Supplemental material

[^{35}S]GTPγS binding assays

Membrane proteins (40 µg) were diluted in a final volume of 250 µl assay buffer composed of 50 mM Tris pH 7.4, 100 mM NaCl, 0.2 mM EGTA, 3 mM $MgCl_2$, 1 mM DTT, 50 µM GDP, 0.5 nM [^{35}S]GTPγS and increasing concentrations (0 nM–100 µM) of DAMGO. Following an incubation period of 120 min at a temperature of 25°C while shaking at 1,000 rpm, the reaction was terminated by rapid filtration through Whatman GF/B filters, followed by six washes with 1 ml of ice-cold washing buffer (50 mM Tris pH 7.4, 100 mM NaCl, 0.2 mM EGTA, 3 mM $MgCl_2$). Subsequently, filters were placed in scintillation vials containing 5 ml liquid scintillation cocktail and decays per minute (dpm) of the filter-bound [^{35}S]GTPγS were measured by a Liquid Scintillation Analyzer.

The basal binding of [^{35}S]GTPγS without stimulation of DAMGO, B_0(DAMGO), the maximum binding of [^{35}S]GTPγS after stimulation of DAMGO, B_{Max}(DAMGO), and the concentration of DAMGO needed to stimulate half maximal [^{35}S]GTPγS binding, EC_{50}(DAMGO), were determined as:

$$B([^{35}S]GTP\gamma S) = B_0([^{35}S]GTP\gamma S) + \frac{B_{Max}([^{35}S]GTP\gamma S) - B_0([^{35}S]GTP\gamma S)}{1 + 10^{(\log EC_{50}(DAMGO) - \log L(DAMGO))}} \quad \text{Equation 1,}$$

where L(DAMGO) denotes the DAMGO concentration and B([^{35}S]GTPγS) the associated binding rate of [^{35}S]GTPγS. Subsequently, DAMGO related net [^{35}S]GTPγS binding per µ-opioid receptor, B_{Net}(DAMGO), was calculated as:

$$B_{Net}(DAMGO) = B_{Max}(DAMGO) - B_0(DAMGO) \quad \text{Equation 2.}$$

The same method was applied to the control experiments with the non-opioid agonist adenosine. The basal [^{35}S]GTPγS binding did not differ statistically significantly between both brain regions and it was also unaffected by *OPRM1* 118A>G SNP as indicated by the absence of any statistically significant main effect of the rm-ANOVA ($p \geq 0.08$; Supplemental material table 2).

Saturation binding assays

All binding experiments were carried out at a constant temperature of 25°C while shaking at 1,000 rpm. After 120 minutes, the membrane bound [^{3}H]DAMGO was separated from unbound [^{3}H]DAMGO by rapid filtration through Whatman GF/B filters (Whatman plc, Brentford, Middlesex, UK), followed by six washes with 1 ml of ice-cold assay buffer.

The number of DAMGO binding sites (B_{Max}) and its affinity (K_D) were assessed by determining the saturation binding of the µ-opioid receptor specific radioligand [^{3}H]DAMGO. To determine total binding, equal volumes of membrane proteins (25 µl) were diluted in a final volume of 250 µl assay buffer (50 mM Tris-HCl pH 7.4) and incubated with at least 10 different concentrations of [^{3}H]DAMGO in the range of 0.07 nM – 14.42 nM. To determine non-specific [^{3}H]DAMGO binding, membrane proteins were incubated with [^{3}H]DAMGO in the range of 0.07 nM – 14.42 nM in the presence of 10 µM naloxone.

The values of K_D([^{3}H]DAMGO) and B_{Max}([^{3}H]DAMGO) were calculated by non-linear regression of the specific binding as:

$$B([^3H]DAMGO) = \frac{B_{Max}([^3H]DAMGO) \bullet F([^3H]DAMGO)}{(K_D([^3H]DAMGO) + F([^3H]DAMGO))} \quad \text{Equation 3,}$$

where B([^{3}H]DAMGO) is the concentration of µ-opioid receptor bound [^{3}H]DAMGO (specific binding), F([^{3}H]DAMGO) the concentration of the free [^{3}H]DAMGO, B_{Max}([^{3}H]DAMGO) the maximum number of [^{3}H]DAMGO binding sites (µ-opioid receptor density) and K_D([^{3}H]DAMGO) the equilibrium dissociation constant of [^{3}H]DAMGO. Specific [^{3}H]DAMGO binding to the membranes was calculated by subtraction of non-specific binding from total binding. The same method was applied to the control

experiments with [^{3}H]Adenosine. Control experiments were performed by incubation of membrane proteins with at least 6 different concentrations of [^{3}H]Adenosine (Amersham, Little Chalfont, Buckinghamshire, UK) in the range of 17-500 nM in the presence and absence of 1 mM adenosine (Sigma-Aldrich, St. Louis, MO, USA).

OPRM1 mRNA expression analysis

Total RNA was isolated from tissue by means of the EZ1 RNA Universal Tissue Kit (Qiagen, Hilden, Germany). *OPRM1* mRNA expression was assessed by means of real-time PCR (RT-PCR). After reverse transcription of RNA (30 ng/µl) using random hexamers and the High capacity cDNA Archive Kit (Applied Biosystems, Darmstadt, Germany), RT-PCR was performed with 50 ng RNA-equivalent in triplicates using the Taqman Universal PCR Master Mix without Amperase® UNG and the validated TaqMan® gene expression assay for the *OPRM1* gene (Hs00168570_m1, Applied Biosystems, Germany). The human *ACTB* (β-Actin) was employed as endogenous housekeeping gene (TaqMan® gene expression assay Hs99999903_m1, Applied Biosystems, Germany). Quantification of mRNA was conducted on a Taqman 7900HT platform (Applied Biosystems, Darmstadt, Germany) by IMGM Laboratories GmbH (Gene Expression Centre Martinsried, Martinsried, Germany).

From raw fluorescence data, a threshold cycle value, *Ct*, corresponding to the cycle number at which the fluorescence signals of the reporter dye passed a fixed threshold (ten standard deviations from the baseline) on the amplification plot was obtained for each sample (Sequence Detection Software, SDS version 2.0; Applied Biosystems). This ensured that the *Ct* value was proportional to the number of RNA copies present at the start of the PCR. Negative controls showed no amplification in the range of $Ct < 40$ indicating the absence of cross-contamination and unspecific amplification of primer or sensor dimers. *OPRM1* mRNA expression was determined by using the comparative *Ct* method as

$$OPRM1-2\,mRNA_{\;expression_{\;relative}} = 2^{(-\Delta\Delta Ct)}$$

$$\Delta\Delta Ct = \left(Ct_{sample} - Ct_{reference}\right)_{Ct_x} - \left(Ct_{sample} - Ct_{reference}\right)_{Ct_{control}}$$

Equation 4,

where Ct_{sample} and $Ct_{reference}$ denote the *Ct* values for the *OPRM1* and *ACTB* mRNA samples, respectively, being the respective means of three analysis repetitions from control and the sample of interest. For comparisons between genotypes and brain regions, a sample with the *Ct* value closest to the median of the *Ct* values of the secondary somatosensory cortex S_{II} of the wild type group was chosen to serve as $Ct_{control}$.

Supplemental material table 1: Subject related data.

Subject	*OPRM1* 118	Sex [m/f]	Age at death [yrs]	pm delay [h]	Storage time [months]	Cause of death
1	GG	f	42	48	28	Respiratory failure
2	AG	f	66	10	28	Myocardial infarction
3	AA	f	59	48	28	Myocardial infarction
4	AA	f	77	6	25	Cardiac failure
5	AA	m	64	72	25	Myocardial infarction
6	AA	m	65	48	25	Endocarditis
7	AA	f	80	24	24	Mamma ca. (no therapy)
8	AG	m	62	9	24	Myocardial infarction
9	AG	m	62	24	23	Renal failure
10	AG	m	73	7	23	Myocardial infarction
11	AA	f	56	7	23	Liver failure
12	AA	m	63	12	23	Hemorrhagic shock
13	AG	f	91	12	23	Hemorrhagic shock
14	AG	m	81	24	22	Myocardial infarction
15	AG	f	51	72	20	Liver failure
16	AG	m	70	8	19	Myocardial infarction
17	AA	m	28	23	17	Hemorrhagic shock
18	AA	m	25	85	16	Sepsis
19	AG	f	39	85	16	Respiratory failure
20	AG	m	47	19	16	Intoxication
21	AA	f	42	20	15	Hemorrhagic shock
22	AA	f	20	19	15	Hemorrhagic shock
23	AG	m	65	24	14	Hemorrhagic shock
24	AA	f	49	4	15	Manual strangulation
25	AA	f	19	26	14	Pulmonary embolism
26	AG	m	47	47	14	Myocardial infarction
27	AA	f	84	46	14	Hemorrhagic shock
28	AA	m	49	28	14	Cardiac failure
29	AG	m	50	67	14	Hemorrhagic shock
30	AG	f	92	17	14	Cardiac failure
31	AG	m	71	67	14	Hemorrhagic shock
32	AG	f	34	15	11	Hemorrhagic shock
33	AG	f	41	11	11	Hemorrhagic shock
34	AG	f	38	48	11	Hemorrhagic shock
35	AA	f	71	39	10	Myocardial infarction
36	GG	f	23	17	9	Multiple organ failure
37	GG	f	68	22	9	Heart failure
	16 AA 18 AG 3 GG	16 m 21 f	55.8±19.7	31.4±23.7	18.0±5.8	

Supplemental material table 2: Basal binding of [^{35}S]GTPγS. Mean values and 95% confidence interval (CI).

			OPRM1	Region		
Test	Value	N	118	S_{II}	Thalamus	Unit
[^{35}S]GTPγS binding	B_0	15	AA	1040 [854,1227]	963 [766,1161]	fmol/ mg protein
		18	G	805 [660,950]	794 [622,965]	