Supplementary Figure Legends

Supplementary Figure 1: Experimental design for viral treatments and behavioral testing.
The scheme shows the exact history of each mouse sub-group in the two cohorts (first cohort in **A** and second cohort in **B**). Behavioral experiments started 6 weeks following viral injection. Naïve mice correspond to mice that were not tested in the lithium CPA experiment (week 6) (see Darcq et al, 2011) prior to the morphine analgesia experiment (week 8). Mice were then tested at week 9 for withdrawal symptoms and killed at week 10 for injection site analysis (GFP fluorescence) and in situ hybridization experiment (week 11). Mice with misplacement of injection site where eliminated from the final analysis (mis-injected, see methods).

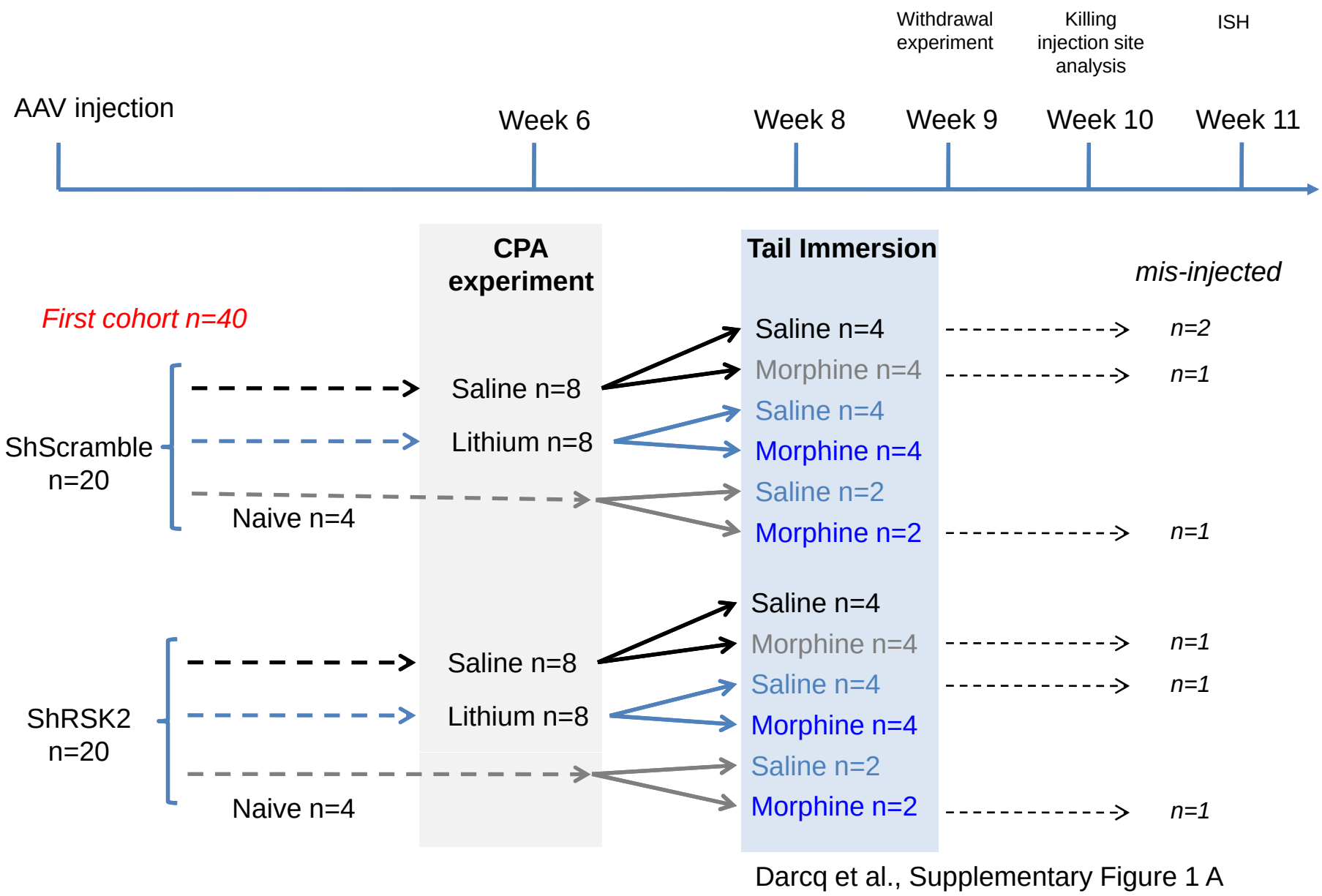
Supplementary Figure 2: Acute morphine activates ERK signaling in the habenula.
Morphine was administered in wild-type mice (n=2) at 5 or 10 mg/kg (i.p.) and samples processed for immunocytochemistry 20 (upper panels) or 40 (lower panels) minutes later. Labeling of the activated form of ERK was performed using a specific antibody reacting with doubly phosphorylated, active ERK (P-ERK) (anti-phospho Thr202-Tyr204 ERK1, cat-9101; Cell Signal Technology, Beverly, MA, USA) (dilution 1: 400), and HRP revelation detected with bright field microscope. Representative sections are shown at the level of the habenula. The activated phosphoERK is detectable following acute morphine in habenula.

Supplementary Figure 3: Morphine analgesia is decreased in the small subgroup of virally-treated animals not pre-exposed to lithium conditioning (naïve).
Naive mice were tested for morphine analgesia (10 mg/kg) in the tail immersion test at 52°C. AAV2-shRSK2-injected naive mice showed reduced morphine-analgesia when compared with control animals injected with shScramble (shScramble saline n=2, ShScramble morphine n=4; ShRSK2 saline n=2 and ShRSK2 morphine n=5). Statistical analysis reveals a significant main effect of morphine [$F(1,9) = 6.106$, $p < .05$] and a main effect of viral treatment [$F(1,9) = 2.889$, $p = 0.1234$]). This result indicates that ShRSK2-treated naïve mice show reduced morphine analgesia in the tail immersion ($p=0.0278$). Further comparisons where performed using larger number of animals (see Figure 4B and C), and confirmed the significant reduction of morphine efficacy in the tail immersion test. Main effect of morphine: ★ $p<0.05$; simple main effect of viral treatment (morphine groups: shScramble vs shRSK2): ☆ $p < 0.05$

Supplementary Figure 4: Physical dependence is not modified following AAV2-shRSK2 injection in the habenula.
Nine weeks following AAV2 injection, mice were treated twice daily for 5 days with either saline or ascending doses of morphine (20–100 mg/kg). On the sixth day, the withdrawal syndrome was precipitated by administration of the antagonist naloxone (1 mg/kg) 2 h after the last morphine injection and mice were observed for 20 min for physical signs of dependence (AAV2-shRSK2, n=18/group; AAV2-shScramble, n=17/group). Many signs are increased in morphine-treated mice (see global withdrawal score shown in Figure 4D). Values represent the mean ± SEM. Main effect of drug: ★★★ $p <0.001$.
!

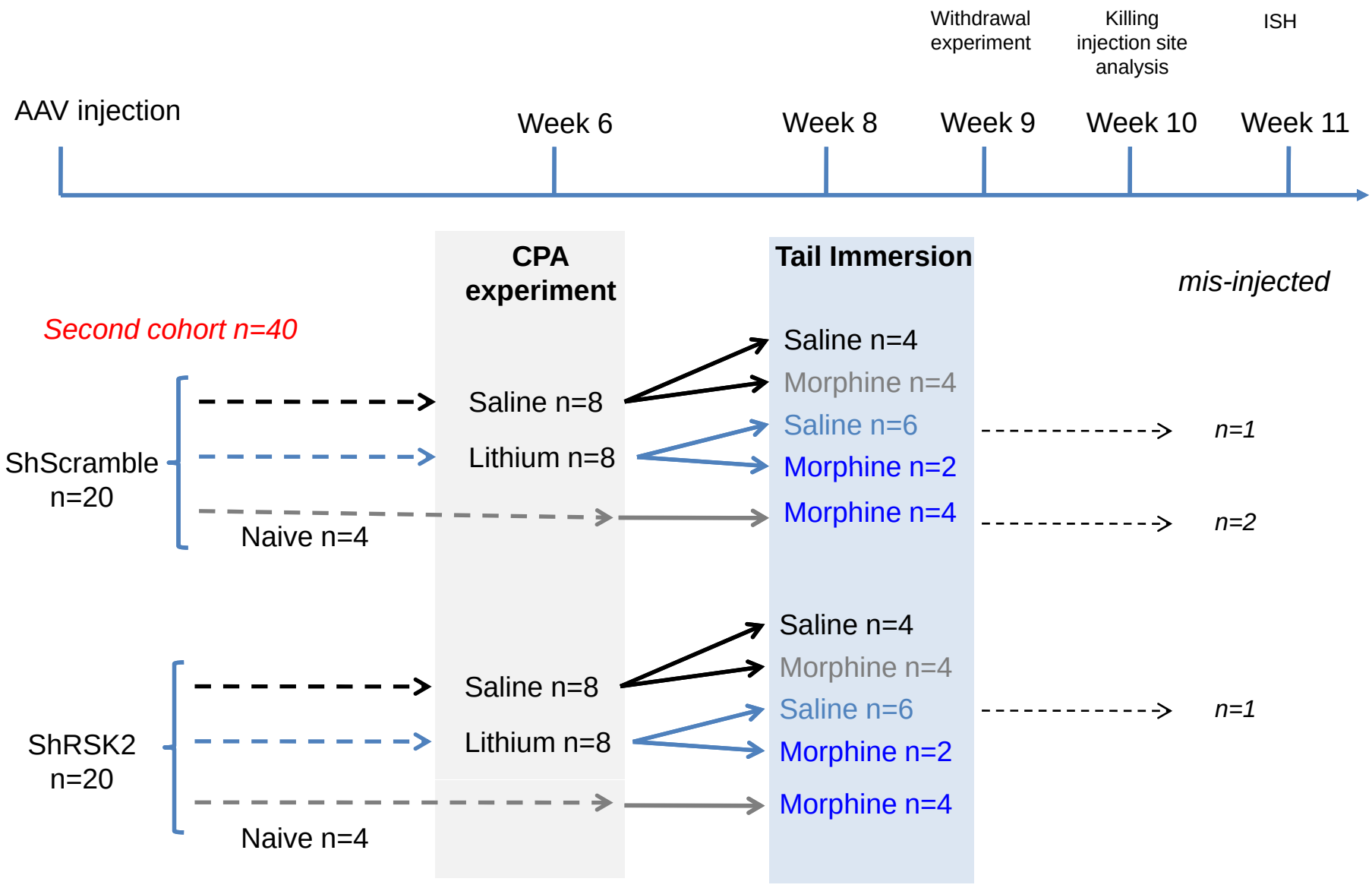
!

Time line of AAV experiments (cohort 1)

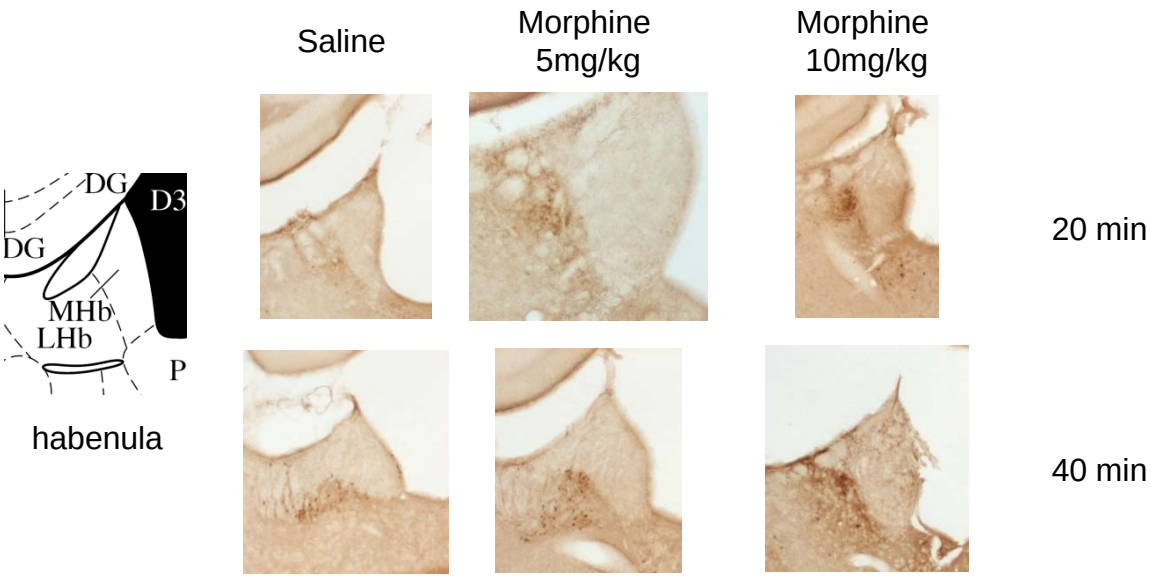


Darcq et al., Supplementary Figure 1 A

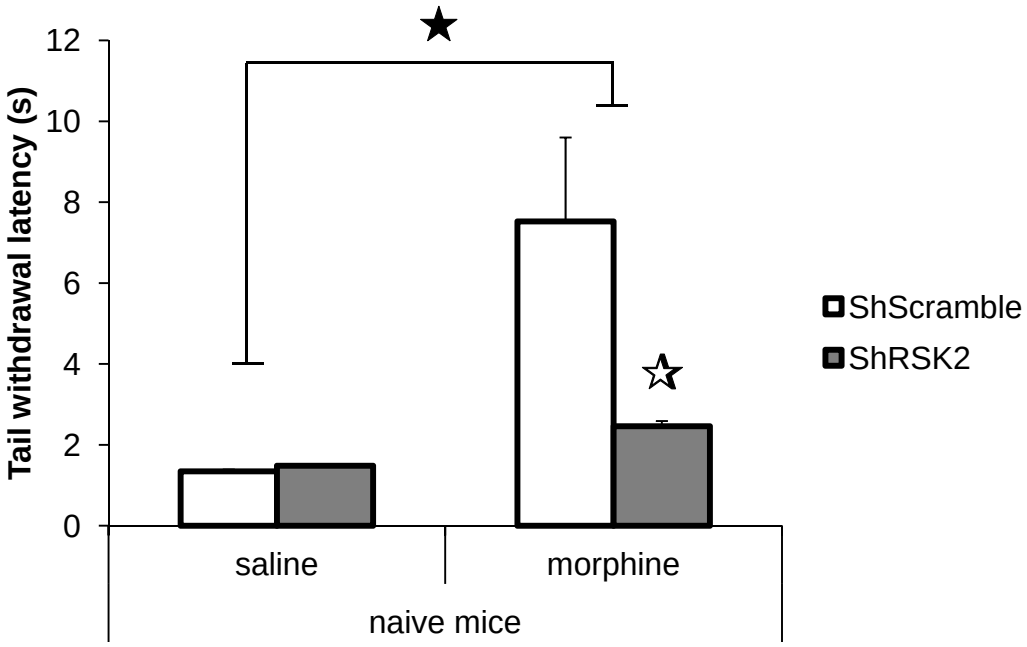
Time line of AAV experiments (cohort 2)



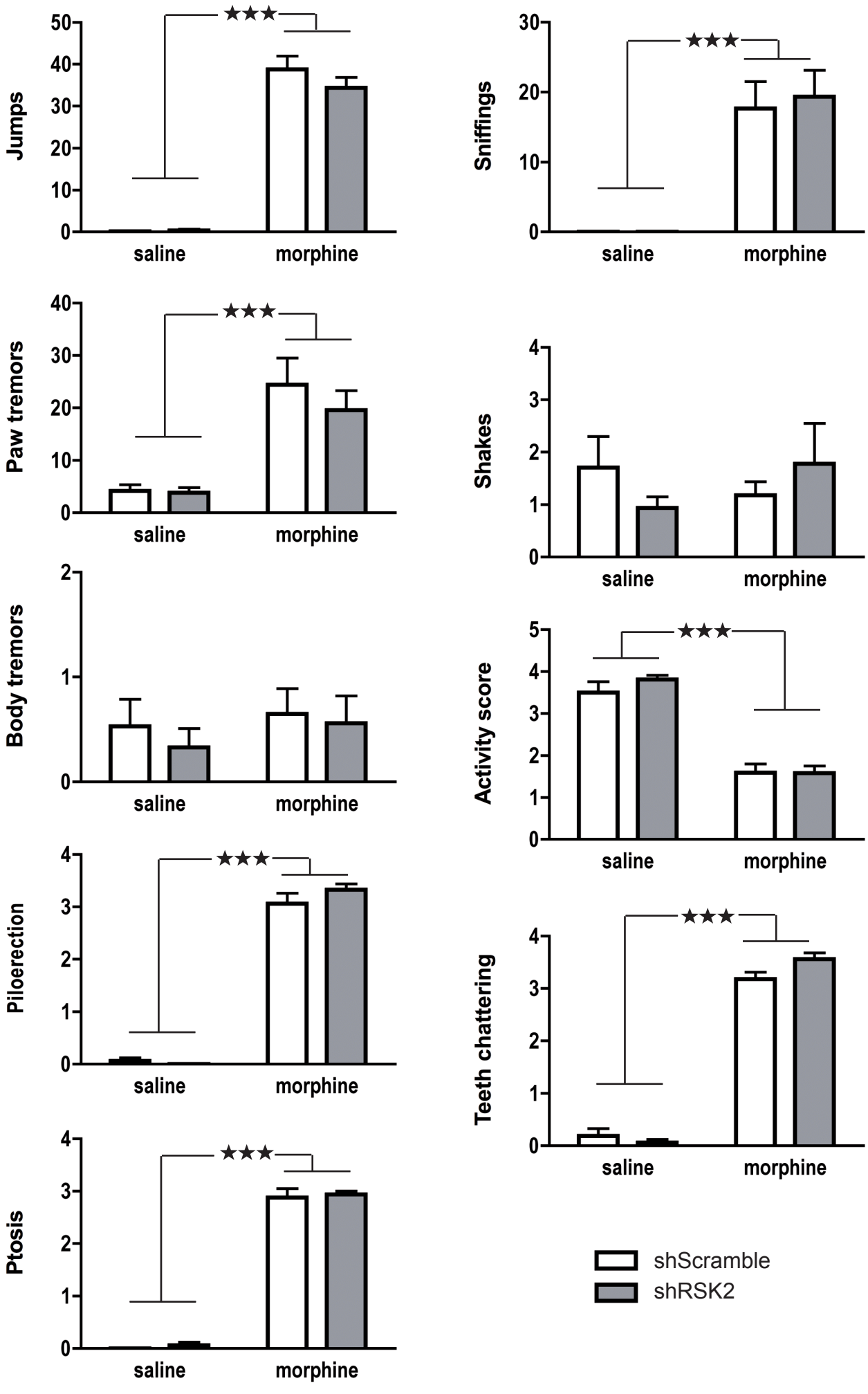
Darcq et al., Supplementary Figure 1 B



Darcq et al., Supplementary Figure 2



Darcq et al., Supplementary Figure 3



Darcq et al., Supplementary Figure 4