



**SUPPLEMENTARY FIG. S5. Effect of *in vivo* NOS inhibition on MOR-mediated activation of NMDARs.** The mice received saline or 7 nmol L-NNA (an inhibitor of NOS) at 30 min before the icv-injection of 10 nmol morphine; subsequently, six mice were sacrificed at the indicated intervals. PAG synaptosomes were obtained and solubilized as described in Materials and Methods section. The MOR proteins were immunoprecipitated, and the associated NR1 subunits, PKC $\gamma$  and HINT1 proteins were determined through Western blotting. The presence of NR1 C1 P-Ser890, NR2A P-Tyr 1325, CaMKII P-Thr286, and total CaMKII was determined through SDS-PAGE followed by Western blotting analysis of PAG synaptosomes. Immunosignals (average optical density of the pixels within the object area/mm<sup>2</sup>, Quantity One Software; Bio-Rad) were expressed as the change relative to the control group (attributed an arbitrary value of 1, *dashed line*). Each bar represents the mean  $\pm$  SEM of the data from three determinations performed in different gel blots. \*Indicates a significant difference with respect to the value of the three corresponding control group, ANOVA-Student-Newman-Keuls test;  $p < 0.05$ . We observed no differences in the levels of the non phosphorylated proteins. SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis.