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Changes in acetylcholinesterase activity and muscarinic receptor bindings in μ -opioid receptor knockout mice

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Abstract

Anatomical evidence indicates that cholinergic and opioidergic systems are co-localized and acting on the same neurons. However, the regulatory mechanisms between cholinergic and opioidergic system have not been well characterized. In the present study, we investigated whether there are compensatory changes of acetylcholinesterase activity and cholinergic receptors in mice lacking μ -opioid receptor gene. The acetylcholinesterase activity was determined by histochemistry assay. The cholinergic receptor binding was carried out by quantitative autoradiography using [³H]-quinuclidinyl benzilate (nonselective muscarinic receptors), *N*-[³H]-methylscopolamine (nonselective muscarinic receptors), [³H]-pirenzepine (M1 subtype muscarinic receptors) and [³H]-AF-DX384 (M2 subtype muscarinic receptors) in brain slices of wild-type and μ -opioid receptor knockout mice. The acetylcholinesterase activity of μ -opioid receptor knockout mice was higher than that of the wild-type in the striatal caudate putamen and nucleus accumbens, but not in the cortex and hippocampus areas. In addition, the bindings in *N*-[³H]-methylscopolamine and [³H]-AF-DX384 of μ -opioid receptor knockout mice were significantly lower when compared with that of the wild-type controls in the striatal caudate putamen and nucleus accumbens. However, there were no significant differences in bindings of [³H]-quinuclidinyl benzilate and [³H]-pirenzepine between μ -opioid receptor knockout and wild-type mice in the cortex, striatum and hippocampus. These data indicate that there are up-regulation of acetylcholinesterase activity and compensatory down-regulation of M2 muscarinic receptors in the striatal caudate putamen and nucleus accumbens of μ -opioid receptor knockout mice.

Keywords

Autoradiography; Histochemistry; M1 and M2 muscarinic receptors; μ -opioid receptor knockout mice

1. Introduction

Opioids including endogenous and exogenous opioids play an important modulatory role in the central nervous system and are well known for their antinociceptive effects, respiratory depression, withdrawal symptoms and abuse potential due to a variety of actions through the brain and spinal cord [25,36]. Several lines of anatomic evidence have been reported that opioidergic and cholinergic receptors were abundantly co-distributed in several brain areas

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such as the cortex, striatum and hippocampus [10,12,31,37,46,48]. Pharmacological studies suggest that central cholinergic neurons mediate many of the signs and symptoms of opioid withdrawal [4]. For instance, cholinergic receptor antagonists have been shown to reduce the expression of several withdrawal symptoms in morphine-dependent animals [30,38,47].

Several studies have indicated that morphine [2] or other opioid receptor agonists [15,22] inhibit acetylcholine release in the brain. For example, administration of μ -opioid receptor agonist DAGO (D -Ala², N -Me-Phe⁴, Gly-ol]enkephalin) resulted in an inhibition of acetylcholine release in the human neocortex [11]. Fentanyl, a synthetic μ -opioid receptor agonist, significantly decreased the acetylcholine release from the rat hippocampus. Naloxone, an μ -opioid receptor antagonist, prevented the inhibitory effect of fentanyl on the acetylcholine release in the hippocampus [39]. These results suggested that there are interactions between cholinergic and opioidergic systems in the brain.

This study was designed to investigate whether there are compensatory changes in acetylcholinesterase activity and cholinergic muscarinic receptors in mice lacking μ -opioid receptor gene. Acetylcholinesterase activity and cholinergic muscarinic receptor bindings were determined.

2. Materials and methods

2.1. Chemicals

[³H]-Quinuclidinyl benzilate (QNB, specific activity: 42.0 Ci/mmol), N -[³H]-methylscopolamine (NMS, specific activity: 83.5 Ci/mmol), [³H]-pirenzepine (PZ, specific activity: 79.0 Ci/mmol) and [³H]-AF-DX384 (specific activity: 100 Ci/mmol) were purchased from New England Nuclear (Boston, MA, USA). Other chemicals were purchased from Sigma (St. Louis, MO, USA).

2.2. Animals

Mu-opioid receptor knockout mice used in this study were developed by Loh et al. [27] and maintained on a 1:1 hybrid genetic background (C57/BL6 and 129/Ola), as described. Mice were maintained in an animal room on a 12-h light/dark cycle and at constant temperature (22 ± 2 °C). All procedures for animal care and breeding were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals and were approved by the University of Mississippi Medical Center Animal Care and Use Committee.

2.3. Sample preparations

Male mice ranging from 8 to 12 weeks old were used in this study. Mice were sacrificed by decapitation, and the brains were removed from the skull and immediately frozen in liquid nitrogen. Coronal sections of 20 μ m thickness were cut in a microtome cryostat (Leica, CM3050S) at -20 °C. The sections were thaw-mounted on a gelatin-coated slides and stored at -80 °C until used.

2.4. Acetylcholinesterase activity by histochemistry assay

Acetylcholinesterase activity was measured by the method as described previously [28]. Prior to incubation, brain sections mounted on the slides were brought to room temperature and blown dry under air for 45 min. The brain slices were immersed in acetone for 15 min and then air-dried at room temperature for 30 min. The dried slices were incubated in a 50 mM acetate buffer (pH 5.0) containing 2 nM acetylthiocholine iodide (substrate), 2 mM copper sulfate and 10 mM glycine at 37 °C for 45 min. Tetraisopropylpyrophosphoramidate was present in the medium at a concentration of 20 μ M to inhibit nonspecific cholinesterase. After incubation, the slides were rinsed by 50 mM acetate buffer (pH 5.0) for five times (45

s each time). Then, slides were transferred in a 1.25% sodium sulfide solution for 1 min and rinsed by 50 mM acetate buffer (pH 5.0) for 5 times (45 s each time). Then, slides were transfer to a 1% silver nitrate solution in distilled water for 1 min and rinsed by 50 mM acetate buffer (pH 5.0) for five times (45 s each time). Finally, the sections were dipped quickly in cold distilled water and blown dry under air at room temperature for at least 30 min and stored at 25 °C until image analysis. Stained brain sections on the slides were scanned and analyzed quantitatively with a Molecular Dynamics personal densitometer (Sunnyvale, CA, USA) as described previously [28]. Optical densities (OD) were used to express staining intensities.

2.5. Autoradiography of muscarinic receptors

[³H]-QNB binding was measured by the method of Schwab et al. [41] with modifications. The brain sections were thawed and dried at room temperature, then incubated with cold 50 mM phosphate buffer (pH 7.4) for 30 min. These sections were dried with cool air and incubated with the final concentration of 1 nM [³H]-QNB for 3 h at room temperature. After incubation, these sections were washed with cold buffer (4 °C) for 5 min twice then rinsed with cold distilled water for 5 s. The wet sections were immediately dried using cool air stream and desiccated overnight. Non-specific binding was determined in presence of 40 μM atropine in the incubation system.

The bindings of [³H]-NMS and [³H]-PZ were performed according to Schwab et al. [41]. The binding of [³H]-AF-DX384 was based on Gattu et al. [14]. The brain sections were thawed and dried at room temperature, then incubated with cold 10 mM phosphate buffer (pH 7.4) for 30 min. These sections were dried with cool air and incubated with the final concentration of 2.5 nM [³H]-NMS, 9 nM [³H]-PZ or 5 nM [³H]-AF-DX384 for 1 h at room temperature. After incubation, these sections were washed with cold buffer (4 °C) for 5 min twice then rinsed with cold distilled water for 5 s. The wet sections were immediately dried using cool air stream and desiccated overnight. Nonspecific binding was determined in the presence of 40 μM atropine.

The slides were placed in the X-ray cassettes with calibration standards and juxtaposed to Cyclone™ Storage Phosphor screen (Packard Instrument, Meriden, CT, USA). After 1 week of exposure at 4 °C for [³H]-QNB and [³H]-PZ, 10 days of exposure at 4 °C for [³H]-NMS and [³H]-AF-DX384, the images on screens were detected by a Packard Cyclone™ Storage Phosphor System and analyzed by the analysis program, ImageQuant 3.3 (Molecular Dynamics).

2.6. Statistical analysis

Data were expressed as mean ± standard errors of the mean (S.E.M.). Statistical analysis was done by one-way ANOVA followed by a post-hoc Student–Newman–Keuls multiple comparison test. A difference was considered significant at $P < 0.05$.

3. Results

3.1. Histochemistry of acetylcholinesterase activity

Representative histochemistry and quantitated data of acetylcholinesterase activity in coronal brain sections of the brain are shown in Fig. 1 and Table 1. The density in striatal complex and hippocampus were analyzed in this study because the enzyme activity is much higher in these areas than others in the brain. The data showed that the acetylcholinesterase activity in μ-opioid receptor knockout mice was significantly higher than that of the wild-type in several brain areas such as striatal caudate putamen (+ 17%), striatal nucleus accumbens (+ 29%) and olfactory tubercle (+ 17%). No significant difference in the

acetylcholinesterase activity in hippocampal formations including CA1–CA3 pyramidal and dentate gyrus granule layers was noted between the genotypes of mice.

3.2. Autoradiography of muscarinic receptors

3.2.1. [³H]-QNB binding—The autoradiograms and quantitated data of [³H]-QNB binding in coronal sections of the brain are shown in Fig. 2 and Table 2. The binding of [³H]-QNB in the cortex, striatum and hippocampal areas was not significantly different between μ -opioid receptor knockout mice and wild-type animals.

3.2.2. [³H]-NMS binding—Representative autoradiograms and quantitated data of [³H]-NMS binding in coronal sections of the brain are shown in Fig. 2 and Table 3. The binding of [³H]-NMS was significantly lower in several brain areas such as striatal caudate putamen (–14%), striatal nucleus accumbens (–10%) and olfactory tubercle (–13%) of μ -opioid receptor knockout mice than that of the wild-type animals. However, no significant difference in the binding of [³H]-NMS in the cortex and hippocampus was noted between the genotypes of mice.

3.2.3. [³H]-PZ binding—Representative autoradiograms and quantitated data of [³H]-PZ binding in coronal sections of the brain are shown in Fig. 2 and Table 4. No significant difference in binding of M1 muscarinic receptor in the cortex, striatum and hippocampal areas of μ -opioid receptor knockout mice was noted when compared with wild-type controls.

3.2.4. [³H]-AF-DX384 binding—Representative autoradiograms and quantitated data of [³H]-AF-DX384 binding in coronal sections of the brain are shown in Fig. 2 and Table 5. The binding of M2 subtype ligand in several brain areas such as striatal caudate putamen (–22%), striatal nucleus accumbens (–23%) and olfactory tubercle (–26%) was significantly lower in μ -opioid receptor knockout than that in the wild-type animals. No significant difference in binding of M2 muscarinic receptor in the cortex and hippocampus was noted between the genotypes of mice.

4. Discussion

Ontogenetic and anatomic evidences have indicated that there is overlapping between acetylcholinesterase and opioid receptors in the striatum during rat developments [21,33]. This relationship persists into adulthood [19,35] and has been identified as predominantly μ -opioid receptors [16,45]. This topologic interaction between acetylcholinesterase and opioid receptors may be related to their interaction in functions. For example, morphine has been shown to increase acetylcholine levels in the central nervous system, and this change is regional specific. Green et al. [18] showed that in mice, 10 mg/kg morphine increased acetylcholine levels significantly in the striatum and at higher doses (300 mg/kg) in the hippocampus. However, some brain areas such as cortex and hypothalamus failed to show acetylcholine release even at the high dose of 300 mg/kg of morphine administration. In rats, the increase in striatal acetylcholine was observed after 30 mg/kg of morphine, and increased levels were measured in the hippocampus after 90 mg/kg of morphine; these effects were shown to be naloxone-reversible. Short-term morphine treatment (3 days) of morphine appears to increase acetylcholine turnover rate initially [3,5], while several studies indicated that longer treatment (2–4 weeks) of morphine appears to result in increased levels or decreased acetylcholine utilization [8]. In addition, morphine withdrawal appears to increase acetylcholine release [1] and morphine dependence appears to increase acetylcholine turnover rate [5]. Our results show that acetylcholinesterase activity was increased in the striatum of μ -opioid receptor knockout mice. This phenomenon may be a

compensatory action caused by losing regulation of acetylcholine release through μ -opioid receptors in mice.

In the present study, we studied the alterations of total muscarinic receptor bindings using muscarinic receptor selective antagonists [3 H]-QNB and [3 H]-NMS as the radiolabelled ligands. [3 H]-QNB and [3 H]-NMS are nonselective ligands to muscarinic receptors, but several studies have confirmed that [3 H]-NMS binding sites are more sensitive to regulation by muscarinic agonists than [3 H]-QNB binding sites [13,26,29] since [3 H]-QNB is a muscarinic antagonist which identifies functional or internalized receptors within the membrane, whereas [3 H]-NMS identifies functional receptors on the cell surface only [20]. Our data indicated that binding of [3 H]-NMS in the striatum of μ -opioid receptor knockout mice was significantly lower than that of the wild-type controls. No difference was found in [3 H]-QNB binding between the two genotypes of mice. The findings indicate that only the muscarinic receptors on the cell surface were altered in mice lacking μ -opioid receptor gene. This result may be associated with the change of stimulation on cell surface of muscarinic receptors by acetylcholine that may be influenced by the change of acetylcholinesterase activity in μ -opioid receptor knockout mice.

In addition, we studied the changes in M1 and M2 subtype muscarinic receptor bindings using subtype selective antagonists [3 H]-PZ and [3 H]-AF-DX384 as the radiolabelled ligands, respectively. Our results indicated that M2 subtype ligand binding in the striatum of μ -opioid receptor knockout mice was significantly lower than that of the wild-type controls. However, no difference was found in M1 subtype ligand binding. This may suggest that the compensatory change of M2 subtype muscarinic receptors is more predominate in the striatum of μ -opioid receptor knockout mice. It has been reported that [3 H]-NMS has higher affinity to M2 subtype muscarinic receptors [6,7]. Therefore, the result that change of [3 H]-NMS was detected in this study also supports the suggestion. Since [3 H]-PZ and [3 H]-AF-DX384 also recognize M4 subtype receptors [44], changes in M4 subtype receptors cannot be excluded in μ -opioid receptor knockout mice.

Anatomic evidence indicated that μ -opioid receptors were widely distributed in brain regions including the cortex, striatum and hippocampus [32]. Activation of μ -opioid receptors can inhibit acetylcholine release in many brain areas including the cortex, striatum and hippocampus [24,39,42]. Several lines of evidence have indicated that the dopaminergic system may be involved in the mechanism of μ -opioid receptors that modulate acetylcholine release in the striatum [9,40]. Dourmap et al. [9] have indicated that stimulation of μ -opioid receptors, by inhibiting the acetylcholine release which stimulates tonically M2-muscarinic receptors located at dopaminergic nerve endings, indirectly increases the striatal DA release. In the present study, only the M2 subtype muscarinic receptor binding was decreased in the striatum of μ -opioid receptor knockout than that of the wild-type animals. These data indicate that there are specific brain regional changes in M2, but not M1, subtype muscarinic receptor binding in mice lacking μ -opioid receptor gene. This compensatory change in M2 subtype muscarinic receptor binding may be caused by the alterations of dopaminergic system in the striatum of μ -opioid receptor knockout mice. This is evidenced by our previous findings that there are compensatory up-regulations in mRNAs of dopaminergic receptor genes and receptor bindings of mice lacking μ -opioid receptor gene [34,43]. In addition, anatomic evidence has indicated that there are interactions between dopaminergic terminals, opioid receptors and acetylcholinesterase in the rat striatum [17,23,33]. These three systems may interact with one another during the development such that one system may guide or control the development of the other [33]. This cross modulation among these three systems may explain why we have found the changes in acetylcholinesterase activity and muscarinic receptor bindings and dopaminergic mRNAs and receptor bindings in the striatum of mice lacking μ -opioid receptor gene but not in the cortex and hippocampus.

However, in order to understand the interactions among μ -opioid receptors and dopaminergic and muscarinic systems, further studies are necessary.

Taken together, the data presented here suggest that there are specific regional brain changes of acetylcholinesterase activity and muscarinic receptor bindings in mice lacking μ -opioid receptor gene. The results obtained indicate that there are compensatory up-regulation of acetylcholinesterase activity and compensatory down-regulation of M2 muscarinic receptors in the striatum of mice that lack μ -opioid receptor gene.

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Fig. 1.
Intensity of acetylcholinesterase activity in coronal sections in naïve wild-type and μ -opioid receptor knockout mice.

Fig. 2. Illustrative autoradiograms of [³H]-QNB, [³H]-NMS, [³H]-PZ and [³H]-AF-DX384 binding in coronal sections in naïve wild-type and μ -opioid receptor knockout mice.

Table 1

Changes in acetylcholinesterase activity in coronal brain sections of the wild-type and μ -opioid receptor knockout mice

Brain region	Intensity of AChE staining (OD)	
	Wild-type	μ -Knockout
Striatal complex		
Caudate putamen	0.65 ± 0.02	$0.75 \pm 0.03^*$
Nucleus accumbens	0.71 ± 0.07	$0.91 \pm 0.05^*$
Olfactory tubercle	0.77 ± 0.03	$0.90 \pm 0.04^*$
Hippocampal formation		
CA1	0.10 ± 0.01	0.09 ± 0.01
CA2	0.10 ± 0.01	0.09 ± 0.01
CA3	0.11 ± 0.01	0.10 ± 0.01
Dentate gyrus	0.10 ± 0.01	0.09 ± 0.01

Values are represented as means $\pm$ S.E.M. from five mice in each group. Statistical analysis was performed using one-way ANOVA following Student–Newman–Keuls test.

* $P < 0.05$ compared with the wild-type mice.

Table 2

Binding of [³H]-quinuclidinyl benzilate to muscarinic cholinergic receptors in coronal brain sections of the wild-type and μ -opioid receptor knockout mice

Brain region	[³ H]-QNB binding (nCi/mg tissue)	
	Wild-type	μ -Knockout
Striatal complex		
Caudate putamen	22.8 $\pm$ 2.0	20.9 $\pm$ 2.3
Nucleus accumbens	22.7 $\pm$ 1.1	22.6 $\pm$ 1.7
Olfactory tubercle	21.8 $\pm$ 3.4	21.3 $\pm$ 2.8
Cortex		
Retrosplenial agranular, Layer I	12.5 $\pm$ 0.3	13.7 $\pm$ 1.9
Retrosplenial agranular, Layer III	11.2 $\pm$ 0.8	12.2 $\pm$ 0.8
Retrosplenial agranular, Layer V	10.0 $\pm$ 1.0	10.7 $\pm$ 0.9
Primary somatosensory cortex, Layer I	16.7 $\pm$ 0.7	17.6 $\pm$ 0.9
Primary somatosensory cortex, Layer III	12.4 $\pm$ 1.1	12.8 $\pm$ 1.5
Primary somatosensory cortex, Layer V	12.3 $\pm$ 0.8	13.5 $\pm$ 1.2
Ectorhinal cortex, Layer I	13.3 $\pm$ 1.0	14.5 $\pm$ 1.0
Ectorhinal cortex, Layer III	12.8 $\pm$ 1.3	13.4 $\pm$ 0.8
Ectorhinal cortex, Layer V	12.1 $\pm$ 1.1	13.0 $\pm$ 1.1
Lateral entorhinal, Layer I	12.5 $\pm$ 1.1	13.6 $\pm$ 1.0
Lateral entorhinal, Layer III	11.9 $\pm$ 0.4	11.8 $\pm$ 1.2
Lateral entorhinal, Layer V	11.8 $\pm$ 0.5	12.4 $\pm$ 0.9
Hippocampal formation		
CA1	15.0 $\pm$ 1.0	15.5 $\pm$ 1.2
CA2	12.1 $\pm$ 1.4	12.3 $\pm$ 0.8
CA3	12.8 $\pm$ 0.9	13.1 $\pm$ 1.8
Dentate gyrus	15.7 $\pm$ 2.0	16.1 $\pm$ 1.9

Values are represented as means $\pm$ S.E.M. from five mice in each group. Statistical analysis was performed using one-way ANOVA following Student–Newman–Keuls test.

Table 3

Binding of *N*-[³H]-methylscopolamine to muscarinic cholinergic receptors in coronal brain sections of the wild-type and μ -opioid receptor knockout mice

Brain region	[³ H]-NMS binding (nCi/mg tissue)	
	Wild-type	μ -Knockout
Striatal complex		
Caudate putamen	24.9 $\pm$ 0.8	21.3 $\pm$ 1.0*
Nucleus accumbens	26.4 $\pm$ 0.4	23.7 $\pm$ 0.8*
Olfactory tubercle	26.3 $\pm$ 0.8	22.8 $\pm$ 0.7*
Cortex		
Retrosplenial agranular, Layer I	15.0 $\pm$ 0.9	13.7 $\pm$ 1.9
Retrosplenial agranular, Layer III	11.5 $\pm$ 0.4	11.7 $\pm$ 0.8
Retrosplenial agranular, Layer V	11.6 $\pm$ 0.6	11.3 $\pm$ 0.7
Primary somatosensory cortex, Layer I	20.1 $\pm$ 0.9	18.5 $\pm$ 1.3
Primary somatosensory cortex, Layer III	12.5 $\pm$ 0.8	11.1 $\pm$ 0.9
Primary somatosensory cortex, Layer V	14.2 $\pm$ 1.0	13.5 $\pm$ 1.4
Ectorhinal cortex, Layer I	16.4 $\pm$ 1.6	18.0 $\pm$ 0.9
Ectorhinal cortex, Layer III	12.9 $\pm$ 0.9	11.9 $\pm$ 0.6
Ectorhinal cortex, Layer V	23.9 $\pm$ 1.4	21.3 $\pm$ 2.3
Lateral entorhinal, Layer I	14.5 $\pm$ 0.8	14.2 $\pm$ 0.9
Lateral entorhinal, Layer III	12.9 $\pm$ 1.1	11.8 $\pm$ 0.4
Lateral entorhinal, Layer V	12.8 $\pm$ 1.0	13.0 $\pm$ 0.5
Hippocampal formation		
CA1	18.1 $\pm$ 1.2	16.9 $\pm$ 0.5
CA2	23.4 $\pm$ 2.3	20.0 $\pm$ 3.2
CA3	16.4 $\pm$ 1.4	15.9 $\pm$ 1.5
Dentate gyrus	17.0 $\pm$ 1.3	16.3 $\pm$ 1.4

Values are represented as means $\pm$ S.E.M. from five individual mice in each group. Statistical analysis was performed using one-way ANOVA following Student–Newman–Keuls test.

* $P < 0.05$ compared with the wide type mice.

Table 4

Binding of [³H]-pirenzepine to M1 muscarinic cholinergic receptors in coronal brain sections of the wild-type and μ -opioid receptor knockout mice

Brain region	[³ H]-PZ binding (nCi/mg tissue)	
	Wild-type	μ -Knockout
Striatal complex		
Caudate putamen	15.6 $\pm$ 0.5	16.0 $\pm$ 1.3
Nucleus accumbens	14.5 $\pm$ 2.5	14.8 $\pm$ 2.9
Olfactory tubercle	13.3 $\pm$ 2.1	14.1 $\pm$ 1.7
Cortex		
Retrosplenial agranular, Layer I	14.6 $\pm$ 1.6	15.6 $\pm$ 2.1
Retrosplenial agranular, Layer III	13.2 $\pm$ 2.1	12.1 $\pm$ 2.1
Retrosplenial agranular, Layer V	10.7 $\pm$ 0.6	10.4 $\pm$ 1.3
Primary somatosensory cortex, Layer I	11.1 $\pm$ 0.6	12.4 $\pm$ 1.7
Primary somatosensory cortex, Layer III	8.0 $\pm$ 0.9	8.2 $\pm$ 0.7
Primary somatosensory cortex, Layer V	7.9 $\pm$ 0.7	9.0 $\pm$ 1.4
Ectorhinal cortex, Layer I	11.1 $\pm$ 0.6	12.4 $\pm$ 1.7
Ectorhinal cortex, Layer III	9.5 $\pm$ 0.7	10.8 $\pm$ 1.6
Ectorhinal cortex, Layer V	9.3 $\pm$ 0.7	10.2 $\pm$ 0.8
Lateral entorhinal, Layer I	13.4 $\pm$ 0.7	14.1 $\pm$ 1.1
Lateral entorhinal, Layer III	10.6 $\pm$ 1.3	11.0 $\pm$ 0.8
Lateral entorhinal, Layer V	8.8 $\pm$ 1.3	9.7 $\pm$ 0.7
Hippocampal formation		
CA1	16.0 $\pm$ 1.1	14.8 $\pm$ 2.4
CA2	13.0 $\pm$ 1.0	12.8 $\pm$ 2.1
CA3	15.0 $\pm$ 0.9	15.7 $\pm$ 0.8
Dentate gyrus	21.1 $\pm$ 1.7	19.3 $\pm$ 2.4

Values are represented as means $\pm$ S.E.M. from five individual mice in each group. Statistical analysis was performed using one-way ANOVA following Student–Newman–Keuls test.

Table 5

Binding of [³H]-AF-DX384 to M2 muscarinic cholinergic receptors in coronal brain sections of the wild-type and μ -opioid receptor knockout mice

Brain region	[³ H]-AF-DX384 binding (nCi/mg tissue)	
	Wild-type	μ -Knockout
Striatal complex		
Caudate putamen	28.7 $\pm$ 2.2	22.5 $\pm$ 3.3*
Nucleus accumbens	27.3 $\pm$ 2.7	21.4 $\pm$ 2.5*
Olfactory tubercle	27.6 $\pm$ 3.0	20.3 $\pm$ 3.8*
Cortex		
Retrosplenial agranular, Layer I	16.9 $\pm$ 1.7	13.7 $\pm$ 3.4
Retrosplenial agranular, Layer III	15.5 $\pm$ 1.0	13.4 $\pm$ 2.0
Retrosplenial agranular, Layer V	16.7 $\pm$ 1.7	15.0 $\pm$ 3.1
Primary somatosensory cortex, Layer I	16.4 $\pm$ 0.4	14.4 $\pm$ 2.8
Primary somatosensory cortex, Layer III	14.5 $\pm$ 1.2	13.1 $\pm$ 1.9
Primary somatosensory cortex, Layer V	16.7 $\pm$ 0.8	14.6 $\pm$ 2.0
Ectorhinal cortex, Layer I	13.2 $\pm$ 1.2	11.4 $\pm$ 1.6
Ectorhinal cortex, Layer III	14.4 $\pm$ 1.7	12.9 $\pm$ 1.6
Ectorhinal cortex, Layer V	16.5 $\pm$ 1.0	15.3 $\pm$ 2.3
Lateral entorhinal, Layer I	6.8 $\pm$ 0.9	8.0 $\pm$ 1.3
Lateral entorhinal, Layer III	9.7 $\pm$ 0.9	10.6 $\pm$ 1.8
Lateral entorhinal, Layer V	12.5 $\pm$ 0.9	13.1 $\pm$ 2.7
Hippocampal formation		
CA1	8.1 $\pm$ 1.3	8.4 $\pm$ 1.5
CA2	8.5 $\pm$ 1.5	8.6 $\pm$ 0.6
CA3	8.7 $\pm$ 1.4	8.3 $\pm$ 0.6
Dentate gyrus	6.5 $\pm$ 0.5	6.2 $\pm$ 1.0

Values are represented as means $\pm$ S.E.M. from five mice in each group. Statistical analysis was performed using one-way ANOVA following Student–Newman–Keuls test.

* $P < 0.05$ compared with the wide type mice.