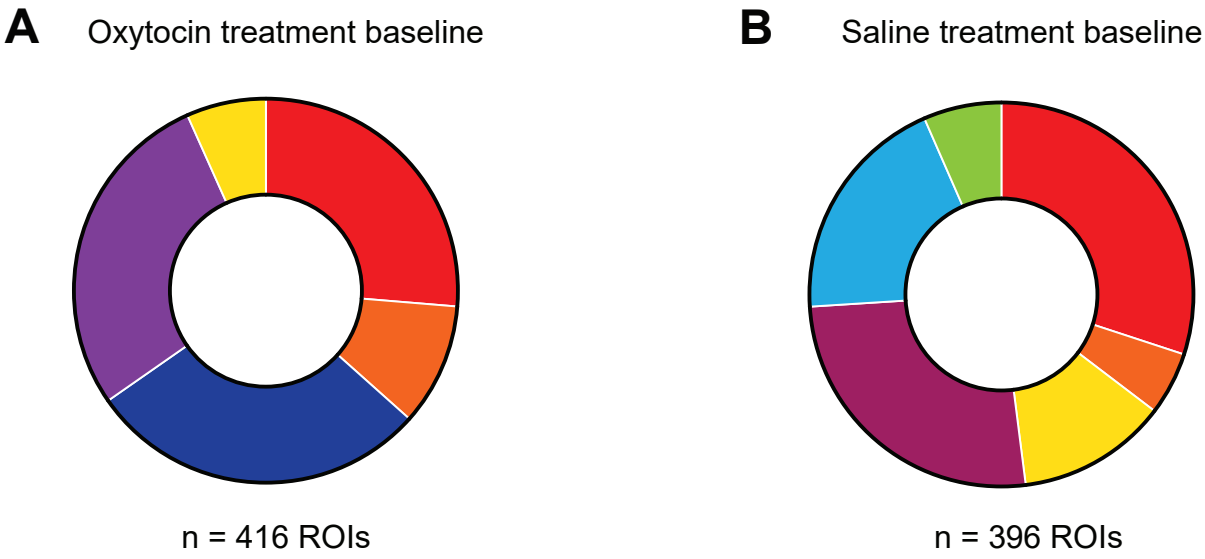

Figure S1: Summary statistics on the recorded ROIs for each animal. Related to Figure 1. (A) Total number of recorded regions of interest (ROIs) during the baseline session prior to oxytocin treatment in 5 rats (n = 416 ROIs). Each color represents ROIs recorded from a single rat. **(B)** Total number of ROIs recorded during the baseline session prior to saline treatment in 6 rats (n = 396 ROIs). Each color represents ROIs recorded from a single rat.

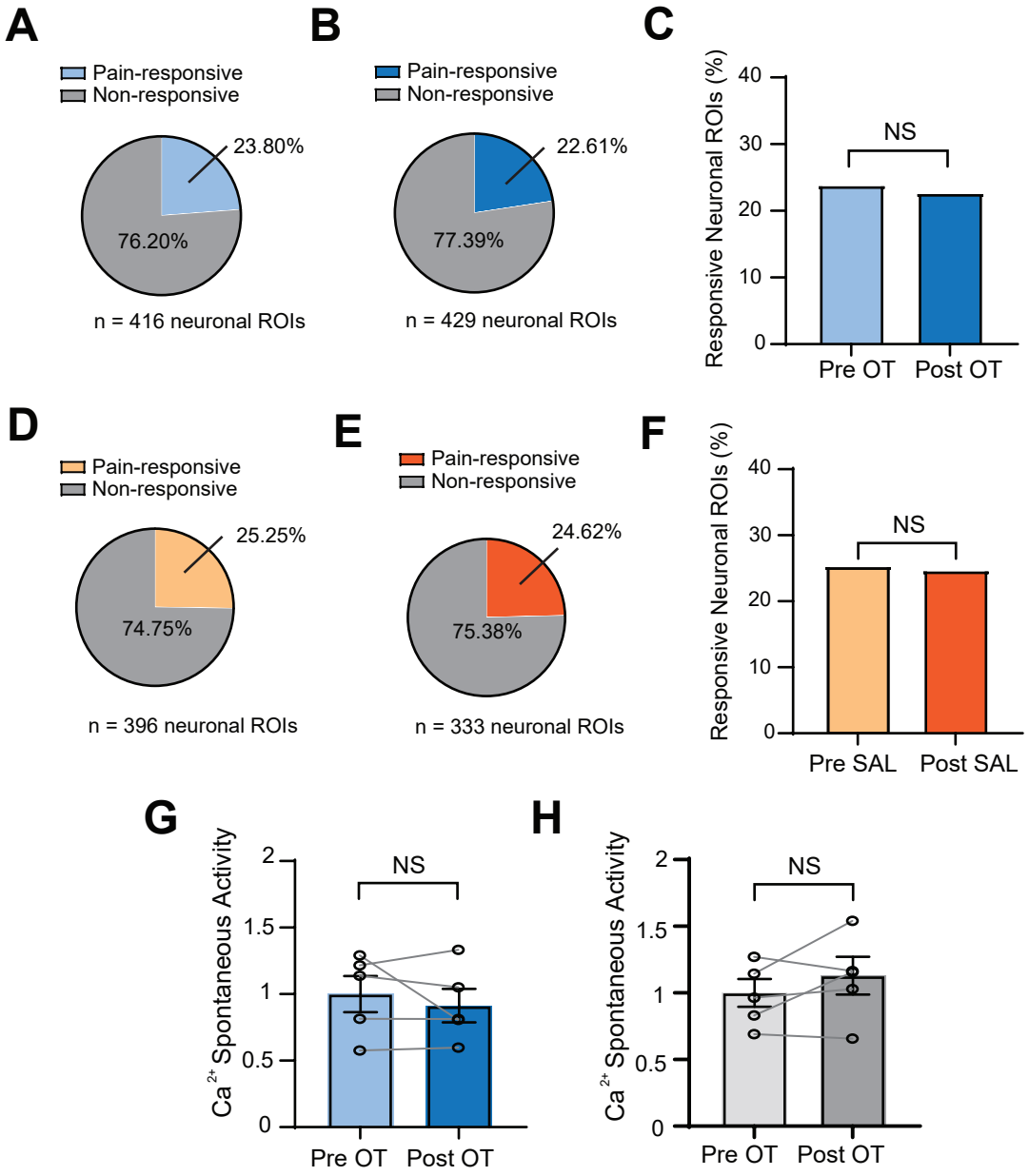

Figure S2: Neuronal activity in the PL-PFC before and after intracranial injections. Related to Figure 1. (A) A total of 23.90% neuronal ROIs in the PL-PFC were pain-responsive pre-oxytocin (OT) (n = 416 neuronal ROIs). **(B)** 22.61% neuronal ROIs in the PL-PFC were pain-reponsive post-OT (n = 429 neuronal ROIs). **(C)** The percentage of pain-responsive neuronal ROIs exhibited no change after OT administration (p = 0.6846, Fisher's exact test). **(D)** 25.25% neuronal ROIs in the PL-PFC were pain-responsive pre-saline (n = 396 neuronal ROIs). **(E)** 24.62% neuronal ROIs in the PL-PFC were pain-responsive post-saline (n = 333 neuronal ROIs). **(F)** The percentage of pain-responsive neuronal ROIs exhibited no change after saline (SAL) administration (p = 0.8639, Fisher's exact test). **(G)** There was no change in spontaneous activity (normalized to pre OT) in pain-responsive neuronal ROIs before and after OT administration (p = 0.4517, paired t-test; n = 5 animals). **(H)** There was no change in spontaneous activity (normalized to pre OT) in all neuronal ROIs before and after OT administration (p = 0.2626, paired t-test; n = 5 animals). For all comparisons, n = 5 OT and n = 6 SAL animals. Error bars are S.E.M.

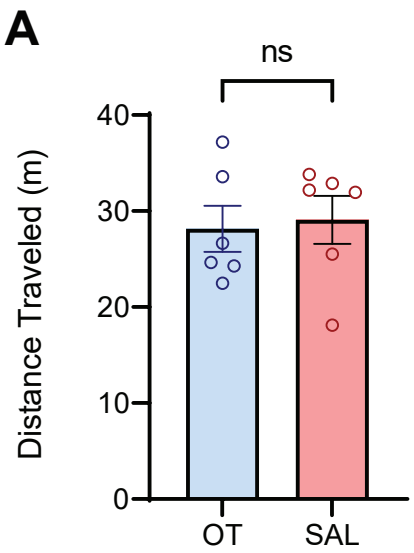

Figure S3: Locomotion following oxytocin (OT) or saline (SAL) injection into the PL-PFC. Related to Figure 2. (A) Intracranial delivery of oxytocin did not alter the total distance traveled relative to that of saline treatment (unpaired t test, p = 0.7935; n = 6 OT and n = 6 SAL).

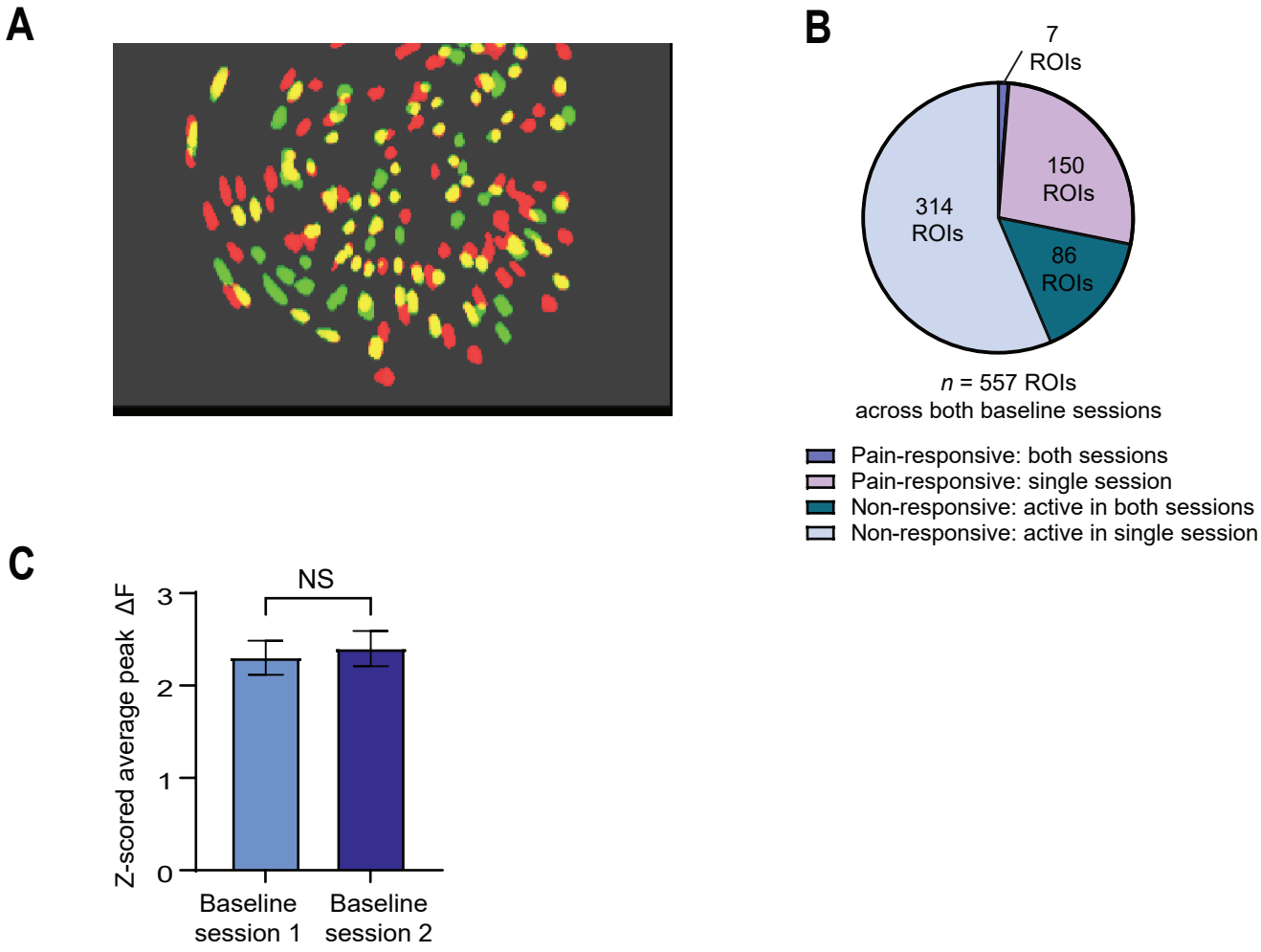

Figure S4: Baseline neuronal activity in the PL-PFC across sessions. Related to Figure 6. (A) Contours of all active neuronal regions of interest (ROIs) matched across two baseline recording sessions on separate days from a single rat. ROIs were considered active when they exhibited changes in fluorescence during the recording session. Red indicates neuronal activity from the first session; green indicates activity from the second session; yellow indicates overlap of activity from both sessions. **(B)** Distribution of pain-responsive and non-responsive neuronal ROIs across sessions in 3 rats. Very few ROIs were considered pain-responsive in both baseline sessions. **(C)** No significant difference in mean peak ΔF was observed in the PL-PFC across baseline sessions in 3 rats ($p = 0.7114$, unpaired t test; $n = 351$ baseline session 1 and $n = 338$ baseline session 2 neuronal ROIs). Error bars are S.E.M.

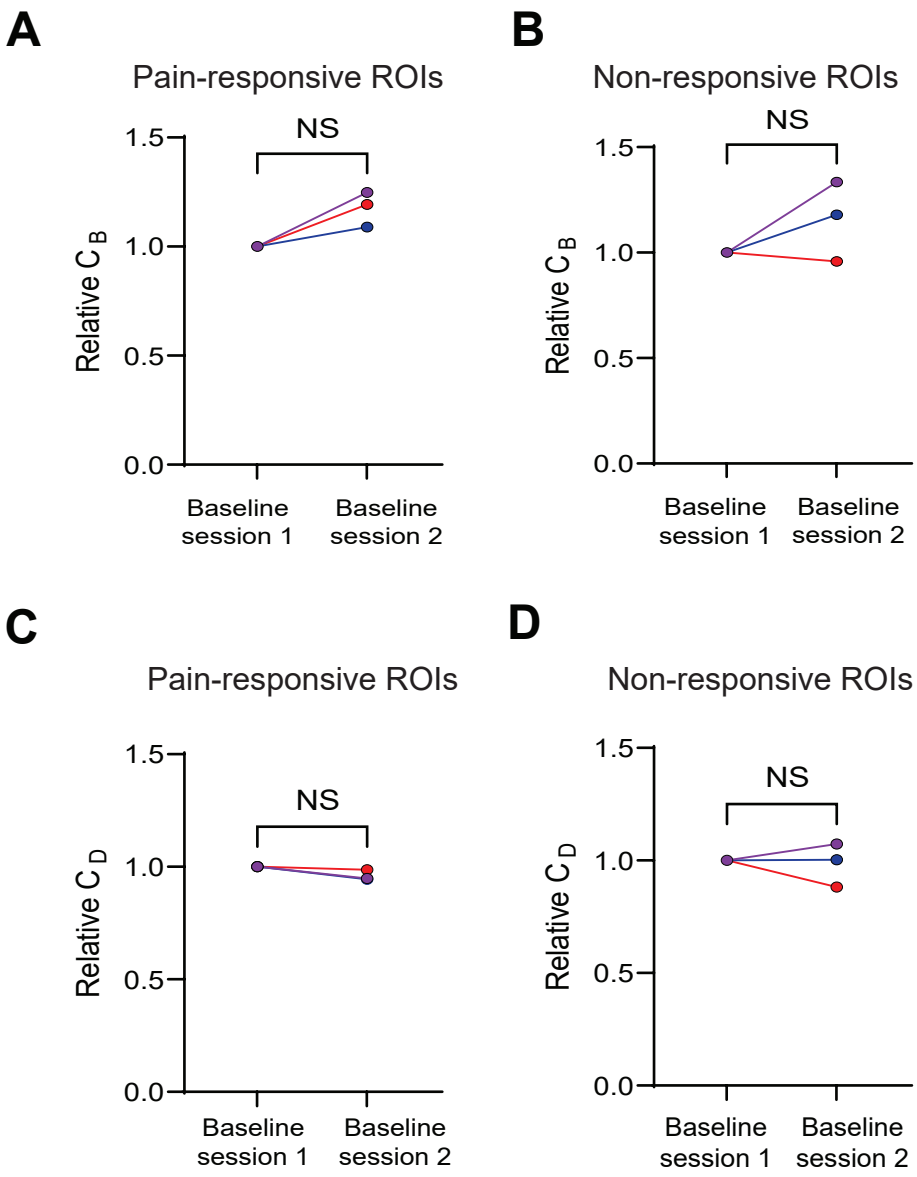

Figure S5: Betweenness and degree centralities are stable across baseline sessions in pain-responsive ROIs. Related to Figure 6. Relative centralities are normalized to baseline session 1. **(A)** Relative betweenness centrality (C_B) of pain-responsive ROIs did not have a significant change between baseline sessions recorded from two separate days ($p = 0.0633$, paired t test). **(B)** Relative C_B of non-responsive ROIs did not have a significant change in baseline recordings across days ($p = 0.2866$, paired t test). **(C)** Relative degree centrality (C_D) of pain-responsive ROIs did not change significantly across days in the baseline condition ($p = 0.0992$, paired t test). **(D)** Relative C_D of non-responsive ROIs experienced no significant change across recording sessions in the baseline condition ($p = 0.8237$, paired t test). n = 3 animals for all comparisons.

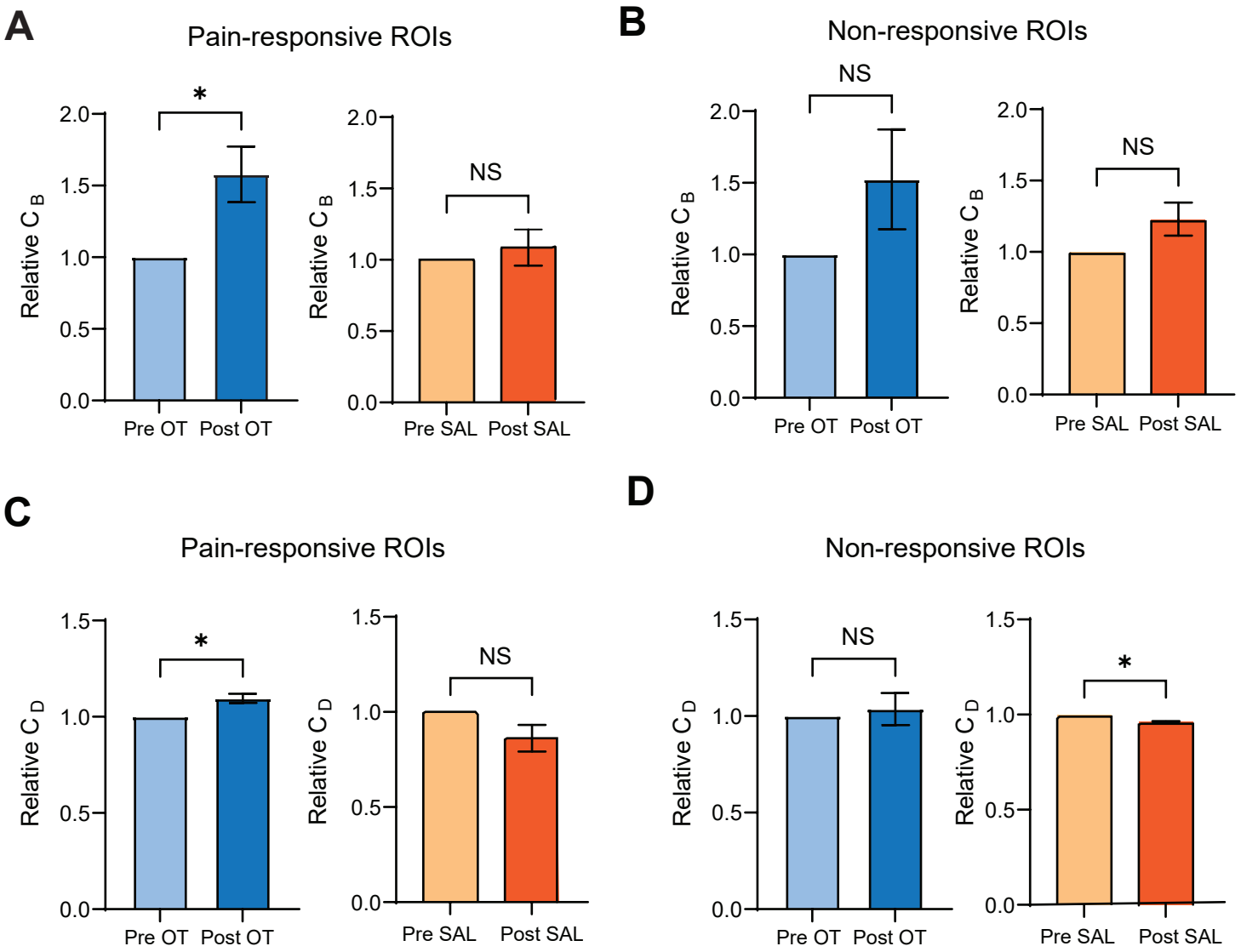

Figure S6: Oxytocin enhances betweenness and degree centralities of pain-responsive ROIs. Related to Figure 6. Relative centralities are normalized to the baseline (pre oxytocin or pre saline condition). **(A)** An increase in relative betweenness centrality (C_B) of pain-responsive ROIs is observed post OT treatment, but not post SAL treatment (paired t test; pre OT versus post OT: $p < 0.05$; pre SAL versus post SAL: $p = 0.6202$). **(B)** No significant change in C_B of non-responsive ROIs was observed after either OT or SAL treatment (paired t test; pre OT versus post OT: $p = 0.2068$; pre SAL versus post SAL: $p = 0.2980$). **(C)** An increase in relative degree centrality (C_D) of pain-responsive ROIs is observed post OT treatment, but not post SAL treatment (paired t test; pre OT versus post OT: $p < 0.05$; pre SAL versus post SAL: $p = 0.2997$). **(D)** While the C_D of non-responsive ROIs demonstrated no change post OT treatment, the C_D exhibited a slight decrease post SAL treatment (paired t test; pre OT versus post OT: $p = 0.6837$; pre SAL versus post SAL: $p < 0.05$). n = 5 OT and n = 2 SAL animals for all comparisons. Error bars are S.E.M.

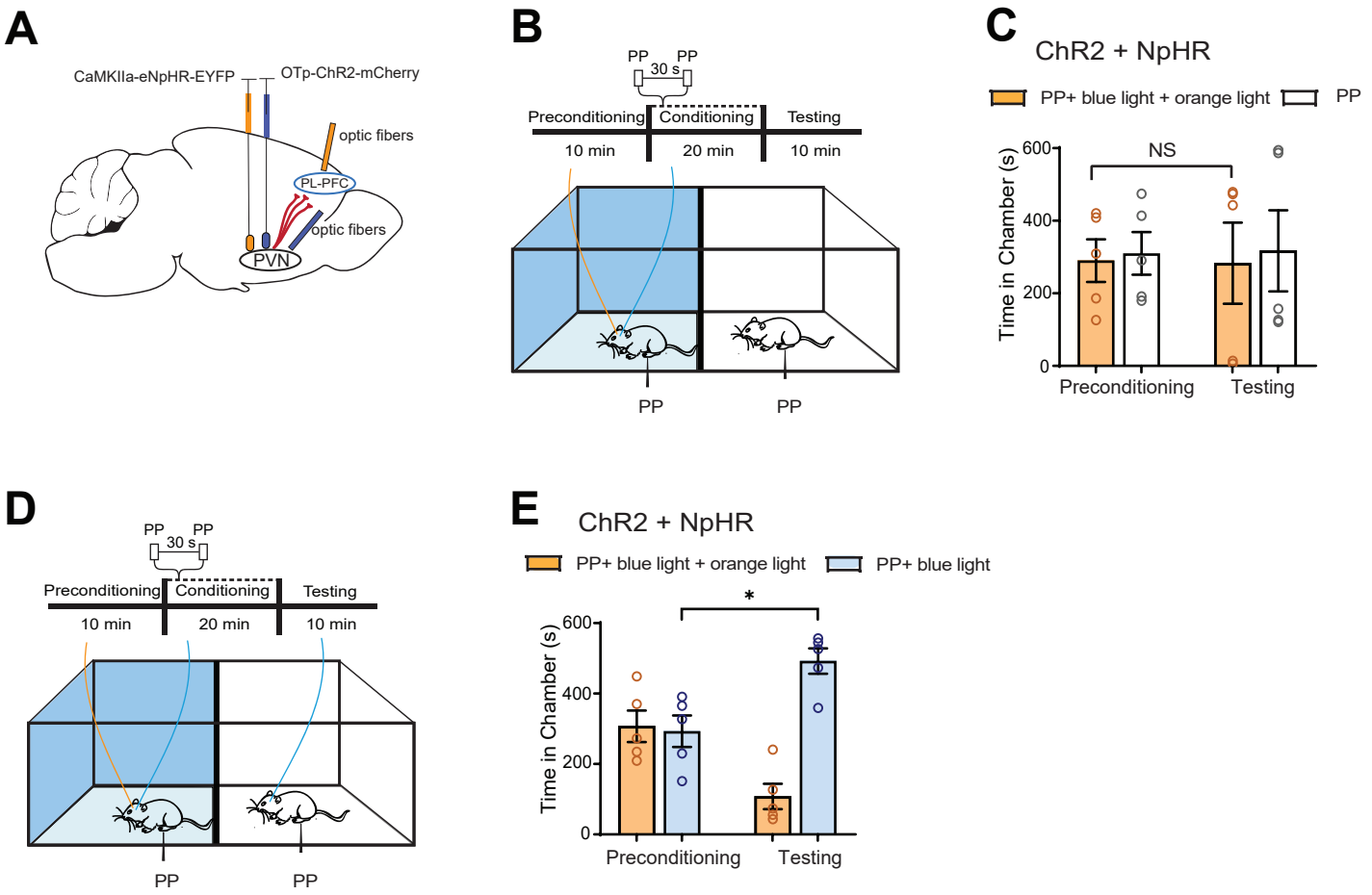

Figure S7: Optogenetic inhibition of axon terminals of PVN neurons in the PL-PFC suppresses the effect of PVN activation. Related to Figure 7. (A) Schematic of the injection of OTp-ChR2-mCherry and CamKIIa-eNpHR-EYFP into the PVN, with optic fibers implanted in the PVN and PL-PFC. **(B)** Schematic of CPP assay. One chamber was paired with simultaneous blue light stimulation of the PVN and orange light stimulation of the PL-PFC, whereas the other chamber was paired with no light treatment. **(C)** ChR2 + NpHR rats demonstrated no preference for the chamber associated with simultaneous blue light and orange light treatment ($p = 0.9982$, two-way ANOVA with repeated measures and Sidak's multiple comparisons test, n = 5 animals). **(D)** Schematic of CPP assay. One chamber was paired with simultaneous blue light stimulation of the PVN and orange light stimulation of the PL-PFC, while the other chamber was paired with only blue light stimulation of the PVN. **(E)** ChR2 + NpHR rats preferred the chamber associated with only blue light treatment of the PVN ($p^* < 0.05$, two-way ANOVA with repeated measures and Sidak's multiple comparisons test, n = 5 animals). Error bars are S.E.M.