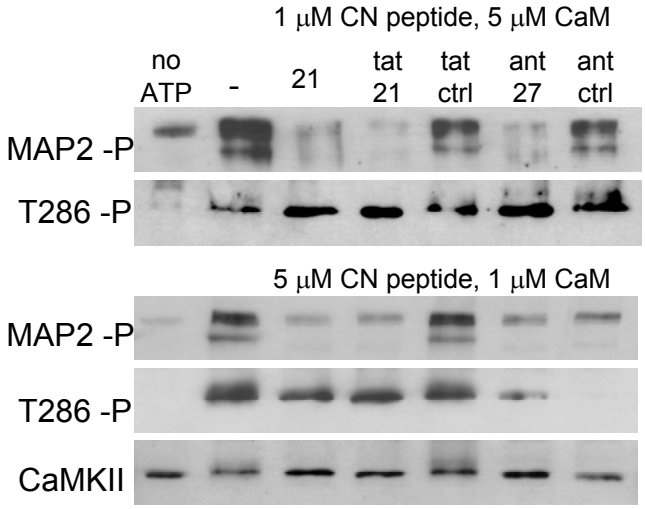
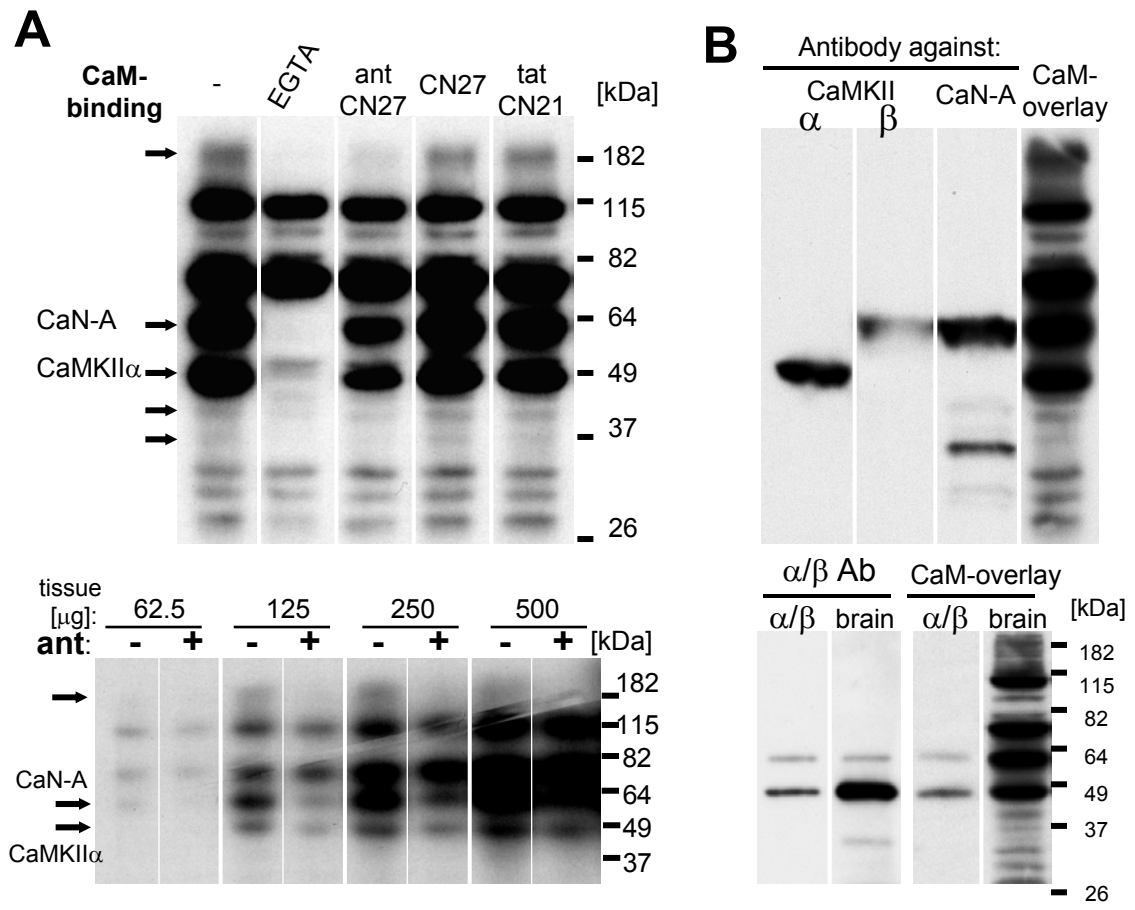
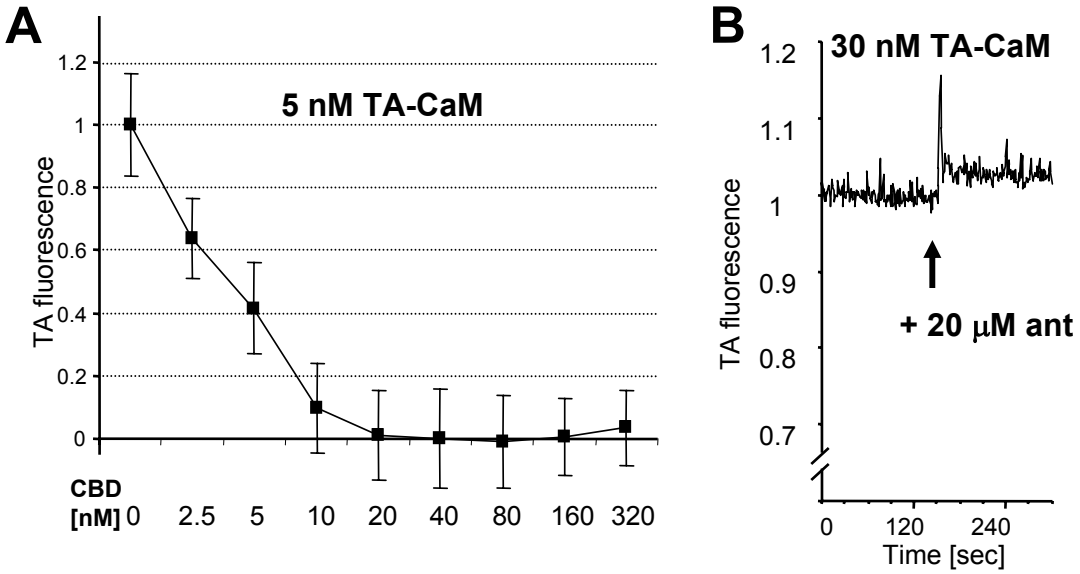


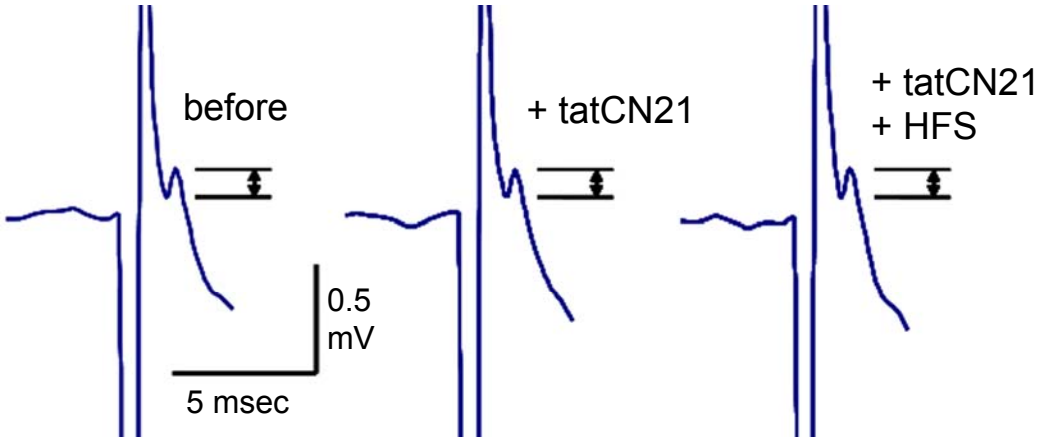
Supplementary Figure 1: Differential effect of tat- and ant-CN fusion peptides on CaMKII substrate- and auto-phosphorylation. Reactions were performed in presence of 1 μM CN peptides and 5 μM CaM (upper panels) or 5 μM CN peptides and 1 μM CaM (lower panels), with 20 nM CaMKII and 10 nM MAP2. Phosphorylation of MAP2 and CaMKII T286 auto-phosphorylation were assessed by Western-analysis. As seen for non-fused CN21 (Vest et al., 2007), tatCN21 effectively blocked MAP2 phosphorylation under all conditions. T286 autophosphorylation is the only CaMKII reaction not effectively blocked by CN21, due to the mechanism of inhibition (Vest et al., 2007), and the same was observed for tatCN21. The tat control peptide had no effect on any phosphorylation. Similar results were obtained for the antCN27 and the ant control peptide, but only at low peptide/high CaM concentration. At high peptide/low CaM, both ant peptides interfered with MAP2- and auto-phosphorylation, as predicted based on their additional CaM-competitive mode of inhibiton generated by ant fusion.



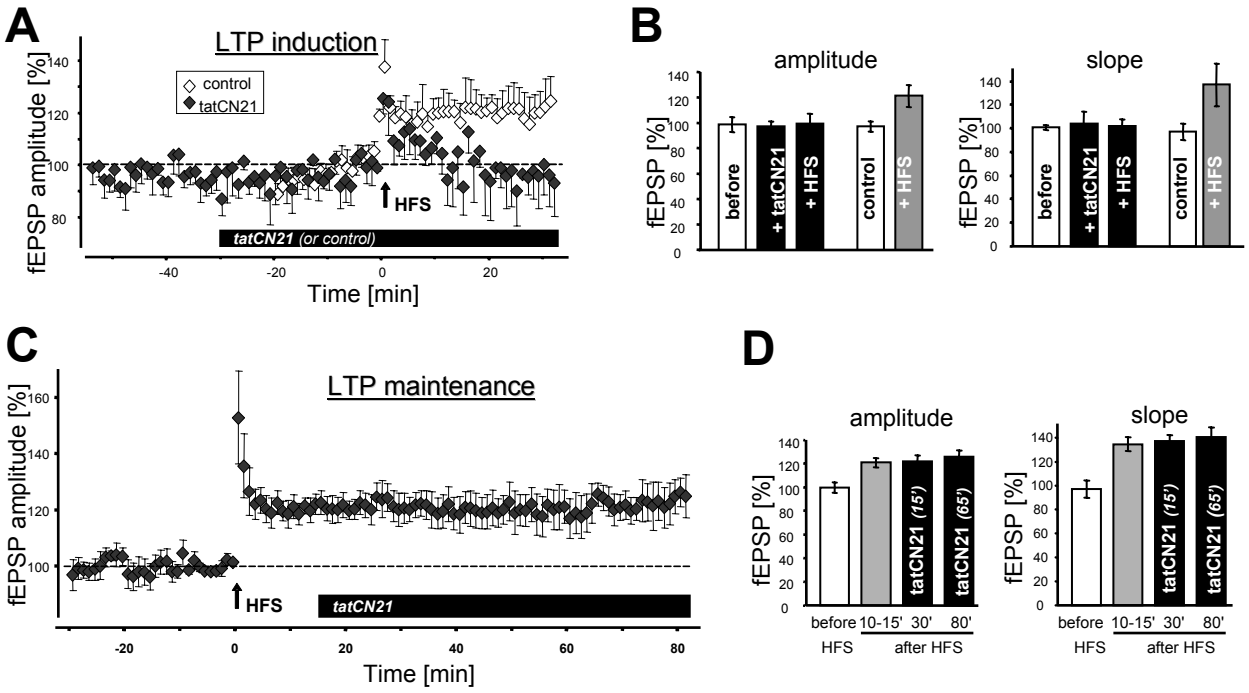
Supplementary Figure 2: (**A**) Ca^{2+}-dependent (arrows) and –independent CaM binding to additional proteins was detected as in Fig. 2B, but after longer exposure. Again, antCN27 (upper panel) and ant (lower panel) interfered specifically with the Ca^{2+}-dependent binding. (**B**) Identification of calcineurin A (CaN-A) and CaMKIIα as the major Ca^{2+}-dependent CaM binding proteins in rat brain extract using specific antibodies. Both proteins were suspected based on the mobility in the gel and their known abundance in brain. The CaN-A and the CaMKIIβ isoform have a very similar mass (~62 kDa) and their bands were not clearly distinguishable. However, CaN-A was identified as the main CaM-binding protein by comparing the signals from biotinylated CaM and an antibody that detects CaMKIIα and β equally well (BD Bioscience), both on brain extract protein and on purified α and β (in a 4:1 mix).



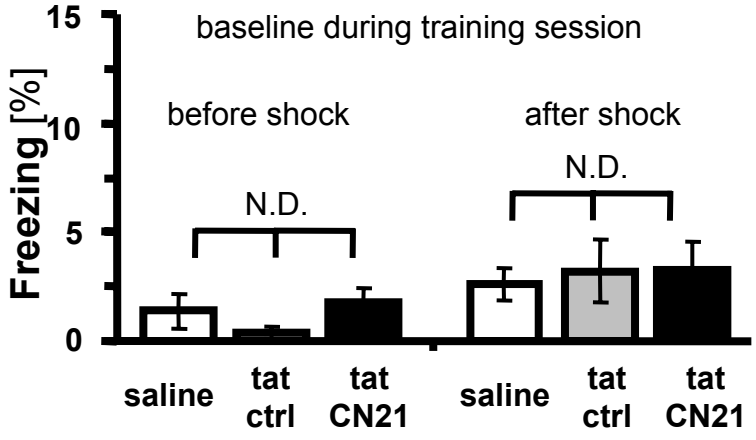
Supplementary Figure 3: TA-CaM fluorescence changes upon peptide addition. (**A**) Titration of 5 nM TA-CaM with increasing concentrations of CBD (CaM binding domain, CaMKII amino acids 290- 309; Calbiochem) indicated a k_D of CBD for CaM binding below 2.5 nM. Error bars show standard deviation. The high affinity prevented determination of the actual k_D by steady state measurement. However, more accurate analysis by Torok et al. (2001), using stopped-flow kinetics, determined a k_D of ~0.05 nM. (**B**) In contrast to CBD, ant does not decrease TA-CaM fluorescence. The slight increase in fluorescence does not allow reliable titration of the binding affinity. However, this enabled the competition experiments of ant with CBD for CaM binding shown in Fig. 2C.



Supplementary Figure 4: Presynaptic fiber potential was not affected by 20 min perfusion perfusion of hippocampal slices with 5 μM tatCN21. Shown are representative samples of the traces used in the quantification shown in Fig. 4C.



Supplementary Figure 5: Perfusion with tatCN21 (5 mM) did not affect basal transmission or LTP maintenance, but efficiently blocked LTP induction. (**A**) tatCN21 did not affect basal transmission but blocked LTP induction by high frequency stimulations (HFS)(n=5), compared to untreated control (n=5). fEPSP amplitude over time is shown as percent of baseline (compare slope shown in Fig. 4A). (**B**) Quantification of average fEPSP amplitude and slope at different time points before and after tatCN21 application and HFS (of the experiments shown in panel A and Fig. 4A) in bar graph representation. (**C**) tatCN21 perfusion 15 min after LTP induction by HFS did not interfere with LTP maintenance (n=5). fEPSP amplitude is shown as percent of baseline (compare slope shown in Fig. 4D). (**D**) Quantification of average fEPSP amplitude and slope at different time points before and after HFS and tatCN21 application (of the experiments shown in panel C and Fig. 4D) in bar graph representation. Error bars indicate s.e.m. in all panels.



Supplementary Figure 6: The tatCtrl and tatCN21 peptides (10 mg/kg i.p.) did not affect baseline freezing during the training session (N.D.; $p>0.4$, ANOVA). Error bars indicate s.e.m.