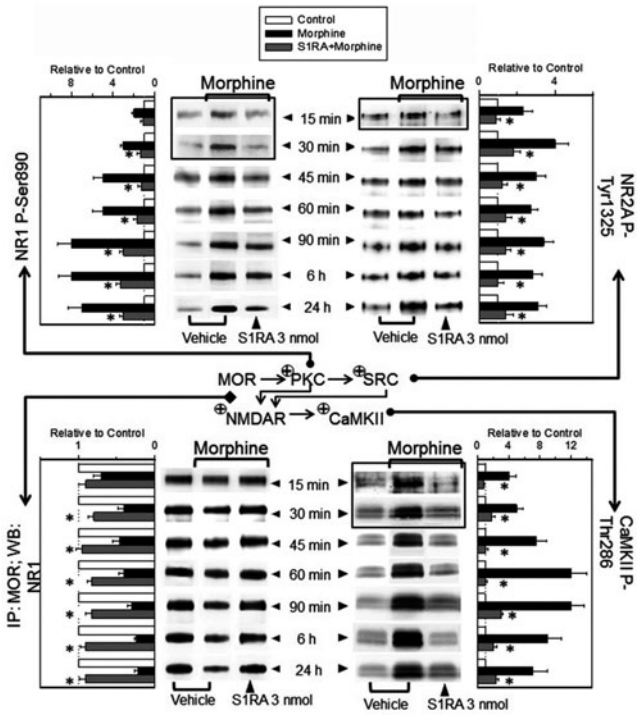

SUPPLEMENTARY FIG. S4. Representative blots describing the effect of the σ1R antagonist S1RA on MOR-mediated activation of NMDARs. Groups of 42 mice each received 10 nmol of morphine alone or 3 nmol of S1RA at 30 min before opioid treatment. Subsequently, for each group receiving morphine, six mice were sacrificed at the indicated intervals. Control mice received saline instead of morphine. PAG synaptosomes were obtained to determine the presence of CaMKII P-Thr286, NR1 C1 P-Ser890, and NR2A P-Tyr 1325. The MOR proteins were immunoprecipitated, and the associated NR1 subunits were determined through Western blotting. The icv administration of 10 nmol morphine increased the function of NMDARs. The opioid administration increased the phosphorylation of the NR1 (PKC on S890) and NR2 subunits (Src on Y1325). Moreover, morphine increased the Thr286 autophosphorylation of the serine and threonine kinase CaMKII. A reduction of the MOR-NR1 association, an index of MOR recruitment of NMDAR activity, was also observed. The administration of 3 nmol of S1RA at 30 min before morphine significantly reduced parameters of NMDAR activity. Immunosignals (average optical density of the pixels within the object area/mm^2; Quantity One Software; Bio-Rad, Madrid, Spain) were expressed as the change relative to the Control group (attributed an arbitrary value of 1). Each bar represents the mean ± SEM of the data from three determinations performed in different gel blots. *For every postopioid, interval indicates a significant difference with respect to the value of the group treated with only morphine, ANOVA-Student–Newman–Keuls test; $p < 0.05$. We observed no differences in the levels of the nonphosphorylated proteins. To increase readability, the frames indicate higher exposition of the blots. Because the groups in the comparison are equally affected through this enhancement, the result remains unaltered. icv, intracerebroventricular; IP, immunoprecipitation; WB, Western blot analysis.