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Supplemental information

Spatial regulation of coordinated excitatory and inhibitory synaptic plasticity at dendritic synapses

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SUPPLEMENTARY FIGURES

Figure S1

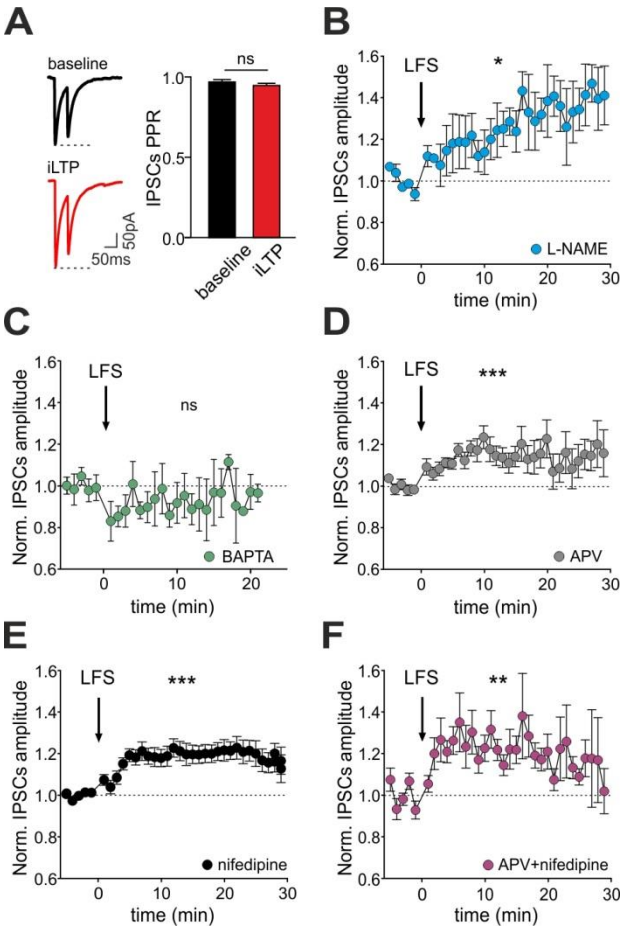

Figure S1 (related to Figure 1): Postsynaptic mechanism and Ca^{2+} dependence of LFS-induced iLTP

A. Left: Representative IPSC paired pulses traces recorded before (baseline) and after (iLTP) the delivery of the LFS. Right: Quantification of the paired pulse ratio (PPR) (before LFS= 0.96 ± 0.02; after LFS= 0.94 ± 0.02; n = 25; p > 0.05, Wilcoxon matched-pairs test). **B.** The nitric oxide synthase blocker L-NAME does not prevent LFS-induced iLTP (at 25-30 min: normalized IPSCs amplitude = 1.40 ± 0.02 of baseline, n = 5, $F_{33,136}$ = 1.6, p < 0.05; one-way ANOVA followed by Tukey's multiple comparison test). **C-E.** Time course of relative IPSC amplitude changes after the delivery of the LFS protocol (arrow) with respect to baseline, in the presence of the fast Ca^{2+} chelator BAPTA (**C**: 0.97 ± 0.04 fold of baseline, n = 4, $F_{25,69}$ = 0.4, p > 0.05), APV (**D**: 1.16 ± 0.01 fold of baseline, n = 11, $F_{33,241}$ = 2.2, p < 0.001), nifedipine (**E**; 1.16 ± 0.01 fold of baseline, n = 21, $F_{33,640}$ = 3.6, p < 0.001), and APV + nifedipine (**F**: 1.13 ± 0.03 fold of baseline, n = 6, $F_{33,162}$ = 2.1, p < 0.01). One-way ANOVA followed by Tukey's multiple comparison test. Values are expressed as mean ± SEM. *p < 0.05, **p < 0.01, ****p < 0.001, ns = not significant.

Figure S2

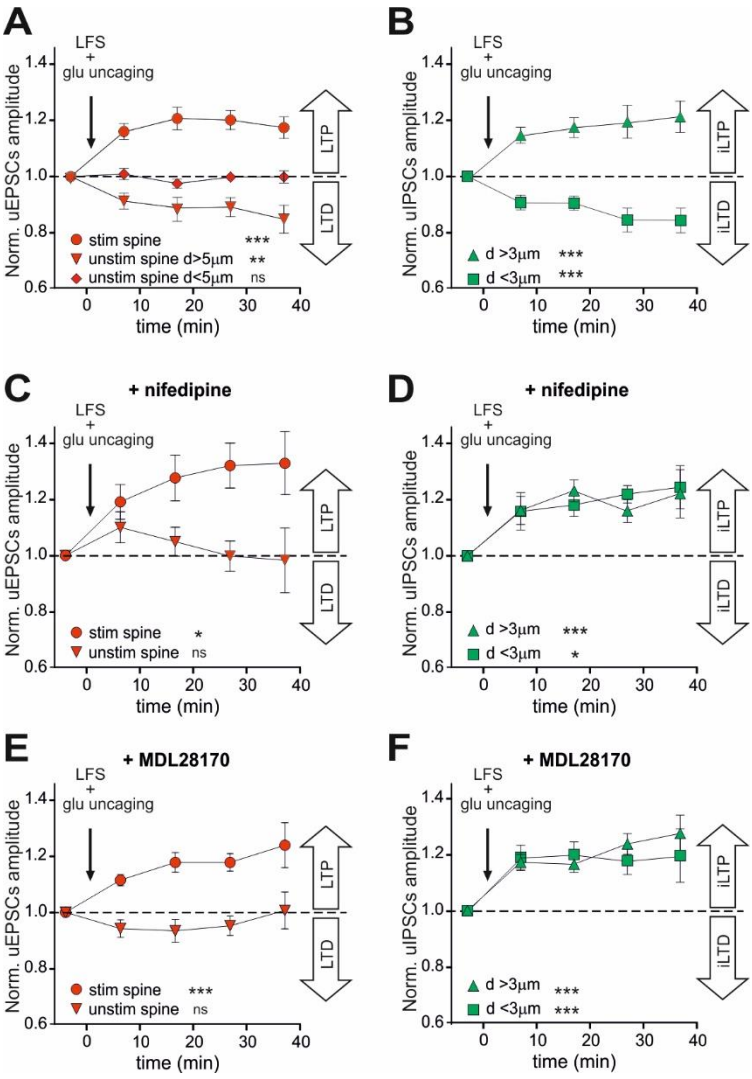

Figure S2 (related to Figure 4): Spatial coordination of the plasticity of excitatory and inhibitory synapses upon single spine LTP

A. After the single-spine LTP protocol ("LFS+glu uncaging"), the stimulated spine (red circles) is selectively potentiated (each datapoint represents at least n = 9 (max 20) synapses from 22 neurons). Normalized uEPSC amplitude at 27 min = 1.20 ± 0.03 fold, $F_{4,74}$=10.6, p< 0.001). Non-photostimulated spines ("unstim") located more distant than 5µm from the stimulated spine are depressed (red triangles, each datapoint represents at least n = 6 (max 15) synapses from 17 neurons); normalized uEPSC amplitude at 27 min= 0.89 ± 0.03 of baseline, $F_{4,53}$ = 5.4, p < 0.001). Spines located within 5µm from the stimulated spine are not affected by the paired Hebbian protocol (red diamonds, each datapoint represents at least n = 3 (max 5) from 5 neurons); normalized uEPSC amplitude= 0.996 ± 0.002 of baseline, $F_{4,14}$ = 1.2, p > 0.05. **B.** After the "LFS+glu uncaging protocol", GABAergic synapses located at d > 3 µm from the stimulated spine are potentiated (diamond, for each datapoint at least n = 7 (max 42) synapses from 21 neurons); normalized uIPSC

amplitude= 1.20 ± 0.06 of baseline, $F_{4,138}$ = 8.2, p< 0.001. GABAergic synapses located at d < 3 µm from the stimulated spine are depressed (square, at least n = 13 (max 34) synapses from 21 neurons); normalized uIPSC amplitude= 0.85 ± 0.04 of baseline, $F_{4,125}$= 5.7, p < 0.001. **C.** Same as in A in presence of nifedipine. Stimulated spine (red circles): each datapoint represents at least n = 4 (max 7) synapses from 7 neurons. uEPSC amplitude at 27 min: 1.32 ± 0.08 fold increase, $F_{4,26}$ = 3.9, p =0.001. Unstim spine (red triangles): each datapoint represents at least n = 3 (max 6) synapses from 7 neurons. $F_{4,20}$ = 0.8, p > 0.05. **D.** Same as in B, in presence of nifedipine. d < 3 µm (squares): each data point represents at least n = 3 (max 9) synapses from 7 neurons. uEPSC amplitude at 27 min = 1.22 ± 0.03 of baseline, $F_{4,27}$ = 4.0, p < 0.05; d > 3 µm (triangles): each data point represents at least n = 4 (max 13) synapses from 7 neurons; uEPSC amplitude at 27 min = 1.16 ± 0.04 of baseline, $F_{4,45}$ = 6.1, p < 0.001. **E.** Same as in A in presence of MDL28170. Stimulated spine, at least n = 3 (max 23) synapses from 24 neurons, $F_{4,78}$ = 11.9, p < 0.001; unstim spine, at least n = 3 (max 19) synapses from 24 neurons, $F_{4,69}$ = 1.2, p > 0.05. **F.** Same as in B, in presence of MDL28170. d < 3 µm, at least n = 5 (max 20) synapses from 24 neurons, $F_{4,71}$ = 7.1, p < 0.001; d > 3, at least n = 6 (max 43) synapses from 24 neurons, $F_{4,162}$ = 16.7, p < 0.001. Values are expressed as mean ± SEM. All the statistical comparison shown here are performed with one-way ANOVA followed Tukey's post-test. *p < 0.05, ***p < 0.001, ns = not significant.

Figure S3

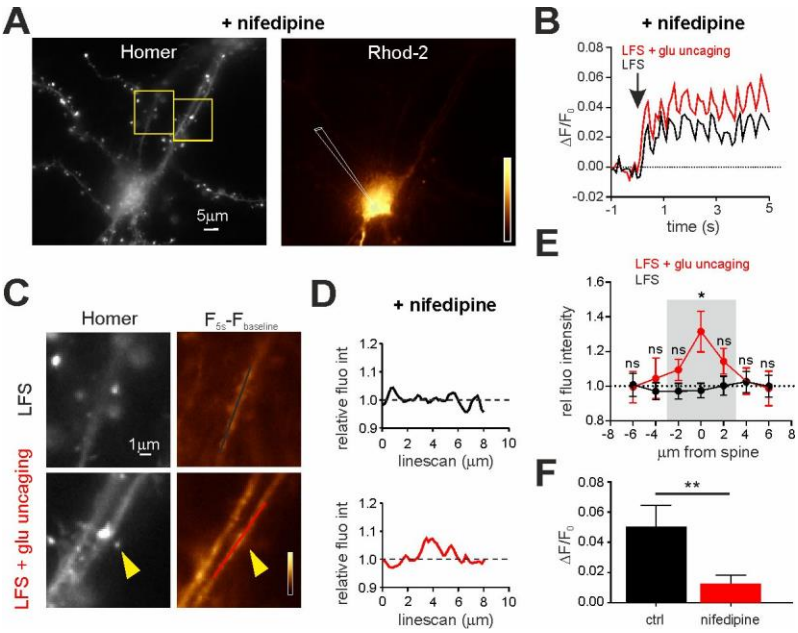

Figure S3 (related to Figure 5): Spatial dynamics of dendritic calcium in the presence of nifedipine during iLTP and LTD

A. Representative epifluorescence image of a Homer1c-GFP expressing neuron (left) loaded with Rhod-2 through the patch pipette (gold, right). Scale bar, 5 µm. **B.** Relative Rhod-2 fluorescence intensity quantified during the LFS protocol (black) and the LFS paired with glutamate uncaging protocol (red), in the presence of nifedipine. Two 4 µm-long portions of dendrites in the same neuron were considered, one centered below a reference spine and one below the stimulated spine. The arrow indicates the beginning of the protocol. **C.** Left: Magnifications of the dendritic portions framed in A, stimulated with LFS (top) or LFS paired with MNI-glutamate uncaging (bottom). The yellow arrowhead indicates the stimulated spine. Scale bar, 1 µm. Right: Gold pseudocolor representation of Rhod-2 fluorescence intensity changes at plateau (5 s) of the stimulating protocols (i.e., LFS, top and LFS paired with glutamate uncaging, bottom) with respect to baseline values (F_{5s}-$F_{baseline}$). The lines indicate the position of the linescans quantified in D. **D**. Relative fluorescence variation induced by "LFS + glu uncaging" protocol with respect to LFS alone, in the presence of nifedipine. The fluorescence intensities quantified along the two linescans in C are normalized to the average fluorescence detected along the linescan in LFS. **E.** Changes in the relative dendritic Rhod-2 fluorescence intensity (as measured in Figure 5D) as a function of the distance from a reference or stimulated spine during the delivery of LFS (black) or the LFS + glu uncaging (red) with nifedipine, respectively. The grey area indicates the range of ± 3µm from the potentiated spine where significant changes in Rhod-2 fluorescence are quantified as compared to the LFS protocol (LFS: n = 13 neurons, LFS+ glu uncaging: n = 13 neurons, $F_{1,168}$ = 5.3, $p < 0.05$, two-way ANOVA followed by Bonferroni's multiple comparison test). Statistical significance for each data point is shown. **F.** Difference of relative fluorescence intensity variation

after the delivery of LFS paired and LFS alone, in the presence of nifedipine (red) or in control (black). Each dendritic region taken in consideration is located within 3 μm from a reference or stimulated spine (control: n = 66, nife: n = 39, $p < 0.01$, Mann Whitney's test). Values are expressed as mean ± SEM. *$p < 0.05$, **$p < 0.01$, ns = not significant, i.e $p > 0.05$.

Figure S4

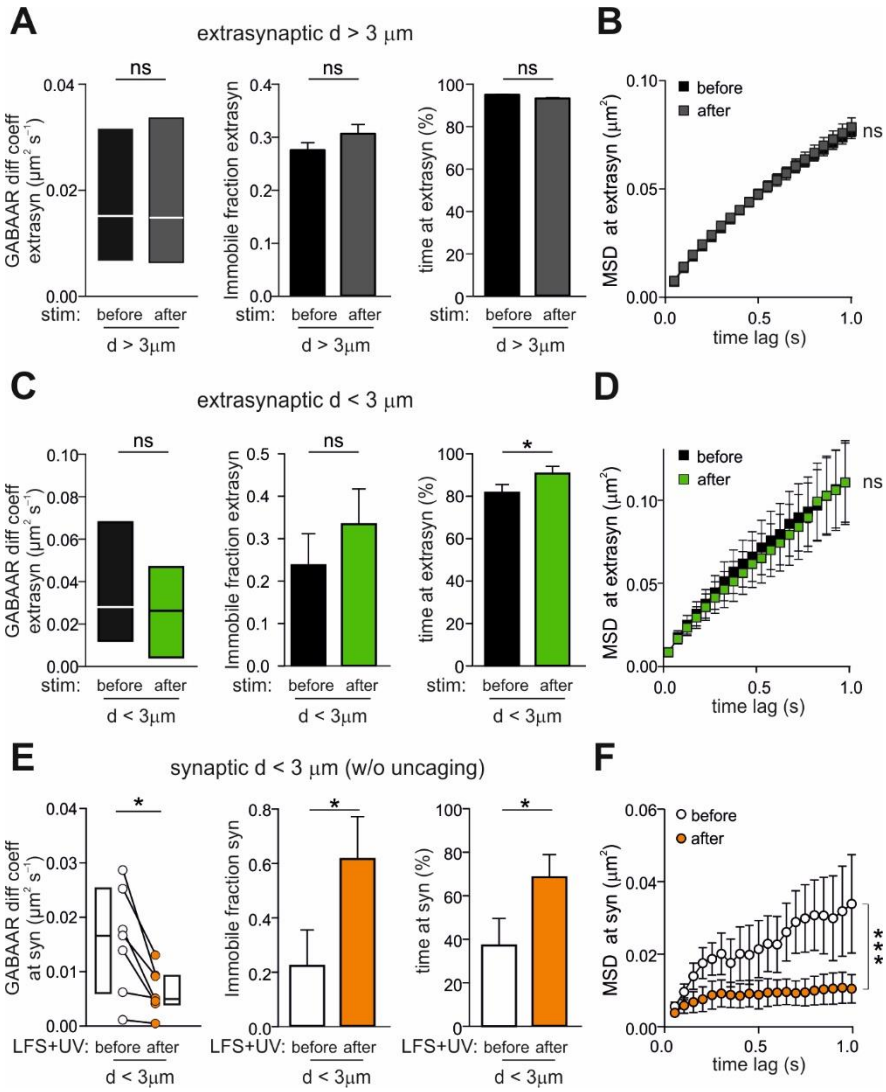

Figure S4 (related to Figure 7): Supplementary data on the modulation of GABAAR lateral mobility upon single spine LTP

A-B. Characterization of the lateral mobility of extrasynaptic GABAARs located at d > 3 µm from the potentiated spine, before (black) and after (grey) the single spine LTP protocol. **A**. Left: Median diffusion coefficient and interquartile range (IQR) (n = 605-739 trajectories from 28 neurons; $p > 0.05$, Mann-Whitney test). Middle: Immobile fraction (n = 592-795 trajectories from 28 neurons; $p > 0.05$, Mann-Whitney test). Right: Percentage of time spent by GABAA receptors in the extrasynaptic compartment (n = 611-761 trajectories; $p > 0.05$, Mann-Whitney test). **B.** MSD versus time plot (n= 614-845 from 28 neurons; $F_{1,28531} = 0.9$, $p > 0.05$, ordinary two-way ANOVA followed by Bonferroni's post hoc test). **C-D.** Characterization of the lateral mobility of extrasynaptic GABAARs located at d < 3 µm from the stimulated spine, before (black) and after (green) the single spine LTP protocol. **C**. Left: Paired median diffusion coefficient (n = 29

trajectories from 18 neurons; $p > 0.05$, paired Wilcoxon test). Middle: Paired IF (n = 29 trajectories from 18 neurons; $p > 0.05$, paired Wilcoxon test). Right: Paired values of percentage time spent by GABAA receptors in the extrasynaptic compartments at $d < 3$ μm from the stimulated spine (n = 29 trajectories from 18 neurons; $p < 0.05$, paired Wilcoxon test). **D.** MSD versus time plot of paired extrasynaptic GABAA receptors close to the potentiated spine ($d < 3$ μm), (n = 22 from 18 neurons, $F_{1,42} = 0.02$, $p > 0.05$, RM two-way ANOVA followed by Bonferroni's post hoc test). **E-F**. Same as in C-D, except for the uncaging. Please note that in this set of experiments the stimulating protocol was LFS + 4Hz UV-light pulses train on a spine (ctrl spine) in absence of MNI-glutamate. Only synaptic GABAAR trajectories localized in the range of 3μm from the ctrl spine were considered. **E**. Left: Paired median diffusion coefficient (before = 0.017 $\mu m^2 s^{-1}$; IQR: 0.006 – 0.025; after = 0.005 $\mu m^2 s^{-1}$; IQR: 0.004 - 0.09; n = 7 from 4 neurons; $p < 0.05$, paired Wilcoxon test). Middle: Paired IF (before = 0.22 ± 0.13; after = 0.62 ± 0.16; n = 7 from 4 neurons; $p < 0.05$, paired Wilcoxon test). Right: Paired values of percentage of time spent by GABAA receptors at synapses close to the control spine (n = 7 from 4 neurons; $p < 0.05$, paired Wilcoxon test). **F.** Paired MSD values of synaptic GABAA receptors close to the control spine ($d < 3$ μm; n = 4 from 4 neurons, $F_{1,60} = 140$, $p < 0.001$, RM two-way ANOVA). Unless stated otherwise, values are expressed as mean ± SEM. *$p < 0.05$, **$p < 0.01$, ns = not significant.