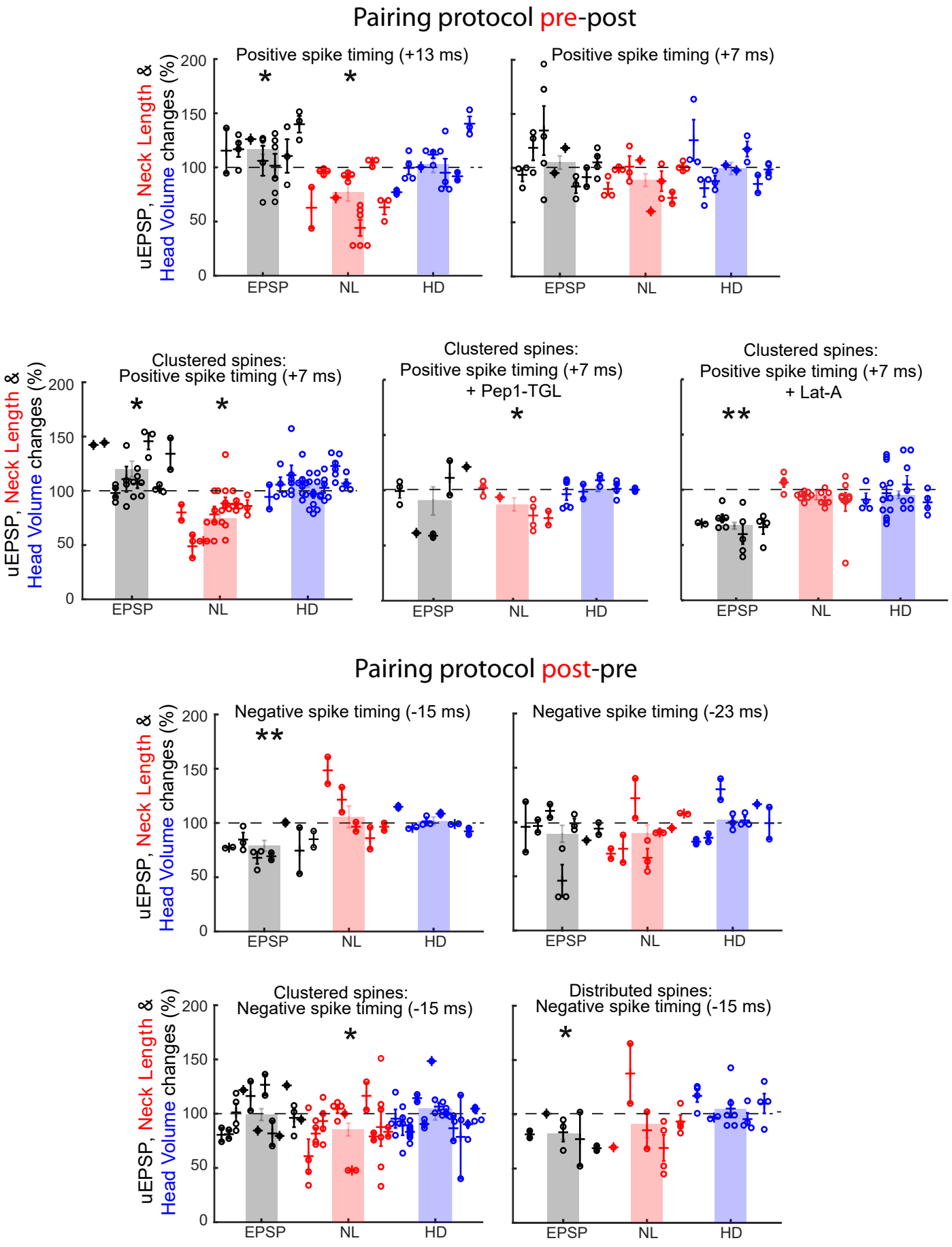
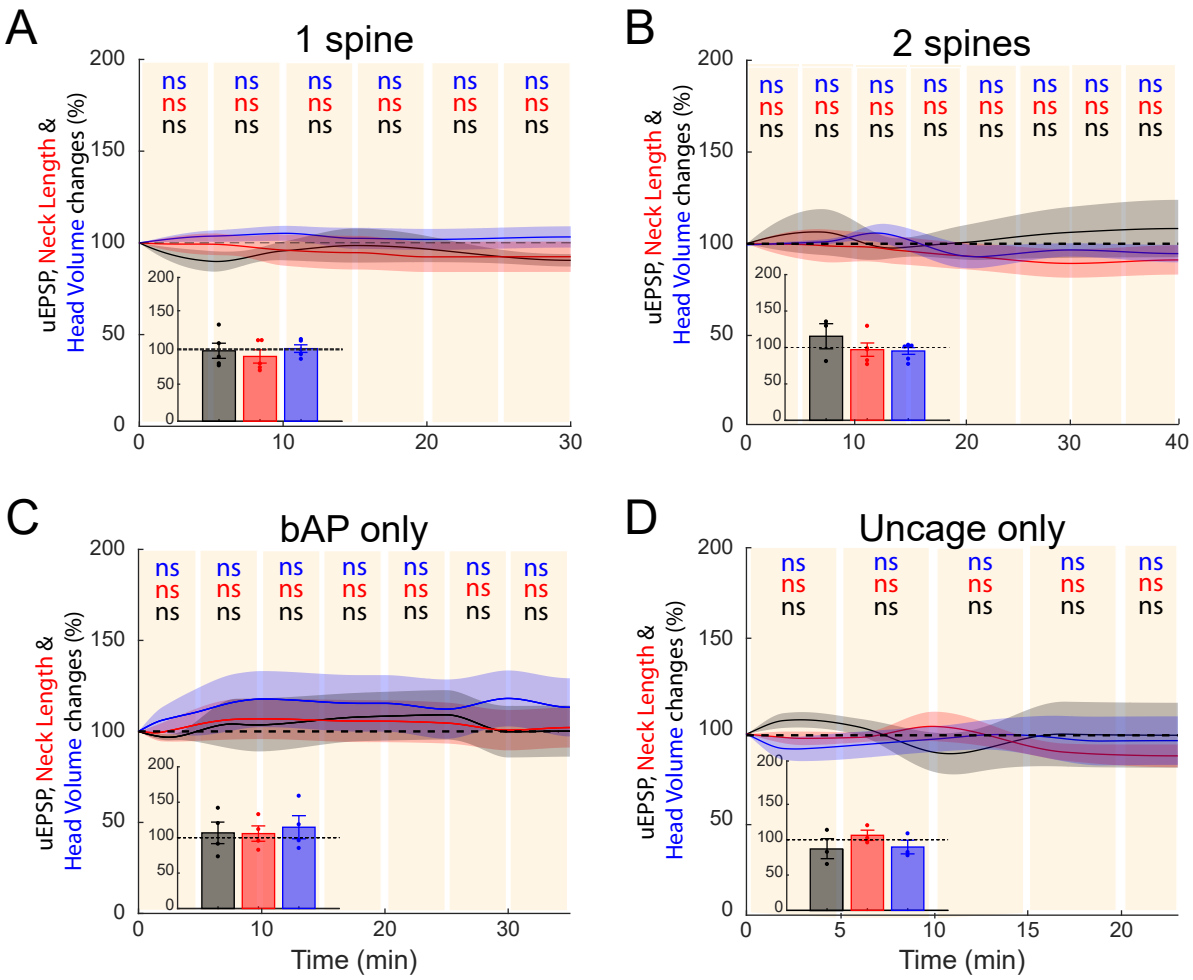
SUPPLEMENTARY INFORMATION

Supplementary Figure1



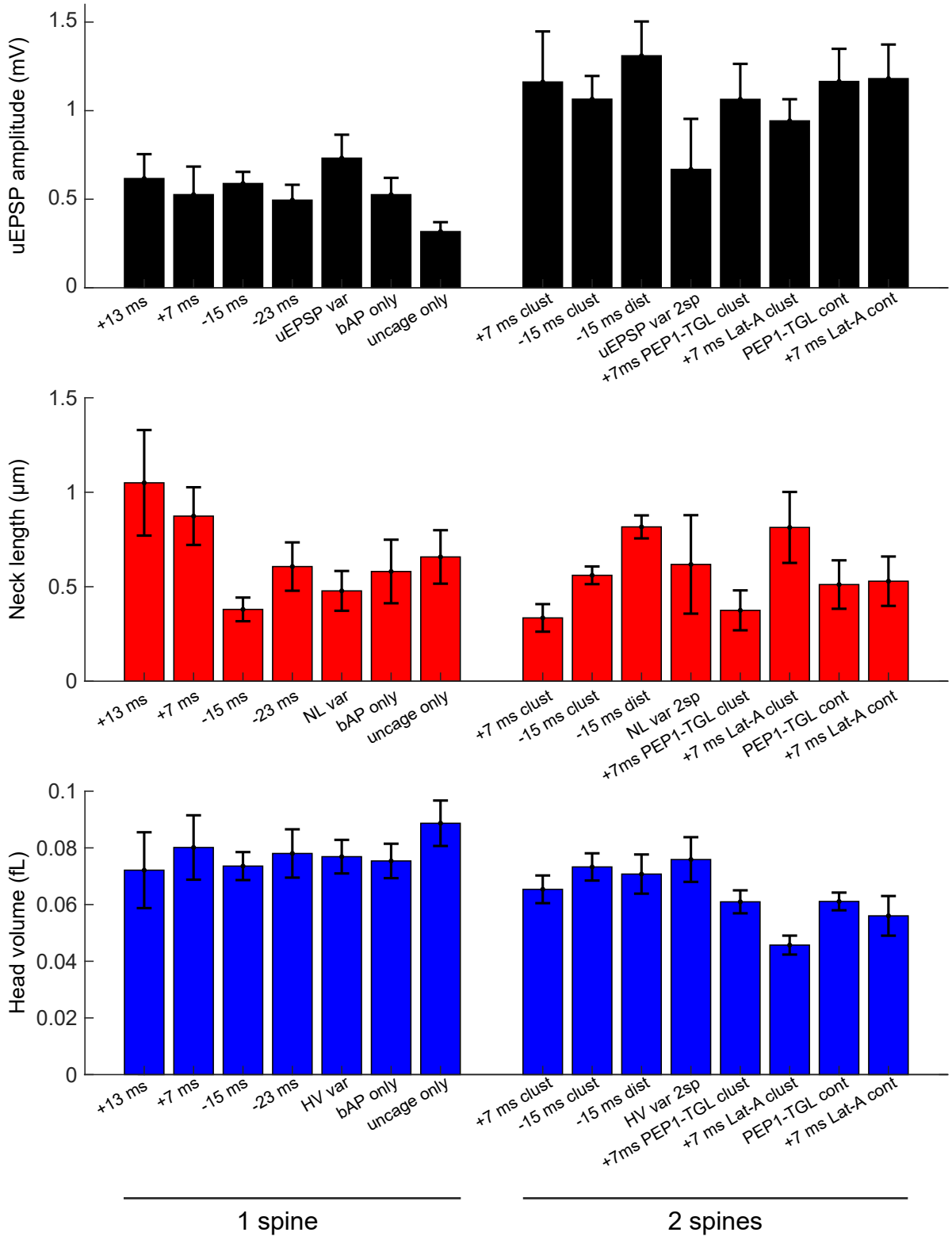
Supplementary Figure 1: Average of all values obtained following STDP induction for uEPSP amplitude, neck length and head volume. Plots showing all the data points for change in uEPSP (black bars and dots), neck length (red bars and dots) and head volume (blue bars and dots) obtained following STDP induction for each protocol we applied. Each column represents data points from a single experiment. Crosses and errors bars indicate the average and SEM for each individual experiment, while the shaded bar graphs represent the average of the mean from each individual experiment. *$P < 0.05$; **$P < 0.01$; Student's paired-t test. Note that statistical significance remains the same whether we consider the maximum uEPSP change and corresponding changes in morphology (Fig. 1-5) or the average of all values obtained following STDP induction for uEPSP amplitude, neck length and head volume.

Supplementary Figure 2



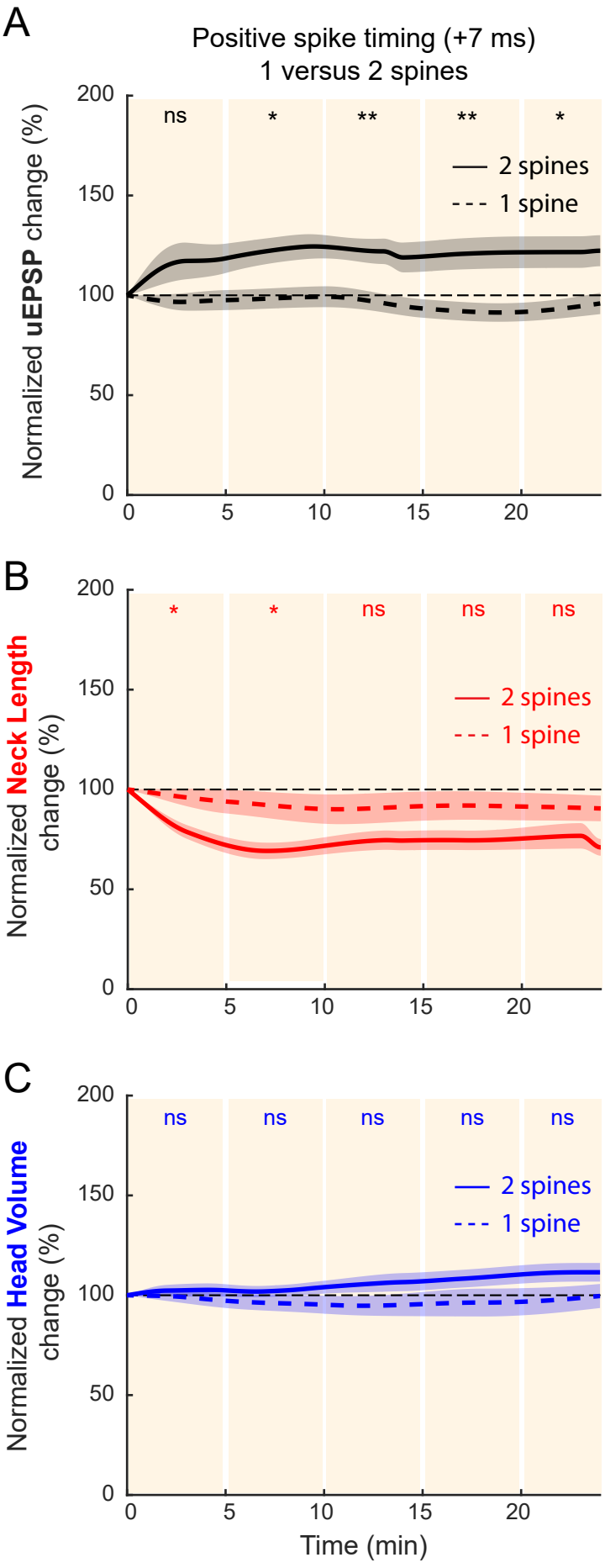
Supplementary Figure 2: Control experiments showing the stability of uEPSP amplitude or spine morphology over time when no pairing protocol was applied. (A-B) Time course of uEPSP amplitude (black line), neck length (red line) and spine head volume (blue line) over the course of ~30 min without any STDP protocol and uncaging 1 (A) and 2 (B) spines approximately every 5 minutes. Insets show maximum changes in uEPSP amplitude (black bar and dots) and concomitant changes in neck length (red bar and dots) and head volume (blue bar and dots) over the course of ~30 min without any STDP protocol. (C-D) Time course of uEPSP amplitude (black line), neck length (red line) and spine head volume (blue line) over the course of ~30 min following bAP only (C) and synaptic stimulation only (D). Insets show maximum changes in uEPSP amplitude (black bar and dots) and concomitant changes in neck length (red bar and dots) and head volume (blue bar and dots) over the course of ~30 min. *ns*, not significant, one-way repeated measures ANOVA followed by post hoc Dunnet's test.

Supplementary Figure 3



Supplementary Figure 3: Actual values for uEPSP amplitude, neck length and head volume. Bar plots showing the initial values for uEPSP amplitude (black bars), neck length (red bars) and head volume (blue bars) for each STDP protocol applied. A one-way ANOVA followed by a post hoc Tukey's multiple comparison test revealed that the uEPSP amplitude was not significantly different when one ($P = 0.65$) or two spines ($P = 0.70$) were activated with two-photon uncaging of glutamate. A significant difference in neck length was only found between a pre-post pairing protocol of +13 ms in one spine and +7 ms in two clustered spines ($P < 0.05$; one-way ANOVA followed by a post hoc Tukey's multiple comparison test). The head volume across all conditions was not significantly different ($P = 0.051$; one-way ANOVA followed by a post hoc Tukey's multiple comparison test). uEPSP var, NL var, HV var and uEPSP var 2sp, NL var 2sp, HV var 2sp correspond to the actual values for uEPSP amplitude, neck length, and head volume, respectively, from the experiment shown in Supplementary Fig. 2A and B; bAP only, and uncage only correspond to the actual values for uEPSP amplitude, neck length, and head volume from the experiment shown in Supplementary Fig. 2C and D, respectively.

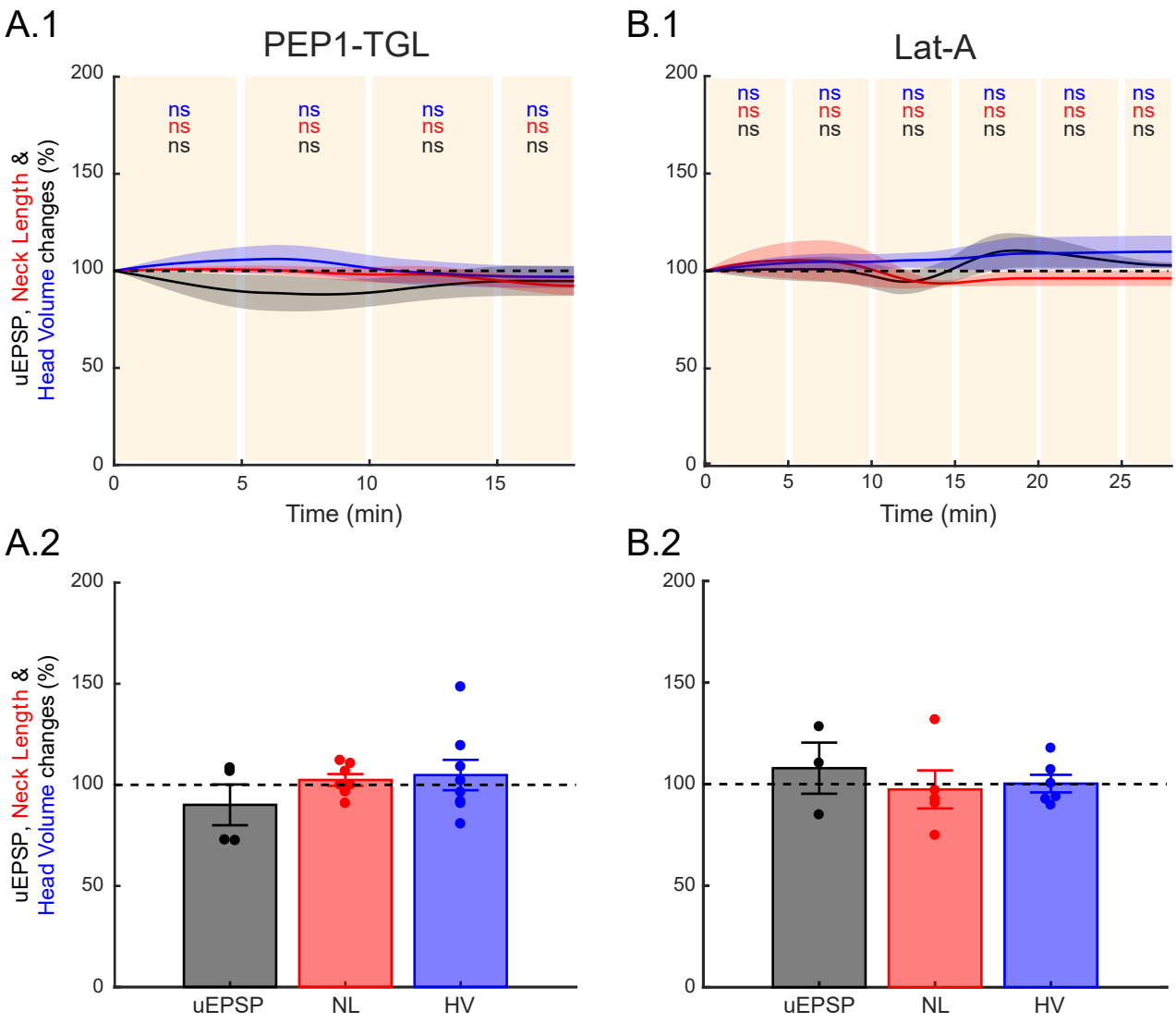
Supplementary Figure 4



Supplementary Figure 4: Induction of t-LTP in single versus two clustered dendritic spines

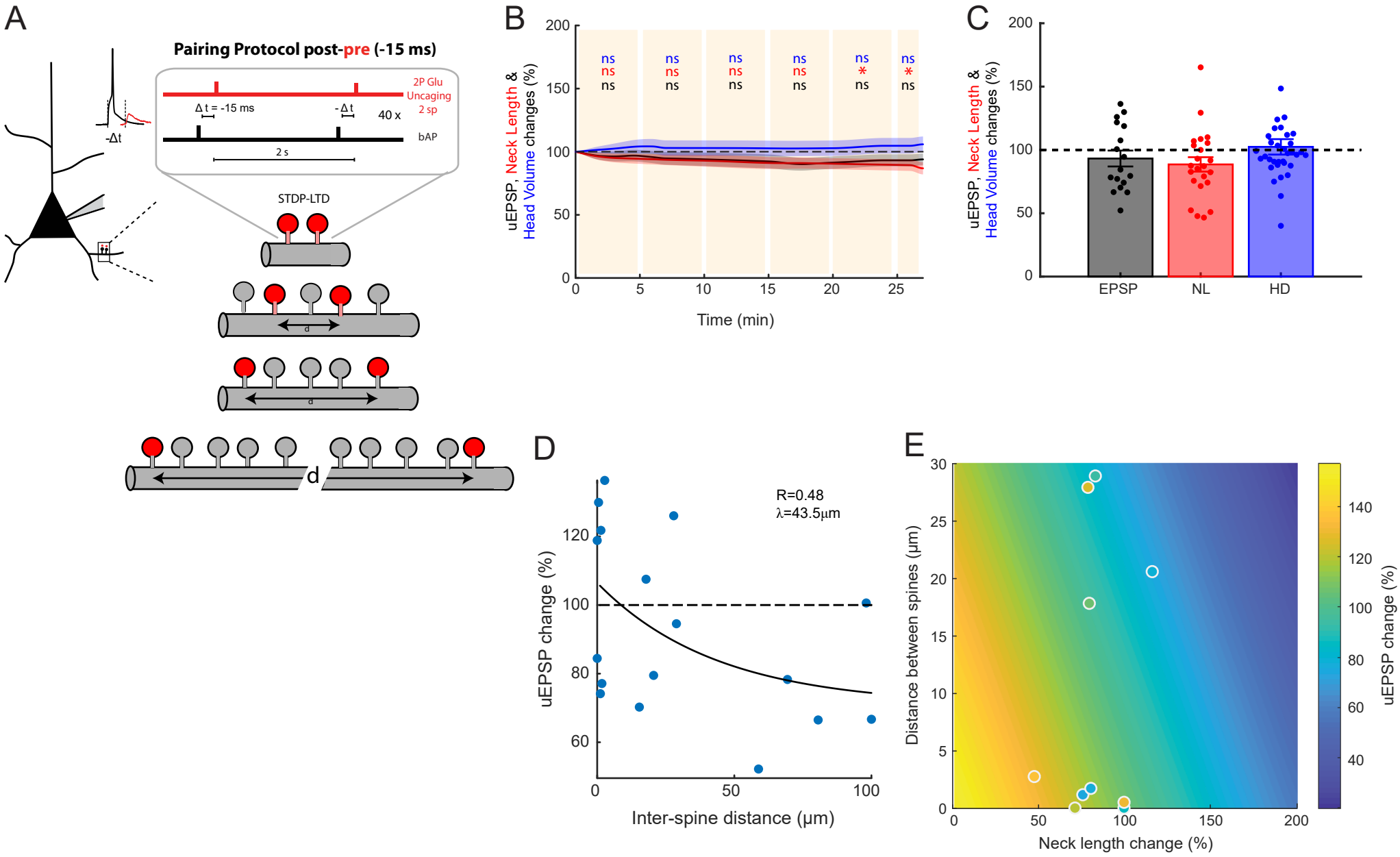
Comparison of the time course of uEPSP amplitude (A), neck length (B) and spine head volume (C) over the course of ~25 min following STDP induction at a pre-post timing of + 7 ms in individual (dashed lines) versus two clustered spines (solid lines) . *ns*, not significant; *P < 0.05; **P < 0.01; two-way ANOVA followed by post hoc Bonferroni.

Supplementary Figure 5



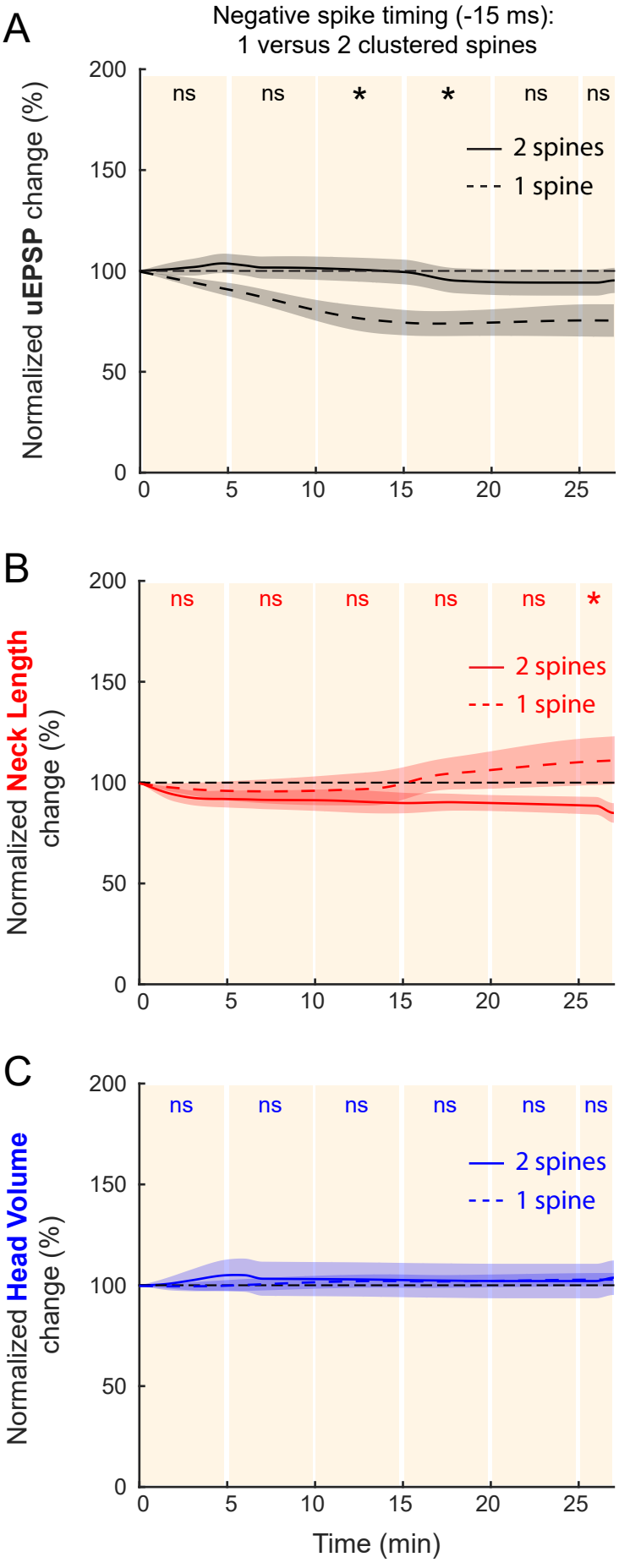
Supplementary Figure 5: Effect of PEP1-TGL and Lat-A on uEPSP amplitude and spine morphology. Time course of uEPSP amplitude (black line), neck length (red line) and spine head volume (blue line) recorded in the presence of 200 µM PEP1-TGL (A.1) or 100 nM Lat-A (B.1) without any STDP induction protocol. *ns*, not significant, one-way repeated measures ANOVA followed by post hoc Dunnet's test. Maximum changes in uEPSP amplitude (black bar and dots) and concomitant changes in neck length (red bar and dots) and head volume (blue bar and dots) of the activated spine recorded in the presence of 200 µM PEP1-TGL (A.2) or 100 nM Lat-A (B.2) without any STDP induction protocol.

Supplementary Figure 6



Supplementary Figure 6: Recovery of t-LTD following a post-pre pairing protocol of -15 ms in two spines when inter-spine distance is greater than 40 µm. and depends on neck length changes change and distance between the two spines. (A) Experimental post-pre induction protocol (pairings of - 15 ms) in two dendritic spines separated by different distances (~from 0 to 100 µm). (B) Time course of uEPSP amplitude (black line), the neck length (red line) and spine head diameter (blue line) of the activated spines for all the inter-spine distances after the induction of t-LTD at pairings of -15 ms. *ns*, not significant; *P < 0.05, one-way repeated measures ANOVA followed by post hoc Dunnet's test. (C) Maximum changes in uEPSP amplitude (black bar and dots) and concomitant changes in neck length (red bar and dots) and head diameter (blue bar and dots) of the two activated spines from each experiment after the induction of t-LTD at a post-pre timing of -15ms. (D) t-LTD recovery (decrease in uEPSP amplitude, less than 100%) is dependent on inter-spine distance. Data points represent mean and SE. We use the extracted value of λ as a boundary between clustered and distributed spines. (E) Color plot showing the relationship between uEPSP change (color coded) and neck length change and distance between two clustered spines following a post-pre t-LTD induction protocol. Note that when a pairing protocol of -15 ms is performed in two spines that are close together, and display neck shrinkage, the result is potentiation (increase in uEPSP amplitude, more than 100%). On the other hand, when the induction protocol is performed in two spines that are further away, without neck length changes, the result is depression (decrease in uEPSP amplitude, less than 100%). The change in uEPSP amplitude was modeled using equation 1 (described in methods).

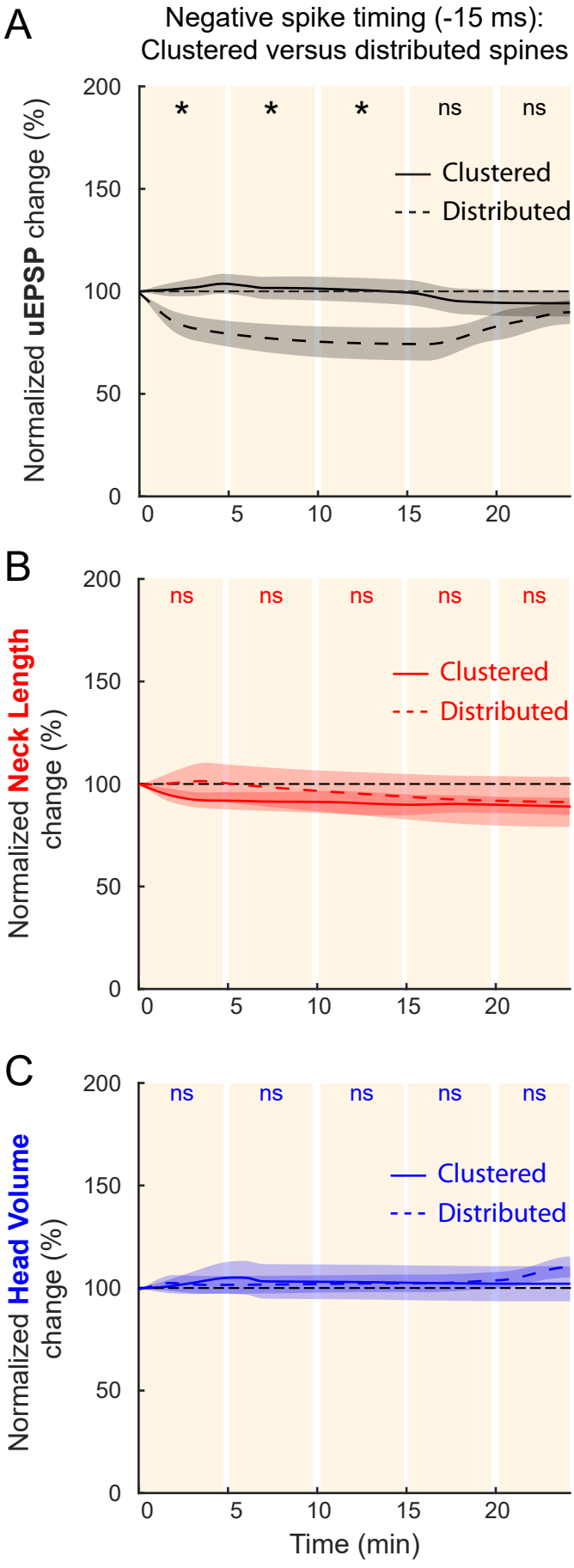
Supplementary Figure 7



Supplementary Figure 7: Induction of t-LTD in single versus two clustered dendritic spines

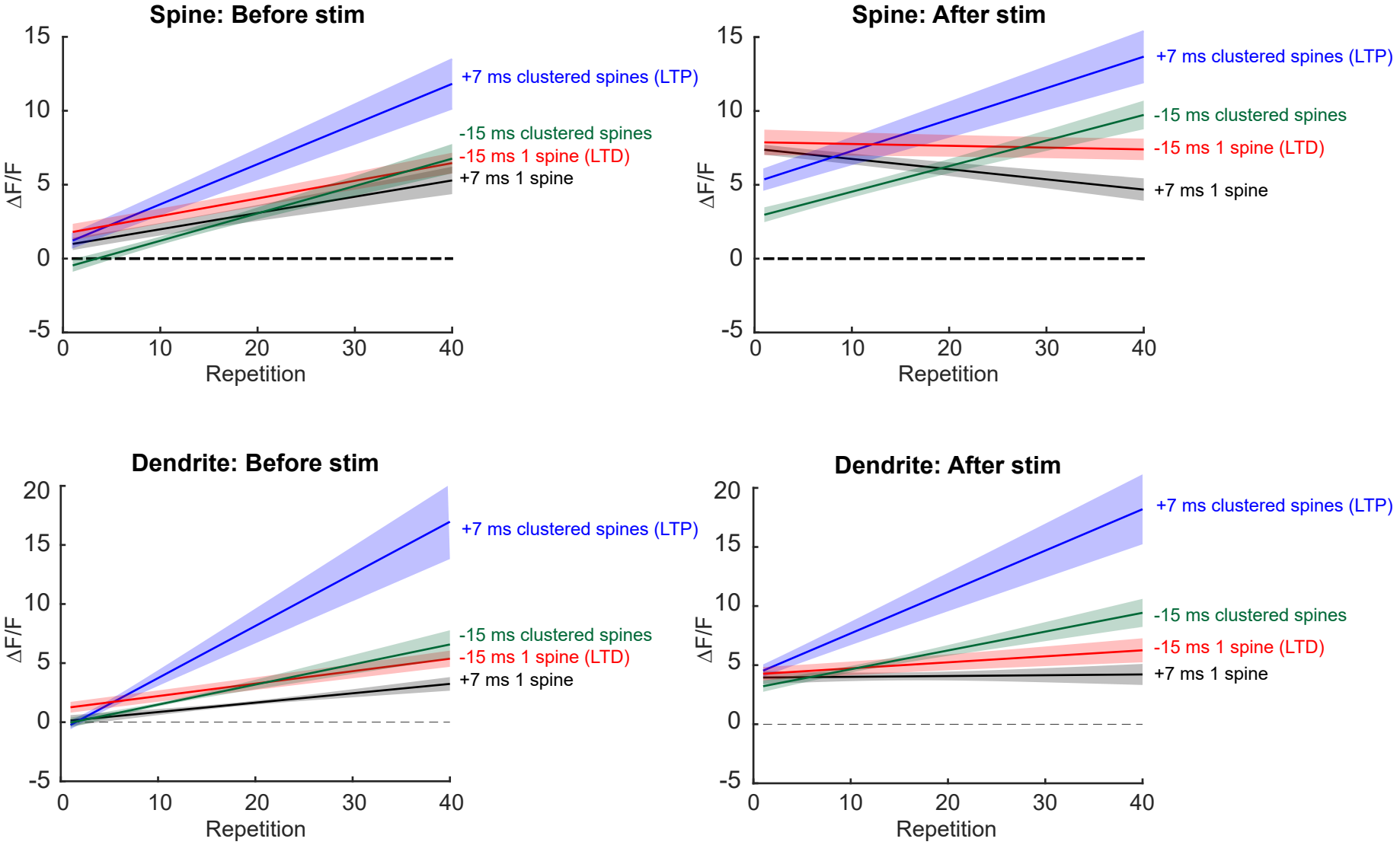
Comparison of the time course of uEPSP amplitude (A), neck length (B) and spine head volume (C) over the course of ~25 min following STDP induction at a pre-post timing of - 15 ms in individual (dashed lines) versus two clustered spines (solid lines) . *ns*, not significant; *P < 0.05; two-way ANOVA followed by post hoc Bonferroni.

Supplementary Figure 8



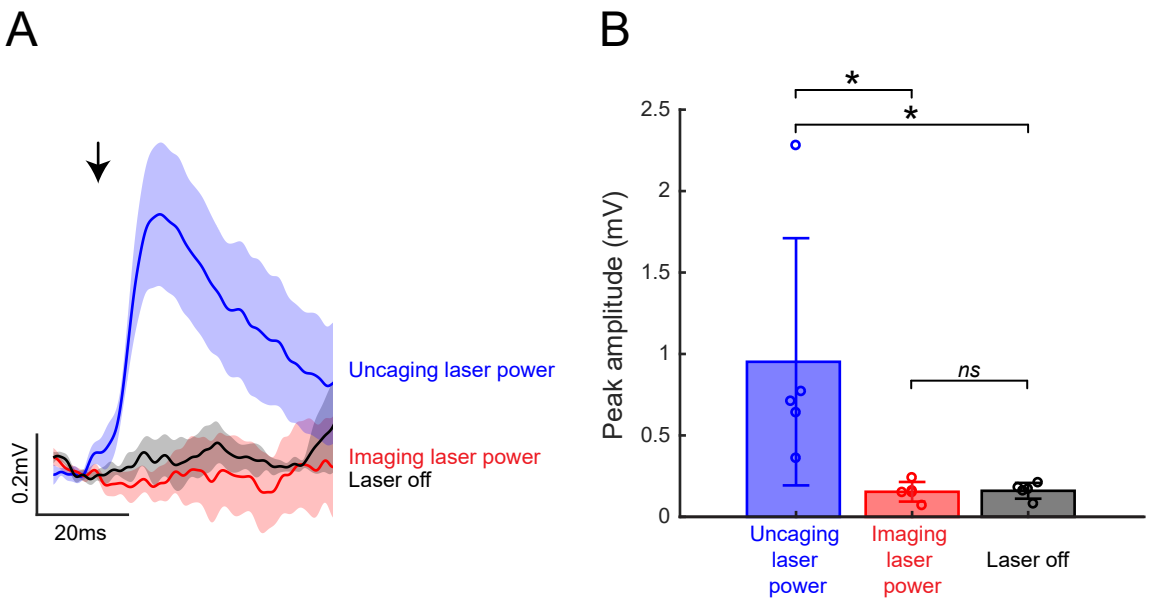
Supplementary Figure 8: Induction of t-LTD in two clustered versus distributed dendritic spines. Comparison of the time course of uEPSP amplitude (A), neck length (B) and spine head volume (C) over the course of ~25 min following STDP induction at a pre-post timing of -15 ms in clustered (solid lines) versus distributed spines (dashed lines) . *ns*, not significant; *P < 0.05; two-way ANOVA followed by post hoc Bonferroni.

Supplementary Figure 9



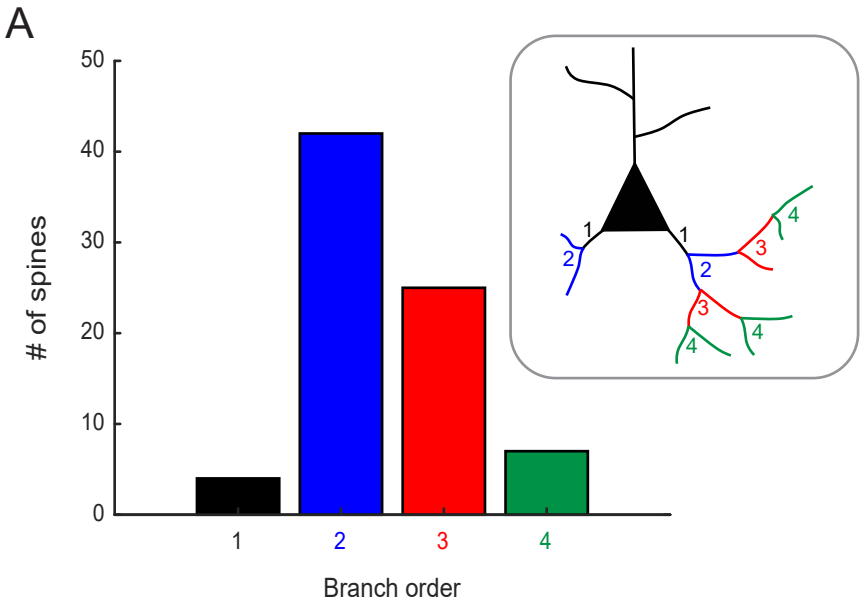
Supplementary Figure 9: Linear fits of the local calcium signals recorded during STDP protocols. Plots showing population averaged linear fits of calcium signals during four different STDP induction protocols: (1) pre-post pairing of +7 ms in 1 spine (black lines); (2) pre-post pairing of +7 ms in two clustered spines (blue lines); (3) post-pre pairing of -15 ms in one spine (red lines); (4) post-pre pairing of -15 ms in two clustered spines (green lines) before (Spine: Before stim; Dendrite: Before stim) and after each pairing (Spine: After stim; Dendrite: After stim) throughout the induction protocol (40 repetitions) in the activated spine(s) and parent dendrite.

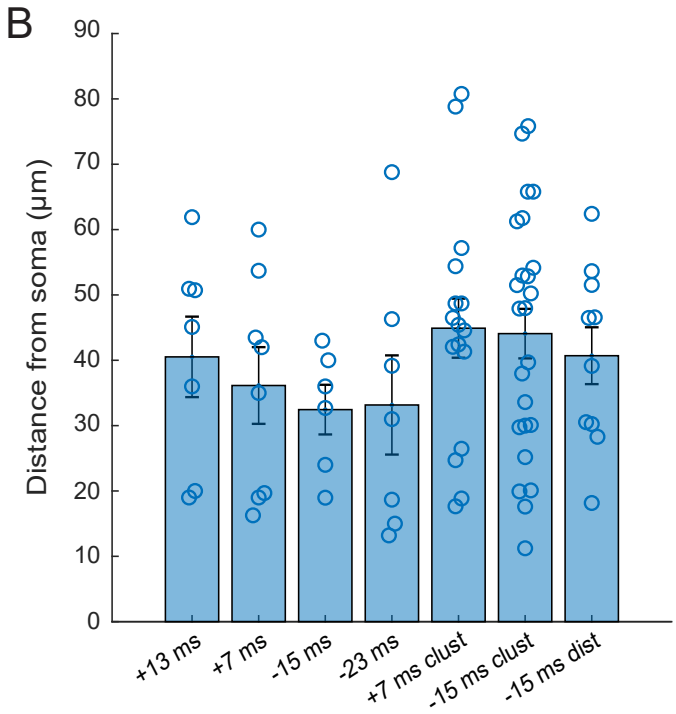
Supplementary Figure 10



Supplementary Figure 10: Imaging laser power does not cause glutamate uncaging-mediated uEPSPs in the soma of L5 pyramidal neurons. (A) Blue trace corresponds to an average of ten depolarizations recorded at the soma caused by uncaging glutamate next to a spine using 4-ms laser pulses of ~25-30mW on sample at 2 second intervals (Uncaging laser power). Note the generation of a clear uEPSP. Red trace corresponds to the average voltage recorded while applying ten 4-ms laser pulses of ~~5 mW on sample at 2 second intervals (Imaging laser power). Note that no uEPSPs were observed. Black trace corresponds to the average voltage recorded a second after the onset of the 4-ms laser pulses, 0mW on sample (Laser off). Shaded area represents the SEM. (B) Plot showing peak amplitude (mV) observed after 2P uncaging of glutamate at Uncaging laser power (Blue), Imaging laser power (red), or with the 2P Laser off (black). N = 5 experiments. *ns*, not significant; *P < 0.05; Student's t-test.

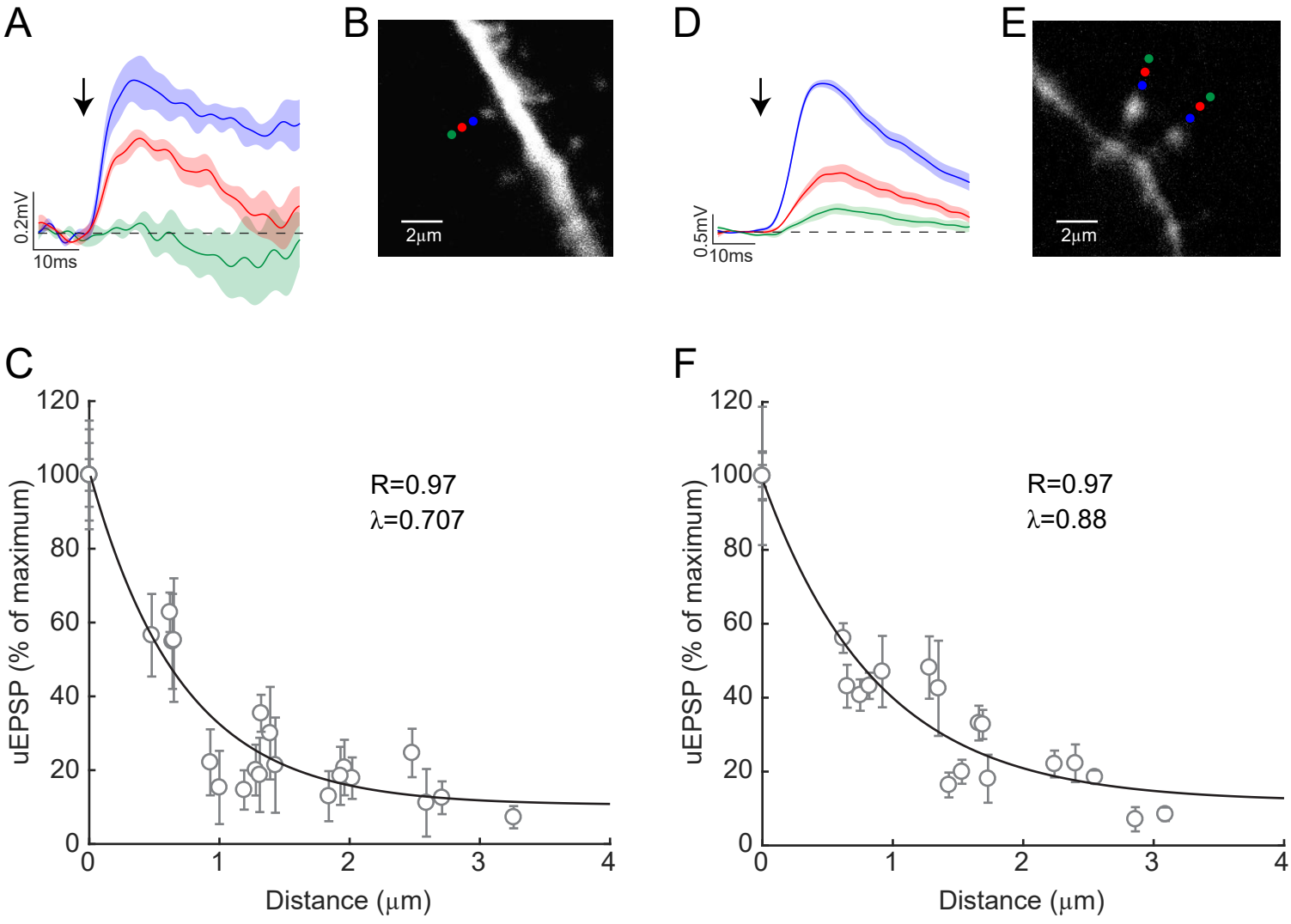
Supplementary Figure 11





Supplementary Figure 11: Morphometric analysis of spines included in this study. (A) Bar plot showing the branching order of spines that were activated in this study. A primary dendrite is one originating from the cell body (branching order labeled as "1" in inset diagram). The branching order increases with each successive branch point (when dendrite splits into two or more branches). (B) Bar graph showing the distance of spines from the soma for each STDP protocol that we applied. Each data point represents the distance from the soma of individual spines). No significant difference was observed across groups (41.5 ± 2.1 μm, p=0.53; one-way ANOVA followed by Tukey's Multiple Comparison Test).

Supplementary Figure 12



Supplementary Figure 12: Spatial resolution of 2P glutamate uncaging in one and two clustered spines. Two-photon activation of single spines: (A) Example uEPSP averaged traces evoked by placing the uncaging spot at the corresponding color coded locations shown in (B). Each trace corresponds to an average of ten depolarizations recorded at the soma. (C) Averaged uEPSP values (normalized to the maximum value obtained in the same experiment) as a function of distance from the closest uncaging spot in the same experiment. Two-photon activation of two clustered spines: (D) Example uEPSP averaged traces evoked by placing the uncaging spots at the corresponding color coded locations shown in (E). Each trace corresponds to an average of ten depolarizations recorded at the soma. (F) Averaged uEPSP values (normalized to the maximum value obtained in the same experiment) as a function of distance from the closest uncaging spot in the same experiment. Shaded area in A and D represents the SEM.