A Common Human μ-Opioid Receptor Genetic Variant Diminishes the Receptor Signaling Efficacy in Brain Regions Processing the Sensory Information of Pain*[S]

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The single nucleotide polymorphism 118A>G of the human μ-opioid receptor gene *OPRM1*, which leads to an exchange of the amino acid asparagine (N) to aspartic acid (D) at position 40 of the extracellular receptor region, alters the *in vivo* effects of opioids to different degrees in pain-processing brain regions. The most pronounced N40D effects were found in brain regions involved in the sensory processing of pain intensity. Using the μ-opioid receptor-specific agonist DAMGO, we analyzed the μ-opioid receptor signaling, expression, and binding affinity in human brain tissue sampled postmortem from the secondary somatosensory area (S_{II}) and from the ventral posterior part of the lateral thalamus, two regions involved in the sensory processing and transmission of nociceptive information. We show that the main effect of the N40D μ-opioid receptor variant is a reduction of the agonist-induced receptor signaling efficacy. In the S_{II} region of homo- and heterozygous carriers of the variant 118G allele ($n = 18$), DAMGO was only 62% as efficient ($p = 0.002$) as in homozygous carriers of the wild-type 118A allele ($n = 15$). In contrast, the number of [^{3}H]DAMGO binding sites was unaffected. Hence, the μ-opioid receptor G-protein coupling efficacy in S_{II} of carriers of the 118G variant was only 58% as efficient as in homozygous carriers of the 118A allele ($p < 0.001$). The thalamus was unaffected by the *OPRM1* 118A>G SNP. In conclusion, we provide a molecular basis for the reduced clinical effects of opioid analgesics in carriers of μ-opioid receptor variant N40D.

The human μ-opioid receptor variant N40D coded by the single nucleotide polymorphism (SNP)[2] 118A>G of the μ-opioid receptor gene, *OPRM1* (dbSNP rs1799971; allelic frequency 8.2–17%) has been found to be associated with diminished opioid effects in experimental (1–5) and clinical (6–9) settings. However, *in vitro* experiments trying to elucidate the underlying molecular mechanisms provided inconsistent results, falling short to support the comparatively strong clinical evidence of decreased opioid effects in carriers of the variant 118G allele.

Among the reported molecular consequences of the 118A>G polymorphism is a three times higher binding affinity of β-endorphin at the N40D μ-opioid receptors expressed in transfected Syrian hamster adenovirus-12-induced tumor cells (AV-12), whereas the affinity of other exogenous opioids was unaffected (10). In contrast, a leftward shift of the potencies of DAMGO and morphine-mediated Ca^{2+} channel inhibition has been shown in N40D variant μ-opioid receptor expressed in rat sympathetic superior cervical ganglion (SCG) neurons (11). However, both findings of a higher ligand affinity and potency were not reproduced in three attempts using N40D variant μ-opioid receptors transfected Cercopithecus aethiops kidney cells (COS), human 293 embryonic kidney cells (HEK293), and again AV-12 cells (12–14). Moreover, they do not explain the decreased clinical opioid potency. As an alternative mechanism, decreased μ-opioid receptor expression caused by the *OPRM1* 118G variant has been demonstrated in transfected HEK293 cells (13) and Chinese hamster ovary cells (CHO) (15). However, decreased μ-opioid receptor expression as the molecular mechanism for the 118A>G variant was contradicted by the report of unchanged receptor expression in N40D variant receptors expressing HEK293 cells (12). Adding to the inconsistency of molecular findings in transfected cell lines, a recent report demonstrated either decreased or unchanged receptor expression in HEK293 and AV-12 cells, respectively, depending on the transfection method (14). Finally, a single investigation assessing *OPRM1* 118A>G effects in postmortem human brain tissue indicated reduced *OPRM1* mRNA levels in carriers of the variant 118G allele (15). However, the effects of the variant 118G allele on μ-opioid receptor protein expression and function were not examined in that investigation. Moreover, whether the analyzed pooled brain tissue from regions such as cortical lobes and pons are to be considered as primary parts of the pain matrix (16) is unknown.

Thus, attempts to identify the molecular mechanism of the clinical functionality of the *OPRM1* 118A>G genetic variant have produced inconsistent or incomplete results that do not satisfyingly explain the clinically observed decreased analgesic effects of opioids in carriers of this variant. This is probably partly attributable to the artificial conditions in non-human non-neuronal cells and partly to the neglect of region-depend-

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ent effects on μ-opioidergic mechanisms in the human brain, which is strongly suggested to play a role for 118A>G by recent fMRI results (17). Specifically, the presence of the *OPRM1* 118A>G SNP decreased the analgesic effects of alfentanil primarily at brain regions processing the sensory dimension of pain whereas brain regions processing the affective dimension appeared to be unaffected. Considering this fMRI evidence for its brain region-specific effects, we analyzed by means of the μ-opioid receptor-specific agonist DAMGO the consequences of the *OPRM1* 118A>G SNP for μ-opioid receptor signaling, expression and ligand binding affinity in human brain tissue from brain regions known to take part in the processing of the sensory dimension of pain. This included the ventral posterior part of the lateral thalamus where the spinothalamic pathway transmitting peripheral noxious information from the dorsal horn of the spinal cord to the thalamus terminates (18). Furthermore, it included the secondary somatosensory cortex (S_{II}) as one of its cortical projection areas processing the sensory dimension of pain (19, 20) where opioid effects on pain-related brain activation have been shown to be affected by the *OPRM1* 118A>G SNP (17).

EXPERIMENTAL PROCEDURES

Tissue Collection and OPRM1 Genetic Testing—Human brain tissue was sampled during autopsy from the secondary somatosensory cortex S_{II} and the ventral posterior part of the lateral thalamus from 95 subjects without known actual opioid treatment or actual or previous drug addiction (ethics approval obtained). DNA was extracted from the brain tissue using the BioRobot® EZ1 work station and the EZ1 DNA Tissue kit (Qiagen, Hilden, Germany). Subsequently, by means of a validated Pyrosequencing™ assay (21), these samples were diagnosed to contain 68 non-carriers, 24 heterozygous, and three homozygous carriers of the variant *OPRM1* 118G allele, of which brain tissue was analyzed from all three homozygous carriers, from 18 heterozygous carriers, and from a random sample of 16 non-carriers of the variant drawn to match the number of carriers (for more subject-related details see supplemental Table S1). Because of limited tissue availability, it was not possible to employ every subject in all experiments. Therefore, the exact sample sizes are indicated at the respective results. Experiments were performed in an investigator-blinded fashion with respect to the sample genotype.

Membrane Preparation—Tissue (1–1.5 g) was homogenized with a fast rotating Ultra-Turrax (Janke & Kunkel GmbH, Staufen i. Breisgau, Germany) in 10–15 volumes of homogenization buffer (10 mM Tris-HCl, pH 7.4, 290 mM D(+)-saccharose). The crude tissue lysate was immediately centrifuged at $1,000 \times g$ at 4 °C for 15 min, and the supernatant again at $45,000 \times g$, at 4 °C for 45 min. The pellet was resuspended in ice-cold isolation buffer (50 mM Tris-HCl, pH 7.4) and stored at −80 °C pending further analysis. Protein concentrations were measured according to the method of Bradford (22).

Assessment of DAMGO-induced μ-Opioid Receptor Signaling Efficacy and Potency—The efficacy (B_{Net}) and potency (EC_{50}) of D-Ala²,N-MePhe⁴,Gly-ol⁵-enkephalin (DAMGO, Sigma-Aldrich) to trigger μ-opioid receptor signaling were assessed by fitting a sigmoidal concentration-response curve to

$[^{35}S]GTP\gamma S$ binding data (for experimental details and equations see supplemental material). The experiments were performed as published previously (23). $[^{35}S]GTP\gamma S$ binding data were acquired by incubation of membrane proteins with 0.5 nM $[^{35}S]GTP\gamma S$ (Amersham Biosciences, Uppsala, Sweden) in the presence of 50 μM GDP (Sigma-Aldrich) and increasing concentrations of DAMGO (0 nM-100 μM). In addition, control experiments were performed under equal conditions with the non-opioid agonist adenosine (0 nM to 100 μM; Sigma-Aldrich) to verify that observed differences between genotype groups were not related to potential confounders, *e.g.* tissue variability.

Assessment of [³H]DAMGO Binding Sites and Affinity—The number of [³H]DAMGO binding sites (B_{Max}) and its affinity (K_D) were assessed by fitting a one site hyperbola to the data of the specific saturation binding of [³H]DAMGO (Amersham Biosciences). Specific [³H]DAMGO binding data were acquired by incubation of membrane proteins with at least 10 different concentrations of [³H]DAMGO in the range of 0.07–14.42 nM in the presence and absence of 10 μM naloxone (Naloxon-Curamed, Curamed, Karlsruhe, Germany). Control experiments were performed by incubation of membrane proteins with at least 6 different concentrations of [³H]adenosine (Amersham Biosciences) in the range of 17–500 nM in the presence and absence of 1 mM adenosine (Sigma-Aldrich).

Furthermore, *OPRM1* mRNA expression was analyzed by means of quantitative real-time PCR (RT-PCR) analysis of *OPRM1* mRNA expression in relation to human *ACTB* (β-actin) mRNA (for experimental details and equations, see supplemental materials).

Statistics—The numerical values of the B_{Net}, EC_{50}, B_{Max}, and K_D were obtained by non-linear regression analyzes of individual data using GraphPad Prism® 5.01 (GraphPad Software, San Diego, CA). *OPRM1* mRNA expression was determined by using the comparative *Ct* method ($2^{-\Delta\Delta Ct}$; for equations see supplemental materials). Because B_{Net}(DAMGO) correlated statistically significantly with B_{Max}([³H]DAMGO) in S_{II} (Pearson correlation: 0.41, $p = 0.018$) and thalamus (0.61, $p < 0.001$), the net effect of agonist binding per μ-opioid receptor was calculated separately for each subject and each brain region as the quotient of DAMGO efficacy and the number of DAMGO binding sites, B_{Net}(DAMGO)/B_{Max}([³H]DAMGO). This allowed for comparison of μ-opioid receptor-G-protein coupling efficacy between brain regions and genotype groups (24–26). Similarly, the B_{Net}(adenosine)/B_{Max}([³H]adenosine) was calculated for the control experiments. Because of the small number of homozygous 118G carriers, hetero- and homozygous carriers of the 118G allele were pooled and subsequent group comparisons were made for two groups (*i.e.* non-carriers of the 118G allele "118AA" and carriers of the "118G" allele "118G"). The region-specific effects of the *OPRM1* 118A>G single nucleotide polymorphism were analyzed by means of multivariate analysis of variance for repeated measures (rm-ANOVA), with region (*i.e.* S_{II} or thalamus; degrees of freedom, df = 1) as within-subject factor and 118A>G (*i.e.* 118AA or 118G; df = 1) as between-subject factor (SPSS 15.0.1.1, SPSS Inc., Chicago, IL). The α-level was set at 0.05. Post-hoc comparisons for statistical main effects were done by *t*-tests. In the case of significant effects of the *ORPM1* 118A>G genotype, the

influence of potential confounders was checked, *e.g.* the subject's sex (*t*-tests), age at death, postmortem delay, and tissue storage period (correlation analyses).

RESULTS

Subject-related Data—The group of homozygous carriers of the wild-type allele (118AA) and the group of hetero- and homozygous carriers of the variant 118G allele (118G) differed not with respect to the subjects' sex (118AA: 6 men and 10 women; 118G: 10 men and 11 women; Chi^2-test: $p = 0.74$), age at death (118AA: 53.2 years (mean), 19–84 years (range); 118G: 57.8 years, 23–92 years; Independent samples *t*-tests: $p = 0.50$), postmortem delay (118AA: 31.7 h, 4–85 h; 118G: 31.1 h, 7–85 h; $p = 0.94$), and tissue storage period (118AA: 18.9 months, 10–28 months; 118G: 17.3 months, 9–28 months; $p = 0.39$).

DAMGO-induced μ-Opioid Receptor Signaling Efficacy and Potency—The efficacy of DAMGO to stimulate [^{35}S]GTPγS binding, B_{Net}(DAMGO), was similar in the S_{II} and thalamus as indicated by the absence of a statistical main effect of rm-ANOVA factor region (F $(1,1,31) = 0.003$, $p = 0.96$; Fig. 1 and Table 1). Similarly, the 118G allele had no effect on the efficacy of DAMGO as indicated by the absence of a statistical main effect of rm-ANOVA factor 118A>G (F $(1,1,31) = 1.78$, $p = 0.19$). However, the genotype influenced significantly the DAMGO efficacy in a brain region-dependent manner (rm-ANOVA interaction region by 118A>G: F $(1,1,31) = 6.78$, $p = 0.014$). Specifically, a genotype effect was seen exclusively in S_{II}, where in hetero- and homozygous 118G carriers DAMGO was only 62% as efficient as in homozygous carriers of the 118A allele (*t* test: t(31) = 3.45, $p = 0.002$). This was not the case for the thalamus, where the DAMGO efficacy was not different between non-carriers and carriers of the variant 118G allele (*t* test: t(31) = 0.39, $p = 0.70$). The potential confounders sex (*t* test: t(31) = -1.30, $p = 0.20$), age at death, postmortem delay, and storage period (Pearson correlation: $p > 0.08$) had no influence on B_{Net}(DAMGO) in S_{II}.

Similar to DAMGO-stimulated [^{35}S]GTPγS binding, the control experiments with the non-opioid agonist adenosine (Fig. 1 and Table 1) showed that the efficacy of adenosine, B_{Net}(adenosine), was similar in both brain regions (rm-ANOVA factor region: F $(1,1,28) = 1.46$, $p = 0.24$) and that it was unaffected by the 118G allele (rm-ANOVA factor 118A>G: F $(1,1,28) = 1.36$, $p = 0.25$). However, in contrast to DAMGO-stimulated [^{35}S]GTPγS binding, the genotype had no significant influence on the adenosine efficacy in a brain region-dependent manner (interaction region by 118A>G: F $(1,1,28) = 0.52$, $p = 0.48$). This further indicates that the observed brain region-dependent differences of [^{35}S]GTPγS binding after DAMGO stimulation between non-carriers and carriers of the 118G allele were indeed related to the 118G allele, and not the result of potential confounders, *e.g.* tissue variability.

The potency of DAMGO to stimulate [^{35}S]GTPγS binding, EC_{50}(DAMGO), was brain region-dependent with a significantly lower potency of DAMGO in S_{II} than in the thalamus as indicated by the higher EC_{50}(DAMGO) in S_{II} (rm-ANOVA factor region: F $(1,1,31) = 13.77$, $p = 0.001$). However, the potency did not differ significantly between homozygous carriers of the

118A allele and hetero- and homozygous carriers of the 118G allele ($p \geq 0.28$; Table 1). In contrast, the control agonist adenosine was equally potent in S_{II} and thalamus of carriers of either genotype as indicated by the absence of any statistically significant main effect of the rm-ANOVA ($p \geq 0.23$; Table 1).

[^{3}H]DAMGO Binding Sites and Affinity—The number of DAMGO binding sites, B_{Max}([^{3}H]DAMGO), was significantly lower in S_{II} than in the thalamus (rm-ANOVA factor region: F $(1,1,31) = 16.28$, $p < 0.001$; Fig. 1 and Table 2). The lower number of DAMGO binding sites was affirmed by lower *OPRM1* mRNA expression in S_{II} compared with the thalamus (rm-ANOVA factor region: F $(1,1,20) = 7.03$, $p = 0.015$). The *OPRM1* 118A>G SNP had no statistically significant effect on the number of DAMGO binding sites ($p \geq 0.46$) and *OPRM1* mRNA-expression levels ($p \geq 0.60$), respectively.

The affinity of [^{3}H]DAMGO at the μ-opioid receptors in S_{II} was significantly lower than in the thalamus as indicated by the higher K_D([^{3}H]DAMGO) in S_{II} (rm-ANOVA factor region: F $(1,1,31) = 8.30$, $p = 0.007$). Again, the *OPRM1* 118A>G SNP had no statistically significant effect on the affinity of [^{3}H]DAMGO ($p \geq 0.16$; Fig. 1 and Table 2).

B_{Max} and K_D of [^{3}H]adenosine were similar in both brain regions and unaffected by the *OPRM1* 118A>G SNP as indicated by the absence of a statistically significant main effect of the rm-ANOVA ($p \geq 0.08$; Table 2).

μ-Opioid Receptor G-protein Coupling Efficacy—Analysis of the ratio B_{Net}(DAMGO)/B_{Max}([^{3}H]DAMGO) confirmed a brain region-dependent μ-opioid receptor G-protein coupling efficacy (rm-ANOVA factor region: F $(1,1,31) = 30.81$, $p < 0.001$; Fig. 1 and Table 3), which was modulated by the 118A>G genotype (rm-ANOVA factor 118A>G: F $(1,1,31) = 8.42$, $p = 0.007$ and rm-ANOVA interaction region by 118A>G: F $(1,1,31) = 19.05$, $p < 0.001$). Post-hoc comparisons showed that B_{Net}(DAMGO)/B_{Max}([^{3}H]DAMGO) in S_{II} of 118G carriers was only 58% as efficient as in 118AA carriers (*t* test: t(31) = 4.31, $p < 0.001$) while it was similar in the thalamus of 118AA and 118G carriers (*t* test: t(31) = 0.23, $p = 0.82$). The potential confounders sex (*t* test: t(31) = -0.39, $p = 0.70$), age at death, postmortem delay, and storage period (Pearson correlation: $p > 0.09$) had no influence on B_{Net}(DAMGO)/B_{Max}([^{3}H]DAMGO) in S_{II}.

The ratio of the control experiments, B_{Net}(adenosine)/B_{Max}([^{3}H]adenosine), was similar in S_{II} and thalamus and, as expected, unaffected by the *OPRM1* 118A>G SNP (rm-ANOVA $p \geq 0.17$; Table 3).

DISCUSSION

We show that the main functional effect of the *OPRM1* 118A>G variant is a decrease in the μ-opioid receptor signaling efficacy in a brain region, S_{II}, known to be an important part of the pain matrix that codes for the pain intensity. Of the so far accumulated knowledge on the molecular mechanism underlying the functional consequences of the variant (10–15), the presented results agree best with the consistent experimental and clinical observations that this variant mainly decreases the opioid effects and/or increases the opioid dosing requirements to achieve analgesia (1–9). The results are also in strong agreement with the fMRI
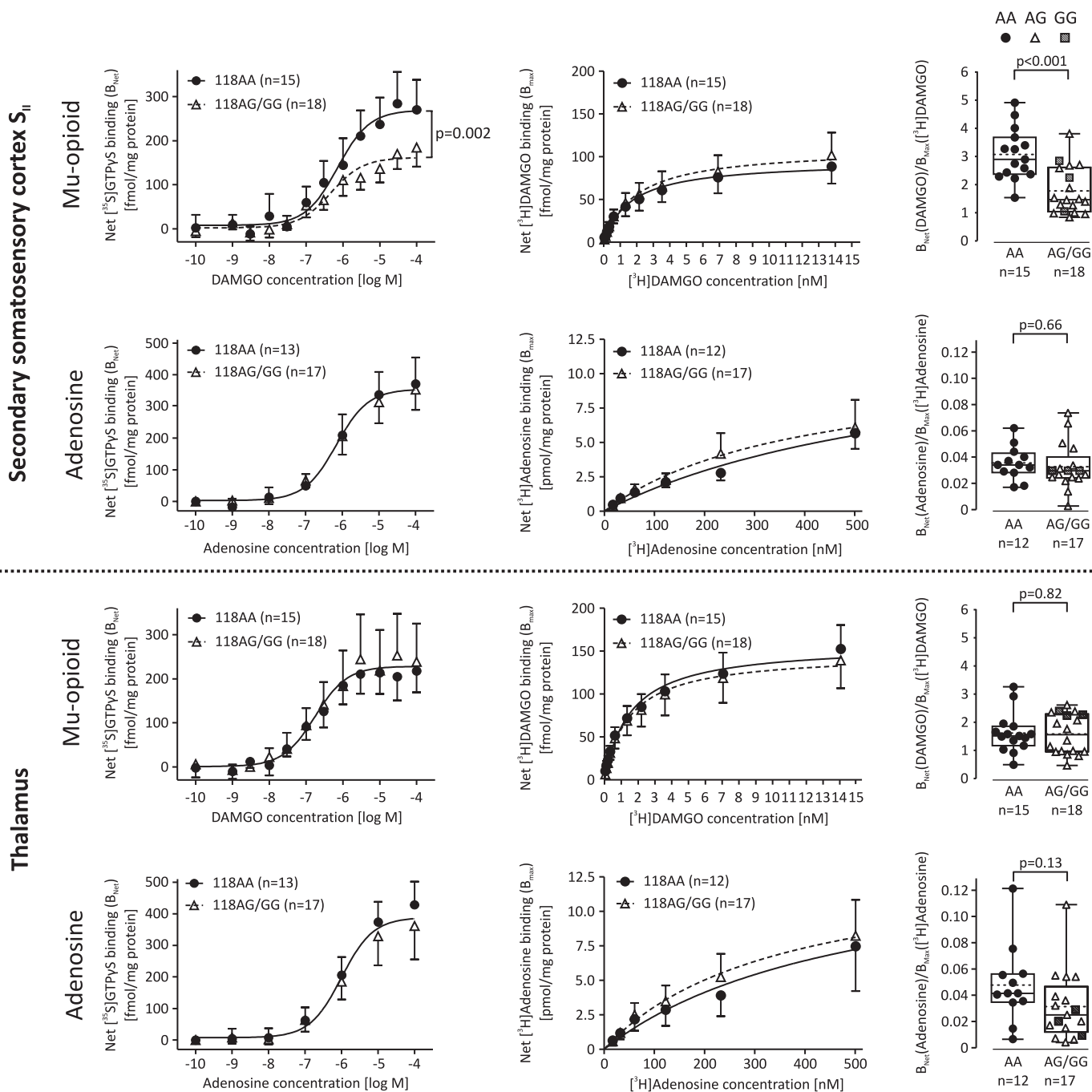
FIGURE 1. Effects of the *OPRM1* 118A>G SNP on the agonist-induced receptor signaling (*left figures*), the agonist binding (*middle figures*), and the receptor-G-protein coupling efficacies (*right figures*) of DAMGO and adenosine in the secondary somatosensory area, S_{II}, and the ventral posterior part of the lateral thalamus. The $[^{35}S]GTP\gamma S$ binding data showed that the efficacy of DAMGO to stimulate the $[^{35}S]GTP\gamma S$ binding was significantly reduced in the S_{II} region of carriers of the variant 118G allele compared with non-carriers (*1st row*, *left figure*; nonlinear fit to mean of data/solid and *dashed lines*, 95% CI/bars). The saturation binding experiments indicated that the different DAMGO efficacies were not related to different amounts of μ-opioid receptor binding sites (*1st row*, *middle figure*; nonlinear fit to mean of data/*solid* and *dashed lines*, 95% CI/bars). This is confirmed by the higher ratio of $B_{Net}(DAMGO)/B_{Max}([^3H]DAMGO)$ in the S_{II} region of carriers of the variant 118G allele compared with non-carriers (*1st row*, *right figure*; median/*solid line*, mean/*dotted line*, min and max/*bars*). The absence of *OPRM1* 118A>G SNP effects on the control experiments with the non-opioid agonist adenosine in the S_{II} region verified that the observed differences of the DAMGO efficacy between genotype groups were not related to μ-opioid receptor independent factors like tissue variability (*2nd row*). In the thalamus, the *OPRM1* 118A>G SNP neither affected the agonist-induced receptor signaling, receptor binding sites, and receptor-G-protein coupling efficacies of DAMGO (*3rd row*) nor of adenosine (*4th row*).

observation that the genetic variant mainly affects the effects of opioid analgesics on the sensory component of pain (17), which is emphasized by the fact that the main molecular effect was presently found in the S_{II} region. Based on the absent effect of the *OPRM1* genotype in the control experiments with the non-opioid agonist adenosine and on the lack of influences of potential confounders, a contribution of factors such as tissue degradation to the differences of μ-opioid receptor signaling/-coupling between non-carriers and carriers of *OPRM1* variant 118G appears to be unlikely.

TABLE 1

[^{35}S]GTPγS binding data after DAMGO and adenosine stimulation in tissue samples from the secondary somatosensory area, S_{II}, and from the ventral posterior part of the lateral thalamus

Mean values and 95% confidence interval (CI) are shown.

Agonist	Value	N	OPRM1 118	S_{II} Value	S_{II} 95% CI	Thalamus Value	Thalamus 95% CI	Unit
DAMGO	B_{Net}[a]	15	AA	283	[236,330]	221	[148,295]	fmol/mg protein
		18[b]	G	176	[133,219]	240	[173,308]	
	EC_{50}[c]	15	AA	1.27	[0.65,1.89]	0.26	[0,0.55]	μM
		18[b]	G	0.94	[0.38,1.51]	0.39	[0.13,0.65]	
Adenosine	B_{Net}	13	AA	373	[306,440]	432	[338,527]	fmol/mg protein
		17[d]	G	345	[286,403]	360	[277,442]	
	EC_{50}	13	AA	1.17	[0.49,1.85]	1.66	[0.73,2.58]	μM
		17[d]	G	0.94	[0.35,1.53]	1.44	[0.63,2.24]	

[a] B_{Net}, maximum net binding of [^{35}S]GTPγS after agonist stimulation. The genotype influenced the DAMGO efficacy in a significant brain region-dependent manner (rm-ANOVA interaction region by 118A>G: $F_{(1,1,31)} = 6.78$, $p = 0.014$). Specifically, in the S_{II} of 118G carriers, DAMGO was only 62% as efficient as in homozygous carriers of the 118A allele (t-test: t(31) = 3.45, $p = 0.002$). In contrast, the DAMGO efficacy in the thalamus was not significantly different between non-carriers and carriers of the variant 118G allele of (t-test: t(31) = 0.39, $p = 0.70$).
[b] Consisting of 15 heterozygous carriers (118AG) and 3 homozygous carriers (118GG) of the 118G allele.
[c] EC_{50}, concentration of agonist producing 50% of the maximum net binding of [^{35}S]GTPγS.
[d] Consisting of 14 heterozygous carriers (118AG) and 3 homozygous carriers (118GG) of the 118G allele.

TABLE 2

[^{3}H]DAMGO binding data, [^{3}H]adenosine binding data, and *OPRM1* mRNA expression data in tissue samples from the secondary somatosensory area, S_{II}, and from the ventral posterior part of the lateral thalamus

Mean values and 95% confidence interval (CI) are shown.

Agonist	Value	N	OPRM1 118	S_{II} Value	S_{II} 95% CI	Thalamus Value	Thalamus 95% CI	Unit
[^{3}H]DAMGO	B_{Max}[a]	15	AA	97	[71,123]	151	[115,186]	fmol/mg protein
		18[b]	G	114	[91,138]	151	[118,183]	
	K_D[c]	15	AA	1.9	[1.3,2.5]	1.4	[0.8,2.0]	nM
		18[b]	G	2.5	[1.9,3.1]	1.8	[1.2,2.3]	
[^{3}H]Adenosine	B_{Max}	12	AA	10.0	[6.6,13.5]	13.0	[7.7,18.4]	pmol/mg protein
		17[d]	G	12.4	[9.5,15.3]	15.7	[11.2,20.2]	
	K_D	12	AA	696	[194,1198]	505	[0,1134]	nM
		17[d]	G	353	[55,652]	348	[52,643]	
OPRM1 mRNA	$2^{-\Delta\Delta Ct}$[e]	11	AA	1.5	[0.7,2.4]	3.0	[1.1,4.8]	—
		11[f]	G	1.4	[0.6,2.2]	3.6	[1.7,5.4]	

[a] B_{Max}, maximum number of agonist binding sites.
[b] Consisting of 15 heterozygous carriers (118AG) and 3 homozygous carriers (118GG) of the 118G allele.
[c] K_D, equilibrium dissociation constant.
[d] Consisting of 14 heterozygous carriers (118AG) and 3 homozygous carriers (118GG) of the 118G allele.
[e] $2^{-\Delta\Delta Ct}$, *OPRM1* mRNA expression in relation to human *ACTB* (β-actin) mRNA.
[f] Consisting of 8 heterozygous carriers (118AG) and 3 homozygous carriers (118GG) of the 118G allele.

TABLE 3

Quotient of the maximum net binding of [^{35}S]GTPγS after agonist stimulation and maximum number of agonist binding sites in tissue samples from the secondary somatosensory area, S_{II}, and from the ventral posterior part of the lateral thalamus

Mean values and 95% confidence interval (CI) are shown.

Value	N	OPRM1 118	S_{II} Value	S_{II} 95% CI	Thalamus Value	Thalamus 95% CI
B_{Net}(DAMGO)/B_{Max}([^{3}H]DAMGO)[a]	15	AA	3.1	[2.6,3.5]	1.6	[1.3,2.0]
	18[b]	G	1.8	[1.3,2.2]	1.6	[1.2,1.9]
B_{Net}(adenosine)/B_{Max}([^{3}H]adenosine)	12	AA	0.036	[0.026,0.045]	0.047	[0.031,0.064]
	17[c]	G	0.033	[0.025,0.041]	0.031	[0.17,0.045]

[a] B_{Net}/B_{Max}, maximum net binding of [^{35}S]GTPγS per receptor after agonist stimulation. Analysis of B_{Net}(DAMGO)/B_{Max}([^{3}H]DAMGO) showed a region-dependent μ-opioid receptor G-protein coupling efficacy (rm-ANOVA factor region: $F_{(1,1,31)} = 30.81$, $p < 0.001$), which was modulated by the genotype (rm-ANOVA factor 118A>G: $F_{(1,1,31)} = 8.42$, $p < 0.001$ and rm-ANOVA interaction region by 118A>G: $F_{(1,1,31)} = 19.05$, $p < 0.001$). Post-hoc comparisons showed that B_{Net}(DAMGO)/B_{Max}([^{3}H]DAMGO) in S_{II} of 118G carriers was only 58% as efficient as in 118AA carriers (t-test: t(31) = 4.31, $p < 0.001$) while it was similar in the thalamus of 118AA and 118G carriers (t-test: t(31) = 0.23, $p = 0.82$).
[b] Consisting of 15 heterozygous carriers (118AG) and 3 homozygous carriers (118GG) of the 118G allele.
[c] Consisting of 14 heterozygous carriers (118AG) and 3 homozygous carriers (118GG) of the 118G allele.

The present selection of brain regions was limited to S_{II} and the thalamus, two brain regions known to be involved in the sensory processing of pain intensity. However, other brain regions as the two here selected may also be affected. This is suggested by the fMRI findings of 118G effects (17) being present also in other brain regions involved in the processing of pain, *e.g.* the posterior insula and S_I. Although the μ-opioid receptor expression differed significantly between the two presently chosen brain regions, we could not reproduce a genotype difference in receptor expression levels as previously shown in human brain samples from pons and cortical lobes (15) and in *OPRM1* 118G-transfected non-brain or non-human cell lines (13–15), respectively. While it is possible that we missed an *OPRM1* 118G effect on μ-opioid receptor protein expression because [^{3}H]DAMGO binds only to agonist states of the μ-opioid receptor (27), the analysis of *OPRM1* mRNA expression affirms the conclusion that μ-opioid receptor expression is unaffected by the *OPRM1* 118A>G SNP in both investigated

brain regions. However, the fact that we missed an *OPRM1* 118G effect on μ-opioid receptor expression may also relate to brain region specific effects of the 118G allele. Regionally limited alterations of μ-opioid receptor expression have already been shown for other SNPs known to affect the opioid system, *e.g.* the *COMT* 472G>A polymorphism (28, 29). Nevertheless, the presented results make a global reduction of receptor expression unlikely as a general molecular mechanism for the reduced analgesia in carriers of *OPRM1* variant 118G.

We would have probably missed a genotype effect if we had analyzed only a single brain region. The importance of brain region-dependent variable μ-opioid receptor expression (30, 31) and function (25, 32) for the functional consequences of the 118A>G variant might be a major reason for the failed or contradictory results of previous investigations in cell cultures (10–14) that contrast with the consistent clinical findings of decreased opioid effects in carriers of variant 118G (1–9). To which extent region-specific expression of μ-opioid receptor splice variants (33, 34) and coupling to G_α and $G_\beta\gamma$ subunits (35, 36), which are known to influence the μ-opioid receptor signaling, contribute to the brain region-dependent effects of the N40D variant, may be addressed in the future.

In conclusion, we provide a molecular basis for the reduced clinical effects of opioid analgesics in carriers of the μ-opioid receptor variant N40D. The variant has region-specific effects on the μ-opioid receptor signaling evident in parts of the pain matrix, consisting of a decreased receptor signaling efficiency after stimulation by exogenous opioid receptor agonists in a brain region known to process the sensory component of pain, *i.e.* pain intensity.

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