

*Supplementary materials for*

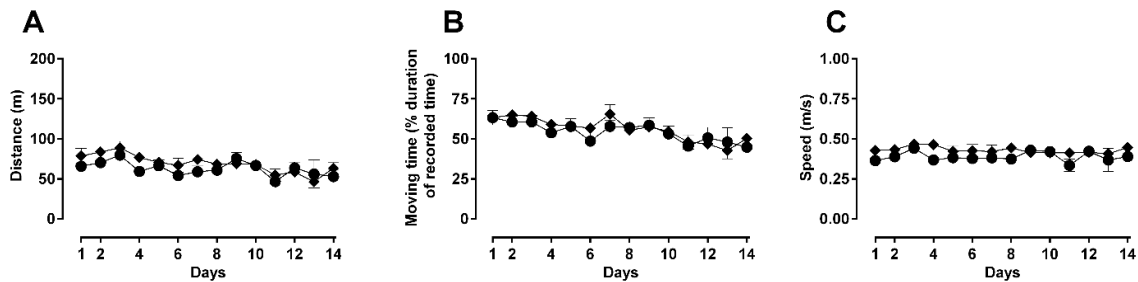
## Profiling the effects of repetitive morphine administration on motor behavior in rats

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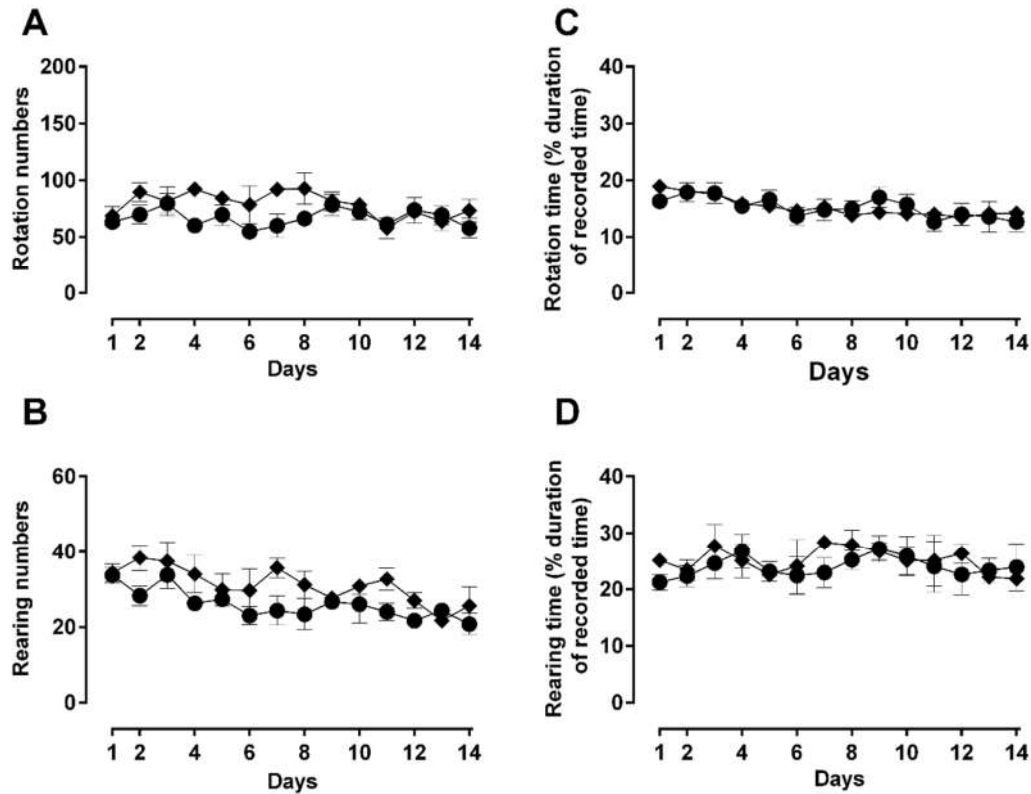
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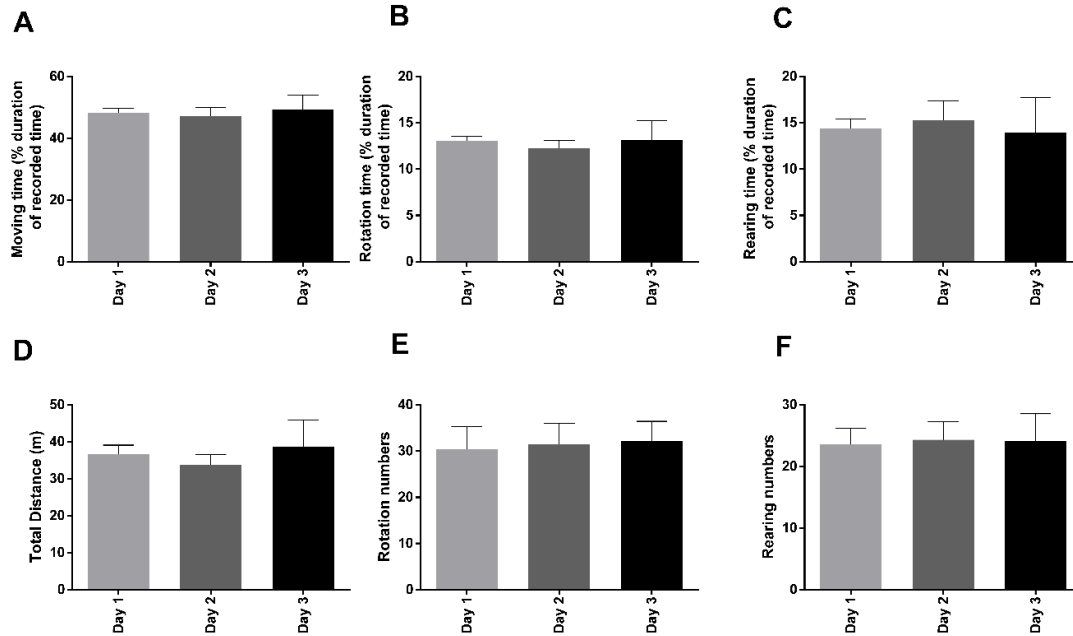
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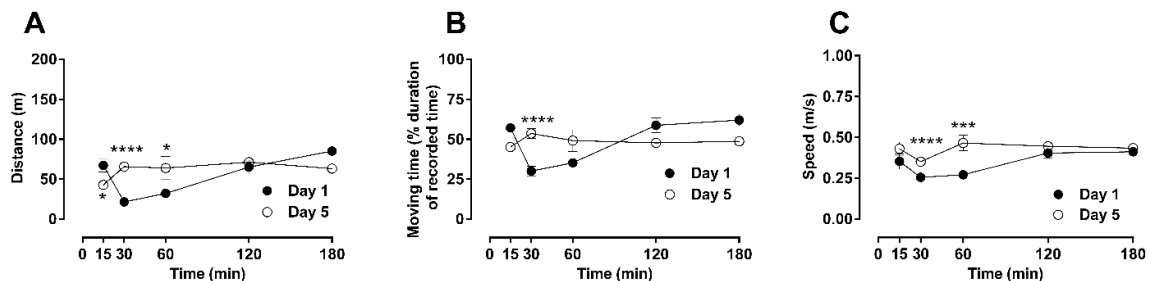
**Supplementary Figure S1.** Basal basic locomotor activities of rats. Open-field locomotor activities at pre-administration (basal) in male Sprague Dawley rats over a period of 14 days. Activities of animals were measured as distance traveled (A), moving time (B), or speed of movement (C). Values are presented as Mean  $\pm$  SEM (n = 6 animals per group). Error bars are sometimes too small to be visible. No statistically significant differences were observed against day 1 using one-way ANOVA with Dunnett's multiple comparisons tests.



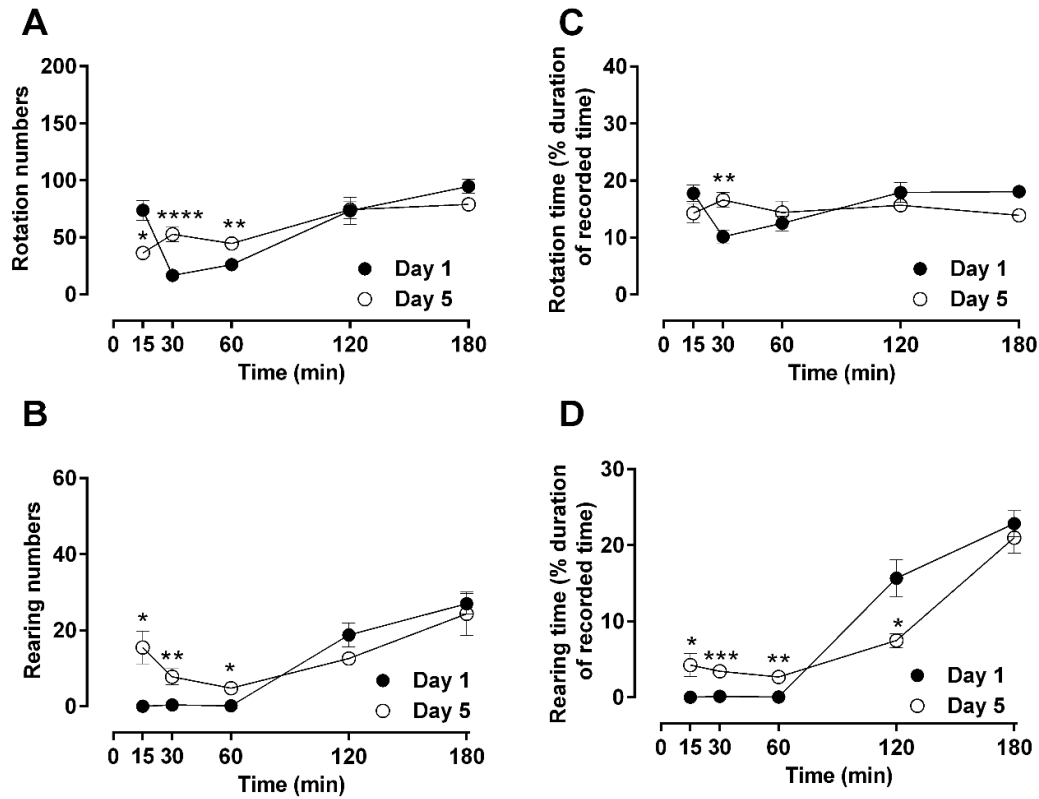
**Supplementary Figure S2.** Basal advanced locomotor activities (rotation and rearing) of rats. Open-field rotation and rearing activities at pre-administration (basal) in male Sprague Dawley rats over a period of 14 days. Activities of animals were measured as rotation no (**A**), rearing no (**B**), rotation time (**C**) and rearing time (**D**). Values are presented as Mean  $\pm$  SEM ( $n = 6$  animals per group). Error bars are sometimes too small to be visible. No statistically significant differences were observed against day 1 using one-way ANOVA with Dunnett's multiple comparisons tests.



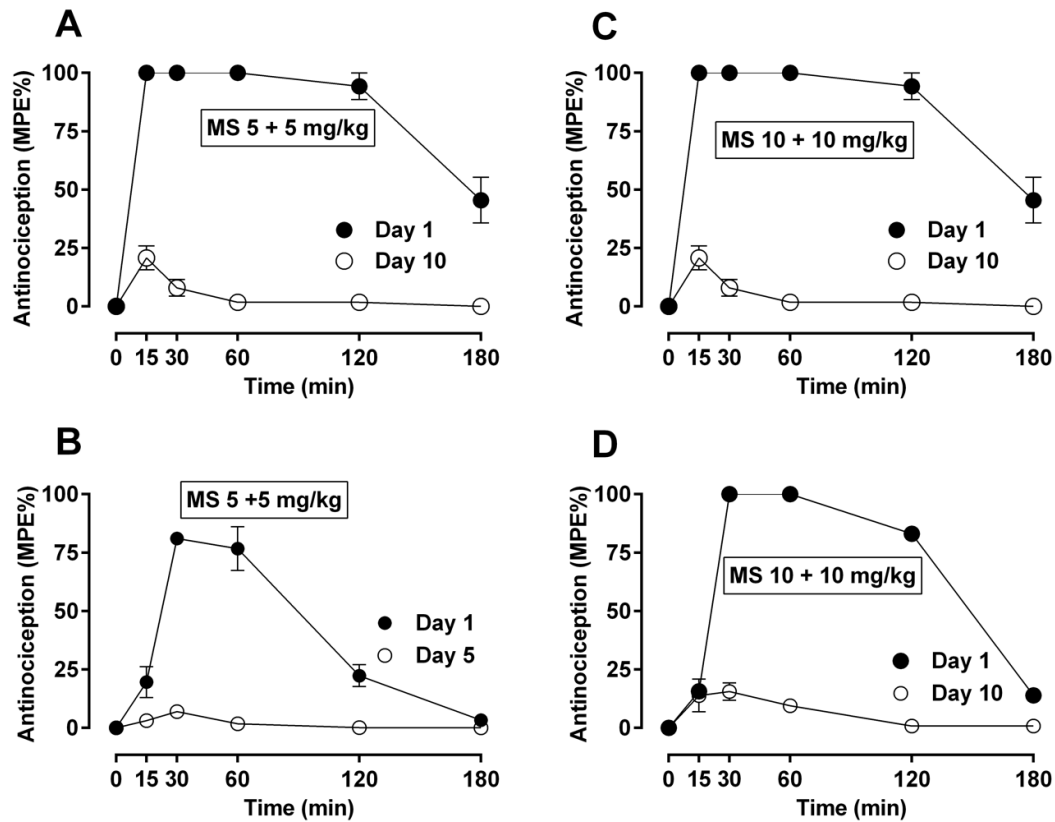
**Supplementary Figure S3.** Locomotor activities of control animals after repeated treatment. Locomotor parameters were recorded in an open-field arena daily over consecutive 3 days after subcutaneous injections of 0.9% w/v saline solution (b.i.d.) in male Sprague Dawley rats, at a daily 30 min mark post-administration during a 5 min recording period. The motor behavior of treated animals was assessed by quantification of moving duration (A), rotation duration (B), rearing duration (C), distance travelled (D), rotation numbers (E), or rearing numbers (F). Values are presented as mean  $\pm$  SEM (n = 6 animals per group). Error bars are sometimes too small to be visible. Statistically significant ( $p < 0.05$ ) differences compared against day 1 were calculated using one-way ANOVA with Dunnett's multiple comparisons test, but no significant changes were recorded.



**Supplementary Figure S4.** Time-resolved basic locomotor activities after repeated morphine treatment (5 mg/kg, b.i.d. over 5 days). Open-field locomotor activities after a single subcutaneous injection of morphine on day 1 or day 5 were measured in male Sprague Dawley rats. Activities of treated animals were measured as distance traveled (A), moving time (B), or speed of movement (C) over a period of 180 min. Statistical significance ( $p < 0.05$ ) at a specific time-point against the effects of day 1 is shown as \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$  and \*\*\*\* $P < 0.0001$  and was calculated using unpaired t-test. Values are presented as mean  $\pm$  SEM (n = 6 animals per group). Error bars are sometimes too small to be visible.



**Supplementary Figure S5.** Time-resolved advanced locomotor activities (rotation and rearing) after repeated morphine administration (5 mg/kg, b.i.d. over 5 days). Open-field turning and rearing activities after a single subcutaneous injection of morphine on day 1 or day 5 were measured in male Sprague Dawley rats. Activities of treated animals were measured as rotation no (A), rearing no (B), rotation time (C) and rearing time (D) over a period of 180 min. Statistically significance ( $p < 0.05$ ) at specific time-point against the effects of day 1 is shown as \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$  and \*\*\*\* $P < 0.0001$  and was calculated using unpaired t-test. Values are presented as mean  $\pm$  SEM ( $n = 6$  animals per group). Error bars are sometimes too small to be visible.



**Supplementary Figure S6.** Antinociceptive effects of daily morphine treated rats. Antinociceptive effects of 5 mg/kg (b.i.d.) and 10 mg/kg (b.i.d.) morphine treated Sprague Dawley rats ( $n = 6$  per group) over the course of 5 to 10 days (groups A and B). The antinociceptive effects were measured using tail-flick (A, C) and hot-plate (B, D) assays. The area under the curves were shown in Fig. 5. Values are presented as mean  $\pm$  SEM ( $n = 6$  animals per group). Error bars are sometimes too small to be visible.