Supplemental Data

A novel knock-in mouse reveals mechanistically distinct forms of morphine tolerance

Johan Enquist, Joseph A Kim, Selena Bartlett, Madeline Ferwerda, Jennifer L Whistler

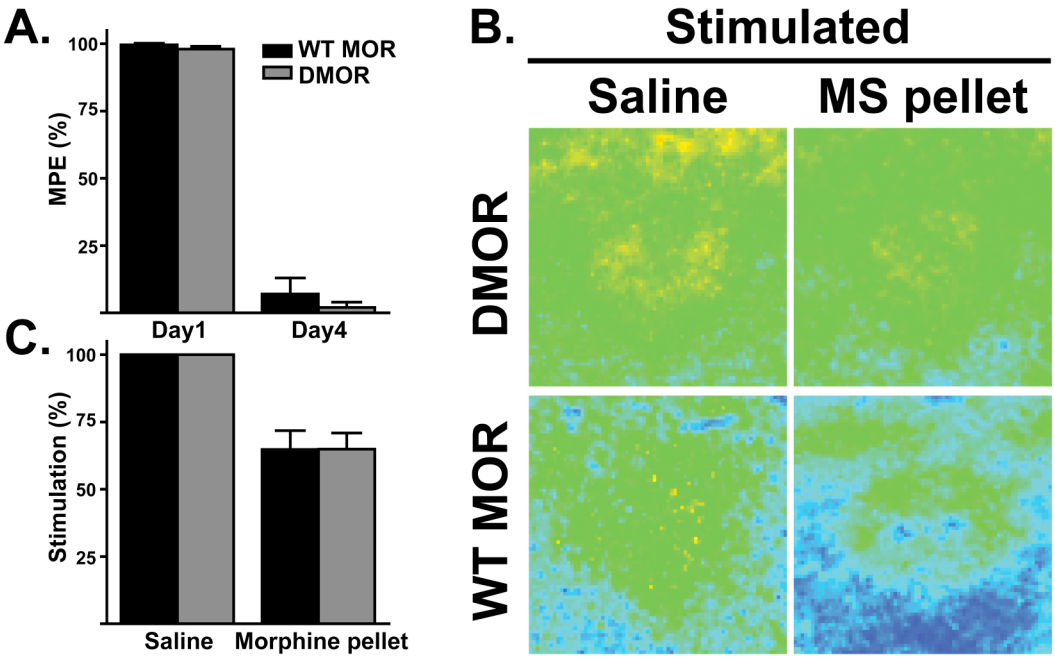
Supplemental Material & Methods

Tolerance Induction Protocol: High Dose Morphine Regimen

WT MOR and DMOR mice were challenged on day 1 with 10 mg/kg morphine s.c. and assessed for antinociception 30 minutes later. Mice were then anesthetized and implanted with 75 mg morphine pellet s.c.. Baseline tailflick latency returned to normal 72 hours post surgery at which point mice were treated with 10 mg/kg morphine s.c. and antinociception was measured 30 minutes later (day 4). Data presented as maximal possible effect MPE ± SEM. 30 WT MOR animals and 47 DMOR animals were implanted with pellets. 12 out of 30 WT MOR, and 47 out of 47 DMOR animals survived until the test day.

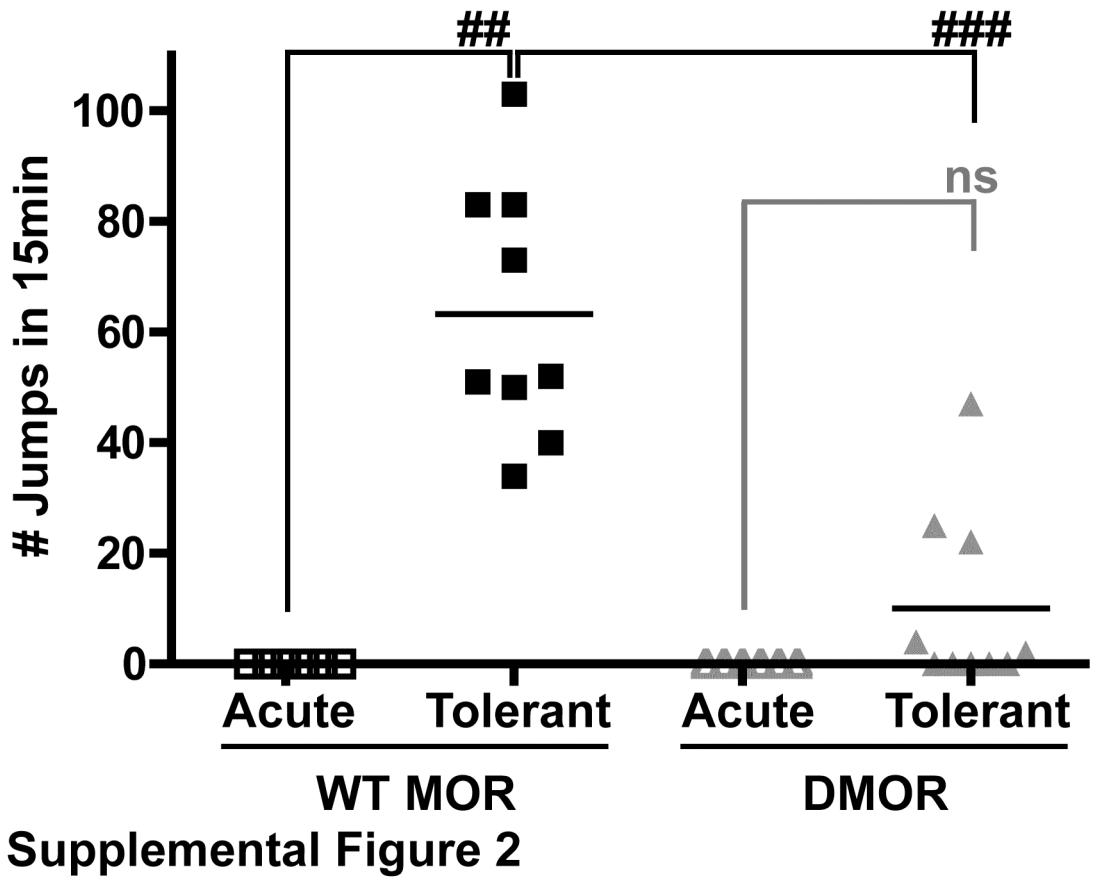
GTPγS binding to slices

Mice were sacrificed by cervical dislocation, and brains were quickly removed, frozen in isopentane on dry ice and stored at −80°C. Coronal sections (12 μm) containing the peri-aqueductal grey area (PAG) were cut on a cryostat at −20°C and thaw mounted onto gelatin-subbed slides. Sections were pre-calibrated to room temperature just before the start of the assay. Slides were rinsed in assay buffer (50 mM Tris-HCl, 3 mM MgCl2, 0.2 mM EGTA, 100 mM NaCl, pH 7.4) at 25ºC for 10 min, then incubated for 15 min in assay buffer containing 2 mM GDP at 25ºC. Slides were then incubated in assay buffer with GDP, 0.05 nM [^{35}S]-GTPγS with 10 μM DAMGO or without ligand at 25ºC for 90 minutes to achieve maximal stimulation of MOR and baseline [^{35}S]-GTPγS respectively. Following agonist incubation, slides were rinsed twice for 2 minutes each in Tris-HCl buffer (50 mM Tris-HCl, pH 7.4) and once in deionized H_2O on ice. Slides were dried overnight and exposed to film for 48 h. Density analysis of film was done using ImageJ; data is presented as percent stimulation over baseline.



Supplemental Figure 1

Supplemental Figure 1: Effect of high dose morphine on antinociception and G protein coupling in peri-aqueductal grey of WT MOR and DMOR mice. On day 1, WT MOR (black bars) and DMOR (grey bars) mice were treated with 10 mg/kg morphine s.c. and assessed for antinociception 30 minutes later (A, day 1). Mice were then anesthetized and implanted with a 75 mg morphine pellet s.c.. Baseline tailflick latency had returned to baseline 72 hours post surgery, at which point mice were treated with 10 mg/kg morphine s.c. and antinociception was measured 30 minutes later (A, day 4). Data presented as maximal possible effect MPE ± SEM. 12 out of 30 WT MOR, and 47 out of 47 DMOR animals survived until the test day. Brain tissue from 3 animals from each group were collected 6 hours after last morphine challenge and rapidly frozen by submersion in 2-methyl-butane on dry ice. Sections of PAG were collected by cryostat sectioning, incubated with or without DAMGO in the presence of [S^{35}]GTP-γ-S and developed by autoradiography. Data is presented as B) color coded density images of PAG from representative animals, or C) as histograms of compiled data showing percent DAMGO stimulated GTP-γ-S recruitment normalized to naïve conditions.



Supplemental Figure 2

Supplemental Figure 2: Morphine dependence after high or moderate morphine in WT MOR and DMOR mice. WT MOR (solid squares), and DMOR (solid triangles) mice were implanted with 75 mg morphine pellets 72 hours prior to test day or left untreated. On test day, morphine naïve (WT MOR Acute – open squares, DMOR Acute - open triangles) or morphine pelleted animals (WT MOR Tolerant, DMOR tolerant) were injected with 10 mg/kg morphine followed by 0.5 mg/kg naloxone 30 minutes later. Withdrawal was scored as number of jumps over 15 minutes (WT MOR 63 ± 8 jumps, DMOR 10 ± 6, $p<0.001$ Mann Whitney; 4 out of 10 DMOR and all WT MOR mice jumped). # Jumps ± SEM are presented. WT MOR versus DMOR statistics by Mann-Whitney nonparametric test, intra genotype statistics by Wilcoxon signed rank test; # $p<0.05$, ## p,0.01, ### $p<0.001$, ns no significant difference; □ WT MOR ACUTE; △ DMOR ACUTE; ■ WT MOR TOLERANT; ▲ DMOR TOLERANT.