receptor (MOPR) gene *OPRM1*. This SNP is associated with higher morphine doses required for postoperative analgesia as well as a variety of drug addiction phenotypes. A mouse model possessing the equivalent substitution (A112G) in the *Oprm1* gene was generated to facilitate mechanistic studies. Mice homozygous for the G112 allele (G/G) displayed lower antinociception to morphine compared with those homozygous for A112 allele (A/A), similar to humans, suggesting that the mice are a good model to further characterize underlying factors contributing to phenotypes associated with this SNP. Here, we compared [³H]DAMGO binding to the MOPR in the brains of A/A and G/G mice using quantitative *in vitro* autoradiography. A/A mice exhibited higher [³H]DAMGO binding than G/G in the cingulate, motor, and insular cortices, nucleus accumbens core and shell, hypothalamus, thalamus, amygdala, periaqueductal gray, superficial gray of superior colliculus, and ventral tegmental area. No genotype differences were observed in somatosensory cortex, caudate putamen, and hippocampus. When males and females were examined separately, A/A mice showed higher [³H]DAMGO binding than G/G mice in more brain regions in males than in females. Radioligand binding using brain membranes also showed higher [³H]DAMGO binding in the cortex and thalamus in A/A mice than G/G mice but no genotype differences in the caudate putamen or hippocampus. Thus, the A112G SNP is associated with reduced MOPR expression in some, but not all, brain regions, and appears to have some sex differences. The elevated MOPR expression in periaqueductal gray and thalamus in A/A mice are consistent with their higher antinociceptive responses to morphine. The higher MOPR levels in nucleus accumbens and/or ventral tegmental area of A/A mice is consistent with the higher morphine-induced hyperactivity and locomotor sensitization observed in these mice. Thus, these results provide some insights into the observed decreased clinical opioid potency in humans with the A118G SNP.

Keywords

A118G; knock-in mouse; mu opioid receptor; N-glycosylation; autoradiography

The mu opioid receptor (MOPR) plays a critical role in modulation of pain and addiction to drugs of abuse. A118G is the most common single nucleotide polymorphism (SNP) in the

coding region of the human MOPR gene (*OPRM1*), in which A at position 118 in exon 1 is replaced by G. This SNP results in the substitution of Asn at position 40 with Asp (N40D) in the N-terminal domain, which removes one of five potential N-linked glycosylation sites of the receptor (Bergen et al., 1997; Bond et al., 1998). The G118 allele frequency varies widely across populations: 1% to 3% in African Americans, 10–14% in both Caucasians and Hispanics, 35–49% in Asians, and 8–21% in other populations [reviewed in (Kreek et al., 2005)]. Clinical studies revealed that subjects with the G118 allele had reduced analgesic responses to morphine for acute postoperative pain (Chou et al., 2006a, b; Sia et al., 2008; Hayashida et al., 2008; Campa et al., 2008). In addition, individuals with the G118 allele had better treatment outcomes for nicotine and alcohol abuse (Oslin et al., 2003; Lerman et al., 2004; Ray and Hutchison, 2007). When treated with naltrexone, alcoholics of European descent with one or two G118 alleles had significantly lower rates of relapse and a longer time to return to heavy drinking following treatment (Oslin et al., 2003). Human subjects with at least one copy of the G allele reported lower alcohol craving and higher alcohol-induced “high,” and the effects of naltrexone on blunting alcohol-induced high were stronger among individuals with the G allele (Ray and Hutchison, 2007). When cigarette smokers underwent nicotine replacement treatment, individual with one or two 118G alleles were more likely to be abstinent at the end of treatment and report less mood disturbance and weight gain (Lerman et al., 2004). Moreover, studies investigating the role of G118 allele in alcohol or other drug dependence have reported positive associations, negative associations, or no associations [for reviews, see (Kreek et al., 2005; Mague and Blendy, 2010)]. Ramchandani et al. (2011) used positron emission tomography and [¹¹C]-raclopride displacement to examine dopamine release following alcohol intake in humans and found that alcohol induced dopamine release in carriers of the G118 allele, but not in A118 homozygotes.

To understand the mechanisms that underlie the alterations caused by A118G SNP observed in humans, extensive molecular, biochemical characterization and behavioral analyses have to be done, which is not feasible in humans. For this purpose, Mague et al. (2009) generated a knock-in mouse line by substituting A112 with G112, producing the mouse equivalent (A112G, N38D) of the human SNP. The mice homologous for G112 allele (G/G mice) displayed reduced antinociceptive responses to morphine than those homologous for A112 allele (A/A mice), similar to humans, suggesting that the mice are a good animal model for the A118G SNP in humans. In addition, G/G mice displayed decreased morphine-mediated hyperactivity and failed to develop locomotor sensitization following repeated morphine administration. The mechanisms underlying the observed differences are not clear. MOPRs in the brains of A/A and G/G mice did not differ in binding affinity for μ -endorphin, morphine, and naloxone (Mague et al., 2009). However, G/G mice had reduced MOPR mRNA expression in some brain regions and lower MOPR protein levels in the thalamus and in the whole brain, compared with A/A mice (Mague et al., 2009).

It should be noted that Ramchandani et al. (2011) have also generated a mouse model of the *OPRM1* A118G SNP by replacing the mouse exon1 with the human exon 1 containing either A118 or G118. Homozygous G118 mice showed a four-fold greater peak dopamine release from the nucleus accumbens in response to an alcohol challenge than homozygous A118 mice.

We hypothesized that the behavioral differences between genotypes may be in part due to alteration in MOPR expression in G/G mice, compared with A/A mice. In the present studies, we investigated the effect of A112G on MOPR expression across a number of brain regions by *in vitro* autoradiography of [³H]DAMGO binding, which allows localization and quantitation of MOPR in specific brain regions (Kitchen et al., 1997).

EXPERIMENTAL PROCEDURES

Animals

An A112G MOPR knock-in mouse line was generated in the C57Bl/6 background by and propagated by heterozygote matings [for detailed description of generation of *Oprm1*^{tm1Jabl} mice, see Mague et al. (2009)]. All mice (8–15 weeks, 18–30 g) were housed and maintained on a 12-h/12-h light/dark cycle with food and water available *ad libitum*. Animals were used in accordance with methods approved by University of Pennsylvania Animal Care and Use Committee.

Materials

[³H]DAMGO (56.0 Ci/mmol) and tritium-sensitive storage phosphor screens were purchased from Perkin Elmer (Boston, MA, USA). Naloxone was kindly provided by the National Institute on Drug Abuse (NIDA). [³H]microscales were obtained from Amersham Co. (Arlington Heights, IL, USA). All other reagent-grade chemicals were obtained from Sigma Chemical Co (St. Louis, MO, USA) or Fisher Scientific (Pittsburgh, PA, USA).

In vitro autoradiography of [³H]DAMGO binding to MOPR

These experiments were performed as described by Kitchen and colleagues (Kitchen et al., 1990; Slowe et al., 1999). Animals were killed by decapitation, and brains were removed and immediately immersed in isopentane in a stainless steel beaker on dry ice. Coronal sections (20 μ m) were cut on a cryostat (Leica CM3050 S) maintained at –20 °C, thaw-mounted onto gelatin-subbed slides, and stored at –80 °C until processed. Sections were thawed, rinsed in 50 mM Tris–HCl buffer (pH 7.4), and incubated with 5 nM [³H]DAMGO in 50 mM Tris–HCl buffer for 1 h at 25 °C. Non-specific binding was assessed in the presence of 10 μ M naloxone. Slides were then rinsed three times (2 min each) in cold 50 mM Tris–HCl buffer, pH 7.4, and once (30 s) in de-ionized H₂O. Slides were dried under a cool stream of air and exposed to tritium-sensitive storage phosphor screens for 1 week in cassettes along with [³H]microscale for calculation of results.

Radioactive images captured on phosphor screens were visualized using a Cyclone Storage Phosphor Scanner (Packard Bioscience), and the data were analyzed using the OptiQuant program was associated with the scanner. Nonspecific binding was subtracted from total binding and the resultant values represented specific [³H]DAMGO binding in fmol/mg as determined using [³H]microscales. Data are reported as mean values $\pm$ SEM of data from six animals, with two or three sections in each brain. Statistical significance of the data was determined by independent two-tailed Student's *t*-test using Prism 3.0.

Brain membrane preparation

Animals were killed by decapitation, brains were removed immediately, and regions of interest were dissected, frozen on dry ice, and stored at –80 °C until use. Tissues were thawed, homogenized in 25 mM Tris–HCl (pH 7.4) containing 1 mM EDTA and 0.1 mM phenylmethylsulfonyl fluoride on ice, and then centrifuged at 100,000 $\times$ *g* for 30 min. Pellets were twice rinsed with 25 mM Tris–HCl buffer and re-suspended in 0.32 M sucrose in 50 mM Tris–HCl (pH 7.0). Suspended membranes were passed through a 26.5 G needle five times and then frozen at –80 °C. Protein contents of membranes were determined by the BCA method (Smith et al., 1985).

[³H]DAMGO binding to the MOPR in brain membranes

Membrane preparations of brain regions were incubated at room temperature for 30 min with 100 mM NaCl and 100 μ M GDP in 50 mM Tris–HCl buffer (pH 7.4) containing 1 mM

EDTA (TE buffer), centrifuged, and washed three times with the TE buffer by resuspension and centrifugation. Membranes were incubated with ~3 nM [³H]DAMGO in TE buffer with 5 mM MgCl₂. Such pretreatment and binding conditions have been shown to convert the MOPR to high agonist affinity states (Nishino et al., 1990; Wong et al., 1994). Nonspecific binding was measured in the presence of naloxone (1 μM). Each binding assay was carried out in duplicate in a final volume of 0.5 ml with 0.1–0.3 mg protein/tube to yield 1500 – 3000 dpm. Incubations were terminated by filtration through Whatman GF/B filters under vacuum. Radioactivity in filters was measured by liquid scintillation counting.

Statistical analysis

For comparison of two groups, two-tailed Student's *t*-test was performed. * *P*<0.05, ** *P*<0.01, *** *P*<0.001 was the level of significance in all statistical analyses.

RESULTS

In vitro autoradiography of [³H]DAMGO binding

In vitro autoradiography of [³H]DAMGO binding to the MOPR was examined across a number of brain regions of A/A and G/G mice. The representative pseudo-color autoradiograms are shown in Fig. 1. Since there is no difference between males and females on the overall anatomical distribution of [³H]DAMGO binding, Fig. 1 shows males AA and GG as representatives. In general, A/A and G/G mice displayed similar regional distribution. Very high levels of MOPR were found in the caudate putamen (CPu), nucleus accumbens (NAc) core and shell, and superficial gray of superior colliculus (SuG) (Table 1). Many other regions exhibited high levels of [³H]DAMGO binding, including thalamus, amygdala, periaqueductal gray (PAG), hypothalamus, ventral tegmental area (VTA), substantial nigra, and cortex (Table 1). This distribution of MOPR in the brain is consistent with previous reports [see (Mansour et al., 1988) for a review]. Quantitation of specific [³H]DAMGO binding was performed by measuring the density of autoradiograms in regions of interest, and calculation was done based on the density of [³H]microscales (Table 1). A/A mice exhibited significantly higher [³H]DAMGO binding than G/G in the cingulate, motor, and insular cortices, NAc core and shell, hypothalamus, amygdala, PAG, SuG, and VTA. No genotype differences were observed in somatosensory cortex, CPu, and hippocampus.

The genotype effects of the *Oprm1* A112G substitution were then examined in males and females separately. As shown in Fig. 2, male A/A mice showed significantly higher [³H]DAMGO binding than G/G mice in the cingulate and insular cortices, NAc core and shell, thalamus, hypothalamus, amygdala, PAG, substantial nigra, and VTA. In contrast, female A/A mice displayed significantly higher [³H]DAMGO binding than G/G mice only in the thalamus, amygdala, PAG, and SuG. Therefore, A112G has more pronounced effects on MOPR levels in males than in females.

[³H]DAMGO binding to the MOPR in brain membranes

To further examine the effect of A112G on MOPR expression, we carried out [³H]DAMGO (3 nM) binding to the MOPR in membranes of four grossly dissected brain regions [thalamus, cortex, hippocampus, and CPu (including NAc; CPu+ NAc)] in A/A and G/G mice. Other brain regions did not yield sufficient amounts of tissues for [³H]DAMGO binding in an individual animal. Compared with A/A mice, G/G mice had decreased specific [³H]DAMGO binding in the thalamus and cortex (Table 2). Further analysis showed that female A/A mice exhibited higher specific [³H]DAMGO binding in thalamus than female G/G mice. In cortex, both male and female A/A mice showed higher binding than G/G mice (Fig. 3). In contrast, no genotype differences were observed in the hippocampus and CPu +NAc (Table 2, Fig. 3).

DISCUSSION

Using quantitative receptor autoradiography and radioligand binding to membranes, we found that G/G mice displayed lower MOPR expression in region-specific manner compared with A/A mice, and there were sex differences in several brain regions. Importantly, MOPR levels were lower in G/G mice in regions involved in antinociception, locomotor activity, and sensitization compared with levels in A/A mice.

In the humanized knock-in mouse model of the OPRM1 A118G SNP generated by Ramchandani et al. (2011), there were no differences in [³H]DAMGO binding in the dorsal striatum, NAc, and VTA between homozygous G118 and A118 mice. The discrepancy between our results and those of Ramchandani et al. (2011) may be due to the replacement of the mouse exon 1 sequence with that of the human in the mouse, which may influence receptor expression.

Reduced MOPR expression in G/G mice is related to genotype difference in MOPR-mediated analgesia

PAG is known to be directly involved in opioid antinociception (Yaksh et al., 1976). The cortical and subcortical brain regions activated by nociceptive stimulation include the anterior cingulate, insular, frontal, and somatosensory cortices, and amygdala, which are often referred to as the “pain matrix” (Ingvar, 1999; Peyron et al., 2000; Brooks and Tracey, 2005). The findings that G/G mice showed reduced MOPR expression in the cingulate and insular cortices, amygdala, and PAG compared with A/A mice were in line with the report that G/G mice showed attenuated morphine-induced antinociception (Mague et al., 2009), which was consistent with decreased analgesic responses to morphine associated with G118 allele in humans (Chou et al., 2006a, b; Sia et al., 2008; Hayashida et al., 2008; Campa et al., 2008).

Reduced MOPR expression in the NAc and/or VTA of G/G mice may contribute to decreased morphine-induced hyperactivity and locomotor sensitization

NAc and VTA were demonstrated to be involved morphine-induced hyperactivity in rats (Bunney et al., 1984). In addition, VTA plays an important role in morphine-induced locomotor sensitization (Kalivas and Duffy, 1987; Vezina et al., 1987). Our finding that G/G mice have lower MOPR expression in NAc and VTA than A/A mice is likely to contribute to the observation that G/G mice displayed decreased morphine-mediated hyperactivity and failed to develop locomotor sensitization following repeated morphine administration (Mague et al., 2009).

Region-specific effects of A112G on MOPR expression

By autoradiography, we found that the A112G substitution resulted in reduced MOPR expression in the cortices, NAc, hypothalamus, amygdala, PAG, SuG, and VTA, but not in the hippocampus and CPu. By receptor binding using brain membranes, we found that compared with A/A mice, G/G mice had lower levels of [³H]DAMGO binding to the MOPR in the cortex and thalamus but had similar levels in the hippocampus and CPu+NAc. When brain regions were dissected for membrane binding, CPu and NAc were dissected together to get sufficient tissues. It is likely that the lack of effects of A112G in the CPu masked the reduction in the NAc because the CPu is much larger. Thus, in general autoradiographic data are consistent with MOPR membrane binding results. In addition, the higher MOPR binding in the thalamus in A/A mice than in G/G mice is also in accord with our previous findings obtained by immunoblotting of the MOPR (Mague et al., 2009).

Region-specific differences in MOPR levels between A118 and G118 have also been demonstrated in humans. In a recent positron emission tomography study using [^{11}C]carfentanil as the MOPR ligand, Ray et al. (2011) reported that smokers heterozygous for the G118 allele had lower levels of MOPR-binding potential in the amygdala, thalamus, and anterior cingulate cortex than those homozygous for the A118 allele, but similar MOPR levels were observed in the ventral striatum/NAc and caudate.

In contrast, Oertel et al. (2009) showed that in human carriers of the G118 allele, K_d and B_{max} values of [^3H]DAMGO binding to the MOPR were similar to those in homozygous A118 carriers in the secondary somatosensory area and ventral posterior part of the lateral thalamus. It is noteworthy that most of the human G118 subjects are heterozygotes.

Region-specific effects of A112G on MOPR protein expression is not related to changes in MOPR mRNA level

A118G/A112G decreased or did not change MOPR mRNA levels depending on brain regions. In human postmortem tissues, A118G lowered levels of MOPR mRNA in the cortical lobes and pons (Zhang et al., 2005). In the mouse, A112G led to lower MOPR mRNA in the cortices, NAc, VTA, hypothalamus, and PAG, but not in the hippocampus (Mague et al., 2009) and thalamus (Mague and Blendy, unpublished data). In the thalamus of G/G mice, the protein level of the MOPR was lower with its mRNA unchanged, compared with A/A mice, indicating that the effects of A112G on MOPR protein levels are independent of changes in MOPR mRNA. On the other hand, A112G induced similar changes in mRNA and protein levels of MOPR in the hippocampus, NAc, VTA, hypothalamus, and PAG; however, no causal relationship was established between mRNA and protein changes. In general, there is no correlation between the expression level of a protein and its mRNA abundance (Gygi et al., 1999).

Variations in N-glycan types and contents in the two MOPR variants may play a role in region-specific effects of A112G on MOPR protein expression

N-glycosylation plays an important role in correct folding of receptors in the endoplasmic reticulum, and hence, their sorting from the endoplasmic reticulum to the plasma membrane. N-glycosylation thereby affects receptor expression [for example, (Fan et al., 1997; Zhang et al., 2001; Li et al., 2007)]. Recently we found that A112G led to reduced MOPR N-glycosylation in the mouse brain (Huang et al., 2012). Similarly, A118G decreased N-glycosylation of the human MOPR expressed in cultured cells and in addition, compromised stability of the human MOPR protein (Huang et al., 2012). If the changes in MOPR protein stability by the SNP are uniform, MOPR levels will be reduced similarly in all the brain regions. However, we found that MOPR levels were lower in some, but not all, brain regions of G/G mice. This may be attributed to variations in N-glycan types and contents in the MOPR in different brain regions. Importantly, the wild-type MOPR has been shown to be differentially modified by N-linked glycans in the CPu and thalamus of the rat and mouse, suggesting that N-glycosylation of MOPR is brain region-specific (Huang et al., 2008, 2012). Indeed, Matsushashi et al. (2003) reported region-specific expression of glycotransferases in adult mouse brain. Thus, we hypothesize that the brain area-specific impact of A112G on MOPR expression may be due to differences in glycan content changes of the MOPR induced by this substitution across brain regions (Huang et al., 2008). This hypothesis remains to be investigated.

The effects of A112G on MOPR protein expression have sex differences

Our autoradiographic data showed that males exhibited genotype differences in MOPR expression in more brain regions than females, suggesting that A112G effect is sexually dimorphic.

Sex differences in N-linked glycosylation have recently been revealed in total serum proteins in humans in that males have higher levels of many types of N-glycans (Knezevic et al., 2009; Stanta et al., 2010). To date, we have examined N-linked glycosylation of the MOPR only in the thalamus and CPu, which show no sex differences. To the best of our knowledge, N-glycan composition has not been studied in any 7-transmembrane receptor. Another factor that may influence sex-specific MOPR protein expression is the hormonal state of females. Female rats had significantly less MOPR immunoreactivity in the caudal ventrolateral PAG than males, with the greatest difference observed during proestrus (Loyd et al., 2008). However, it remains to be studied whether A112- and G112-MOPRs are differentially modulated by sex-related factors. Epigenetic regulation may also play a role in sex differences. MOPR gene expression is epigenetically programmed in the brain, and methyl-CpG-binding protein 2 (MeCP2) regulates the epigenetic program during DNA methylation and chromatin remodeling of the MOPR promoter (Hwang et al., 2009). Neonatal females express significantly higher levels of MeCP2 than males in the amygdala [reviewed in (Jessen and Auger, 2011)]. MeCP2 and other epigenetic factors may regulate A112- and G112-MOPR differentially between sexes.

CONCLUSION

Mice with *oprm1* A112G, the equivalent of the *OPRM1* A118G, exhibited lower MOPR expression in some, but not all, brain regions. The brain region-specific N-glycosylation may contribute to differential effects of A112G on MOPR expression. The observed reduction in MOPR expression in brain regions by the A112G is likely to be involved in attenuated morphine-induced antinociception, hyperactivity, and locomotor sensitization. Our findings provide some insights into the observed genotype differences in behavioral responses between A/A and G/G mice, which may be extended to the differences between genotypes in humans.

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Abbreviations

A/A	homozygous for A112 allele
CPu	caudate putamen
G/G	homozygous for G112 allele
MeCP2	methyl-CpG-binding protein 2
MOPR	mu opioid receptor
NAc	nucleus accumbens
<i>OPRM1</i> and <i>oprm1</i>	human and mouse MOPR genes, respectively
PAG	periaqueductal gray
SNP	single nucleotide polymorphism
SuG	superficial gray of superior colliculus
VTA	ventral tegmental area

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Fig. 1.

Pseudo-color autoradiograms of $[^3\text{H}]\text{DAMGO}$ (~5 nM) binding to the mu opioid receptor in coronal sections of A/A and G/G male mouse brain. Sections were incubated with ~5 nM $[^3\text{H}]\text{DAMGO}$ for 1 h at room temperature, washed, dried, and exposed to tritium-sensitive phosphor screens. Non-specific binding (N.S) was performed in the presence of 10 μM naloxone. Exposure time was 1 wk. The color bars are pseudo-color representation of black and white screen images in nCi/mg tissue equivalents. All the sections were processed in parallel throughout binding experiments and for development of autoradiograms.

Abbreviations: cc, cingulate cortex; mc, motor cortex; sc, somatosensory cortex; ic, insular cortex; cpu, caudate putamen; nac, nucleus accumbens; hip, hippocampus; thl, thalamus; hyp, hypothalamus; amg, amygdala; sug, superficial gray of superior colliculus; pag, periaqueductal gray; sn, substantia nigra; vta, ventral tegmental area.

Fig. 2.

Brain regions that show significant genotype differences in [³H]DAMGO binding level in (A) male and (B) female mice. The data from male and female mice were analyzed separately. Data are the mean±SEM of six animals, two or three sections from each brain. * $P<0.05$, ** $P<0.01$, *** $P<0.001$ different from A/A by the nonpaired two-tailed Student's t -test.

Fig. 3. (A–D) Binding data in Table 2 are separately presented for male and female mice. Data are presented as specific binding/tube (dpm) (mean $\pm$ SEM, $n=4-6$, each in duplicate). * $P < 0.05$, ** $P < 0.01$ compared with A/A by the nonpaired two-tailed Student's t -test.

Table 1

[³H]DAMGO binding is decreased in some, but not all, brain regions in A112G knock-in (G/G) mice, compared with A/A mice. Male and female data were combined

Region	<u>[³H]DAMGO binding (fmol/mg)</u>	
	A/A	G/G
Cingulate cortex	169.9±4.4	145.2±4.4 ^{***}
Motor cortex	124.4±4.3	109.5±4.1 [*]
Somatosensory cortex	113.6±3.2	104.6±4.3
Insular cortex	169.2±4.4	143.9±6.5 ^{**}
CPu	311.0±14.9	295.0±9.4
NAc shell	414.8±12.3	333.2±11.9
NAc core	357.9±14.5	288.2±9.3 ^{**}
Hippocampus	89.7±2.5	84.5±2.3
Thalamus	222.5±7.3	164.3±4.4 ^{***}
Hypothalamus	155.5±4.9	135.5±4.9 ^{**}
Amygdala	217.8±6.4	171.2±3.4 ^{***}
PAG	203.9±5.7	170.8±4.4 ^{***}
Substantia nigra	167.7±6.7	154.1±5.2
SuG	326.4±5.8	253.6±5.7 ^{***}
VTA	171.0±3.5	132.6±3.4 ^{***}

Quantitation was done on both left and right sides of each section. Data are expressed as mean±SEM of 12 animals, two or three sections from each brain.

^{*} $P<0.05$,

^{**} $P<0.01$,

^{***} $P<0.001$ different from A/A by the nonpaired two-tailed Student's *t*-test.

Table 2

Binding of [³H]DAMGO to the MOPRs in brain membranes of A/A and G/G mice. Binding was conducted with 3 nM [³H]DAMGO on membranes of the thalamus (0.1 mg proteins/tube), cortex (0.3 mg proteins/tube), hippocampus (0.2 mg proteins/tube), and CPu+NAc (0.1 mg proteins/tube). Nonspecific binding was defined by 1 μ M naloxone

Region	<u>[³H]DAMGO specific binding (dpm)</u>	
	A/A	G/G
Cortex	2858±150.4	2050±152.1 ***
Thalamus	1880±137.3	1259±139.5 **
Hippocampus	1603±174.4	1215±97.5
CPu+NAc	1698±212.8	1608±79.9

Data are presented as specific binding/tube (dpm) for each sample run in duplicate (mean±SEM, *n*=9–11).

**
P<0.01,

P<0.001 compared with A/A by the non-paired two-tailed Student's *t*-test.