Title: Long-term reduction of kappa opioid receptor function by norbinaltorphimine in peripheral sensory neurons requires c-Jun N-terminal kinase activity and new protein synthesis.

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Supplemental Figure Legends

Supplemental Figure 1: Long-term reduction in U50488-mediated inhibition of PGE_2-stimulated thermal allodynia in the rat hindpaw by norBNI is mediated by activation of JNK. Rats received intraplantar (i.pl.) injections of the JNK inhibitor, SP600125 (1 µg) or vehicle (Veh) 24 h and 30 min before injection of norBNI (30 ng, i.pl.) or vehicle. Two days (**A**), and 7 days (**B**) after norBNI injection, rats received co-injections of PGE_2 (0.3 µg, i.pl.) with either U50488 (0.1 µg, i.pl.) or vehicle. Paw withdrawal latencies (PWL) in response to a radiant heat stimulus applied to the ventral surface of the hindpaw were measured in duplicate before and at 5 min intervals after the last injection for 20 min. Data are expressed as the change in PWL (sec) from pre-injection baseline and represent the mean ± SEM of six rats per group Negative values for PWL reflect allodynia (increased sensitivity to the heat stimulus) and positive values reflect antinociception (decreased sensitivity).

Supplemental Figure 2: Long-term reduction of Sal-A-, but not DPDPE-, mediated inhibition of PGE2-stimulated thermal allodynia by norBNI. Rats were injected with norBNI (30 ng, i.pl.) or

vehicle nine days before co-injection of PGE_2 (0.3 µg, i.pl.) and the selective KOR agonist, Salvinorin-A (Sal-A, 0.1 µg, i.pl.) or two days before co-injection of PGE_2 (0.3 µg, i.pl.) and the selective DOR agonist, DPDPE (20 µg, i.pl.). PWL in response to a radiant heat stimulus applied to the ventral surface of the hindpaw was measured in duplicate before and at 5 min intervals after the last injection. Data are expressed as the change in PWL (in sec) from pre-injection baseline and represent the mean ± SEM of 6 animals.

Supplemental Figure 3: Dose-response for long-term inhibitory effect of norBNI on U50488-mediated antinociception. Rats were injected (i.pl.) with the indicated doses of norBNI (0.3, 3, or 30 ng) or vehicle (Veh). Two days later rats received injections (i.pl.) of PGE_2 (0.3 µg) with U50488 (0.1 µg) or vehicle. PWL in response to application of a radiant heat stimulus to the ventral surface of the hindpaw was measured in duplicate before and at 5 min intervals following the last injection. **A)** Data are expressed as the mean ± SEM of the area under the time course curves (AUC) for each group of 6 animals. ** $P < 0.05$, compared to veh/PGE_2 . **B)** Data are expressed as the change in PWL (in sec) from pre-injection baseline and represent the mean ± SEM of 6 animals. The 3 ng dose of norBNI was chosen for the experiment to surmount norBNI occupancy of KOR with U50488 as shown in Figure 3.

Supplemental Figure 4: Pre-treatment with the KOR agonist, U50488, blocked the long-term inhibitory effect of norBNI. Rats received injections (i.pl.) of vehicle, U50488 (100 µg, 10,000xK_i), norBNI (3 ng, 100xK_i), or U50488 (100 µg) with norBNI (3 ng). Two (**A**) and seven (**B**) days later, U50488-mediated inhibition of PGE_2-stimulated thermal allodynia was measured. Rats received co-injections (i.pl.) of PGE_2 (0.3 µg) with U50488 (0.1 µg). PWL in response to application of a radiant heat stimulus to the ventral surface of the hindpaw was measured in duplicate before and at 5 min intervals

following the co-injection of PGE_2 plus U50488. Data are expressed as the change in PWL (in sec) from pre-injection baseline and represent the mean ± SEM of 6 animals.

Supplemental Figure 5: Long term reduction of U50488-mediated inhibition of PGE_2-stimulated thermal allodynia by norBNI is peripherally mediated. Rats received injections (i.pl.) of norBNI (30 ng) or vehicle (Veh). Two **(A)** and seven **(B)** days later, Rats received co-injections (i.pl.) of PGE_2 (0.3 µg) with U50488 (0.1 µg) in either the contralateral or the ipsilateral hindpaw. PWL in response to application of a radiant heat stimulus to the ventral surface of the hindpaw was measured in duplicate before and at 5 min intervals following the co-injection of PGE_2 plus U50488. Data are expressed as the change in PWL (in sec) from pre-injection baseline and represent the mean ± SEM of 6 animals.

Supplemental Figure 6: Washout of norBNI from cultures of peripheral sensory neurons. Cells were pre-treated with norBNI (3 nM) or vehicle for 15 min and washed thoroughly for 30 min. Then cells were treated with norBNI (3 nM) or vehicle with PGE_2 (1 µM) and U50488 (100 nM) for 15 min before measuring cAMP levels. Data are expressed as the percentage of PGE_2-stimulated cAMP levels and represent the mean ± SEM of four experiments. Data were analyzed with one-way ANOVA followed by Dunnett's post hoc test. ***$P < 0.001$ compared with vehicle pre-treated cells.

Supplemental Figure 7: The long-term inhibitory effect of norBNI in vivo is blocked by inhibitors of protein translation. Rats received injections (i.pl.) of cycloheximide, CHX (25 µg; **A,B**), rapamycin (RAPA, 12 µg; **C,D**) or vehicle 30 min prior to and 24 h following injection (i.pl.) of norBNI (30 ng) or vehicle. Two **(A,C)** and seven days **(B,D)** following norBNI injection, rats received co-injections (i.pl.) of PGE_2 (0.3 µg) with U50488 (0.1 µg) or vehicle. PWL in response to application of a radiant heat stimulus to the ventral surface of the hindpaw was measured in duplicate before and at 5 min intervals

following the last injection. Data are expressed as the change in PW (in sec)L from pre-injection baseline and represent the mean ± SEM of 6 animals.

Supplemental Figure 8: Long-term reduction of U50488-mediated antinociception by norBNI was not blocked by the protein synthesis inhibitor, cycloheximide, given 5-6 days after norBNI injection. Rats were injected (i.pl.) with norBNI (30 ng) or vehicle (Veh). Five and six days following norBNI administration, rats were injected (i.pl.) with cycloheximide (CHX, 25 µg) or vehicle. On day 7, rats were co-injected (i.pl.) with PGE_2 (0.3 µg) and U50488 (0.1 µg) and PWL in response to a radiant heat stimulus applied to the ventral surface of the hindpaw was measured in duplicate before and every 5 min for 20 min after the last injection. **A)** Data are expressed as the mean ± SEM of the area under the time course curves (AUC) for each group of 6 animals and were analyzed with one-way ANOVA and Dunnett's post hoc test. $^{**}P < 0.01$ compared with norBNI-vehicle treated rats, $^{\dagger}P < 0.05$ compared with norBNI-CHX treated rats. **B)** Data are expressed as the change in PWL from pre-injection baseline values and represent the mean ± SEM of 6 animals.

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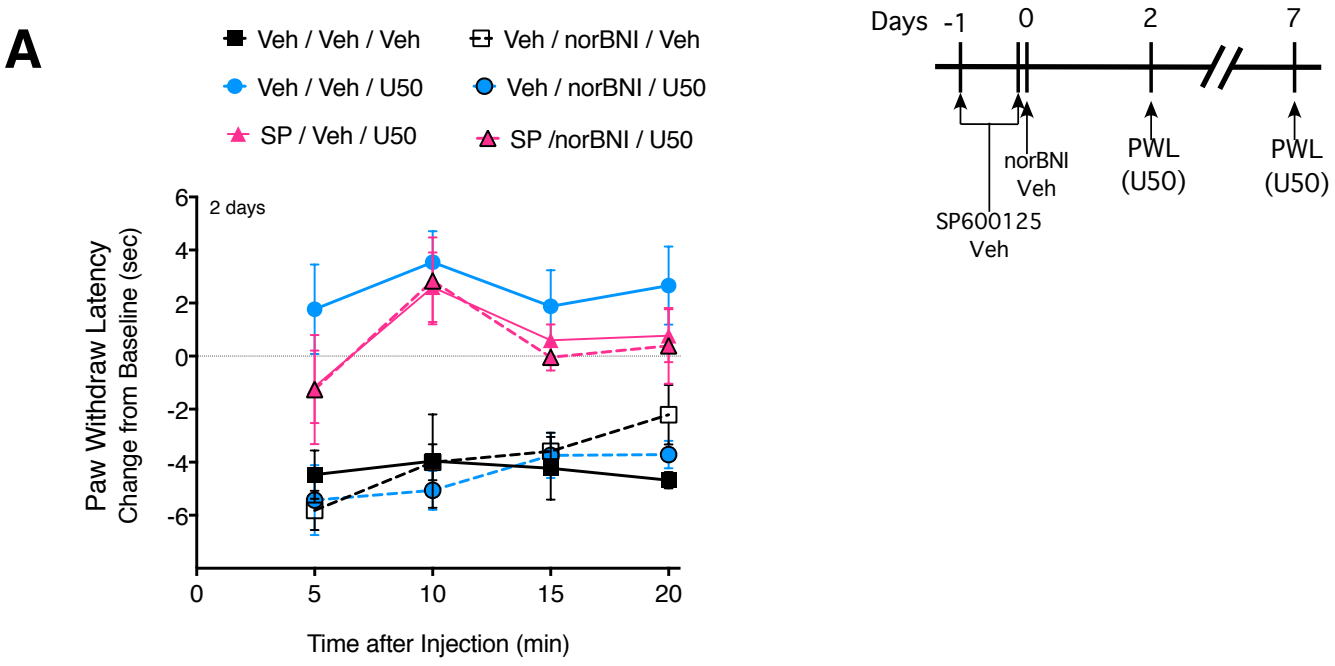

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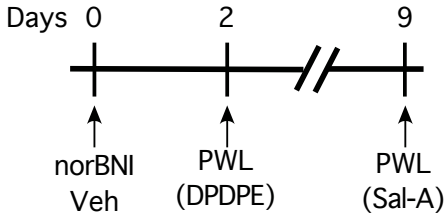

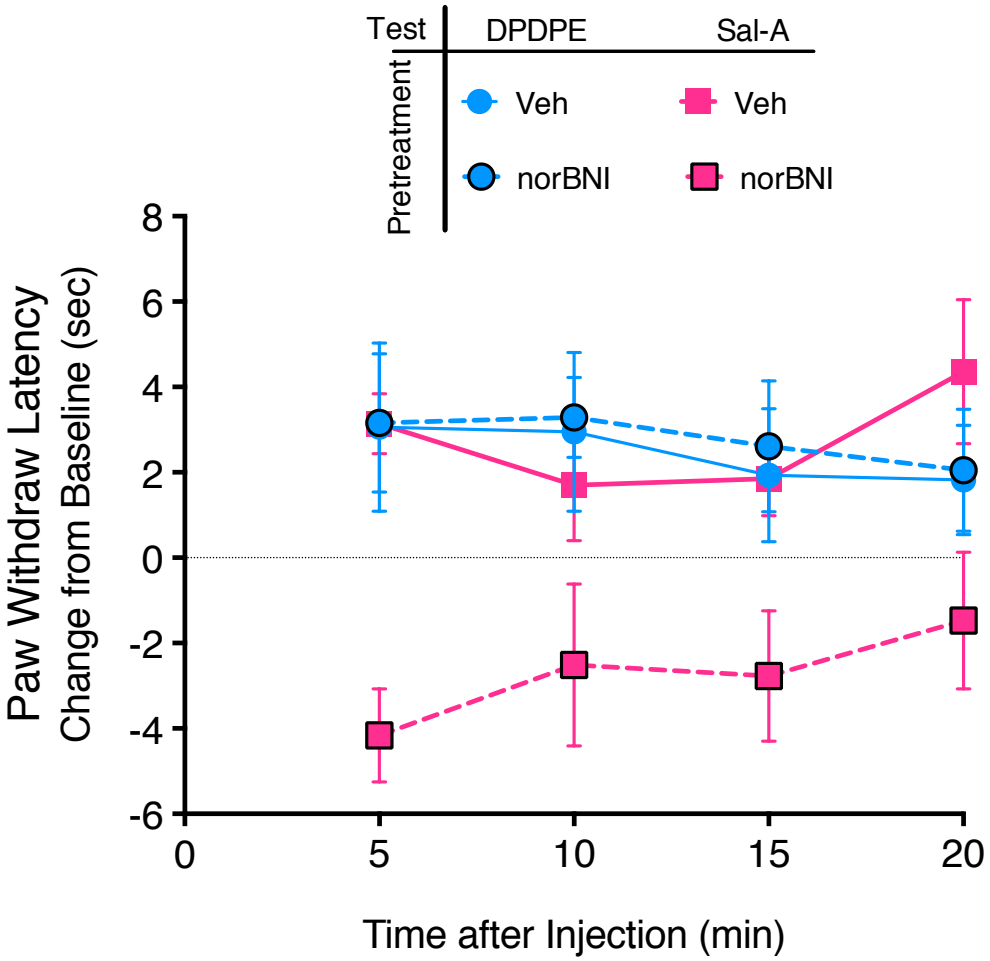

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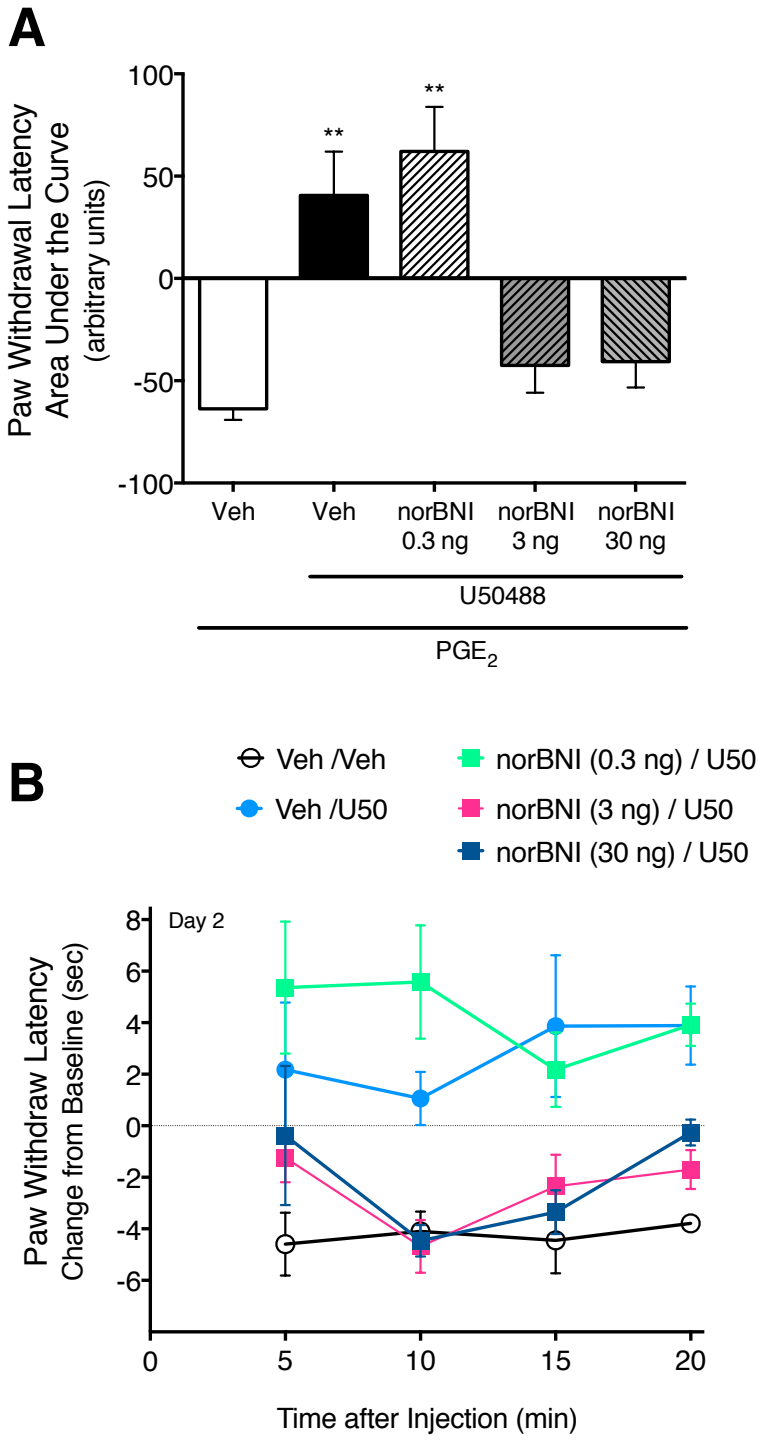

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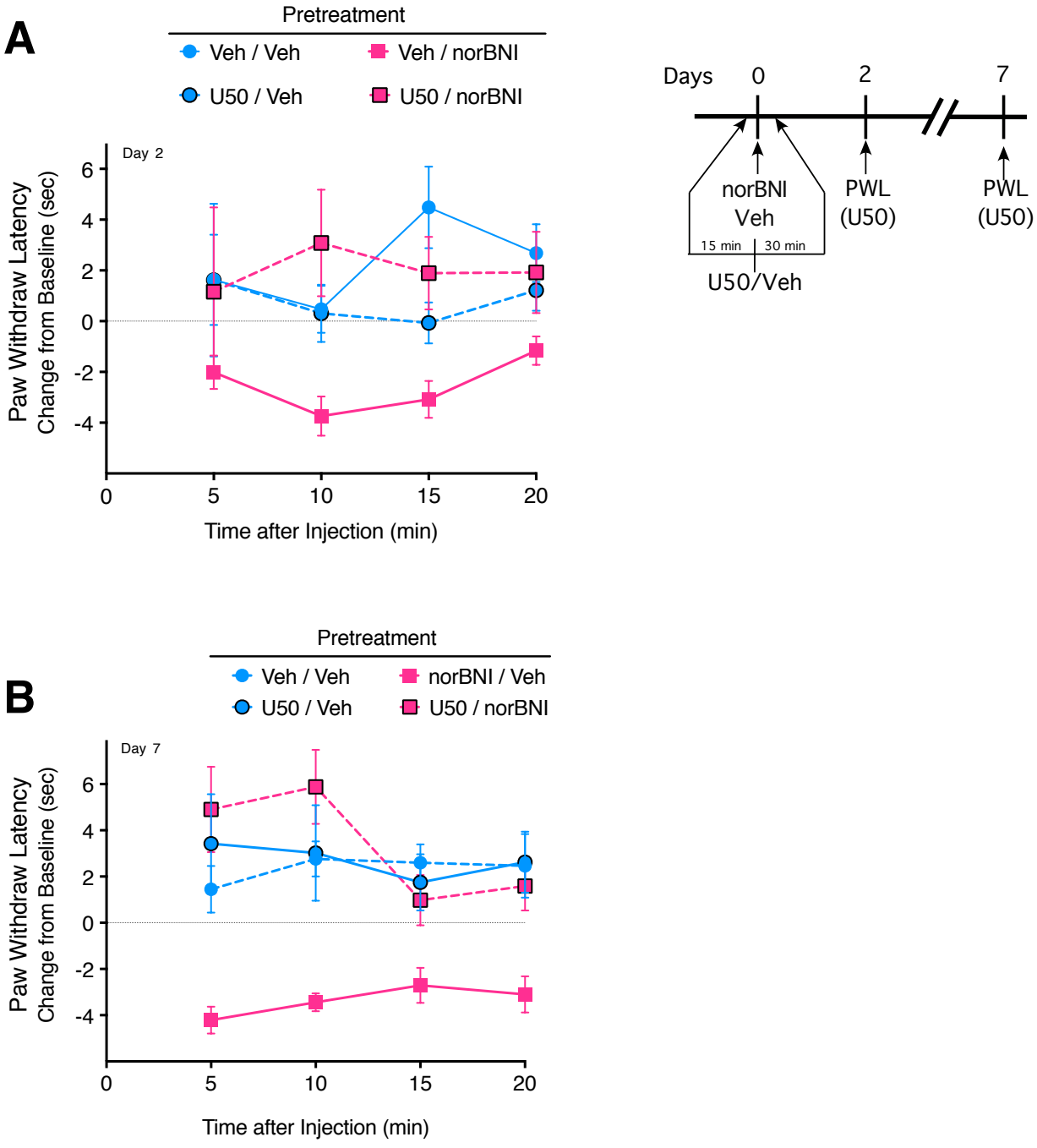

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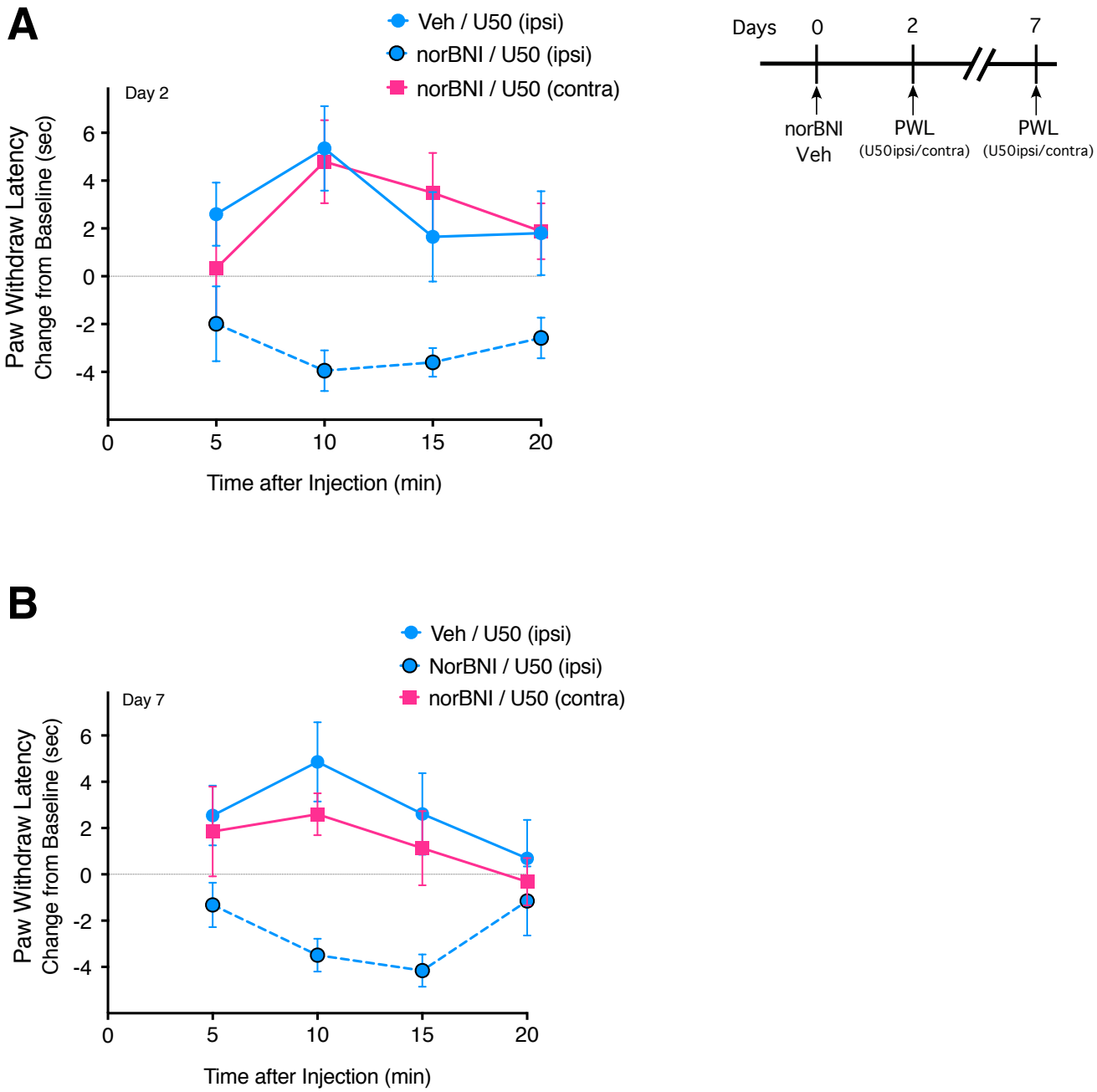

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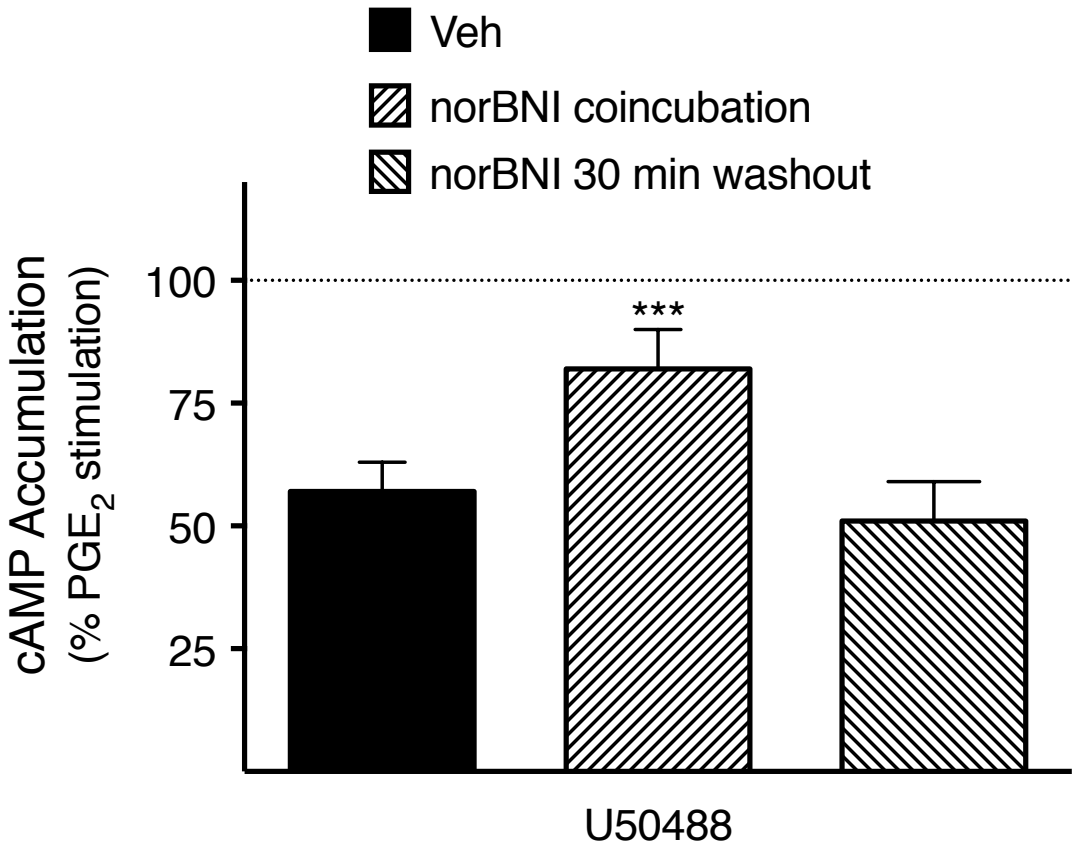

Supplemental Figure 6

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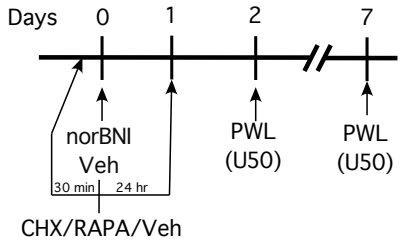

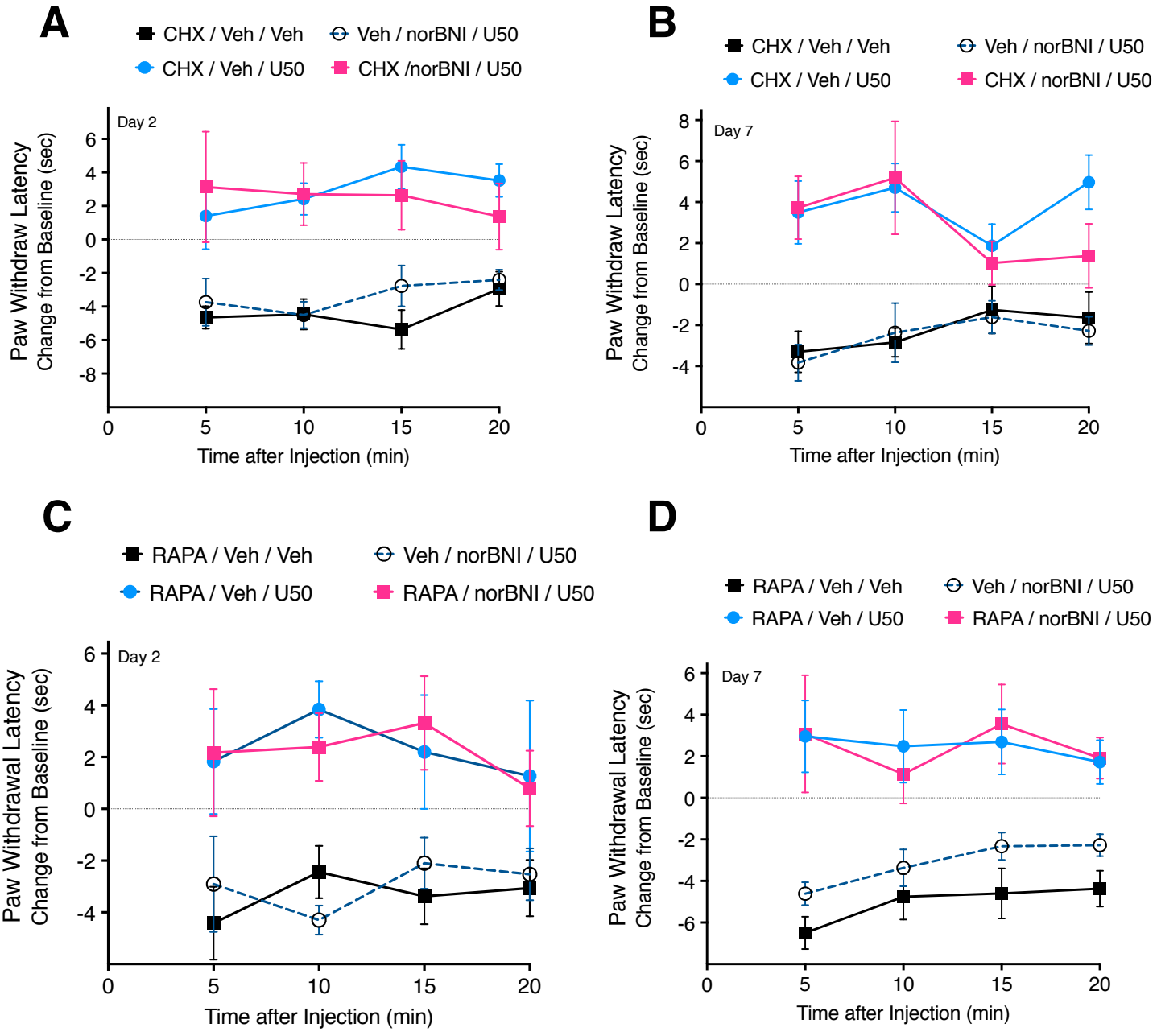

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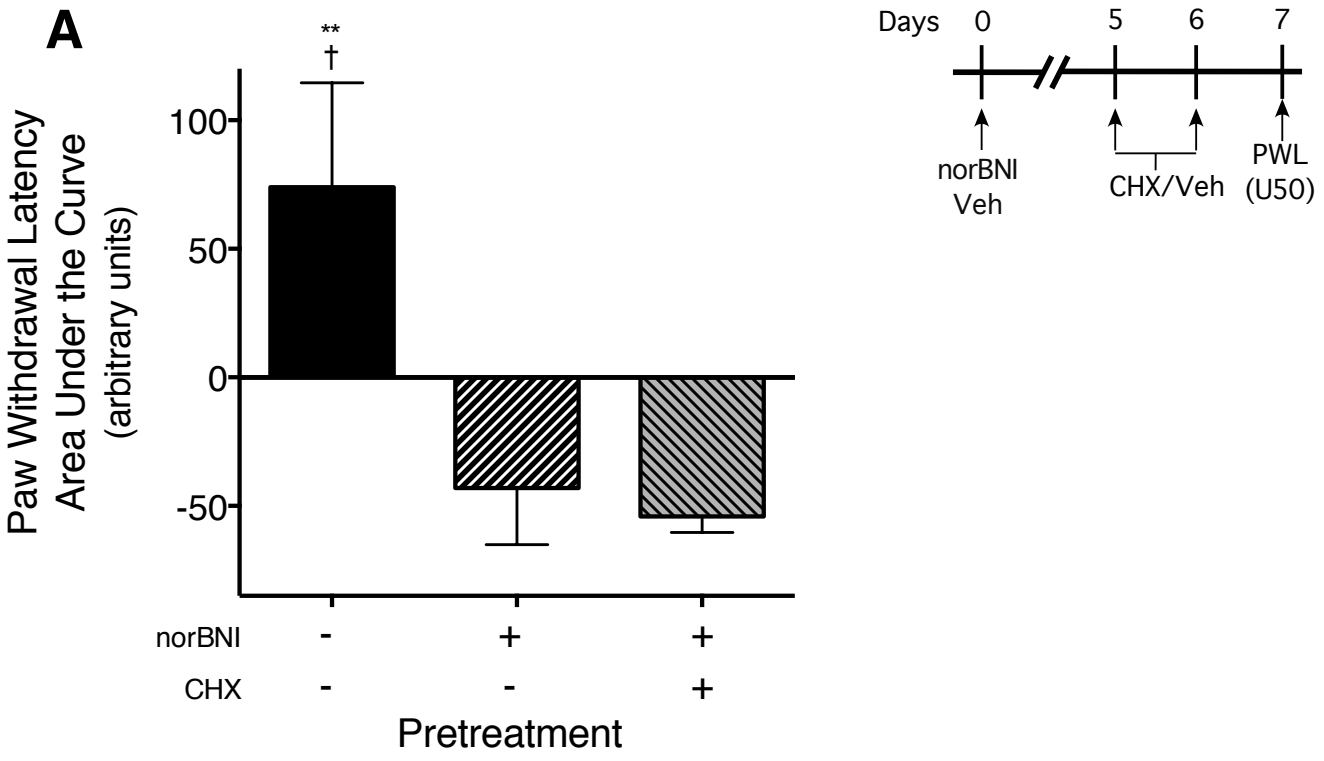

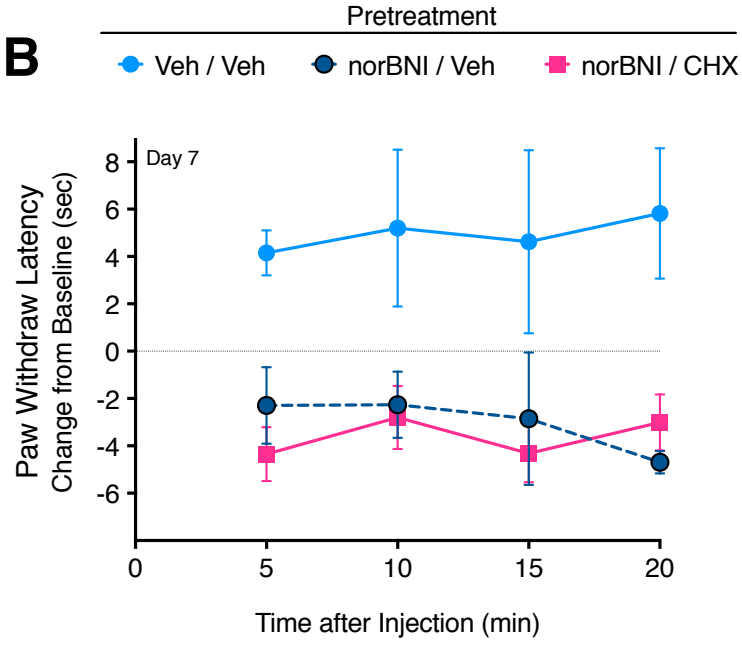

Supplemental Figure 8