Figures and figure supplements

Adult-born granule cells modulate CA2 network activity during retrieval of developmental memories of the mother

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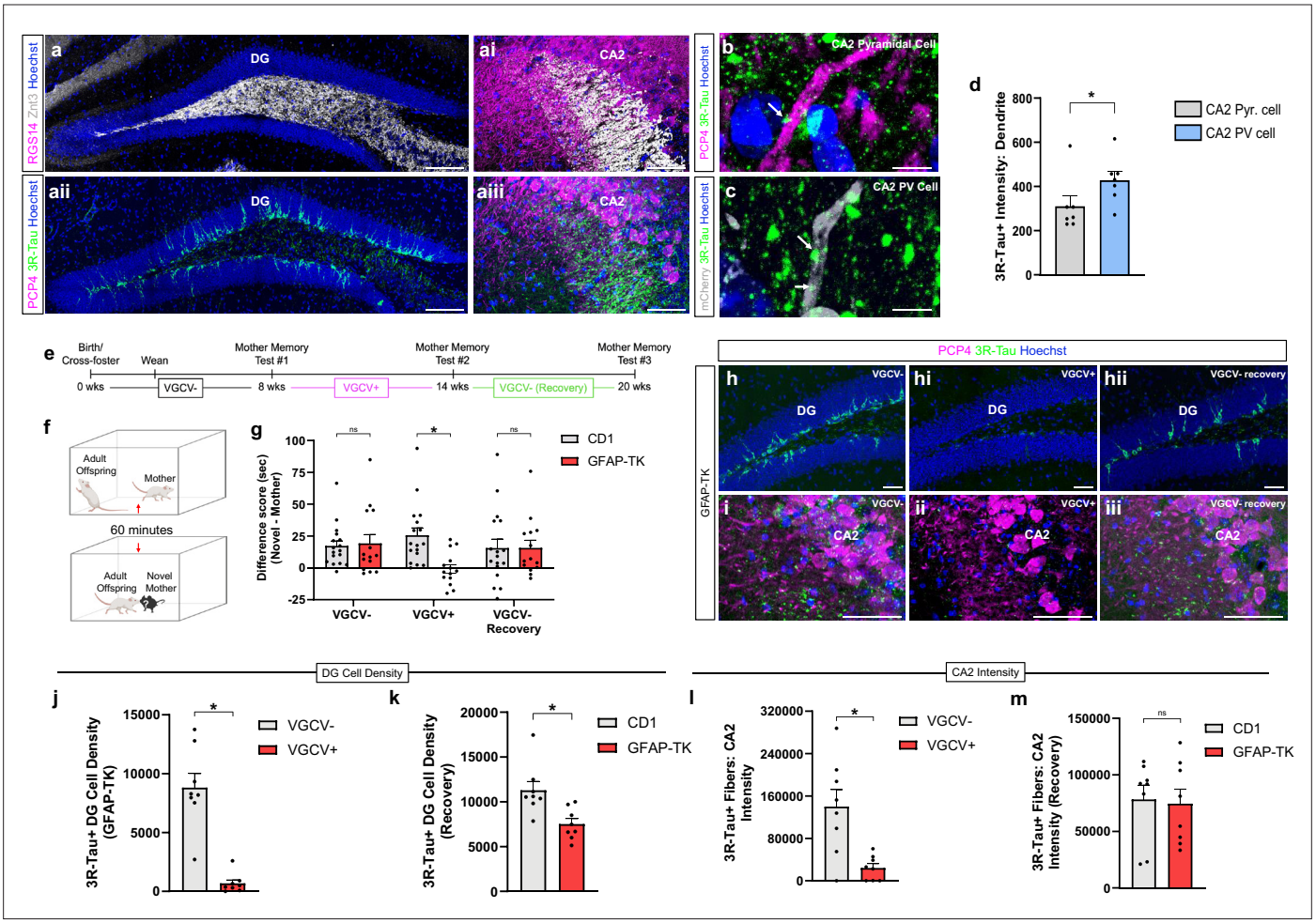

Figure 1. Adult-born granule cells support retrieval of developmental remote memories of the mother. (**a-aiii**) Confocal images of dentate gyrus granule cells, both mature (ZnT3+) and adult-born (3R-Tau+), and their robust projections to CA2. Confocal images of adult-born granule cell (abGC) 3R-Tau+axons (arrows) (**b**) near proximal dendrite of a CA2 PCP4 + pyramidal cell and (**c**) near proximal dendrite of a CA2 mCherry + PV+ interneuron. (**d**) abGC fibers (3R-Tau+) in the CA2 are more abundant near proximal dendrites (n=7; paired t-test: t_6=3.163, p=0.0195) of PV + interneurons (mCherry+) than those of pyramidal cells (PCP4+). (**e**) Timeline for behavioral experiment. (**f**) Schematic demonstrating direct social interaction assay used at three experimental time points. (**g**) Ablation of abGCs abolishes difference in investigation time between mother and novel mother (CD1: n=17; TK: n=14; two-way RM ANOVA: Genotype x Drug: $F_{2,58}$ = 5.352, p=0.0074; Šídák's test p=0.0021). After removing valganciclovir (VGCV) from rodent chow and allowing adult neurogenesis to recover over the course of 6 weeks, mice were able to discriminate between mother and novel mother again (p>0.999). (**h-hii**) Confocal images of abGCs in the DG at three different drug time points. VGCV administration produces a dramatic loss of (**h-hii**) abGCs (3R-Tau + cells) in DG (VGCV-: n=8; VGCV+: n=8; unpaired t-test: t_{14}=6.588, p<0.0001) and (**i-iii**) 3R-Tau+ fibers in CA2 (VGCV: n=8; VGCV+: n=8; unpaired t-test: t_{14}=3.488, p=0.0036). 6 weeks after removing VGCV from the rodent chow, DG abGC number (**j, k**) undergoes a 70% recovery (CD1: n=8; TK: n=8; unpaired t-test: t_{14}=3.218, p=0.0062), and (**l, m**) 3R-Tau+ fibers present in CA2 undergo a complete recovery (CD1: n=8; TK: n=8; unpaired t-test: t_{14}=0.7271, p=0.4791). *p<0.05, bars represent mean + SEM. Scale bars = 200 μm for **a, h, i**; 2 μm for **b, c**. ns = not significant.

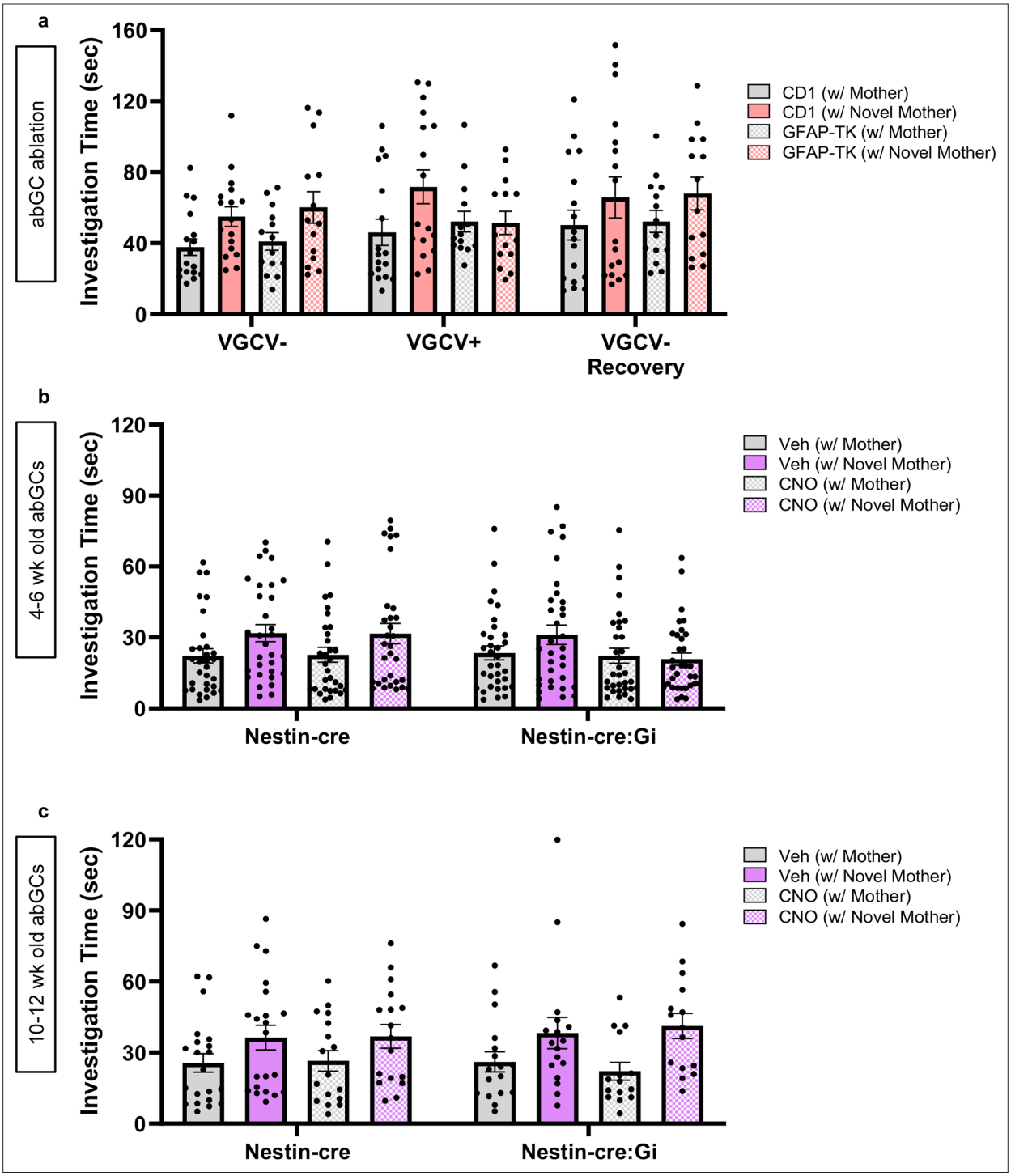

Figure 1—figure supplement 1. Total investigation times during social interaction. (**a**) Total investigation times of the mother and a novel mother were assessed for CD1 and GFAP-TK mice before (VGCV-), during (VGCV+), and after valganciclovir (VGCV) administration (VGCV- Recovery) (CD1: n=17; TK: n=14; RM ANOVA: Genotype x Drug: $F_{6,116}$ = 1.672, p=0.1339; Drug: $F_{1.173}$ = 5.515, p=0.0072). (**b**) Total investigation times of the mother and a novel mother were quantified for Nestin-cre mice and Nestin-cre:Gi mice with a 4–6-week-old population of inhibitory DREADD + adult-born granule cells (abGCs) (Nestin-cre: n=31; Nestin-cre:Gi: n=33; Mixed-effects ANOVA: Genotype x Drug x Stimulus: $F_{1,56}$ = 4.290, p=0.0430; Tukey's test: Nestin-cre Veh Mother vs Novel p=0.0004, Nestin-cre CNO Mother vs Novel p=0.0274, Nestin-cre:Gi Veh Mother vs Novel p=0.0326, Nestin-cre:Gi CNO Mother vs Novel p=0.9904). (**c**) Total investigation times of the mother and a novel mother were quantified for Nestin-cre mice and Nestin-cre:Gi mice with a 10–12-week-old population of inhibitory DREADD + abGCs (Nestin-cre: n=21; Nestin-cre:Gi: n=17; Mixed-effects ANOVA: Genotype x Drug x Stimulus: $F_{1,12}$ = 1.204, p=0.2941; Drug: $F_{1,40}$ = 60.46, p<0.0001). Bars represent mean + SEM. This figure contains data that relates to both main ***Figures 1 and 2***.

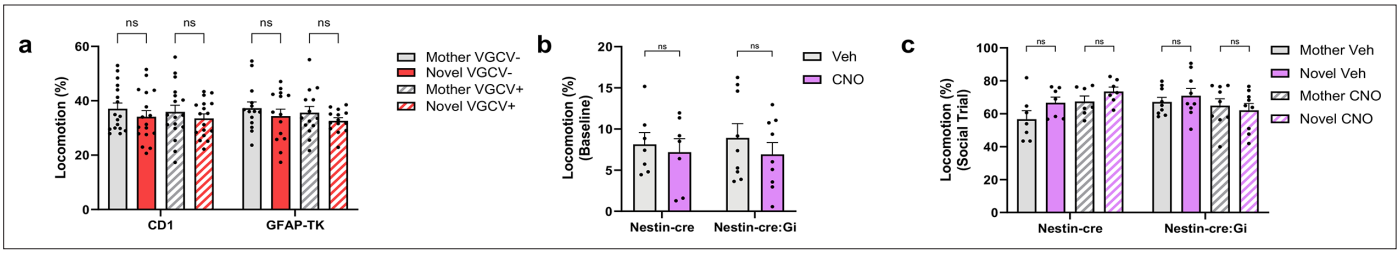

Figure 1—figure supplement 2. Valganciclovir (VGCV) or clozapine-N-oxide (CNO) administration does not alter locomotion. (**a**) GFAP-TK mice display normal locomotion during social interaction after 6 weeks of VGCV administration (CD1: n=17; TK: n=14; three-way ANOVA: Genotype x Drug x Stimulus: $F_{1,29}$ = 0.01998, p=0.8886). CNO administration has no effect on locomotion during (**b**) baseline (Nestin-cre: n=7; Nestin-cre:Gi: n=9; two-way RM ANOVA: Genotype x Drug: $F_{1,14}$ = 0.02291, p=0.8818) or (**c**) social stimulus trials (Nestin-cre: n=7; Nestin-cre:Gi: n=9; three-way ANOVA: Genotype x Drug x Stimulus: $F_{1,14}$ = 0.06126, p=0.8081) in Nestin-cre and Nestin-cre:Gi mice. A significant two-way interaction was observed during social trials, but all post hoc tests failed to reach significance (two-way ANOVA: Genotype x Drug: $F_{1,14}$ = 7.180, p=0.0180). Bars represent mean + SEM. ns = not significant. This figure contains data that relates to both main ***Figures 1 and 2***.

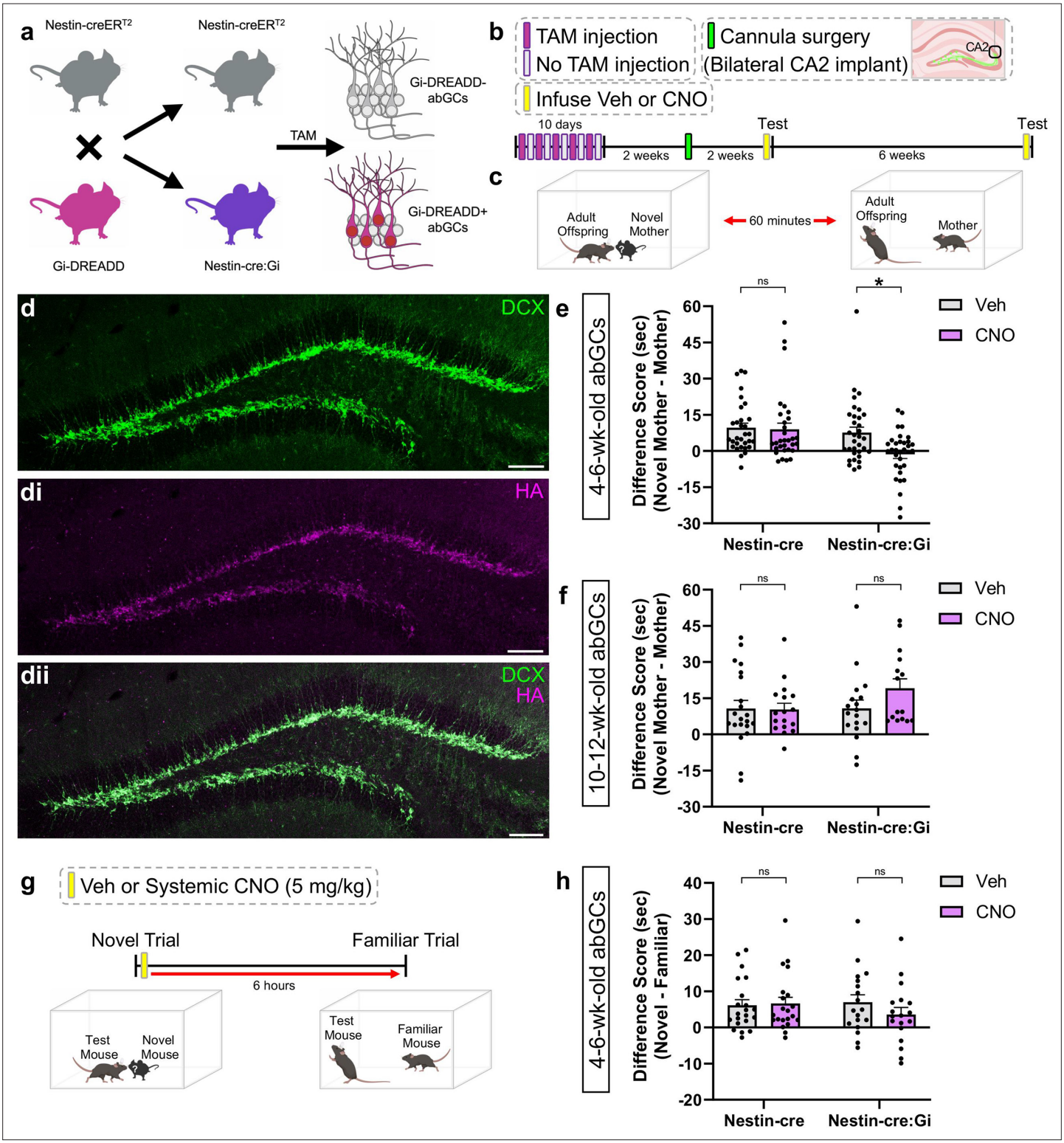

Figure 2. Inhibiting 4–6-week-old abGC projections to CA2 prevents discrimination between mothers and novel mothers. (**a**) Schematic of breeding to produce Nestin-cre:Gi double transgenic offspring. (**b**) Timeline outlining tamoxifen administration, cannula implantation, and behavioral testing. (**c**) Schematic demonstrating direct social interaction testing. (**d-dii**) Confocal images depicting 4–6-week-old Gi-DREADD + abGCs in the DG. (**e**) Inhibiting 4–6-week-old adult-born granule cell (abGC) projections to CA2 with clozapine-N-oxide (CNO) prevents discrimination between mother and novel mother (Nestin-cre: n=31; Nestin-cre:Gi: n = 33; two-way RM ANOVA: Genotype x Drug: $F_{1,62}$ = 4.042, p=0.0487; Šídák's test p=0.0057). (**f**) Inhibiting 10–12-week old abGC projections to CA2 does not influence discrimination between mother and novel mother Nestin-cre: n=21 (Veh), n=17

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(CNO); Nestin-cre:Gi: n=18 (Veh), n=15 (CNO); Mixed-effects ANOVA: Genotype x Drug:$_{1,67}$ = 1.669, Pp=0.2008. (**g**) Schematic for experiment assessing abGC contributions to social memory consolidation. (**h**) Systemic inhibition of abGCs during memory consolidation has no significant effect on social memory (Nestin-cre: n=21; Nestin-cre:Gi: n=18; two-way RM ANOVA: Genotype x Drug: $F_{1,37}$ = 1.238, p=0.2730). *p<0.05, bars represent mean + SEM. ns = not significant. Scale bars in d=200 µm.

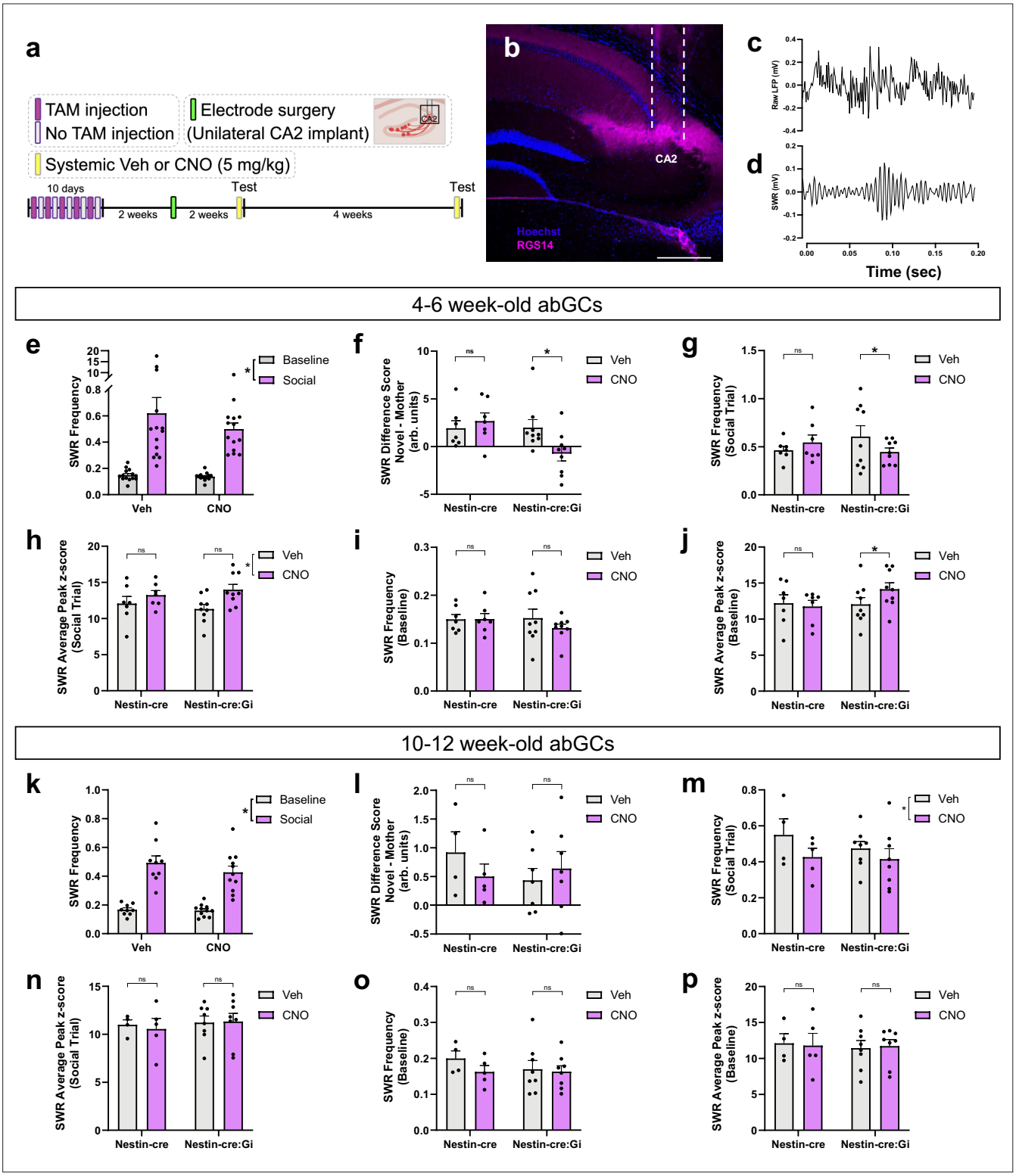

Figure 3. Adult-born neurons influence CA2 sharp wave ripple (SWR) changes associated with discrimination between novel mother and mother. (**a**) Timeline of experiment demonstrating tamoxifen injection schedule and electrode implantation in CA2. (**b**) Confocal image demonstrating accuracy of electrode placement. (**c**) Example of SWR trace and (**d**) filtered trace recorded from CA2. (**e**) CA2 SWR frequency is increased during social interactions, regardless of whether the stimulus is the novel mother or mother (Veh: n=14; CNO: n=14; 2-way RM ANOVA: Stimulus: $F_{1,13} = 46.40$, $p<0.0001$). (**f**) Inhibiting 4–6-weekold adult-born granule cells (abGCs) with clozapine-N-oxide (CNO) prevents characteristic SWR production patterns present during exposure to novel mothers vs mothers (Nestin-cre: n=7; Nestin-cre:Gi: n=9; Mixed-effects ANOVA: Genotype x Drug: $F_{1,110} = 18.6529$, $p<0.0001$;

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Tukey's test p<0.0001). (**g**) Inhibiting 4–6-week old abGCs significantly reduces social trial SWR production (Nestin-cre: n=7; Nestin-cre:Gi; n=9; Mixed-effects ANOVA: Genotype x Drug: $F_{1,228}$ = 16.2465, *P*<0.0001; Tukey's test p=0.0018). (**h**) Inhibiting 4–6 week old abGCs increases SWR peak amplitude during social interaction trials (Nestin-cre: n=7; Nestin-cre:Gi: n=9; Mixed-effects ANOVA: Genotype x Drug: $F_{1,238}$ = 3.69, p=0.0559) and (**j**) baseline (Nestin-cre: n=7; Nestin-cre:Gi: n=9; Mixed-effects ANOVA: Genotype x Drug: $F_{1,237}$ = 6.0322, p=0.01477; Tukey's test p=0.0133). (**i**) Changes in baseline SWR production did not reach significance (Nestin-cre: n=7; Nestin-cre:Gi: n=9; Mixed-effects ANOVA: Genotype x Drug: $F_{1,238}$ = 3.5101, p=0.0622). (**k**) At the 10–12 week abGC time point, CA2 SWR frequency is increased during social interactions, regardless of stimulus (Veh: n=9; CNO: n=11; Mixed-effects ANOVA: Stimulus: $F_{1,10}$ = 133.1, p<0.0001). (**l**) Inhibiting 10–12-week old abGCs has no influence on characteristic SWR production patterns present during exposure to the novel mother vs mother (Nestin-cre: n=4 (Veh), n=5 (CNO); Nestin-cre:Gi: n=7 (Veh and CNO); Mixed-effects ANOVA: Genotype x Drug: $F_{1,82}$ = 1.7431, p=0.1904), nor on (**m**) SWR production during social trials (Nestin-cre: n=4 (Veh), n=5 (CNO) Nestin-cre:Gi: n=8 (Veh and CNO); Mixed-effects ANOVA: Genotype x Drug: $F_{1,170}$ = 0.4496, p=0.503), or (**o**) during baseline recording (Nestin-cre: n=4 (Veh), n=5 (CNO); Nestin-cre:Gi: n=8 (Veh and CNO); Mixed-effects ANOVA: Genotype x Drug: $F_{1,170}$ = 2.0552, p=0.15352). (**n**) Inhibiting 10–12-week old adult-born granule cells (abGCs) has no influence on SWR peak amplitude social interaction trials (Nestin-cre: n=4 (Veh), n=5 (CNO); Nestin-cre:Gi: n=8 (Veh and CNO); Mixed-effects ANOVA: Genotype x Drug: $F_{1,18}$ = 0.9744, p=0.3250) or (**p**) during baseline (Nestin-cre: n=4 (Veh), n=5 (CNO); Nestin-cre:Gi: n=8 (Veh and CNO); Mixed-effects ANOVA: Genotype x Drug: $F_{1,172}$ = 0.540, p=0.8165), *p<0.05, bars represent mean + SEM. Scale bar in b=1500 µm. ns = not significant.

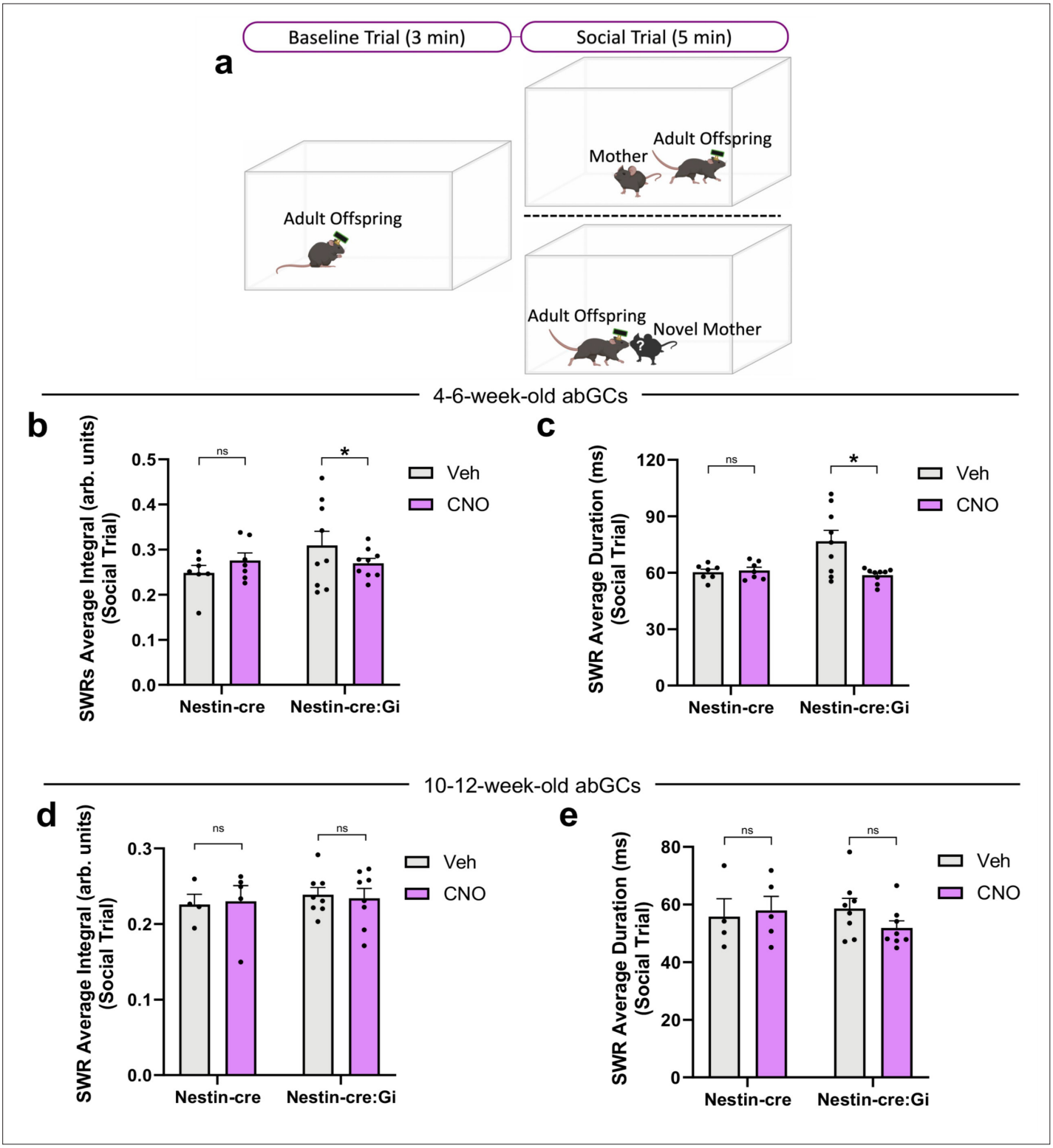

Figure 3—figure supplement 1. 4–6-week-old a adult-born granule cells (bGCs) influence multiple features of CA2 sharp wave ripples (SWRs). (**a**) Schematic of electrophysiological recordings during baseline and social trials during the 4–6-week-old abGC timepoint. (**b**) Silencing 4–6-week old abGCs reduces average CA2 SWR integral (Nestin-cre: n=7; Nestin-cre:Gi: n=9; Mixed-effects ANOVA: Genotype x Drug: $F_{1,236}$ = 12.83, p=0.0004017; Tukey's test p=0.0099). (**c**) Silencing 4–6-week old abGCs reduces average CA2 SWR duration (Nestin-cre: n=7; Nestin-cre:Gi: n=9; Mixed-effects ANOVA: Genotype x Drug: $F_{1,223}$ = 24.077, p<0.0001; Tukey's test p<0.0001). (**d**) Silencing 10–12-week old abGCs has no effect on average CA2 SWR integral but a significant interaction is observed (Nestin-cre: n=4 (Veh), n=5 (CNO); Nestin-cre:Gi: n=8 (Veh and CNO); Mixed-effects ANOVA: Genotype

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x Drug: $F_{1,172}$ = 4.0061, p=0.0469; Tukey's test p=0.7457). (**e**) Silencing 10–12-week old abGCs does not reduce average CA2 SWR duration but a significant interaction is observed (Nestin-cre: n=4 (Veh), n=5 (CNO); Nestin-cre:Gi: n=8 (Veh and CNO); Mixed-effects ANOVA: Genotype x Drug: F_{1172} = 8.5609, p=0.003898; Tukey's test p=0.1439). *p<0.05, bars represent mean + SEM. ns = not significant.

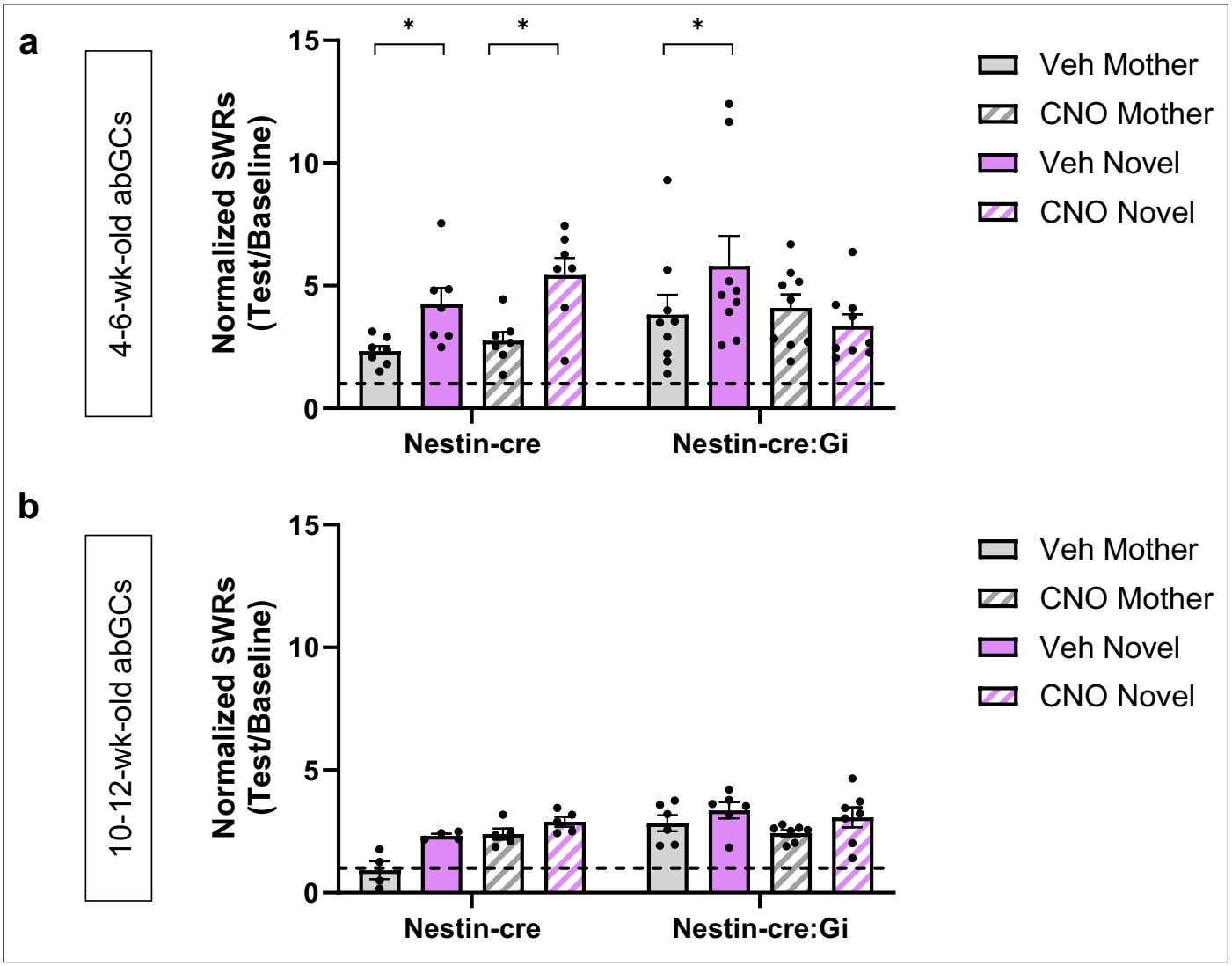

Figure 3—figure supplement 2. Normalized sharp wave ripple (SWR) generation. (**a**) Silencing 4–6-week-old adult-born granule cells (bGCs) alters CA2 SWR generation (Nestin-cre: n=7; Nestin-cre:Gi: n=9; Mixed-effects ANOVA: Genotype x Drug x Stimulus: $F_{1,234}$ = 13.3621, p=0.000317; Tukey's test: Nestin-cre Veh Mother vs Novel p=0.0042, Nestin-cre CNO Mother vs Novel p< 0.0001, Nestin-cre:Gi Veh Mother vs Novel p0.0003, Nestin-cre:Gi CNO Mother vs Novel p=0.7190). (**b**) Silencing 10–12 week-old abGCs has no influence on CA2 SWR generation (Nestin-cre: n=5; Nestin-cre:Gi: n=7; Mixed-effects ANOVA: Genotype x Drug x Stimulus: $F_{1,161}$ = 2.1093, p=0.14834; Stimulus: $F_{1,161}$ = 33.0013, p<0.0001). *p<0.05, bars represent mean + SEM.

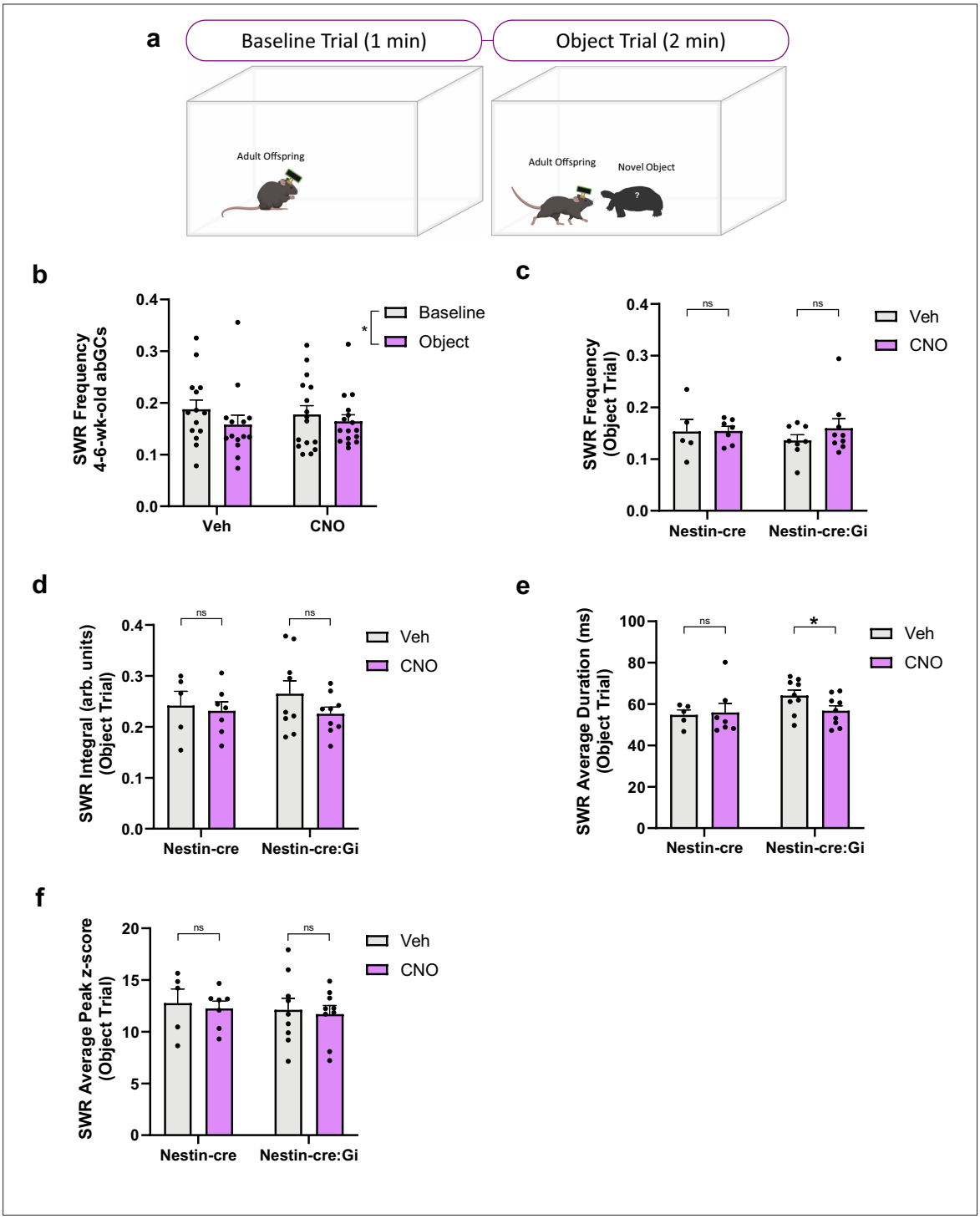

Figure 3—figure supplement 3. Nonsocial stimuli suppress CA2 sharp wave ripple (SWR) frequency. (**a**) Schematic of electrophysiological recordings during baseline and exposure to nonsocial object (plastic toy animal) trials during the 4–6-week old abGC timepoint. (**b**) Nonsocial stimuli slightly inhibit CA2 SWR frequency (Veh: n=14; CNO: n=16; 2-way RM ANOVA: Stimulus: $F_{1,28}$ = 7.760, p=0.0095). (**c**) Silencing 4–6-week old adult-born granule cells (abGCs) has no influence on CA2 SWR frequency during object exposure (Nestin-cre: n=5 (Veh), n=7 (CNO); Nestin-cre:Gi: n=8 (Veh), n=9 (CNO); Mixed-effects ANOVA: Genotype x Drug: $F_{1,95}$ = 0.7761, p=0.3806), (**d**) average integral (Nestin-cre: n=5, n=7 (CNO); Nestin-cre:Gi: n=9 (Veh and CNO); Mixed-effects ANOVA: Genotype x Drug: $F_{1,108}$ = 0.1653, p=0.2027). (**e**) Silencing 4–6-week old abGCs reduces average SWR duration during object trials (Nestin-cre: n=5 (Veh), n=7 (CNO); Nestin-cre:Gi: n=9 (Veh and CNO); Mixed-effects ANOVA: Genotype x Drug: $F_{1,106}$ = 7.9118, p=0.005849; Tukey's test p=0.0094). (**f**) Silencing 4–6-week old abGCs has no effect on SWR peak z-score (Nestin-cre: n=5 (Veh), n=7 (CNO); Nestin-cre:Gi: n=9 (Veh and CNO); Mixed-effects ANOVA: Genotype x Drug: $F_{1,111}$ = 0.0003, p=0.9874). *p<0.05, bars represent mean + SEM. ns = not significant.

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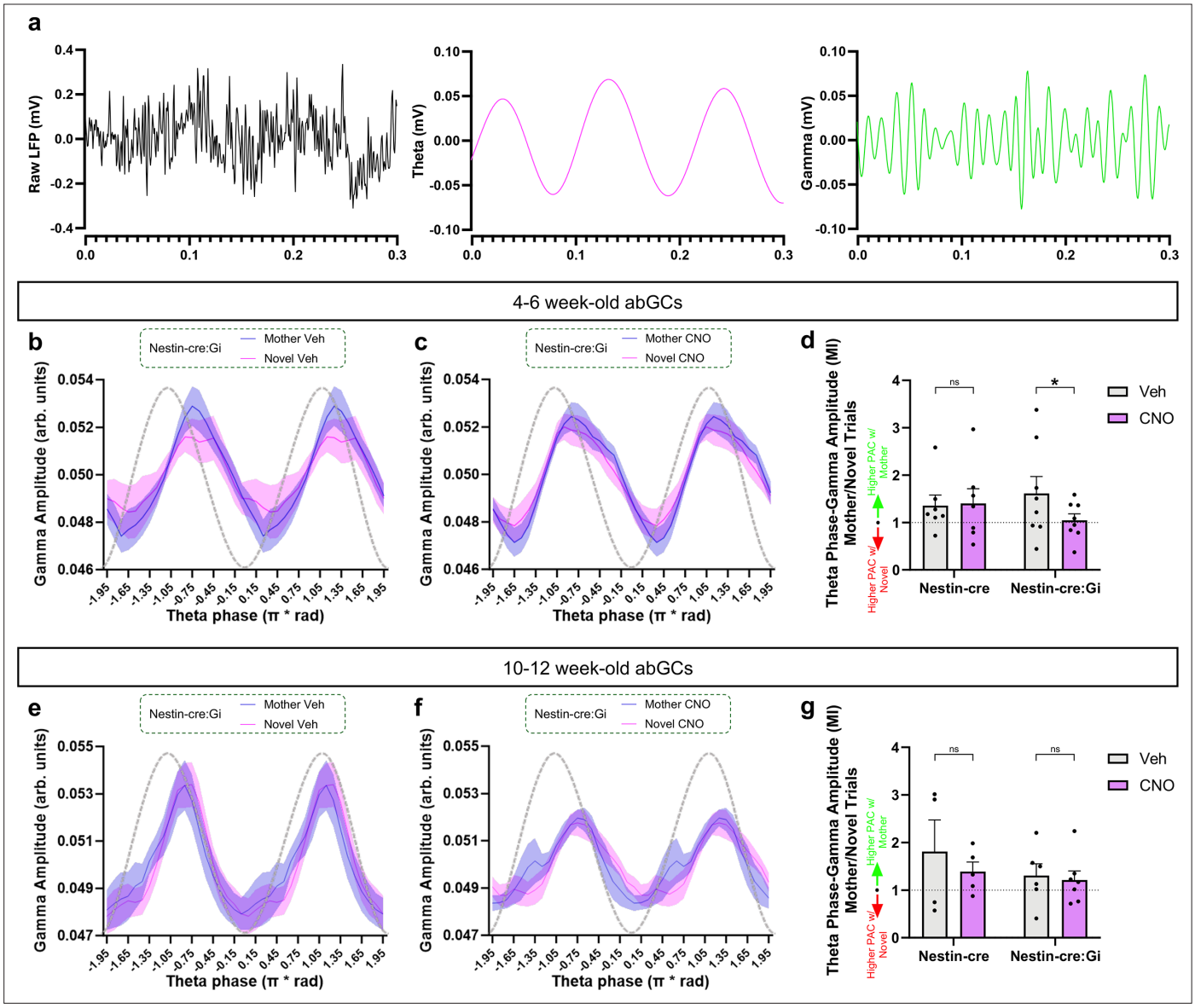

Figure 4. Adult-born neurons influence CA2 phase-amplitude coupling (PAC) changes associated with retrieval of developmental memories of the mother. (**a**) Example trace of raw local field potential (LFP) (black), 4–12 Hz theta oscillation (magenta), and 50–100 Hz gamma oscillation (green). (**b,c**) Graphs demonstrating mid-gamma amplitude modulation across theta phases in Nestin-cre:Gi mice. (**d**) Inhibiting 4–6-week old adult-born granule cells (abGCs) abolishes the increase in theta-mid-gamma PAC present during exposure to the mother (Nestin-cre: n=7; Nestin-cre:Gi: n=8; Mixed-effects ANOVA: Genotype x Drug: $F_{1,103}$ = 4.6729, p=0.03296; Šídák's test p=0.0219). (**e,f**) Graphs demonstrating mid-gamma amplitude modulation across theta phases in Nestin-cre:Gi mice. (**g**) Inhibiting 10–12-week old abGCs has no influence on theta-mid-gamma PAC during exposure to the mother (Nestin-cre: n=4 (Veh), n=5 (CNO); Nestin-cre:Gi: n=8 (Veh), n=7 (CNO); Mixed-effects ANOVA: Genotype x Drug: $F_{1,18}$ = 0.2616, p=0.6153). *p<0.05, bars represent mean + SEM.

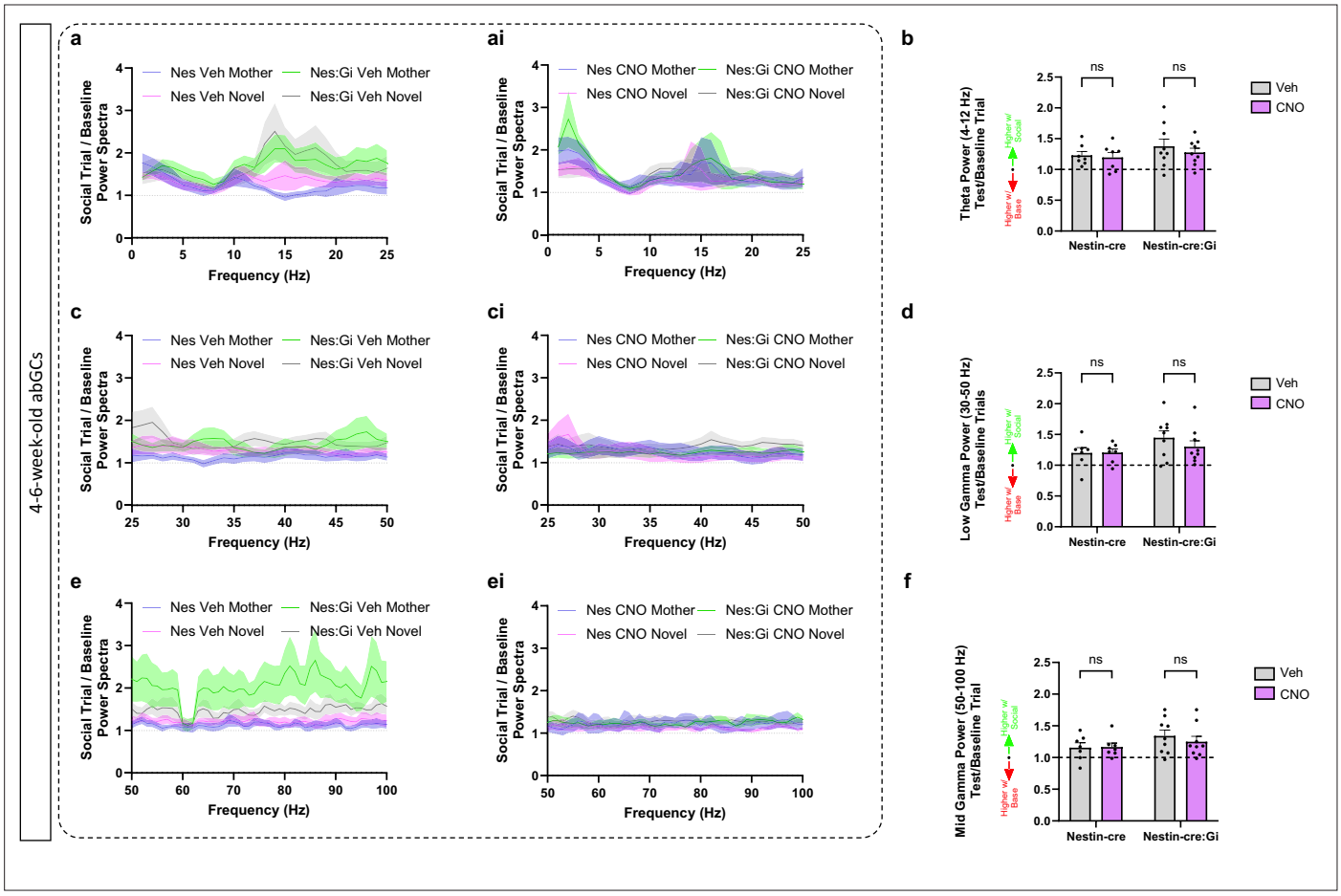

Figure 4—figure supplement 1. 4–6-week old adult-born granule cell (abGC) inhibition does not influence theta and gamma power during social interaction. (**a,ai,c,ci,e,ei**) Normalized power spectra graphs (Social stimulus/baseline). (**b**) Silencing 4–6-week old abGCs has no influence on theta power (Nestin-cre: n=7; Nestin-cre:Gi: n=9; Mixed-effects ANOVA: Genotype x Drug: $F_{1,14}$ = 0.2303, p=0.6387). (**d**) Silencing 4–6-week old abGCs has no influence on low-gamma power (Nestin-cre: n=7; Nestin-cre:Gi: n=9; Mixed effects ANOVA: Genotype x Drug: $F_{1,14}$ = 0.7979, p=0.3868). (**f**) Silencing 4–6-week old abGCs has no influence on mid-gamma power (Nestin-cre: n=7; Nestin-cre:Gi: n=9; Mixed-effects ANOVA: Genotype x Drug: $F_{1,28}$ = 0.3702, p=0.5478). Bars represent mean + SEM for **a-ai,cci,e-ei**; + SEM for **b**,**d**,**e**. ns = not significant.