Supplementary Fig. 1: C3a-C3aR signaling positively regulates the size of VGLUT1+ puncta in the cortex close to the infarct. (A, B) Average area of synapsin I+ puncta in the proximal peri-infarct and contralesional cortex (mean ± SEM; C3aR+/+ n=10, C3aR−/− n=14, WT n=13, C3a-GFAP n=12). (C) Representative low magnification wide-field image of positive synapsin I staining in the peri-infarct
motor cortex. (D) Low magnification wide-field image of synapsin I negative control staining. Scale bar, 100 µm. (D’) Confocal image of region squared in D. Scale bar 10 µm. (E, F) Average area of VGLUT1+ puncta in the proximal peri-infarct and contralesional cortex (mean ± SEM; C3aR+/+ n=6, C3aR−/− n=6, WT n=6, GFAP-C3a n=7). (G) Representative low magnification wide-field image of positive VGLUT1 staining in the peri-infarct motor cortex. (H) Low magnification wide-field image of VGLUT1 antibody negative control staining. Scale bar, 100 µm. (H’) Confocal image of region squared in H. (D’, H’) Brightness of the source image was increased to visualize tissue outline. Scale bar 10 µm. One-way ANOVA with Sidak’s planned comparisons: ** P<0.01, *** P<0.001, **** P<0.0001 for ipsi vs. contra comparisons; # P<0.05, ## P<0.001 for between-genotype comparisons. contra – contralesional cortex; ipsi M – ipsilesional motor cortex; ipsi S – ipsilesional somatosensory cortex.
Supplementary Fig. 2: C3aR−/− mice have decreased numbers of synapsin I+ puncta in the cortex distant from the infarct. Density (A, B) and average area (C, D) of synapsin I+ puncta in the distal peri-infarct and contralesional cortex (mean ± SEM; C3aR+/+ n=10, C3aR−/− n=14, WT n=13, GFAP-C3a n=12). One-way ANOVA with Sidak’s planned comparisons: * P<0.05, ** P<0.01, *** P<0.001, **** P<0.0001 for ipsi vs. contra comparisons; # P<0.05, ## P<0.01 for between-genotype comparisons. contra – contralesional cortex; ipsi M – ipsilesional motor cortex; ipsi S – ipsilesional somatosensory cortex.
Supplementary Fig. 3: Naïve C3aR<sup>-/-</sup> and GFAP-C3a mice have normal number and size of synapsin I<sup>+</sup> puncta in the cortex and striatum. Density (A, B) and average area (C, D) of synapsin I<sup>+</sup> puncta in the cortex of naïve mice (mean ± SEM; n= 6 mice/group). S – somatosensory cortex; M – motor cortex; Str – dorsal striatum.
**Supplementary Fig 4:** Plasticity marker GAP-43 in peri-infarct cortex is predominantly localized in the neuronal compartment. (A-D) Orthogonal projections of Z-stack confocal images double-stained for GAP43 (red) and a phenotyping markers (green): (A) synaptophysin, (B) β3-tubulin, (C) GFAP and (D) S100β. Scale bar 10 µm. Crosshair lines are placed so as not to obscure fine double-positive objects. Small insets marked with ‘ and ’ represent 2D zoom-in of squared areas in respective images. Scale bar, 2 µm. Arrows indicate objects with high degree of co-localization in 3D; Arrowheads – indicate partially co-localized objects located in close apposition. (E) Results of quantitative co-localization analysis.
Supplementary Fig. 5: C3aR signaling upregulates expression of GAP-43 in the cortex distant from the infarct. Density (A, B) and average area (C, D) of GAP-43+ puncta in the distal peri-infarct and contralesional cortex (C3aR+/+ n=10, C3aR−/− n=14, WT n=13, GFAP-C3a n=12). In A, C and D data are presented as mean ± SEM and analyzed by one-way ANOVA with Sidak’s planned comparisons, and in B data are presented as median ± IQR and analyzed by Kruskal-Wallis with Dunn’s planned comparisons: * P<0.05, ** P<0.01, *** P<0.001, **** P<0.0001 for ipsi vs. contra comparisons; # P<0.05, ## P<0.01, ### P<0.001 for between-genotype comparisons. contra – contralesional cortex; ipsi M – ipsilesional motor cortex; ipsi S – ipsilesional somatosensory cortex. (E) Representative low magnification wide-field image of positive GAP-43 staining in the peri-infarct motor cortex. (F) Low magnification wide-field image of GAP-43 negative control staining. Scale bar, 100 μm. (F') Confocal image of region squared in F. Brightness of the source image was increased to visualize tissue outline. Scale bar, 10 μm.
Supplementary Fig. 6: *Intranasal administration of C3a does not lead to a systemic anaphylactic response.* Time course of body temperature changes following intranasal administration of C3a or PBS in unchallenged WT mice. Mean ± SEM.
Supplementary Fig. 7: C3a treatment results in increased size of pre-synaptic terminals in the peri-infarct motor cortex. (A) Average area of synapsin I$^+$ puncta in the proximal peri-infarct and contralesional cortex (mean ± SEM; n=14 mice/treatment group). (B) Average area of VGLUT1$^+$ puncta in the proximal peri-infarct and contralesional cortex (mean ± SEM; n=6 mice/treatment group). One-way ANOVA with Sidak’s planned comparisons: * P<0.05, ** P<0.01, *** P<0.001, **** P<0.0001 for ipsilesional vs. contralesional hemisphere comparisons; # P<0.05, ## P<0.01, ### P<0.001 for between-treatment comparisons. (C) Average area of GAP-43$^+$ puncta in the proximal peri-infarct and contralesional cortex (median ± IQR; n=14 mice/treatment group). Kruskal-Wallis test with Dunn’s planned comparisons: ** P<0.01, *** P<0.001, **** P<0.0001 for I vs. C comparisons; C – contralesional cortex; I – ipsilesional cortex.
Supplementary Fig. 8: Increased synapsin I expression in peri-infarct cortex at 21 days post stroke is associated with functional recovery. (A, B) Scatter plots and linear regression fit of association between density of synapsin I⁺ puncta and change in performance between days 7 and 21 post stroke in (A) grid walking test and (B) cylinder test. (C, D) Scatter plots and linear regression fit of association between size of synapsin I⁺ puncta and change in performance between days 7 and 21 post stroke in (C) grid walking test (n=14/treatment group) and (D) cylinder test (n=10/treatment group).