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Supplemental information

Synaptic protein interaction networks encode experience by assuming stimulus-specific and brain-region-specific states

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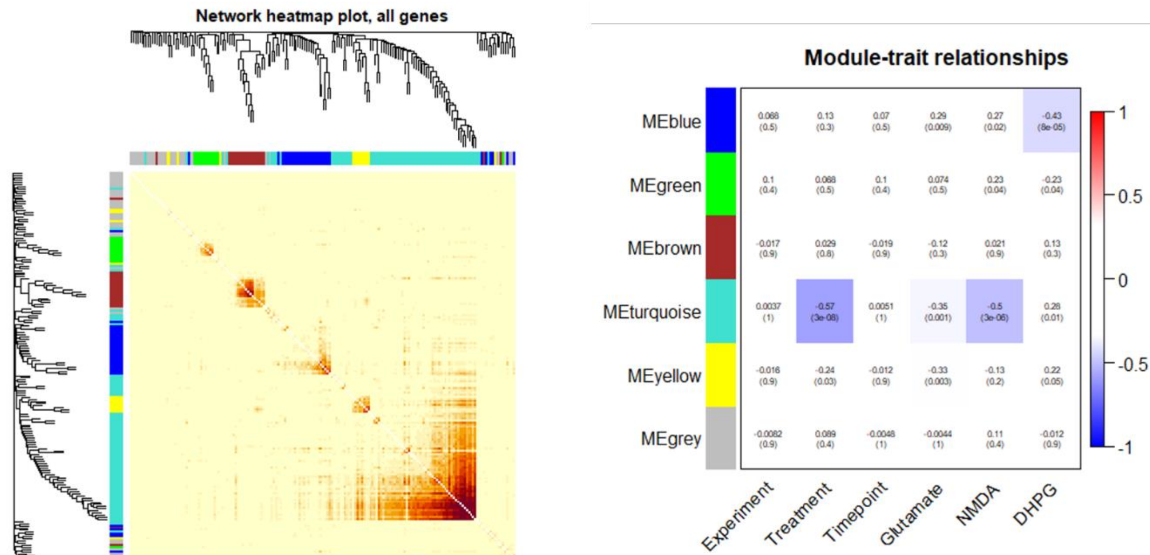
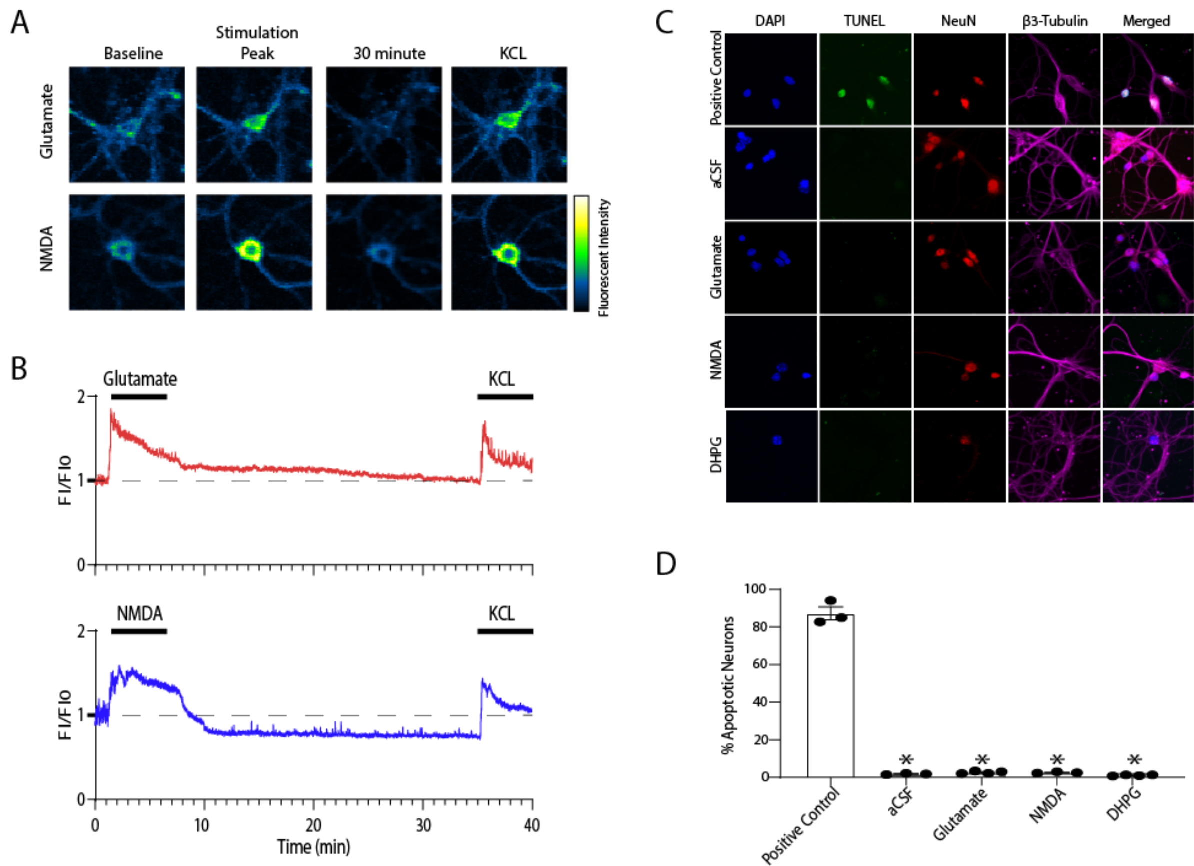
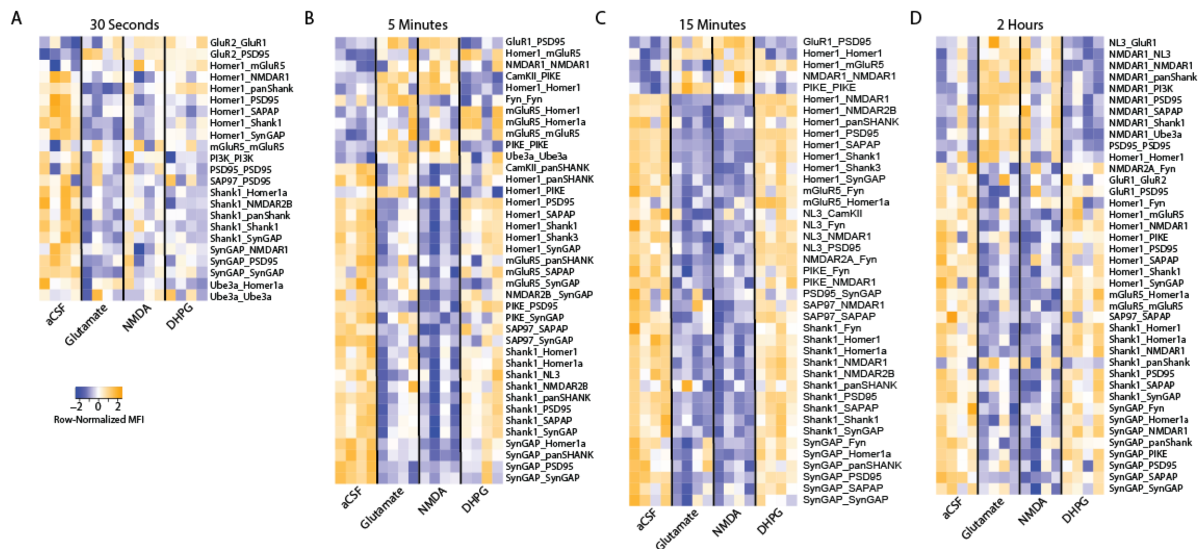


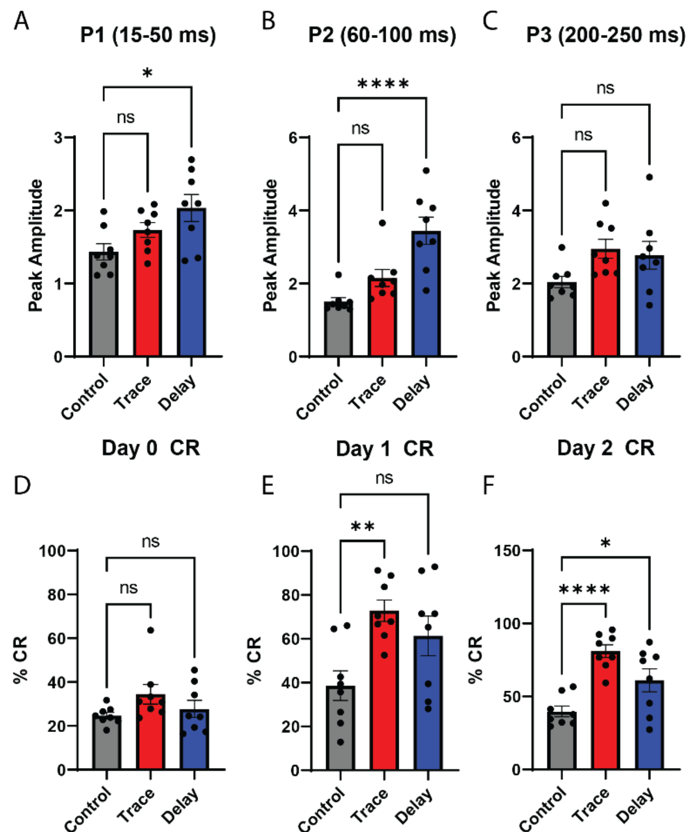
Supplementary Figure 1: Correlation network analysis of the timecourse experiment. A) A topological overlap matrix (TOM) plot showing the correlation of each PiSCES with every other PiSCES over the 48 biological replicates comprising the timecourse experiment show in Figure 1. The CNA program clusters all PiSCES based on correlated behavior (red = high correlation, yellow = low correlation), and assigns clusters into modules indicated by colored bars below the cladogram. B) Module-trail correlation table showing the correlation coefficient (top number in each box) and p-value (bottom number in each box) for the correlation between each color-coded module's eigenvector and experimental variables shown at the bottom of the table. Colored boxes indicate higher correlation coefficients. Note the turquoise module is correlated strongly with both "treatment" (coded ACSF = 0, Glutamate = 1, NMDA = 1, DHPG = 1) and "NMDA" (coded 0,0,1,0), while the blue module is correlated most strongly with DHPG (coded 0,0,0,1).



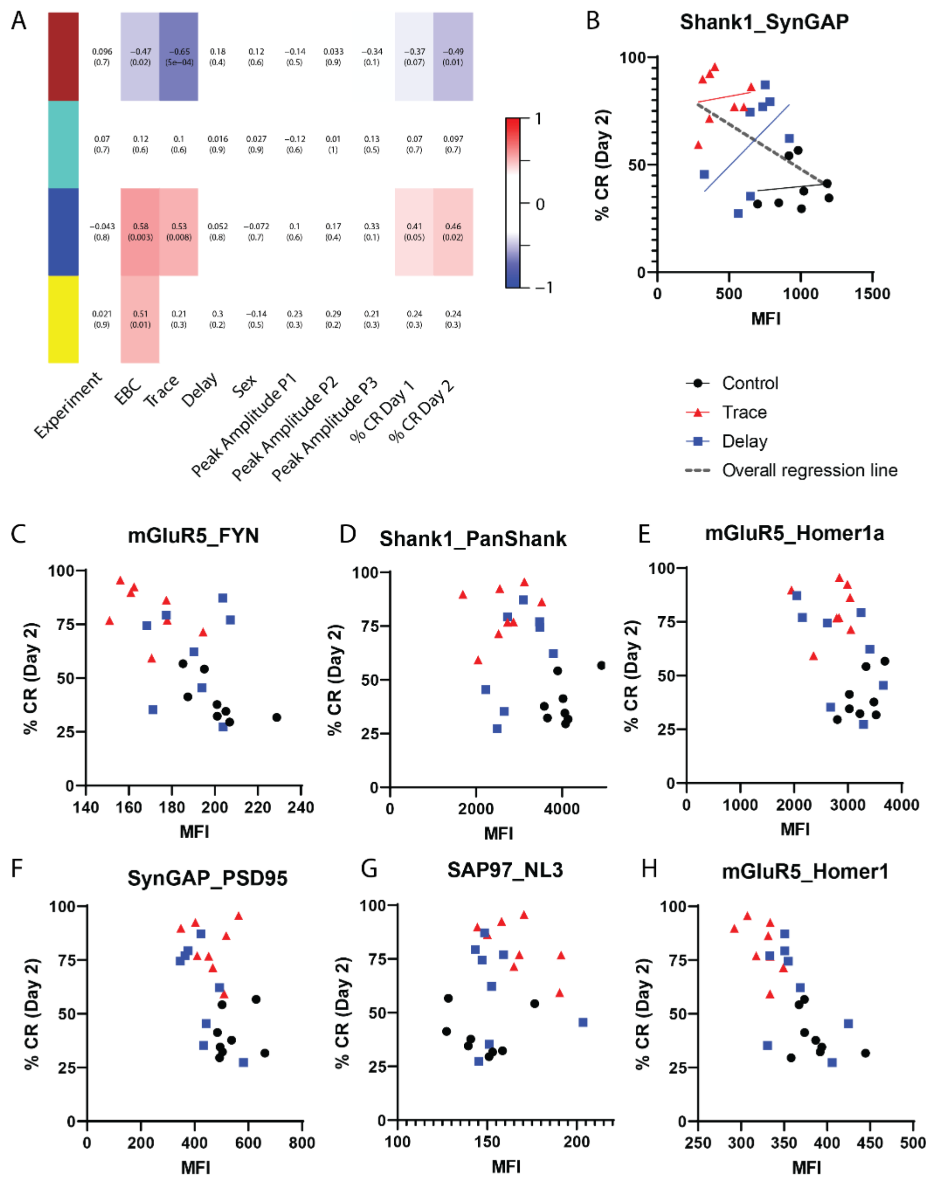
Supplementary Figure 2: NMDA and DHPG treatments do not induce excitotoxicity. A) Example of Ca2+ imaging experiment showing baseline, stimulation peak (~2-3 minutes following NMDA), 30 minutes after wash-out, and the peak of a second KCL stimulation. B) Averaged FI/F0 traces for glutamate and NMDA stimulations. N= 28-29 neurons. C) TUNEL staining showing lack of apoptosis two hours following aCSF, glutamate, NMDA, or DHPG treatment. D) Quantification of data shown in C, ANOVA: $F_{(4,12)}=720.4$, $p<0.0001$; * $p<0.05$ vs. positive control by Bonferroni-corrected post-hoc test, N=3-4 experiments per condition, <500 cells counted per experiment.



Supplementary Figure 3: Heatmap of row-normalized MFIs for all PiSCES ANC∩CNA significant at each of the timepoints shown in Figure 1. Data are expressed as row-normalized MFI.



Supplementary Figure 4: Eyeblink conditioning data by individual. The amplitude of the A) P1, B) P2 and C) P3 components of the eyeblink response on day 2, or the percent of trails that elicited a conditioned response on D) day 0, E) day 1 and F) day 2 of conditioning is shown for control (black), trace (red) and delay (blue) EBC. Data points represent individual animals, error bars represent SEM. Amplitude data are expressed as fold-change over the baseline period immediately prior to the CS. Significance was assessed by ANOVA followed by Dunnet's post-hoc test, as indicated by astersisks, NS = not significant. A) P1 component: $F_{(2, 21)} = 4.74$, p=0.0199; B) P2 component $F_{(2, 21)} = 14.12$, P=0.0001; C) P3 component $F_{(2, 21)} = 2.93$, P=0.0752 NS; D) Day 0 (baseline) % CR: $F_{(2, 21)} = 2.013$, P=0.1586 NS; E) Day 1 % CR: $F_{(2, 21)} = 6.014$, P=0.0086 F) Day 2 % CR: $F_{(2, 21)} = 13.52$, P=0.0002. Data in P2 and P3 are identical to that shown in Fig 5.



Supplementary Figure 5: Correlation between animal behavior and protein complex abundance. A) Module-trait relationship table from CNA analysis showing the brown and blue modules (color-coded at left) significantly correlated with % CR on both day 1 and day 2, but not with amplitude, sex or experimental replicate. The modules also correlated with the presence of EBC (coded control = 0, delay = 1, trace = 1), and only trace EBC (coded control = 0, delay = 0, trace = 1). B) Example of the relationship between %CR on day 2 and the median fluorescent intensity (MFI) of a single PiSCES,

Shank1_SynGAP. Regression lines are shown for all individuals (Dashed grey line, slope = -0.042, $F_{(1,22)}$=7.27, p=0.013), as well as for animals within the Control (black, slope= 0.0064 , $F_{(1,6)}$ 0.063 P = 0.809 NS), delay (blue, slope= 0.06828, $F_{(1,6)}$= 2.42 P = 0.171 NS) and trace (red, slope = 0.012, $F_{(1,6)}$=0.114, P=0.75 NS) groups. C-H) Examples of other PiSCES that were ANC∩CNA significant for EBC (as shown in Figure 5), plotted against %CR for individual animals.