SUPPLEMENTAL INFORMATION

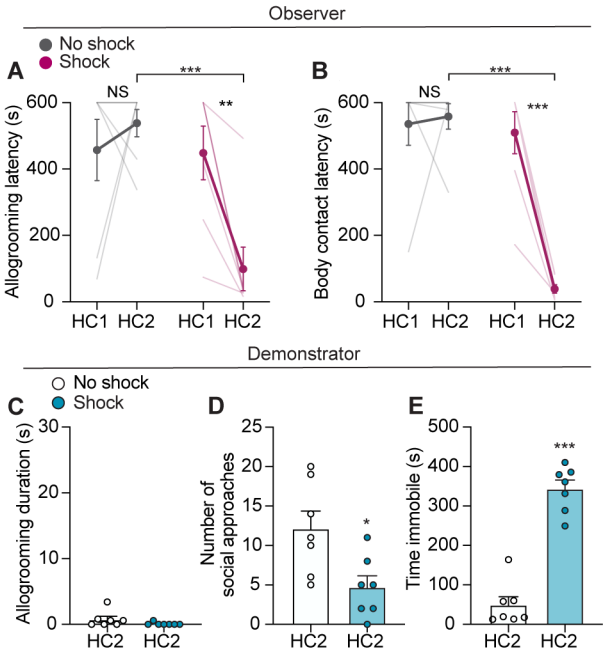

Figure S1. Other-directed affiliation latency by observers and demonstrator behaviors in DIA paradigm (Related to Figure 1)

(A and B) Allogrooming latency (A) and body contact latency (B) of observer mice directed toward demonstrators under shock or no shock conditions during HC1 and HC2.

(C-E) Allogrooming duration (C), number of social approaches (D) and time immobile (E) by shocked demonstrators in HC2.

Statistical tests: (A-E). Two-tailed paired Student's *t*-tests were used for within group comparisons (HC1 vs. HC2) and two-tailed unpaired Student's *t*-tests were used for comparison between two groups. **P* < .05, ***P* < .01, ****P* < .001. NS, not significant. Data represents mean ± s.e.m. Statistical details are provided in Table S2.

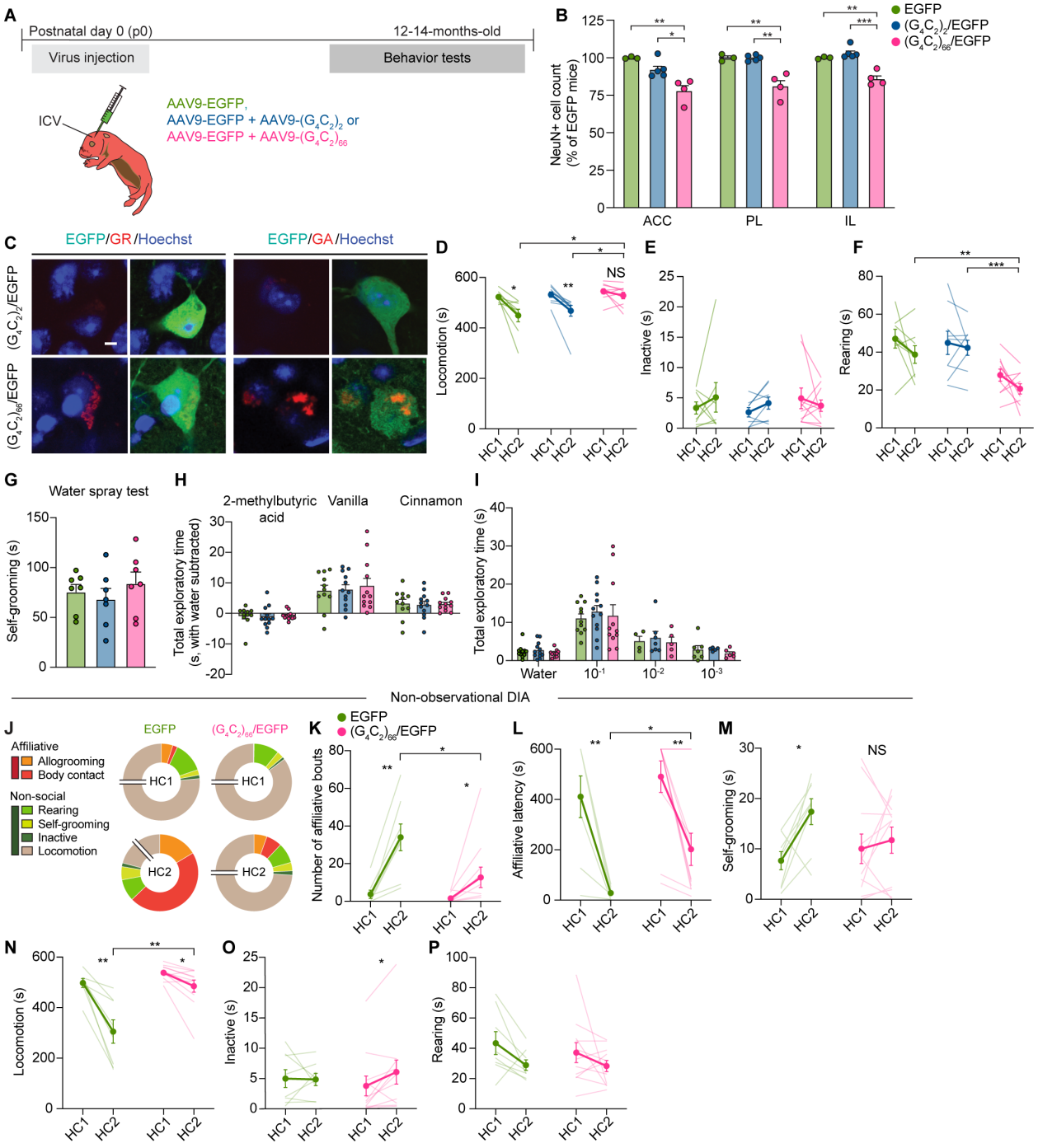

Figure S2. Pathological and behavioral phenotypes of aged $(G_4C_2)_{66}$/EGFP mice (Related to Figure 2)

(A) Timeline and schematic of bilateral intracerebroventricular (ICV) viral injections at postnatal day 0 (p0) and behavioral assays at advanced ages (12-14 months).

(B) Quantification of NeuN-positive cells in the anterior cingulate (ACC, Cg1/Cg2), prelimbic (PL) and infralimbic (IL) cortical subregions.

(C) Presence of dipeptide repeat (DPRs) proteins poly(GR) and poly(GA) and their co-expression with EGFP in 12-month-old $(G_4C_2)_{66}$/EGFP mice. Scale bar, 5 μm.

(D-F) Total locomotion (D), inactive (E), and rearing (F) duration by observers during HC1 and HC2 in observational DIA tests.
(G) Total time self-grooming during water-spray test.
(H) Total time sniffing attractive and aversive olfactory stimuli (discrimination).
(I) Total time sniffing various dilutions of vanilla scent (sensitivity).
(J) Proportions of time aged $(G_4C_2)_{66}$/EGFP and EGFP observer mice spent engaging in other-directed affiliative and non-social behaviors. Observers did not witness demonstrator mice receiving foot shocks during DIA tests (non-observational). Breaks in pie charts indicate 4 minutes of locomotion.
(K and L) Number of bouts (K) and latency (L) of affiliative behaviors exhibited by aged $(G_4C_2)_{66}$/EGFP and EGFP observers toward distressed demonstrators in non-observational DIA tests.
(M) Total self-grooming of aged observer mice in non-observational DIA tests.
(N-P) Total locomotion (D), inactive (E), and rearing (F) duration by observers during HC1 and HC2 in non-observational DIA tests.
Statistics: (B, G-I), one-way ANOVAs with Bonferroni's post-hoc tests. (D-F, K-P), two-tailed paired Student's *t*-tests were used for within group comparisons (HC1 vs. HC2) and two-tailed unpaired Student's *t*-tests were used for comparison between two groups. $*P < .05$, $**P < .01$, $***P < .001$. NS, not significant. Data represents mean ± s.e.m. Statistical details are provided in Table S2.

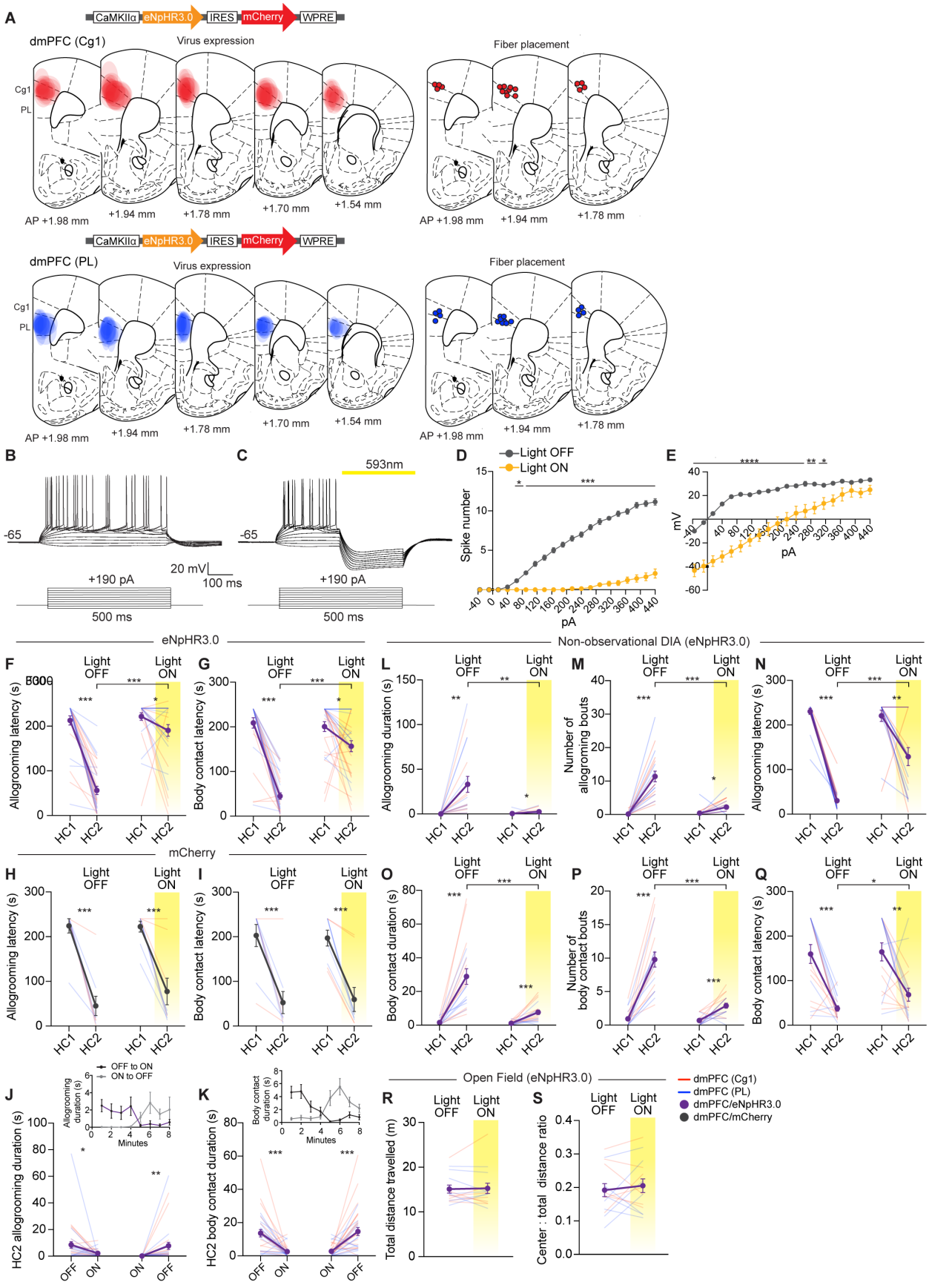
A
CaMKIIα
eNpHR3.0
IRES
mCherry
WPRE
dmPFC (Cg1)
Virus expression
Fiber placement
Cg1
PL
AP +1.98 mm
+1.94 mm
+1.78 mm
+1.70 mm
+1.54 mm
dmPFC (PL)
B
C
593nm
-65
+190 pA
500 ms
20 mV
100 ms
D
Light OFF
Light ON
Spike number
pA
E
mV
pA
eNpHR3.0
Non-observational DIA (eNpHR3.0)
mCherry
Light OFF
Light ON
F
Allogrooming latency (s)
G
Body contact latency (s)
L
Allogrooming duration (s)
M
Number of allogrooming bouts
N
H
I
O
Body contact duration (s)
P
Number of body contact bouts
Q
HC1
HC2
J
OFF to ON
ON to OFF
Minutes
HC2 allogrooming duration (s)
K
HC2 body contact duration (s)
Open Field (eNpHR3.0)
R
Total distance travelled (m)
S
Center : total distance ratio
dmPFC (Cg1)
dmPFC (PL)
dmPFC/eNpHR3.0
dmPFC/mCherry

Figure S3. Virus expression, optic fiber placements, slice electrophysiology validation, and additional behavioral analyses of *in vivo* optogenetics experiments (Related to Figure 4)
(A) Viral construct and schematic representations of virus expression (left) and fiber placements (right) throughout the dmPFC (targeting primarily Cg1 or PL) in consecutive coronal brain sections.
(B and C) Representative traces showing injected current steps (500 ms, +25 pA steps) reliably evoked action potentials in dmPFC neurons (B) and, in the same neuron, eNpHR3.0 activation by yellow light (593 nm, 250 ms) inhibited evoked action potentials (C).
(D) Summary of AP spike numbers elicited by step current injections in the absence and presence of yellow light from (C).
(E) Quantification of current voltage relationship from (C) in the absence (measured at 250 ms) vs. presence (measured at 500 ms) of yellow light.
(F and G) Allogrooming latency (F) and body contact latency (G) by eNpHR3.0-transduced observer mice during HC1 and HC2 (light OFF vs. light ON) in observational DIA tests.
(H and I) Allogrooming latency (H) and body contact latency (I) by mCherry-transduced observer mice during HC1 and HC2 (light OFF vs. light ON) in observational DIA tests.
(J and K) Total allogrooming or body contact duration of eNpHR3.0-transduced observer mice during 4-minute laser ON or 4-minute laser OFF phases of ON-OFF and OFF-ON counterbalanced photostimulation schemes during HC2 of observational DIA tests. Insets, time courses of allogrooming (J) or body contact (K) duration (per minute) during ON-OFF and OFF-ON transitions.
(L-N) Allogrooming duration (L), number of bouts (M), and latency (N) during HC1 and HC2 (light OFF vs. light ON) by eNPHR3.0-transduced observers in non-observational DIA tests.
(O-Q) Body contact duration (O), number of bouts (P), and latency (Q) during HC1 and HC2 (light OFF vs. light ON) by eNPHR3.0-transduced observers in non-observational DIA tests.
(R and S) Total distance travelled (R) and center to total distance ratio (S) by eNPHR3.0-transduced observers during open field test with light OFF vs. light ON for dmPFC inhibition.
Statistics: (D and E): Two-way ANOVA with Bonferroni's post-hoc tests. (F-S): Two-tailed paired Student's *t*-tests were used for within group comparisons (HC1 vs. HC2, Light OFF vs. Light ON) and two-tailed unpaired Student's *t*-tests were used for comparison between two groups. $*P < .05$, $**P < .01$, $***P < .001$. Data represents mean ± s.e.m. Statistical details are provided in Table S2.

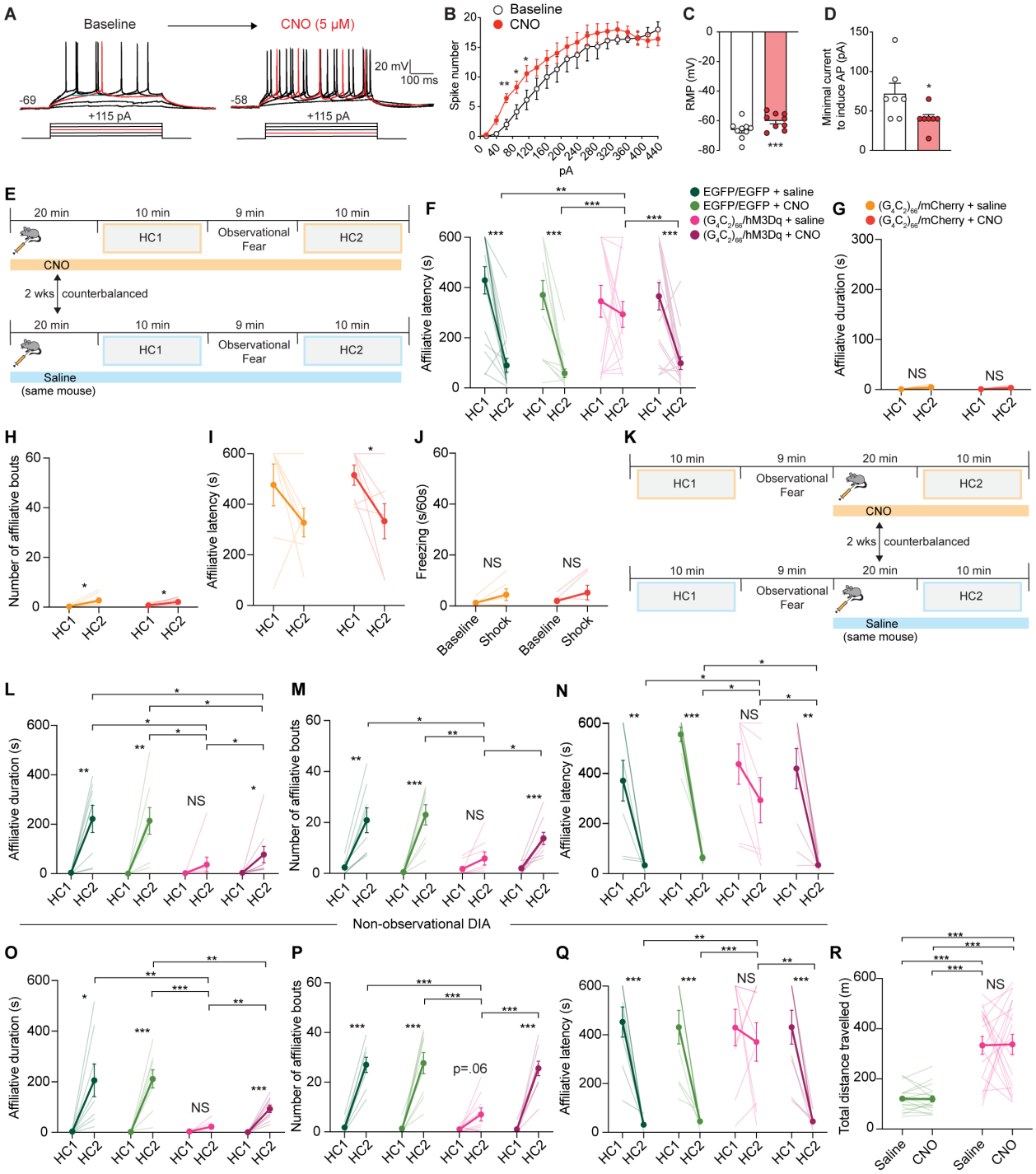

Figure S4. Slice electrophysiological validation and additional data of *in vivo* chemogenetics experiments (Related to Figure 4)

(A) Representative traces showing voltage responses to a series of current pulses with or without bath-applied CNO. Red traces indicate the minimal currents that induced APs.

(B-D) Evoked APs (B), summary of resting membrane potential (C) and minimal current to induce APs (D) in the absence and presence of CNO. RMP, resting membrane potential.

(E) Timeline and schematic for *in vivo* observational DIA chemogenetic experiments. CNO was administered (i.p.) to observer mice 20 min before behavioral tests.
(F) Affiliative latency by $(G_4C_2)_{66}$/hM3Dq or control EGFP/EGFP observers towards demonstrators in saline or CNO conditions in observational DIA tests.
(G-I) Affiliative duration (G), number of bouts (H), and latency (I) by $(G_4C_2)_{66}$/EGFP observers transduced with control AAV-mCherry ($(G_4C_2)_{66}$/mCherry) towards demonstrators in saline or CNO conditions in observational DIA tests.
(J) Average freezing time during baseline (minutes 1-5) or shock (minutes 5-9) phase of OFC by $(G_4C_2)_{66}$/mCherry observers after saline or CNO injections.
(K) Timeline and schematic of a modified *in vivo* chemogenetic observational DIA paradigm. CNO was administered to observer mice immediately after OFC. 20 minutes later, observers were reunited with distressed demonstrators for measurement of affiliative behaviors in HC2.
(L-N) Affiliative duration (L), number of bouts (M), and latency (N) by $(G_4C_2)_{66}$/hM3Dq or EGFP/EGFP observers towards demonstrators in saline or CNO conditions in the modified observational DIA tests.
(O-Q) Affiliative duration (O), number of bouts (P), and latency (Q) by $(G_4C_2)_{66}$/hM3Dq or EGFP/EGFP observers towards demonstrators in saline or CNO conditions in non-observational DIA tests.
(R) Total distance travelled during open field test with saline or CNO i.p. injections.
Statistics: (B): Two-way repeated measures ANOVA with Bonferroni's post-hoc tests. (C, D, F-J, L-R): Two-tailed paired Student's *t*-tests were used for within group comparisons (Baseline vs. CNO, HC1 vs. HC2, Baseline vs. Shock, Saline vs. CNO) and two-tailed unpaired Student's *t*-tests were used for comparison between two groups. $*P < .05$, $**P < .01$ $***P < .001$. NS, not significant. Data represents mean ± s.e.m. Statistical details are provided in Table S2

	Cg1			PL		
	EGFP	$(G_4C_2)_2$/EGFP	$(G_4C_2)_{66}$/EGFP	EGFP	$(G_4C_2)_2$/EGFP	$(G_4C_2)_{66}$/EGFP
Resting Potential, mV	-70.65 ± 1.10	-70.16 ± 1.03	-66.94 ± 1.52	-68.78 ± 1.04	-70.44 ± 1.83	-68.11 ± 1.66
Threshold, mV	-38.46 ± .76	-39.48 ± .75	-41.93 ± 2.54	-38.95 ± .67	-37.91 ± .66	-39.25 ± 1.47
Half-width, ms	0.76 ± .02	0.78 ± .04	0.64 ± .03	0.89 ± .05	0.77 ± .03	0.92 ± .05
fAHP, mV	-5.80 ± .97	-6.43 ± .44	-9.42 ± .18	-3.87 ± .63	-5.47 ± 1.03	-7.69 ±1.19*
mAHP, mV	-13.41 ± .99	-15.07 ± 1.16	-13.30 ± .96	-8.8 ± 1.09	-12.76 ± 2.76	-12.81 ± 1.28
sAHP, mV	-1.53 ± .26	-2.34 ± .27	1.71 ± .55	-2.07 ± .28	-1.64 ± .44	-1.64 ± .36
Amplitude, mV	80.83 ± 2.94	80.33 ± 2.0	82.98 ± 2.74	81.78 ± 1.99	77.46 ± 2.17	85.63 ± 2.19$^{\#}$
Rise slope, mV	162.87 ± 19.08	145.49 ± 7.45	167.39 ± 10.77	145.49 ± 9.65	144.32 ± 13.26	157.01 ± 11.24
Decay slope, mV	-58.98 ± 2.88	-58.62 ± 2.55	-75.98 ± 6.76*	-51.53 ± 2.66	-55.21 ± 3.25	-53.19 ± 3.28
Rheobase, pA	83.75 ± 6.25	82.86 ± 9.24	140 ± 11.18**	70 ± 5.19	67.27 ± 6.27	151.54 ± 20.24***
Spike Number (215pA)	9.75 ± .45	9.43 ± .72	4.17 ± .40***	12.1 ± .53	13.27 ± .94	5.00 ± .97***
Input Resistance, MΩ	156.3 ± 6.32	166.9 ± 13.72	100.3 ± 7.58**	226.8 ± 16.32	215.7 ± 14.99	143.8 ± 13.21**
Capacitance, pF	144.5 ± 11.62	145.9 ± 14.90	143.4 ± 17.65	189.2 ± 10.11	165.7 ± 15.45	174.1 ± 13.75

Table S1. Passive and active membrane properties of dmPFC LV pyramidal neurons from aged mice (Related to Figure 3)

RMP, resting membrane potential; AP, action potential; fAHP, fast afterhyperpolarization; mAHP, medium afterhyperpolarization; sAHP, slow afterhyperpolarization. Statistical tests: One-way-ANOVAs with Bonferroni's post-hoc tests. *$P < .05$, **$P < .01$ ***$P < .001$ vs. EGFP and $(G_4C_2)_2$/EGFP. $^{\#}P < .05$ vs. $(G_4C_2)_2$/EGFP. Data represents mean ± s.e.m. Statistical details are provided in Table S2.