RESEARCH ARTICLE

Figures and figure supplements

Dorsal raphe nucleus to anterior cingulate cortex 5-HTergic neural circuit modulates consolation and sociability

Laifu Li *et al*

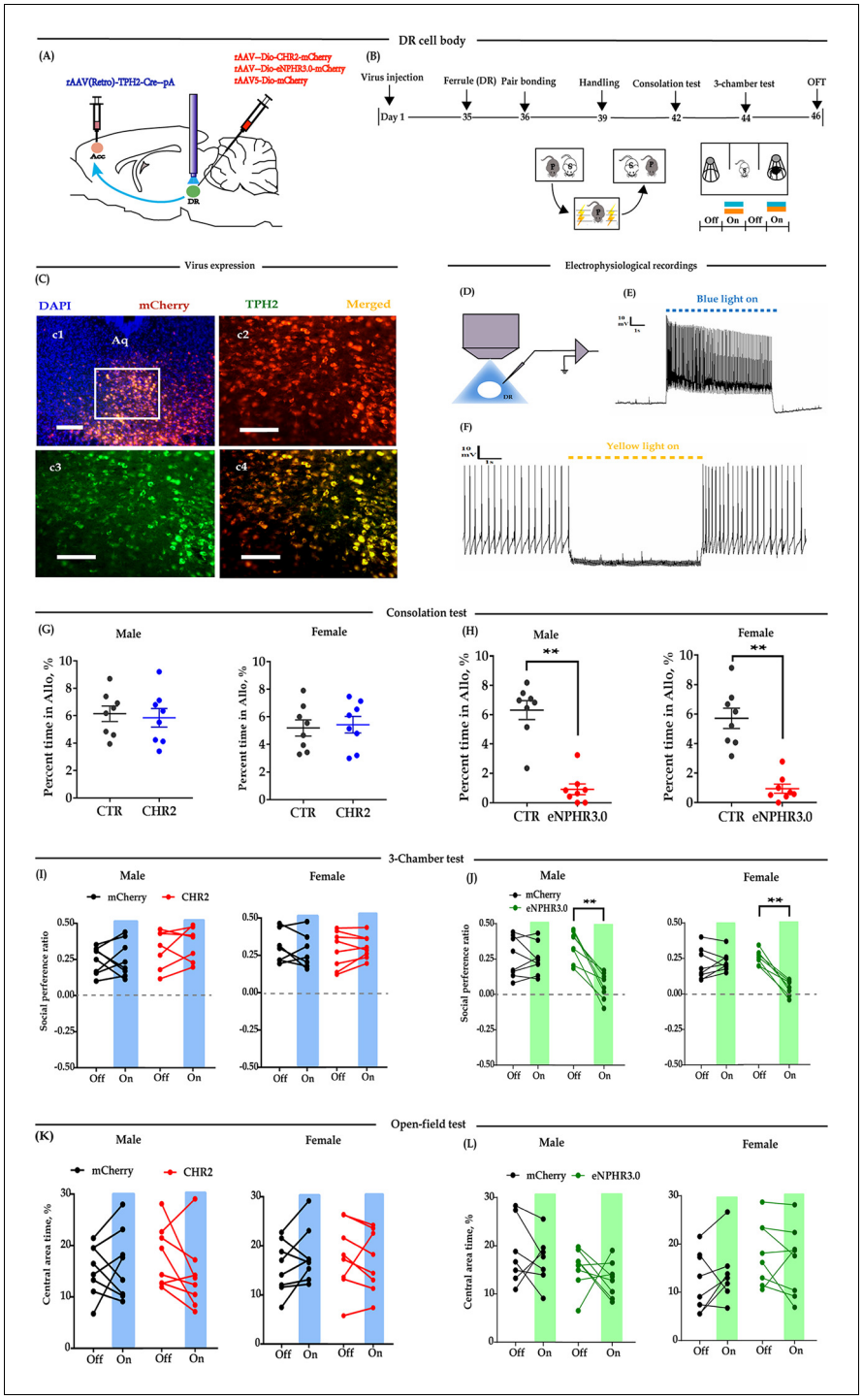

Figure 1. Optogenetic bidirectional modulation of 5-HT neuron in the DR in the DR-ACC neural circuit. (**A**) Schematic of optogenetic manipulation. (**B**) Timeline of experiments. (**C**) Immunohistological image showing virus expression in the DR (c1) and amplified images in the left box showing the mCherry, TPH2, and the colocalization of the two ('c2-c4'). (**D**) Electrophysiological recording model. (**E and F**) Representative traces from electrophysiological recordings showing photostimulation (**E**) and photoinhibition of a 5-HT neuron (**F**). (**G**) Quantification of allogrooming time in the consolation test of the CHR2 and control animals (n = 8 in each group; CHR2 vs CTR, independent samples t-test and Bayesian independent samples t-test; male: $t_{(14)}$ = 0.340, p=0.739, BF_{+0} = 0.445 with median posterior δ = 0.104, 95% CI = [−0.673 to 0.930]; female: $t_{(14)}$ = −0.279, p = 0.785, BF_{+0} = 0.439 with median posterior δ = −0.085, 95% CI = [−0.906 to 0.694]). (**H**) Quantification of allogrooming time in the

Figure 1 continued on next page

Figure 1 continued

consolation test of the eNPHR3.0 and control animals (n = 8 in each group; CHR2 vs eNPHR3.0, independent samples t-test and Bayesian independent samples t-test; male: $t_{(14)}$ = 7.293, p < 0.001, BF_{+0} = 6000.583; female: $t_{(14)}$ = 6.327, p < 0.001, BF_{+0} = 1562.921). (**I**) Quantification of social preference ratio in the three-chamber test of the CHR2 and control animals (n = 7 in CHR2 groups, one male and one female were excluded from analysis due to immobility; n = 8 in CTR groups; two-way repeated measures ANOVA along with two-way Bayesian repeated measures ANOVA; male: group: $F_{(1, 13)}$ = 3.042, p = 0.105, $BF_{(incl)}$ = 1.184; light: $F_{(1, 13)}$ = 0.531, p = 0.479, $BF_{(incl)}$ = 0.425; group × light: $F_{(1, 13)}$ = 0.246, p = 0.628, $BF_{(incl)}$ = 0.479; female: group: $F_{(1, 13)}$ = 2.088, p = 0.172, $BF_{(incl)}$ = 0.790; light: $F_{(1, 13)}$ = 0.180, p = 0.678, $BF_{(incl)}$ = 0.426; group × light: $F_{(1, 13)}$ = 0.233, p = 0.638, $BF_{(incl)}$ = 0.358). (**J**) Quantification of social preference ratio in the three-chamber test of the eNPHR3.0 and control animals (n = 8 in each group; two-way repeated measures ANOVA along with two-way Bayesian repeated measures ANOVA; male: group: $F_{(1, 14)}$ = 4.084, p = 0.063, $BF_{(incl)}$ = 1.236; light: $F_{(1, 14)}$ = 28.361, p < 0.001, $BF_{(incl)}$ = 25.390; group × light: $F_{(1, 14)}$ = 22.959, p < 0.001, $BF_{(incl)}$ = 87.850; post-hoc comparisons (Tukey): mCherry_Off vs mCherry_On, p = 0.981; eNPHR_Off vs eNPHR_On, p<0.001; female: group: $F_{(1, 14)}$ = 11.892, p = 0.004, $BF_{(incl)}$ = 4.965; light: $F_{(1, 14)}$ = 22.678, p < 0.001, $BF_{(incl)}$ = 7.067; group × light: $F_{(1, 14)}$ = 33.771, p < 0.001, $BF_{(incl)}$ = 623.339; post-hoc comparisons (Tukey): mCherry_Off vs mCherry_On, p = 0.879; eNPHR_Off vs eNPHR_On, p < 0.001). (**K**) Quantification of time spent in the central area in the open-field test of the CHR2 and control animals (n = 8 in each group; two-way repeated measures ANOVA along with two-way Bayesian repeated measures ANOVA; male: group: $F_{(1, 14)}$ = 0.009, p = 0.465, $BF_{(incl)}$ = 1.184; light: $F_{(1, 14)}$ = 0.808, p = 0.384, $BF_{(incl)}$ = 0.442; group × light: $F_{(1, 14)}$ = 2.266, p = 0.155, $BF_{(incl)}$ = 0.964; female: group: $F_{(1, 14)}$ = 0.240, p = 0.632, $BF_{(incl)}$ = 0.602; light: $F_{(1, 14)}$ = 0.341, p = 0.568, $BF_{(incl)}$ = 0.371; group × light: $F_{(1, 14)}$ = 2.192, p = 0.161, $BF_{(incl)}$ = 0.910). (**L**) Quantification of time spent in the central area in the open-field test of the eNPHR3.0 and control animals (male_mCherry, n = 7 (one was excluded from analysis due to immobility); male_eNPHR3.0, n = 8; female_mCherry, n = 8; male_eNPHR3.0, n = 8; two-way repeated measures ANOVA along with two-way Bayesian repeated measures ANOVA; male: group: $F_{(1, 13)}$ = 6.326, p = 0.026, $BF_{(incl)}$=1.935; light: $F_{(1, 13)}$ = 1.176, p = 0.298, $BF_{(incl)}$=0.605; group × light: $F_{(1, 13)}$ = 0.039, p = 0.846, $BF_{(incl)}$ = 0.578; post-hoc comparisons (Tukey): mCherry_Off vs mCherry_On, p = 0.928; eNPHR_Off vs eNPHR_On, p = 0.785; female: group: $F_{(1, 14)}$ = 0.794, p = 0.388, $BF_{(incl)}$ = 0.660; light: $F_{(1, 14)}$ = 0.632, p = 0.440, $BF_{(incl)}$ = 0.402; group × light: $F_{(1, 14)}$ = 3.390, p = 0.087, $BF_{(incl)}$ = 1.352; post-hoc comparisons (Tukey): mCherry_Off vs mCherry_On, p = 0.928; eNPHR_Off vs eNPHR_On, p = 0.785). Scale bars, 100 μm. Error bars are ± SEM. **p < 0.01. For raw data in this figure, please refer to ***Figure 1—source data 1***. ACC: anterior cingulate cortex; Aq: aqueduct; ANOVA: analysis of variance; CTR: control; DR: dorsal raphe nucleus; TPH2: tryptophan hydroxylase 2; 5-HT: serotonin.

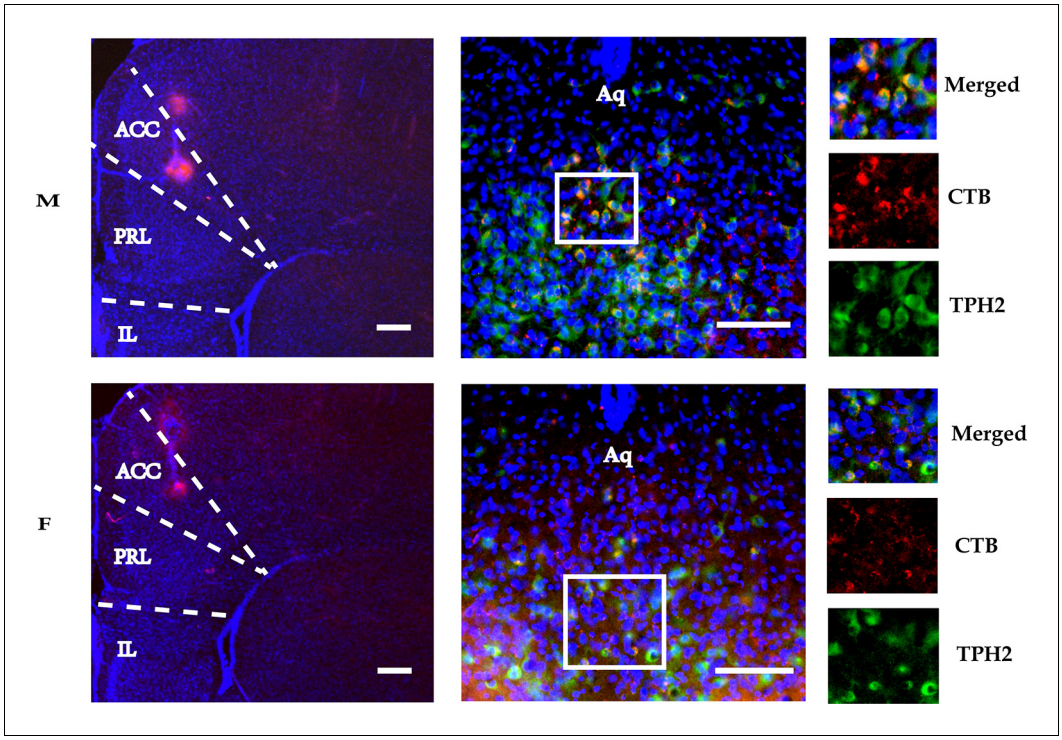

Figure 1—figure supplement 1. The histology of CTB injecting into the right ACC of male (upper row) and female voles (lower row). The right panels show colocalization of $TPH2^{+}$ neurons (green) and CTB (red) in the DR. Scale bars, 100 μm. M: male; F: female; ACC: anterior cingulate cortex; DR: dorsal raphe nucleus; TPH2: tryptophan hydroxylase 2; Aq: aqueduct.

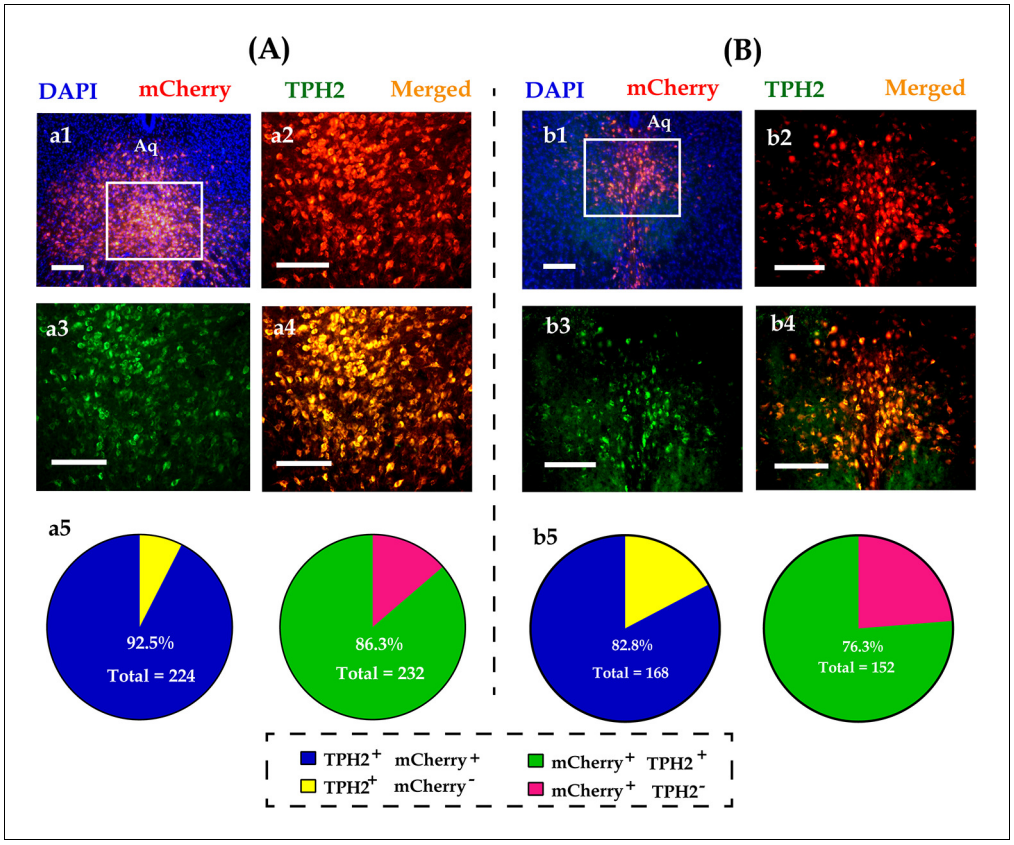

Figure 1—figure supplement 2. Immunohistological images showing colocalization of opsins (mCherry, red), TPH2+ neurons (green), and DAPI (blue) in the DR of male (**A**) and (**B**) female voles. (a1 and b1): Merged image of DAPI and mCherry; (a2–a4, b2–b4): amplified images in the left box showing the mCherry, TPH2, and the colocalization of mCherry and TPH2; (a5, b5): quantification rates of mCherry neurons colabeled with TPH2 (left pies) and TPH2 neurons colabeled with mCherry (right pies); *n* = 3 in each sex. Scale bars, 100 μm. Aq: aqueduct; DR: dorsal raphe nucleus; TPH2: tryptophan hydroxylase 2; DAPI: 4′,6-diamidino-2-phenylindole.

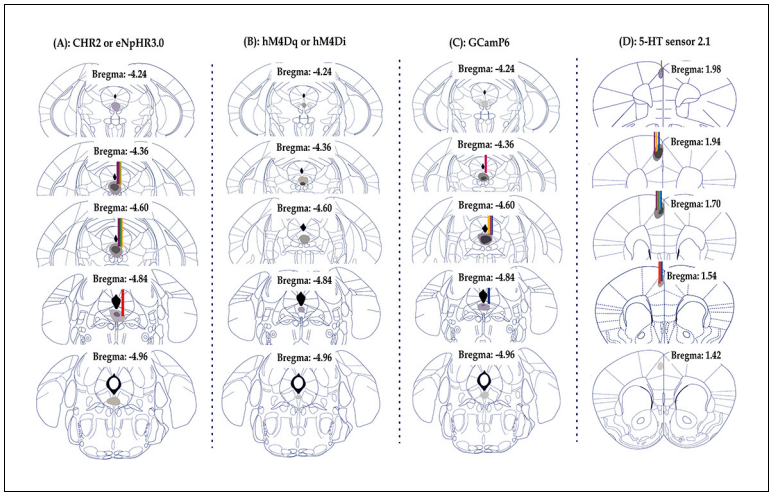

Figure 1—figure supplement 3. Schematics depicting virus spread (shades) and optic fiber placements (lines) for recording and functional manipulation experiments, related to ***Figures 1***, ***3***, ***4*** and ***5*** (A, B, C, and D, respectively).

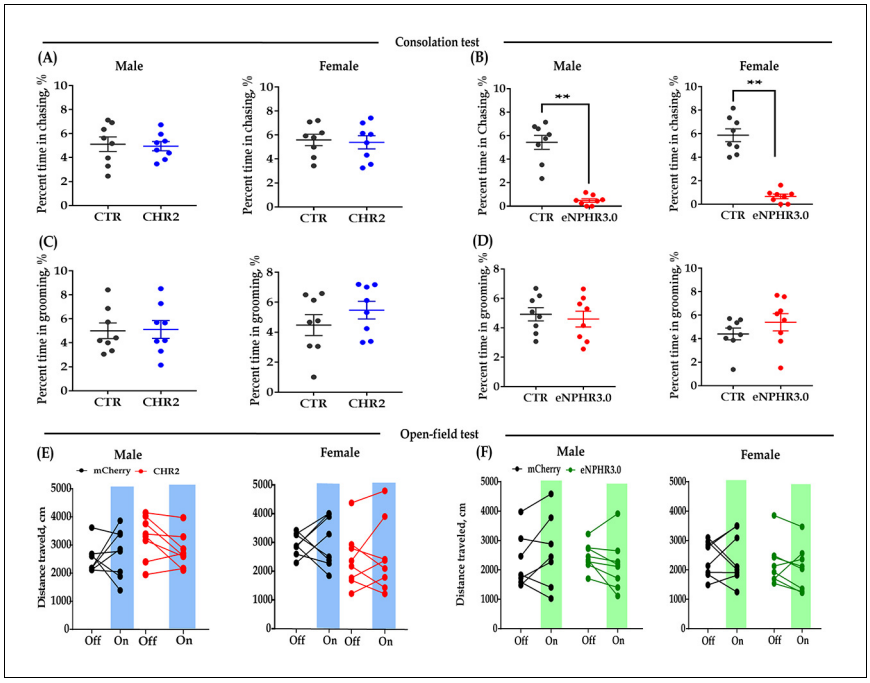

Figure 1—figure supplement 4. Effect of bidirectional optogenetic modulation of DR 5-HT neuron activities in the DR-ACC neural circuit on some control behaviors. (**A**) Quantification of chasing time in the consolation test of the CHR2 and control animals (n = 8 in each group; independent samples t-test and Bayesian independent samples t-test, two tailed; male: $t_{(14)}$ = 0.224, p = 0.826, BF_{+0} = 0.435 with median posterior δ = 0.068, 95% CI = [−0.714 to 0.884]; female: $t_{(14)}$ = 0.277, p = 0.786, BF_{+0} = 0.439 with median posterior δ = 0.084, 95% CI = [−0.695 to 0.905]). (**B**) Quantification of chasing time in the consolation test of the eNPHR3.0 and control animals (N = 8 in each group; independent samples t-test and Bayesian independent samples t-test, one tailed; male: $t_{(14)}$ = 8.086, p<0.001, BF_{+0} = 16993.904; female: $t_{(14)}$ = −1.092, p = 0.293, BF_{+0} = 53961.336). (**C**) Quantification of selfgrooming time in the consolation test of the CHR2 and control animals (n = 8 in each group; independent samples t-test and Bayesian independent samples t-test, two-tailed; male: $t_{(14)}$ = −0.114, p = 0.911, BF_{+0} = 0.430 with median posterior δ = −0.035, 95% CI = [−0.840 to 0.754]; female: $t_{(14)}$ = −1.092, p = 0.293, BF_{+0} = 0.635 with median posterior δ = −0.345, 95% CI = [−1.268 to 0.431]). (**D**) Quantification of selfgrooming time in the consolation test of the eNPHR3.0 and control animals (n = 8 in each group; independent samples t-test and Bayesian independent samples t-test, two-tailed; male: $t_{(14)}$ = 0.470, p = 0.645, BF_{+0} = 0.596 with median posterior δ = 0.325, 95% CI = [0.016 to 1.701]; female: $t_{(14)}$ = −1.118, p = 0.282, BF_{+0} = 0.245 with median posterior δ = 0.168, 95% CI = [0.007 to 0.689]). (**E**) Quantification of total distance traveled in the 5-min open-field test of the CHR2 and control animals (n = 8 in each group; two-way repeated measures ANOVA along with two-way Bayesian repeated measures ANOVA; male: group: $F_{(1, 14)}$ = 0.024, p = 0.878, $BF_{(incl)}$ = 0.435; light: $F_{(1, 14)}$ = 0.359, p = 0.558, $BF_{(incl)}$ = 0.384; group × light: $F_{(1, 14)}$ = 2.266, p = 0.154, $BF_{(incl)}$ = 1.062; female: group: $F_{(1, 14)}$ = 1.828, p = 0.198, $BF_{(incl)}$ = 0.831; light: $F_{(1, 14)}$ = 0.156, p = 0.36099, $BF_{(incl)}$ = 0.360; group × light: $F_{(1, 14)}$ = 1.769e^{-4}, p = 0.990, $BF_{(incl)}$ = 0.447). (**F**) Quantification of total distance traveled in the 5-min open-field test of the eNPHR3.0 and control animals (male_mCherry, n = 7 (one was excluded from analysis due to immobility); male_eNPHR3.0, n = 8; female_mCherry, n = 8; male_eNPHR3.0, n = 8; two-way repeated measures ANOVA along with two-way Bayesian repeated measures ANOVA; male: group: $F_{(1, 13)}$ = 0.049, p = 0.827, $BF_{(incl)}$ = 0.582; light: $F_{(1, 13)}$ = 0.011, p = 0.918, $BF_{(incl)}$ = 0.338; group × light: $F_{(1, 13)}$ = 3.518, p = 0.083, $BF_{(incl)}$ = 1.330; female: group: $F_{(1, 14)}$ = 0.261, p = 0.618, $BF_{(incl)}$ = 0.569; light: $F_{(1, 14)}$ = 0.251, p = 0.624, $BF_{(incl)}$ = 0.363; group × light: $F_{(1, 14)}$ = 0.339, p = 0.569, $BF_{(incl)}$ = 0.469). Data are presented as mean ± SE, **p<0.01. For raw data, please refer to ***Figure 1—figure supplement 4—source data 1***. ACC: anterior cingulate cortex; ANOVA: analysis of variance; CTR: control; DR: dorsal raphe nucleus; 5-HT: serotonin.

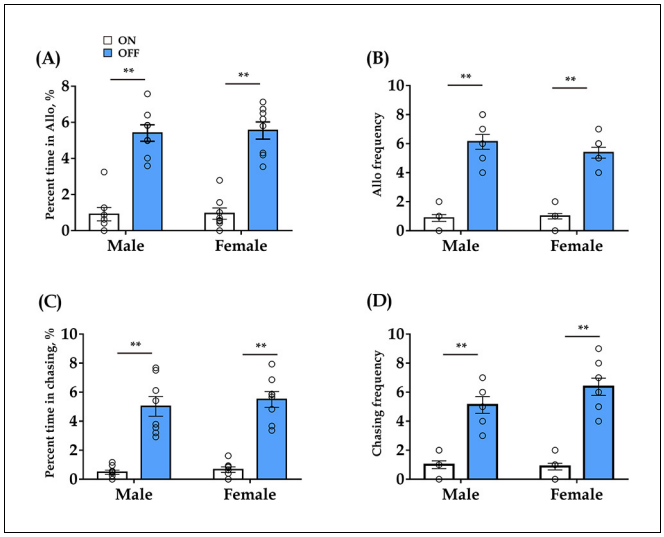

Figure 1—figure supplement 5. Effect of optogenetic inhibition of DR 5-HT neurons in the DR-ACC neural circuit does not elicit long-lasting effects (< 24 hr) on allogrooming and chasing behavior in the consolation test. (**A**) Time spent in allogrooming (male: $t_{(7)} = -11.707$, $p < 0.001$, $BF_{+0} = 142.924$; female: $t_{(7)} = -7.427$, $p<0.001$, $BF_{+0} = 400.207$). (**B**) Allogrooming frequency (male: $t_{(7)} = -8.104$, $p<0.001$, $BF_{+0} = 634.047$; female: $t_{(7)} = -9.501$, $p<0.001$, $BF_{+0} = 318.031$). (**C**) Time spent in chasing (male: $t_{(7)} = -6.063$, $p<0.001$, $BF_{+0} = 435.381$; female: $t_{(14)} = -7.548$, $p<0.001$, $BF_{+0} = 4796.167$). (**D**) Chasing frequency (male: $t_{(7)} = -7.105$, $p<0.001$, $BF_{+0} = 4796.167$; female: $t_{(14)} = -8.072$, $p<0.001$, $BF_{+0} = 620.600$). The data for 'light on' are derived from '***Figure 1H***'. *N* = 8 in each group. Paired sample *t*-test and Bayesian paired samples *t*-test, one-tailed. Data are presented as mean ± SE, **p<0.01. For raw data, please refer to ***Figure 1—figure supplement 5—source data 1***. ACC: anterior cingulate cortex; DR: dorsal raphe nucleus; CTR: control; 5-HT: serotonin.

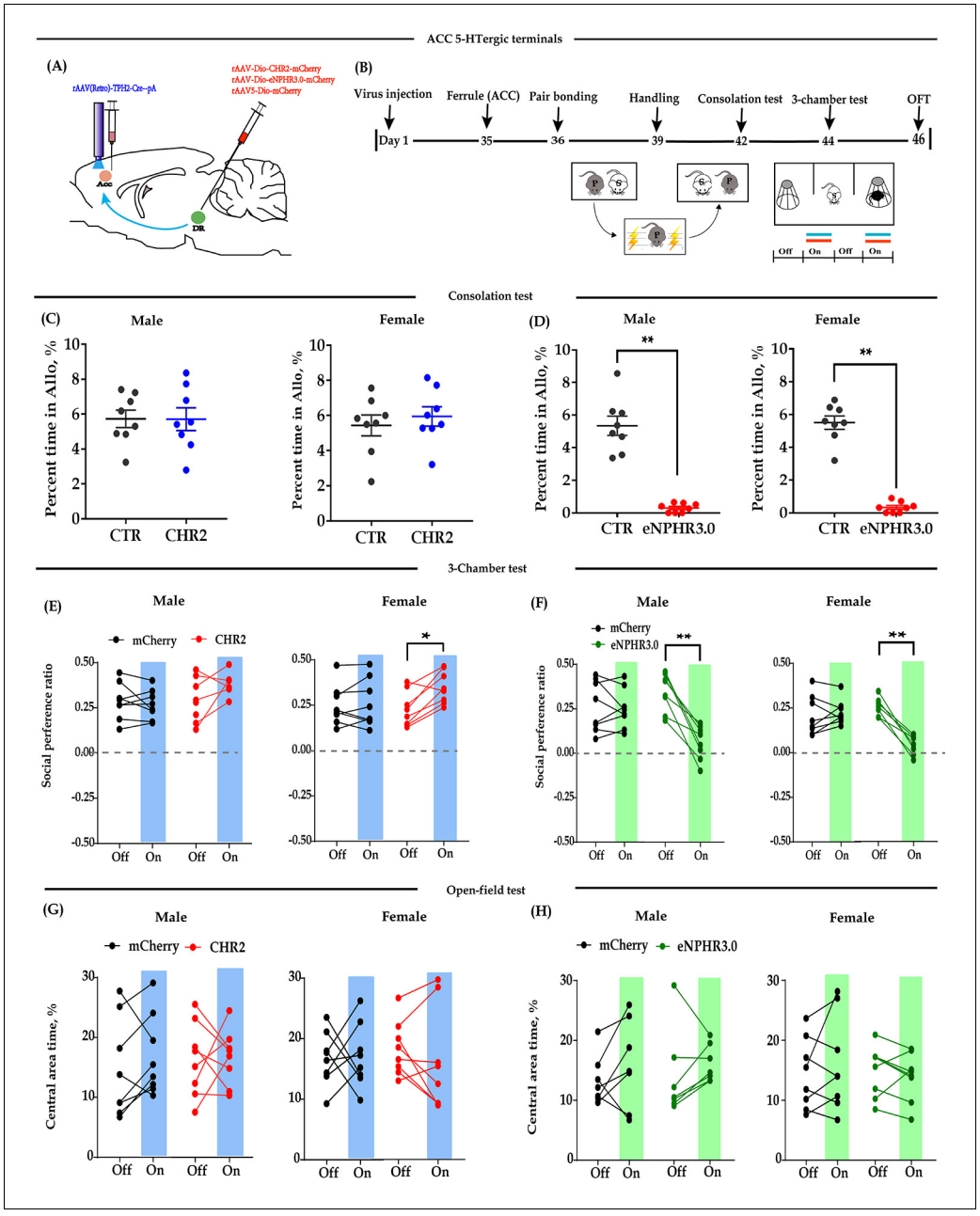

Figure 2. Optogenetic bidirectional modulation of 5-HT terminals within the ACC in the DR-ACC neural circuit. (**A**) Schematic of optogenetic manipulation. (**B**) Timeline of experiments. (**C**) Quantification of time spent in allogrooming in the consolation test of the CHR2 and control animals (n = 8 in each group; CHR2 vs CTR, independent samples t-test and Bayesian independent samples t-test; male: $t_{(14)} = 0.011$, p = 0.992, $BF_{+0} = 0.428$ with median posterior $\delta = 0.003$, 95% CI = [−0.792 to 0.800]; female: $t_{(14)} = -0.630$, p = 0.539, $BF_{+0} = 0.489$ with median posterior $\delta = -0.194$, 95% CI = [−1.054 to 0.575]). (**D**) Quantification of time spent in allogrooming in the consolation test of the eNPHR3.0 and control animals (n = 8 in each group; CHR2 vs CTR, independent samples t-test and Bayesian independent samples t-test; male: $t_{(14)} = 8.44$, p<0.001, $BF_{+0} = 26556.455$; female: $t_{(14)} = 12.174$, p<0.001, $BF_{+0} = 1.577 \times e^{+6}$). (**E**) Quantification of social preference ratio in the three-chamber test of the CHR2 and control animals (n = 8 in each group; two-way repeated measures ANOVA along with two-way Bayesian repeated measures ANOVA; male: group: $F_{(1, 14)} = 0.443$, p = 0.516, $BF_{(incl)} = 0.624$; light: $F_{(1, 14)} = 5.764$, p = 0.031, $BF_{(incl)} = 1.763$; group × light: $F_{(1, 14)} = 3.556$, p = 0.080, $BF_{(incl)} = 1.239$; female: group: $F_{(1, 14)} = 3.985$, p = 0.066, $BF_{(incl)} = 1.151$; light: $F_{(1, 14)} = 3.704$, p = 0.075, $BF_{(incl)} = 1.097$; group × light: $F_{(1, 14)} = 6.297$, p = 0.025, $BF_{(incl)} = 4.186$; post-hoc comparisons (Tukey): mCherry_Off vs mCherry_On, p = 0.975; CHR2_Off vs CHR2_On, p = 0.033). (**F**) Quantification of social preference ratio in the three-chamber test of the eNPHR3.0 and control

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animals (n = 8 in each group; two-way repeated measures ANOVA along with two-way Bayesian repeated measures ANOVA; male: group: $F_{(1, 14)}$ = 9.763, p = 0.007, $BF_{(incl)}$ = 3.374; light: $F_{(1, 14)}$ = 13.168, p = 0.003, $BF_{(incl)}$=9.588; group × light: $F_{(1, 14)}$ = 10.843, p = 0.005, $BF_{(incl)}$ = 13.892; post-hoc comparisons (Tukey): mCherry_Off vs mCherry_On, p = 0.995; eNPHR_Off vs eNPHR_On, p = 0.001; female: group: $F_{(1, 14)}$ = 6.912, p = 0.020, $BF_{(incl)}$ = 1.545; light: $F_{(1, 14)}$ = 93.330, p < 0.001, $BF_{(incl)}$ = 423.687; group × light: $F_{(1, 14)}$ = 65.062, p < 0.001, $BF_{(incl)}$ = 20440.982; post-hoc comparisons (Tukey): mCherry_Off vs mCherry_On, p = 0.679; eNPHR_Off vs eNPHR_On, p < 0.001). (**G**) Quantification of time spent in the central area in the open-field test of the CHR2 and control animals (n = 8 in each group; two-way repeated measures ANOVA along with two-way Bayesian repeated measures ANOVA; male: group: $F_{(1, 14)} = 4.189 \times e^{-4}$, p = 0.984, $BF_{(incl)}$ = 0.483; light: $F_{(1, 14)}$ = 0.842, p = 0.374, $BF_{(incl)}$ = 0.489; group × light: $F_{(1, 14)}$ = 0.501, p = 0.491, $BF_{(incl)}$ = 0.486; female: group: $F_{(1, 14)}$ = 0.014, p = 0.906, $BF_{(incl)}$ = 0.457; light: $F_{(1, 14)}$ = 0.050, p = 0.826, $BF_{(incl)}$ = 0.335; group × light: $F_{(1, 14)}$ = 0.177, p = 0.681, $BF_{(incl)}$ = 0.440). (**H**) Quantification of time spent in the central area in the open-field test of the eNPHR3.0 and control animals (male: n = 7, two animals (one from each group) were excluded from analysis due to immobility; female n = 8; two-way repeated measures ANOVA along with two-way Bayesian repeated measures ANOVA; male: group: $F_{(1, 12)}$ = 0.091, p = 0.769, $BF_{(incl)}$ = 0.566; light: $F_{(1, 12)}$ = 8.130, p = 0.015, $BF_{(incl)}$ = 4.162; group × light: $F_{(1, 12)}$ = 0.802, p = 0.388, $BF_{(incl)}$ = 0.550; post-hoc comparisons (Tukey): mCherry_Off vs mCherry_On, p = 0.532; eNPHR_Off vs eNPHR_On, p = 0.086; female: group: $F_{(1, 14)}$ = 0.276, p = 0.607, $BF_{(incl)}$ = 0.578; light: $F_{(1, 14)}$ = 0.580, p = 0.459, $BF_{(incl)}$ = 0.404; group × light: $F_{(1, 14)}$ = 2.234, p = 0.157, $BF_{(incl)}$ = 0.871). Error bars are ± SEM. *p < 0.05, **p < 0.01. For raw data in this figure, please refer to ***Figure 2—source data 1***. ACC: anterior cingulate cortex; ANOVA: analysis of variance; DR: dorsal raphe nucleus; CTR: control; 5-HT: serotonin.

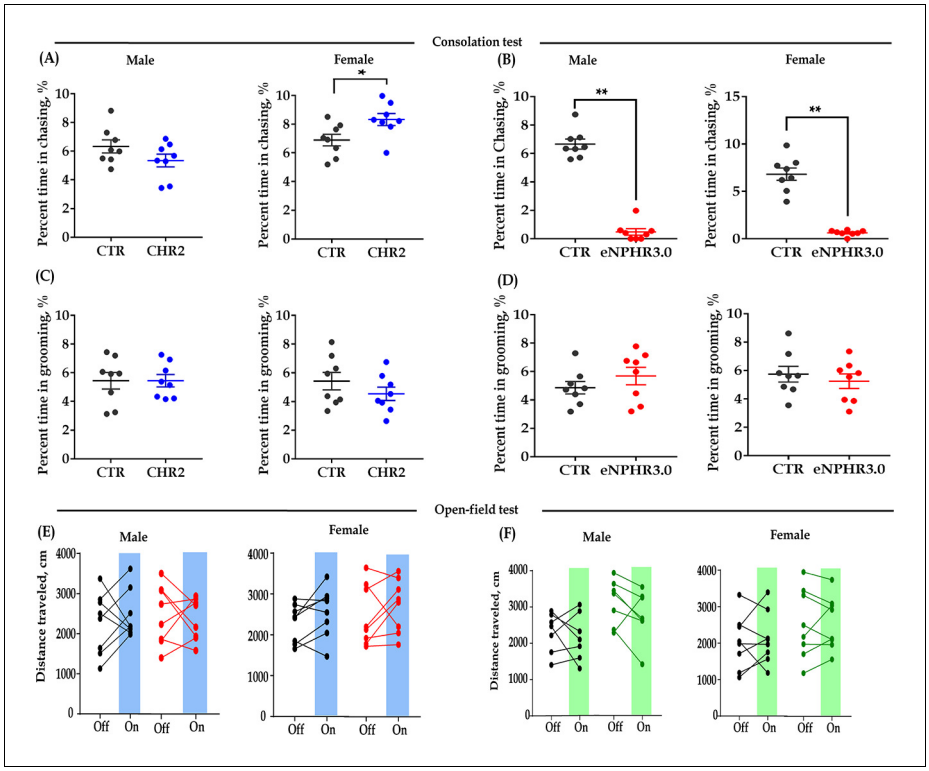

Figure 2—figure supplement 1. Effect of bidirectional optogenetic modulation of ACC 5-HT terminals in the DR-ACC neural circuit on some control behaviors. (**A**) Quantification of chasing time in the consolation test of the CHR2 and control animals (n = 8 in each group; independent samples t-test and Bayesian independent samples t-test, two-tailed; male: $t_{(14)}$ = 1.542, p = 0.145, BF_{+0} = 0.920 with median posterior δ = 0.506, 95% CI = [−0.301 to 1.496]; female: $t_{(14)}$ = −2.458, p = 0.028, BF_{+0} = 2.585 with median posterior δ = −0.884, 95% CI = [−2.006 to 0.050]). (**B**) Quantification of chasing time in the consolation test of the eNPHR3.0 and control animals (n = 8 in each group; independent samples t-test and Bayesian independent samples t-test, two-tailed; male: $t_{(14)}$ = 14.635, p<0.001, BF_{+0} = 1.407e+7; female: $t_{(14)}$ = 9.384, p<0.001, BF_{+0} = 82683.230). (**C**) Quantification of selfgrooming time in the consolation test of the CHR2 and control animals (n = 8 in each group; independent samples t-test and Bayesian independent samples t-test, two-tailed; male: $t_{(14)}$ = 8.651e^{-4}, p = 0.999, BF_{+0} = 0.428 with median posterior δ = 0.000, 95% CI = [−0.796 to 0.797]; female: $t_{(14)}$ = 1.145, p = 0.271, BF_{+0} = 0.660 with median posterior δ = 0.364, 95% CI = [−0.415 to 1.294]). (**D**) Quantification of selfgrooming time in the consolation test of the eNPHR3.0 and control animals (n = 8 in each group; independent samples t-test and Bayesian independent samples t-test, two-tailed; male: $t_{(14)}$ = −0.438, p = 0.668, BF_{+0} = 0.332 with median posterior δ = 0.214, 95% CI = [0.009 to 0.814]; female: $t_{(14)}$ = 0.654, p = 0.524, BF_{+0} = 0.691 with median posterior δ = 0.357, 95% CI = [0.019 to 1.137]). (**E**) Quantification of total distance traveled in the 5-min open-field test of the CHR2 and control animals (n = 8 in each group; two-way repeated measures ANOVA along with two-way Bayesian repeated measures ANOVA; male: group: $F_{(1, 14)}$ = 1.077, p = 0.317, $BF_{(incl)}$ = 0.656; light: $F_{(1, 14)}$ = 0.076, p = 0.787, $BF_{(incl)}$ = 0.330; group × light: $F_{(1, 14)}$ = 0.581, p = 0.458, $BF_{(incl)}$ = 0.502; female: group: $F_{(1, 14)}$ = 0.304, p = 0.590, $BF_{(incl)}$ = 0.542; light: $F_{(1, 14)}$ = 3.241, p = 0.093, $BF_{(incl)}$ = 1.138; group × light: $F_{(1, 14)}$ = 0.006, p = 0.939, $BF_{(incl)}$ = 0.416). (**F**) Quantification of total distance traveled in the 5-min open-field test of the eNPHR3.0 and control animals (male: n = 7 in each group, two animals (one from each group) were excluded from analysis due to immobility; female: n = 8 in each group; two-way repeated measures ANOVA along with two-way Bayesian repeated measures ANOVA; male: group: $F_{(1, 12)}$ = 5.832, p = 0.033, $BF_{(incl)}$ = 2.450; light: $F_{(1, 12)}$ = 1.990, p = 0.184, $BF_{(incl)}$ = 0.679; group × light: $F_{(1, 12)}$ = 0.483, p = 0.500, $BF_{(incl)}$ = 0.540; female: group: $F_{(1, 14)}$ = 1.642, p = 0.221, $BF_{(incl)}$ = 0.857; light: $F_{(1, 14)}$ = 0.178, p = 0.680, $BF_{(incl)}$ = 0.356; group × light: $F_{(1, 14)}$ = 0.067, p = 0.799, $BF_{(incl)}$ = 0.417). Data are presented as mean ± SE, **p<0.01. For raw data, please refer to ***Figure 2—figure supplement 1—source data 1***. ACC: anterior cingulate cortex; ANOVA: analysis of variance; DR: dorsal raphe nucleus; CTR: control; 5-HT: serotonin.

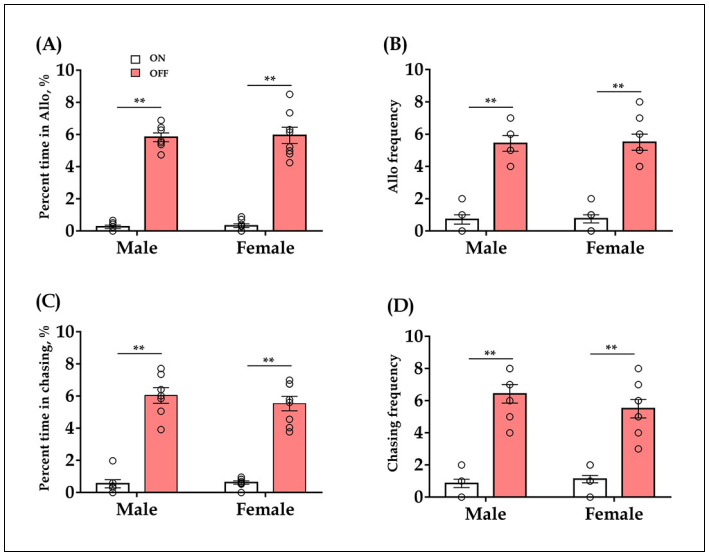

Figure 2—figure supplement 2. Effect of optogenetic inhibition of ACC 5-HT terminals in the DR-ACC neural circuit does not elicit long-lasting effects (<24 hr) on allogrooming and chasing behaviors in the consolation test. (**A**) Time spent in allogrooming (male: $t_{(6)} = -19.792$, $p < 0.001$, $BF_{+0} = 21609.467$; female: $t_{(7)} = -10.404$, $p < 0.001$, $BF_{+0} = 2475.291$). (**B**) Allogrooming frequency (male: $t_{(6)} = -11.210$, $p < 0.001$, $BF_{+0} = 1392.685$; female: $t_{(7)} = -7.333$, $p < 0.001$, $BF_{+0} = 374.389$). (**C**) Time spent in chasing (male: $t_{(6)} = -12.448$, $p < 0.001$, $BF_{+0} = 1144.562$; female: $t_{(7)} = -9.858$, $p < 0.001$, $BF_{+0} = 917.640$). (**D**) Chasing frequency (male: $t_{(7)} = -9.750$, $p < 0.001$, $BF_{+0} = 362.702$; female: $t_{(7)} = -7.344$, $p<0.001$, $BF_{+0} = 188.706$). *N* = 8 in each group. The data for 'light on' are derived from '***Figure 2D'***. Paired sample *t*-test and Bayesian paired samples *t*-test, one-tailed. Data are presented as mean ± SE, **p < 0.01. For raw data, please refer to ***Figure 2—figure supplement 2—source data 1***. ACC: anterior cingulate cortex; DR: dorsal raphe nucleus; CTR: control; 5-HT: serotonin.

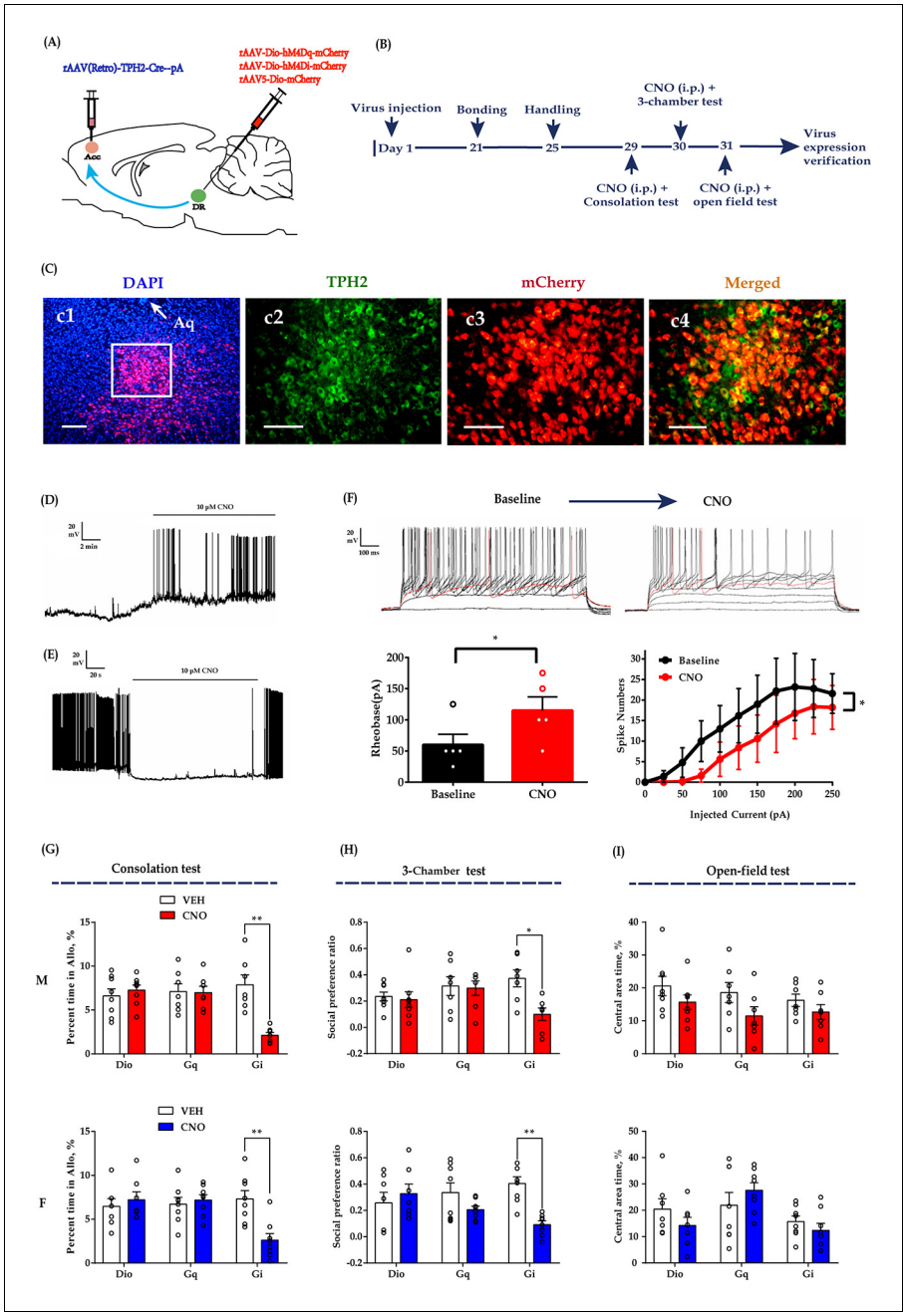

Figure 3. Chemogenetic modulation of DR 5-HT neuron activities in the DR-ACC neural circuit. (**A**) Schematic of chemogenetical manipulations. (**B**) Timeline of experiments. (**C**) Immunohistological image showing virus expression in the DR (c1) and amplified images in the left white box showing the mCherry, TPH2, and the colocalization of the two (c2–c4). (**D**) Representative trace from a Gq-DREADD neuron. (**E**) Representative trace from a Gi-DREADD-transfected neuron. (**F**) Quantification of spike rheobase and spike numbers under current step injections in Gi-DREADD-transfected neurons (n = 5 neurons; spike rheobase: paired t-test, $t_{(4)}$ = 4.491, p = 0.0109; two-way repeated measures ANOVA; spike numbers: treatment: $F_{(1, 4)}$ = 8.734, p = 0.0417, current: $F_{(10, 40)}$ = 8.989, p<0.0001; treatment ×current: $F_{(10, 40)}$ = 4.013, p = 0.0008). (**G**) Quantification of allogrooming time in the consolation test (two-way repeated measures ANOVA along with two-way Bayesian repeated measures ANOVA; male: treatment: $F_{(1, 19)}$ = 6.300, p = 0.021, $BF_{(incl)}$ = 2.007; group: $F_{(2, 19)}$ = 5.434, p = 0.014, $BF_{(incl)}$ = 0.777; treatment ×group: $F_{(1, 19)}$ = 8.433, p = 0.002, $BF_{(incl)}$ = 215.751; post-hoc comparisons (Tukey): Gi_Saline vs Gi_CNO, p = 0.002; Gq_Saline vs Gq_CNO, p = 1; Dio_Saline vs Dio_CNO, p = 0.991). Female: treatment: $F_{(1, 20)}$ = 4.570, p = 0.045, $BF_{(incl)}$ = 1.120; group: $F_{(2, 20)}$ = 2.884, p = 0.079, $BF_{(incl)}$ = 0.816; treatment × group: $F_{(1, 20)}$ = 10.778, p< 0.001, $BF_{(incl)}$ = 154.421. Post-hoc comparisons (Tukey): Gi_Saline vs Gi_CNO, p < 0.001; Gq_Saline

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vs Gq_CNO, p = 0.996; Dio_Saline vs Dio_CNO, p = 0.977. (**H**) Quantification of social preference ratio in the three-chamber test (two-way repeated measures ANOVA along with two-way Bayesian repeated measures ANOVA; male: treatment: $F_{(1, 19)}$ = 4.707, p = 0.043, $BF_{(incl)}$ = 2.261; group: $F_{(2, 19)}$ = 1.387, p = 0.274, $BF_{(incl)}$ = 0.365; treatment × group: $F_{(1, 19)}$ = 2.990, p = 0.074, $BF_{(incl)}$ = 2.562; post-hoc comparisons (Tukey): Gi_Saline vs Gi_CNO, p = 0.046; Gq_Saline vs Gq_CNO, p = 1; Dio_Saline vs Dio_CNO, p = 1; female: treatment: $F_{(1,20)}$ = 11.687, p = 0.003, $BF_{(incl)}$ = 9.390; group: $F_{(2, 20)}$ = 0.205, p = 0.817, $BF_{(incl)}$ = 0.272; treatment × group: $F_{(1, 20)}$ = 8.923, p = 0.002, $BF_{(incl)}$ = 42.856; post-hoc comparisons (Tukey): Gi_Saline vs Gi_CNO, p < 0.001; Gq_Saline vs Gq_CNO, p = 0.307; Dio_Saline vs Dio_CNO, p = 0.892). (**I**) Quantification of time spent in the central area in the open-field test (two-way repeated measures ANOVA along with two-way Bayesian repeated measures ANOVA; male: treatment: $F_{(1, 19)}$ = 4.305, p = 0.052, $BF_{(incl)}$ = 4.438; group: $F_{(2, 19)}$ = 2.064, p = 0.155, $BF_{(incl)}$ = 0.384; treatment × group: $F_{(1, 19)}$ = 0.164, p = 0.850, $BF_{(incl)}$ = 0.300; post-hoc comparisons (Tukey): Gi_Saline vs Gi_CNO, p = 0.963; Gq_Saline vs Gq_CNO, p = 0.605; Dio_Saline vs Dio_CNO, p = 0.838; female: treatment: $F_{(1, 20)}$ = 0.288, p = 0.597, $BF_{(incl)}$ = 0.313; group: $F_{(2, 20)}$ = 4.419, p = 0.026, $BF_{(incl)}$ = 2.335; treatment × group: $F_{(1, 20)}$ = 2.242, p = 0.132, $BF_{(incl)}$ = 1.146; post-hoc comparisons (Tukey): Gi_Saline vs Gi_CNO, p = 0.959; Gq_Saline vs Gq_CNO, p = 0.726; Dio_Saline vs Dio_CNO, p = 0.711). Male_Dio, *n* = 8; female_Dio, *n* = 7; male_Gq, *n* = 7; female_Gq, *n* = 8; male_Gi, *n* = 7; female_Gi, *n* = 8. Error bars are ± SEM. Scale bars, 100 μm. *p < 0.05, **p < 0.01. For raw data in this figure, please refer to ***Figure 3—source data 1***. ACC: anterior cingulate cortex; Aq: aqueduct; ANOVA: analysis of variance; DR: dorsal raphe nucleus; TPH2: tryptophan hydroxylase 2; M: male; F: female; 5-HT: serotonin.

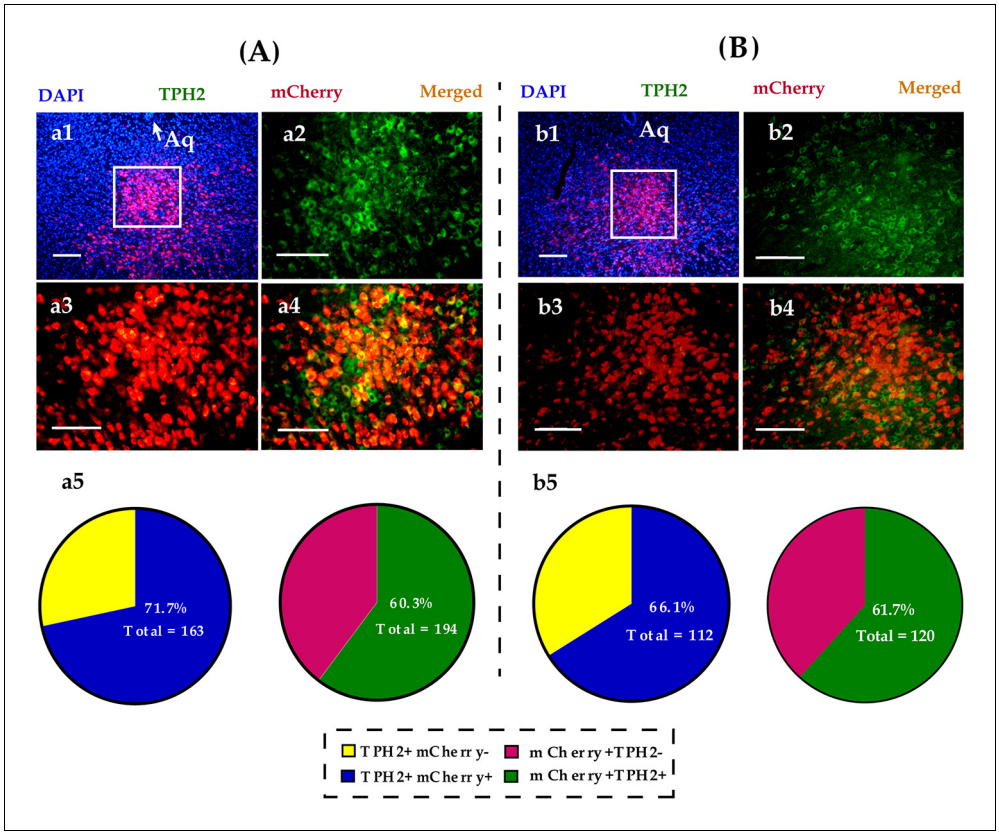

Figure 3—figure supplement 1. Immunohistological image showing colocalization of DREADD (mCherry, red), TPH2+ neurons (green), and DAPI (blue) in the DR of male (**A**) and (**B**) female voles. (a1 and b1) Merged image of DAPI and mCherry; (a2–a4, b2–b4) amplified images in the left box showing the mCherry, TPH2, and the colocalization of mCherry and TPH2; (a5, b5) quantification rates of mCherry neurons colabeled with TPH2 (left pies) and TPH2 neurons colabeled with mCherry (right pies), *n* = 3 in each sex. Scale bars, 100 μm. Aq: aqueduct; DR: dorsal raphe nucleus; TPH2: tryptophan hydroxylase 2; DAPI: 4′,6-diamidino-2-phenylindole.

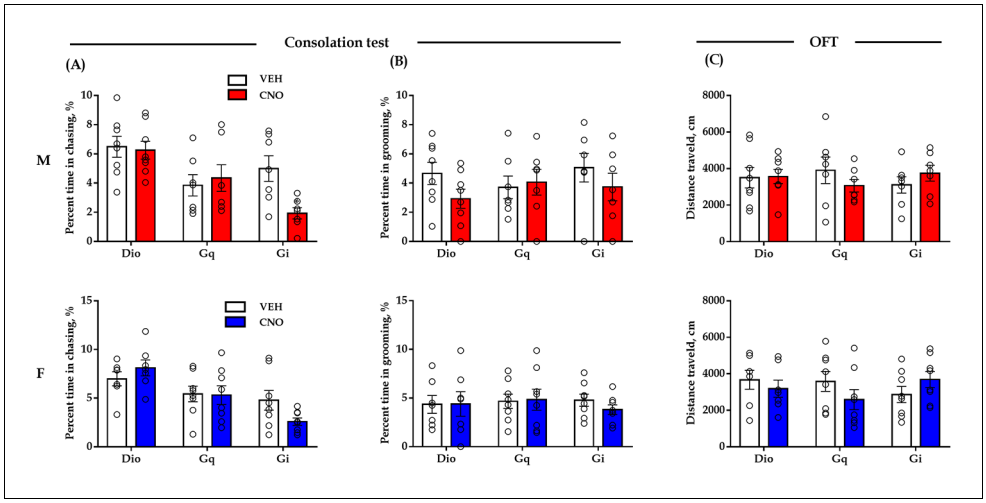

Figure 3—figure supplement 2. Effect of chemogenetic modulation of DR 5-HT neuron activities in the DR-ACC neural circuit on some control behaviors. (**A**) Quantification of chasing time in the consolation test (male: treatment: $F_{(1, 19)} = 2.118$, $p = 0.162$, $BF_{(incl)} = 0.757$; group: $F_{(2, 19)} = 11.238$, $p<0.001$, $BF_{(incl)} = 7.968$; treatment × group: $F_{(1, 19)} = 2.765$, $p = 0.088$, $BF_{(incl)} = 2.286$; post-hoc comparisons (Tukey): Gi_Saline vs Gi_CNO, $p = 0.122$; Gq_Saline vs Gq_CNO, $p = 0.998$; Dio_Saline vs Dio_CNO, $p = 1$; female: treatment: $F_{(1, 20)} = 0.436$, $p = 0.517$, $BF_{(incl)} = 0.363$; group: $F_{(2, 20)} = 9.228$, $p = 0.001$, $BF_{(incl)} = 15.063$; treatment × group: $F_{(1, 20)} = 2.564$, $p = 0.102$, $BF_{(incl)} = 1.396$; post-hoc comparisons (Tukey): Gi_Saline vs Gi_CNO, $p = 0.300$; Gq_Saline vs Gq_CNO, $p = 1$; Dio_Saline vs Dio_CNO, $p = 0.902$). (**B**) Quantification of selfgrooming time in the consolation test (male: treatment: $F_{(1, 19)} = 1.957$, $p = 0.178$, $BF_{(incl)} = 0.824$; group: $F_{(2, 19)} = 0.270$, $p = 0.766$, $BF_{(incl)} = 0.278$; treatment × group: $F_{(1, 19)} = 0.980$, $p = 0.393$, $BF_{(incl)} = 0.525$; female: treatment: $F_{(1, 20)} = 0.115$, $p = 0.739$, $BF_{(incl)} = 0.307$; group: $F_{(2, 20)} = 0.189$, $p = 0.829$, $BF_{(incl)} = 0.237$; treatment × group: $F_{(1, 20)} = 0.229$, $p = 0.797$, $BF_{(incl)} = 0.309$). (**C**) Quantification of total distance traveled in the 5-min open-field test (male: treatment: $F_{(1, 19)} = 0.005$, $p = 0.945$, $BF_{(incl)} = 0.289$; group: $F_{(2, 19)} = 0.028$, $p = 0.236$, $BF_{(incl)} = 0.384$; treatment × group: $F_{(1, 19)} = 0.979$, $p = 0.394$, $BF_{(incl)} = 0.556$; female: treatment: $F_{(1, 20)} = 0.299$, $p = 0.591$, $BF_{(incl)} = 0.337$; group: $F_{(2, 20)} = 0.231$, $p = 0.796$, $BF_{(incl)} = 0.249$; treatment × group: $F_{(1, 20)} = 1.926$, $p = 0.172$, $BF_{(incl)} = 1.191$). Male_Dio, $n = 8$; female_Dio, $n = 7$; male_Gq, $n = 7$; female_Gq, $n = 8$; male_Gi, $n = 7$; female_Gi, $n = 8$. Two-way repeated measures ANOVA along with two-way Bayesian repeated measures ANOVA. Error bars are ± SEM. For raw data in this figure, please refer to ***Figure 3—figure supplement 2—source data 1***. ACC: anterior cingulate cortex; Aq: aqueduct; ANOVA: analysis of variance; DR: dorsal raphe nucleus; TPH2: tryptophan hydroxylase 2; M: male; F: female; 5-HT: serotonin.

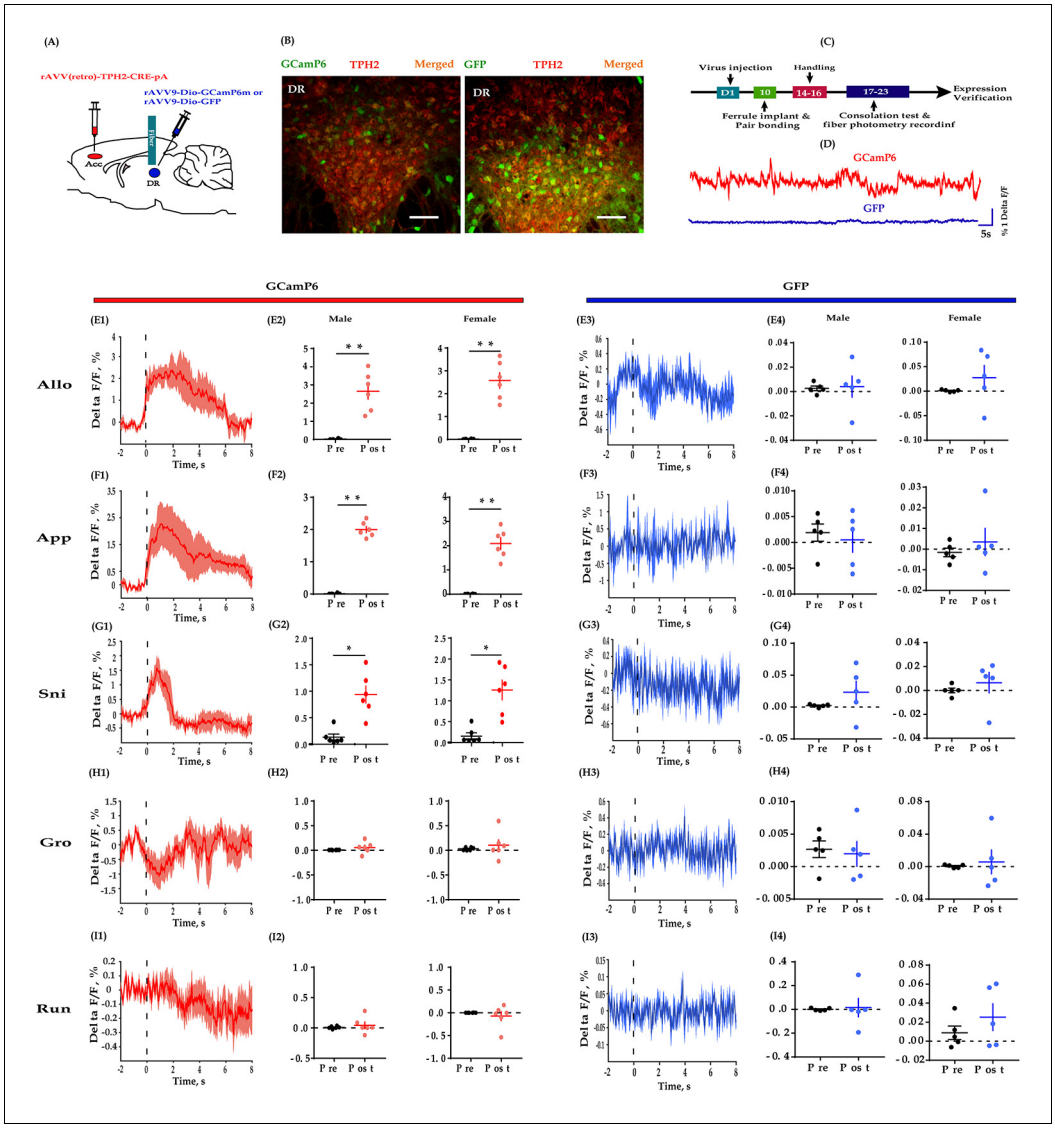

Figure 4. Fiber photometry recording DR 5-HT neural dynamics during the consolation test. (**A**) Schematic diagrams depicting the virus injection and recording sites. (**B**) Histology showing the expression of GCaMP6 (left) and GFP control (right) in the DR. (**C**) Experimental timeline for photometry experiments. (**D**) Representative fluorescence changes of GCaMP6 (red line) and GFP (blue line) during photometry recordings. (**E1–I1**) Representative peri-event plot of GCaMP6 fluorescence signals aligned to onsets of various behaviors (for all peri-event plots, the red line denotes the mean signals of four to six bouts of behaviors, whereas the red shaded region denotes the SEM). (**E2**) Quantification of change in GCaMP6 fluorescence signals before and after allogrooming (n = 6 in each group; male: $t_{(5)} = -5.967$, p = 0.002, $BF_{+0} = 24.488$ with median posterior $\delta = -1.904$, 95% CI = [−3.749,–0.424]; female: $t_{(5)} = -7.420$, p<0.01, $BF_{+0} = 52.689$ with median posterior $\delta = -2.397$, 95% CI = [−4.610,–0.625]). (**F2**) Quantification of change in GCaMP6 fluorescence signals before and after approaching (n = 6 in each group; male: $t_{(5)} = -19.871$, p<0.001, $BF_{+0} = 2233.691$; female: $t_{(5)} = -8.448$, p < 0.001, BF_{+0} = 84.470 with median posterior $\delta = -2.747$, 95% CI = [−5.225,–0.767]). (**G2**) Quantification of change in GCaMP6 fluorescence signals before and after sniffing (n = 6 in each group; male: $t_{(5)} = -3.689$, p = 0.011, $BF_{+0} = 6.449$ with median posterior $\delta = -1.221$, 95% CI = [−2.576,–0.138]; female: $t_{(5)} = -3.689$, p = 0.014, $BF_{+0} = 5.312$ with median posterior $\delta = -1.137$, 95% CI = [−2.434,–0.101]). (**H2**) Quantification of change in GCaMP6 fluorescence signals before and after selfgrooming (n = 6 in each group; male: $t_{(5)} = -1.032$, p = 0.350, $BF_{+0} = 0.559$ with median posterior $\delta = -0.300$, 95% CI = [−1.076, 0.383]; female: $t_{(5)} = -0.707$, p = 0.511, $BF_{+0} = 0.456$ with median posterior $\delta = -0.205$, 95% CI = [−0.904, 0.466]). (**I2**) Quantification of change in GCaMP6 fluorescence signals before and after running (n = 6 in each group; male: $t_{(5)} = -1.032$, p = 0.350, $BF_{+0} = 0.559$ with median posterior $\delta = -0.300$, 95% CI = [−1.076, 0.383]; female: $t_{(5)} = -0.707$, p = 0.511, $BF_{+0} = 0.456$ with median posterior δ =

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−0.205, 95% CI = [−0.904, 0.466]). (**E3–I3**) Representative peri-event plot of GFP signals aligned to onsets of various behavioral events (for all peri-event plots, the blue line denotes the mean signals of four to six bouts of behaviors, whereas the blue shaded region denotes the SE). (**E4**) Quantification of change in GFP fluorescence signals before and after allogrooming (n = 5 in each group; male: $t_{(4)}$ = −0.145, p = 0.885, BF_{+0} = 0.401 with median posterior δ = −0.047, 95% CI = [−0.3787, 0.675]; female: $t_{(4)}$ = −1.085, p = 0.339, BF_{+0} = 0.610 with median posterior δ = −0.32, 95% CI = [−1.198,–0.406]). (**F4**) Quantification of change in GFP fluorescence signals before and after approaching (n = 5 in each group; male: $t_{(4)}$ = −1.211, p = 0.293, BF_{+0} = 0.667 with median posterior δ = −0.371, 95% CI = [−1.260, 0.377]; female: $t_{(4)}$ = −0.723, p = 0.510, BF_{+0} = 0.488 with median posterior δ = −0.220, 95% CI = [−1.026, 0.499]). (**G4**) Quantification of change in GFP fluorescence signals before and after sniffing (n = 5 in each group; male: $t_{(4)}$ = 1.001, p = 0.373, BF_{+0} = 0.577 with median posterior δ = 0.306, 95% CI = [−0.427, 1.156]; female: $t_{(4)}$ = −0.687, p = 0.530, BF_{+0} = 0.479 with median posterior δ = −0.209, 95% CI = [−1.009, 0.510]). (**H4**) Quantification of change in GFP fluorescence signals before and after selfgrooming (n = 5 in each group; male: $t_{(4)}$ = 0.237, p = 0.825, BF_{+0} = 0.407 with median posterior δ = 0.072, 95% CI = [−0.647, 0.819]; female: $t_{(4)}$ = −0.350, p = 0.744, BF_{+0} = 0.418 with median posterior δ = −0.106, 95% CI = [−0.865, 0.610]). (**I4**) Quantification of change in GFP fluorescence signals before and after running (n = 5 in each group; paired t-test and Bayesian paired samples t-test, two-tailed; male: $t_{(4)}$ = −0.202, p = 0.850, BF_{+0} = 0.404 with median posterior δ = −0.061, 95% CI = [−0.806, 0.659]; female: $t_{(4)}$ = −1.813, p = 0.144, BF_{+0} = 52.689 with median posterior δ = 0.560, 95% CI = [−1.580,–0.251]). Error bars are ± SEM. Scale bars, 100 μm. *p<0.05, **p<0.01. Paired samples t-test along with Bayesian paired samples t-test. For raw data in this figure, please refer to ***Figure 4—source data 1***. ACC: anterior cingulate cortex; GFP: green fluorescent protein; TPH2: tryptophan hydroxylase 2; Aq: aqueduct; Allo: allogrooming; Sni: sniffing; App: approaching; Gro: selfgrooming; Run: running; 5-HT: serotonin.

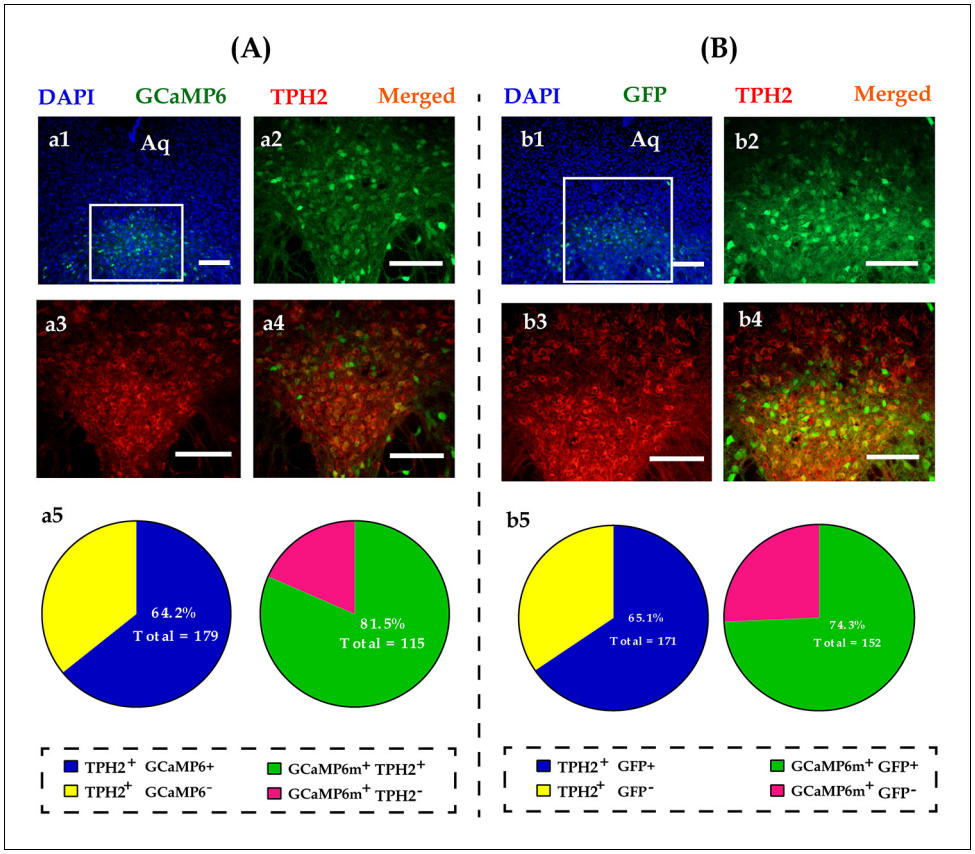

Figure 4—figure supplement 1. Representative viral infection images of GCaMp6 (A) and virus control of GFP (B). (**a1**) Immunohistological image showing GCaMP6 expression in the DR; (**a2–a4**) amplified images in the left box showing the GCaMP6 (green), TPH2 (red), and the colocalization (yellow) of the two; (**a5**) quantification rates of GCaMP6 neurons colabeled with TPH2 (left pie) and TPH2 neurons colabeled with GCaMP6 (right pie), *N* = 3; (**b1**) immunohistological image showing GFP expression in the DR; (**b2–b4**) amplified images in the left box showing the GFP (green), TPH2 (red), and the colocalization (yellow) of the two; (**b5**) quantification rates of GFP neurons colabeled with TPH2 (left pie) and TPH2 neurons colabeled with GFP (right pie), *n* = 3 in each sex. Scale bars, 100 μm. Aq: aqueduct; DR: dorsal raphe nucleus; TPH2: tryptophan hydroxylase 2; GFP: green fluorescent protein.

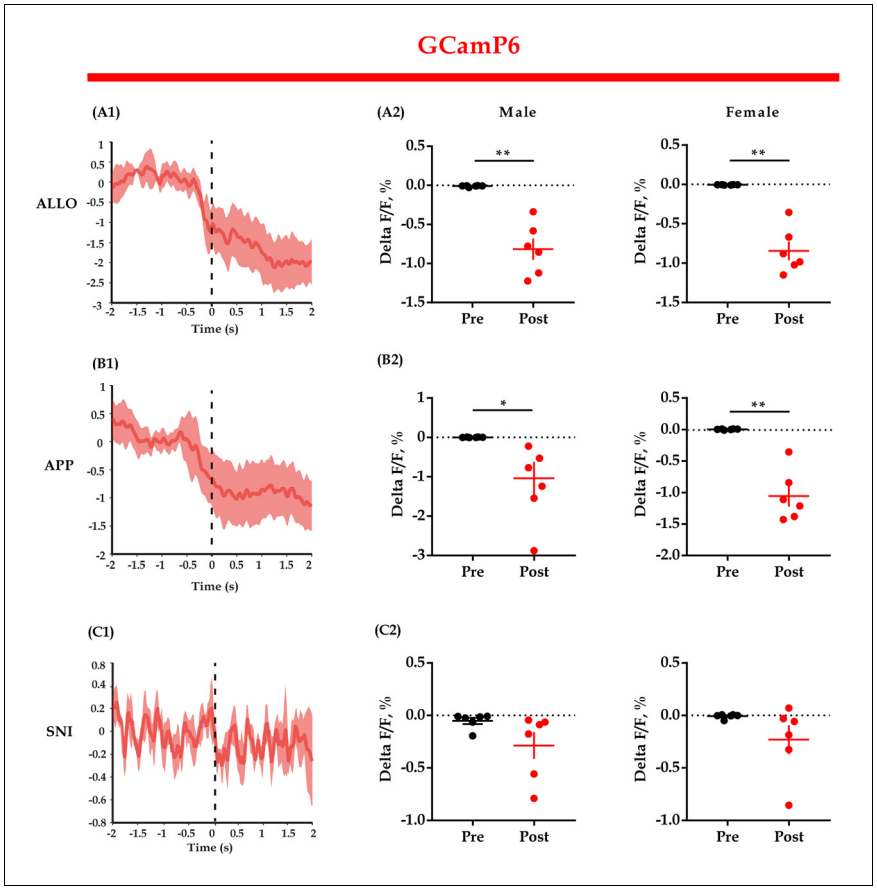

Figure 4—figure supplement 2. GCaMP6 fluorescent signals align to the end of some behaviors. (**A1, B1, C1**) Representative peri-event plot of GCaMP6 fluorescence signals aligned to the end of allogrooming, social approaching, and sniffing (for all peri-event plots, the red line denotes the mean signals of four to six bouts of behaviors, whereas the red shaded region denotes the SE). (**A2, B2, C2**) Quantification of change in GCaMP6 fluorescence signals (n = 6 in each group; paired t-test and Bayesian paired samples t-test, two-tailed; A2: male: $t_{(5)}$ = 5.984, p<0.001, BF_{+0} = 49.388 with median posterior δ = 1.912, 95% CI = [0.453, 3.760]; female: $t_{(5)}$ = 7.105, p<0.001, BF_{+0} = 90.205 with median posterior δ = 2.292, 95% CI = [0.598, 4.424]; B2: male: $t_{(5)}$ = 3.170, p = 0.012, BF_{+0} = 6.839 with median posterior δ = 0.982, 95% CI = [0.139, 2.153]; female: $t_{(5)}$ = 6.459, p<0.001, BF_{+0} = 64.386 with median posterior δ = 2.073, 95% CI = [0.514, 4.040]; C2: male: $t_{(5)}$ = 1.611, p = 0.084, BF_{+0} = 1.589 with median posterior δ = 0.528, 95% CI = [0.041, 1.363]; female: $t_{(5)}$ = 1.724, p = 0.073, BF_{+0} = 1.778 with median posterior δ = 0.557, 95% CI = [0.046, 1.415]). Data are presented as mean ± SE, *p<0.05, **p<0.01. For raw data, please refer to ***Figure 4—figure supplement 2—source data 1***. ALLO: allogrooming; APP: approaching; SNI: sniffing.

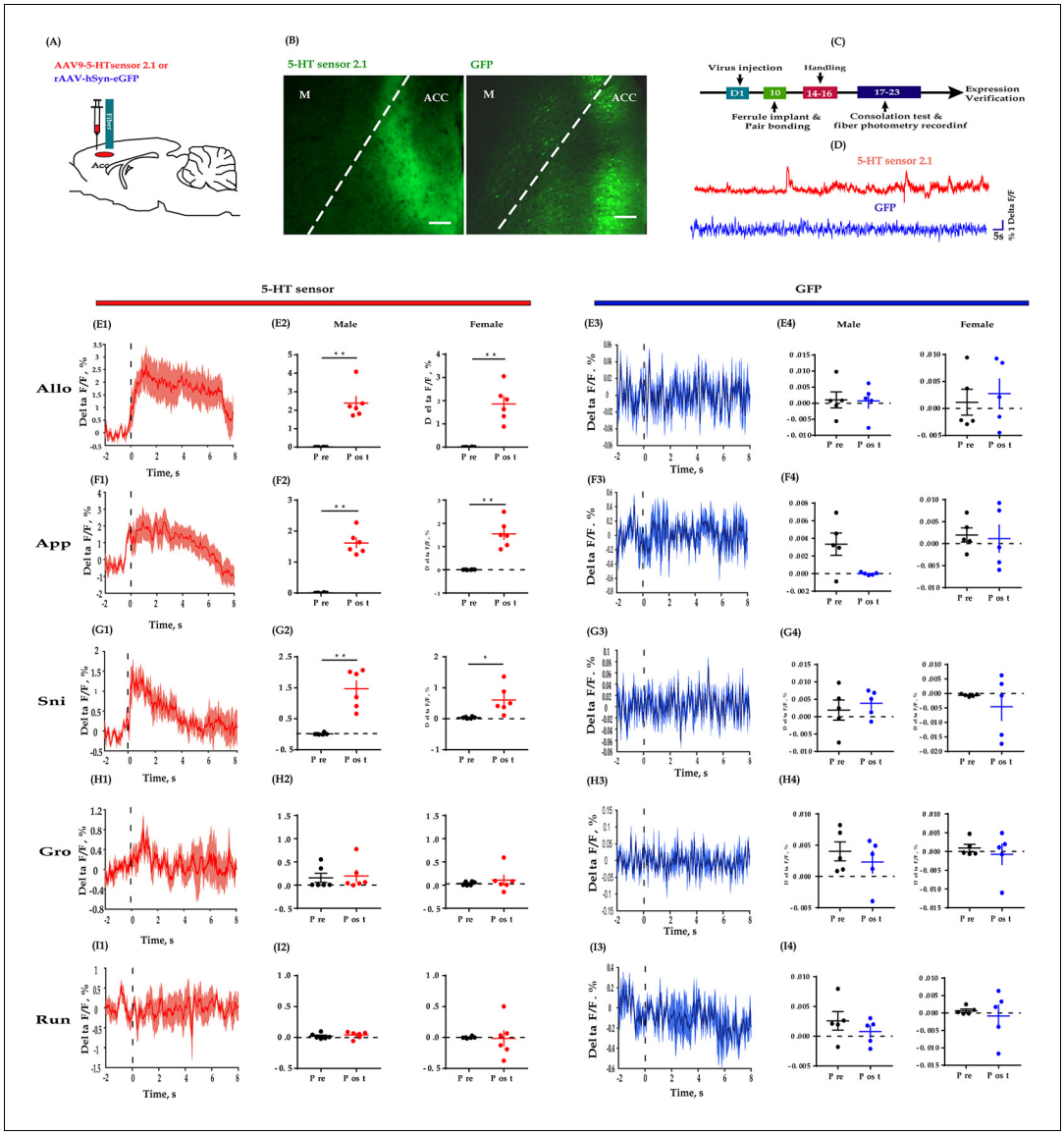

Figure 5. Fiber photometry recording dynamics of 5-HT within the ACC during the consolation test. (**A**) Schematic diagrams depicting the virus injection and recording sites. (**B**) Histology showing the expression of 5-HT sensor (left) and GFP control (right) within the ACC. (**C**) Experimental timeline for photometry experiments. (**D**) Representative fluorescence changes of 5-HT sensor (red line) and GFP (blue line) during photometry recordings. (**E1–I1**) Representative peri-event plot of 5-HT fluorescence signals aligned to onsets of various behaviors (for all peri-event plots, the red line denotes the mean signals of four to six bouts of behaviors, whereas the red shaded region denotes the SEM). (**E2**) Quantification of change in 5-HT fluorescence signals before and after allogrooming (n = 6 in each group; male: $t_{(5)} = -6.687$, p = 0.001, $BF_{+0} = 36.393$ with median posterior $\delta = -2.148$, 95% CI = [−4.174,–0.523]; female: $t_{(5)} = -6.038$, p = 0.002, $BF_{+0} = 25.495$ with median posterior $\delta = -1.928$, 95% CI = [−3.790,–0.433]). (**F2**) Quantification of change in 5-HT fluorescence signals before and after approaching (n = 6 in each group; male: $t_{(5)} = -10.551$, p < 0.001, $BF_{+0} = 193.396$ with median posterior $\delta = -3.460$, 95% CI = [−6.490,–1.060]; female: $t_{(5)} = -6.496$, p = 0.001, $BF_{+0} = 32.865$ with median posterior $\delta = -2.083$, 95% CI = [−4.061,–0.497]). (**G2**) Quantification of change in 5-HT fluorescence signals before and after sniffing (n = 6 in each group; male: $t_{(5)} = -5.863$, p = 0.002, $BF_{+0} = 23.053$ with median posterior $\delta = -1.868$, 95% CI = [−3.687,–0.409]; female: $t_{(5)} = -3.030$, p = 0.029, $BF_{+0} = 3.108$ with median posterior $\delta = -0.921$, 95% CI = [−2.070, 0.000]). (**H2**) Quantification of change in 5-HT fluorescence signals before and after selfgrooming (n = 6 in each group; male: $t_{(5)} = -0.215$, p = 0.838, $BF_{+0} = 0.381$ with median posterior $\delta = -0.062$, 95% CI = [−0.753, 0.609]; female: $t_{(5)} = -0.738$, p = 0.494, $BF_{+0} = 0.464$ with median posterior $\delta = -0.214$, 95% CI = [−0.953, 0.458]). (**I2**) Quantification of change in 5-HT fluorescence signals before and after running (n = 6 in each group; male: $t_{(5)} = -0.627$, p = 0.558,

Figure 5 continued on next page

Figure 5 continued

BF_{+0} = 0.438 with median posterior δ = −0.182, 95% CI = [−0.908,–0.488]; female: $t_{(5)}$ = 0.129, p = 0.903, BF_{+0} = 0.376 with median posterior δ = 0.037, 95% CI = [−0.636, 0.722]). (**E3–I3**) Representative peri-event plot of GFP signals aligned to onsets of various behavioral events (for all peri-event plots, the blue line denotes the mean signals of four to six bouts of behaviors, whereas the blue shaded region denotes the SE). (**E4**) Quantification of change in GFP fluorescence signals before and after allogrooming (*n* = 5 in each group; male: $t_{(4)}$ = 0.082, p = 0.939, BF_{+0} = 0.399 with median posterior δ = 0.025, 95% CI = [−0.700, 0.760]; female: $t_{(4)}$ = −0.340, p = 0.751, BF_{+0} = 0.610 with median posterior δ = −0.103, 95% CI = [−0.861,–614]). (**F4**) Quantification of change in GFP fluorescence signals before and after approaching (*n* = 5 in each group; male: $t_{(4)}$ = −0.836, p = 0.450, BF_{+0} = 0.520 with median posterior δ = −0.255, 95% CI = [−1.078, 0.469]; female: $t_{(4)}$ = 0.861, p = 0.438, BF_{+0} = 0.528 with median posterior δ = 0.263, 95% CI = [−0.462, 1.090]). (**G4**) Quantification of change in GFP fluorescence signals before and after sniffing (*n* = 5 in each group; male: $t_{(4)}$ = 2.686, p = 0.055, BF_{+0} = 2.077 with median posterior δ = 0.843, 95% CI = [−0.104, 2.086]; female: $t_{(4)}$ = 0.208, p = 0.845, BF_{+0} = 0.405 with median posterior δ = 0.063, 95% CI = [−0.657, 0.808]). (**H4**) Quantification of change in GFP fluorescence signals before and after selfgrooming (*n* = 5 in each group; male: $t_{(4)}$ = 0.822, p = 0.457, BF_{+0} = 0.516 with median posterior δ = 0.251, 95% CI = [−0.473, 1.071]; female: $t_{(4)}$ = 0.653, p = 0.549, BF_{+0} = 0.471 with median posterior δ = 0.199, 95% CI = [−0.519, 0.994]). (**I4**) Quantification of change in GFP fluorescence signals before and after running (*n* = 5 in each group; male: $t_{(4)}$ = 1.415, p = 0.230, BF_{+0} = 0.777 with median posterior δ = 0.435, 95% CI = [−0.331, 1.366]; female: $t_{(4)}$ = 0.510, p = 0.637, BF_{+0} = 0.442 with median posterior δ = 0.155, 95% CI = [−0.561, 0.932]). Error bars are ± SEM. Scale bars, 100 μm. *p < 0.05, **p < 0.01; paired samples *t*-test along with Bayesian paired samples *t*-test. For raw data in this figure, please refer to ***Figure 5—source data 1***. ACC: anterior cingulate cortex; M: motor cortex; TPH2: tryptophan hydroxylase 2; Allo: allogrooming; Sni: sniffing; App: approaching; Gro: selfgrooming; Run: running; GFP: green fluorescent protein; 5-HT: serotonin.

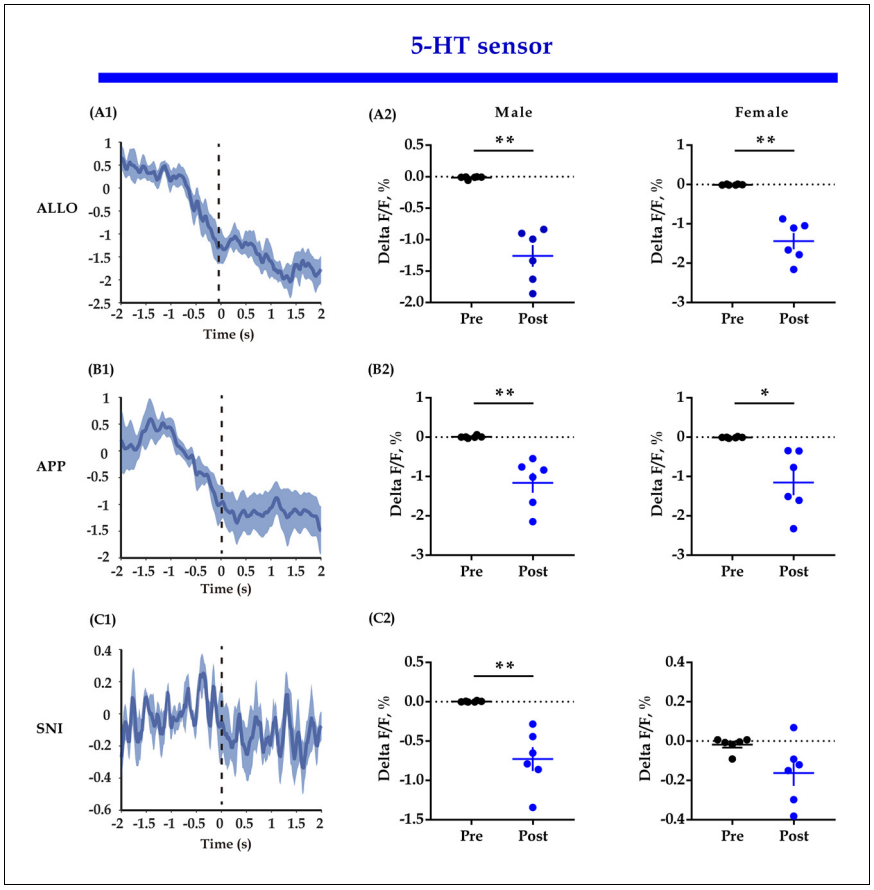

Figure 5—figure supplement 1. 5-HT sensor fluorescent signals align to the end of some behaviors. (**A1, B1, C1**) Representative peri-event plot of 5-HT sensor fluorescence signals aligned to the end of allogrooming, social approaching, and sniffing (for all peri-event plots, the blue line denotes the mean signals of four to six bouts of behaviors, whereas the blue shaded region denotes the SE). (**A2, B2, C2**) Quantification of change in 5-HT sensor fluorescence signals (n = 6 in each group; paired t-test and Bayesian paired samples t-test, two-tailed; A2: male: $t_{(5)}$ = 7.479, p<0.001, BF_{+0} = 108.402 with median posterior δ = 2.419, 95% CI = [0.648, 4.646]; female: $t_{(5)}$ = 6.994, p<0.001, BF_{+0} = 6.994 with median posterior δ = 2.255, 95% CI = [0.584, 4.357]; B2: male: $t_{(5)}$ = 4.489, p = 0.003, BF_{+0} = 19.042 with median posterior δ = 1.411, 95% CI = [0.273, 2.891]; female: $t_{(5)}$ = 3.558, p = 0.008, BF_{+0} = 9.436 with median posterior δ = 1.106, 95% CI = [0.174, 2.366]; C2: male: $t_{(5)}$ = 4.912, p = 0.002, BF_{+0} = 25.426 with median posterior δ = 1.552, 95% CI = [0.322, 30134]; female: $t_{(5)}$ = 2.151, p = 0.042, BF_{+0} = 2.701 with median posterior δ = 0.674, 95% CI = [0.066, 1.620]). Data are presented as mean ± SE, *p<0.05, **p<0.01. For raw data, please refer to ***Figure 5—figure supplement 1—source data 1***. ALLO: allogrooming; APP: approaching; SNI: sniffing; 5-HT: serotonin.

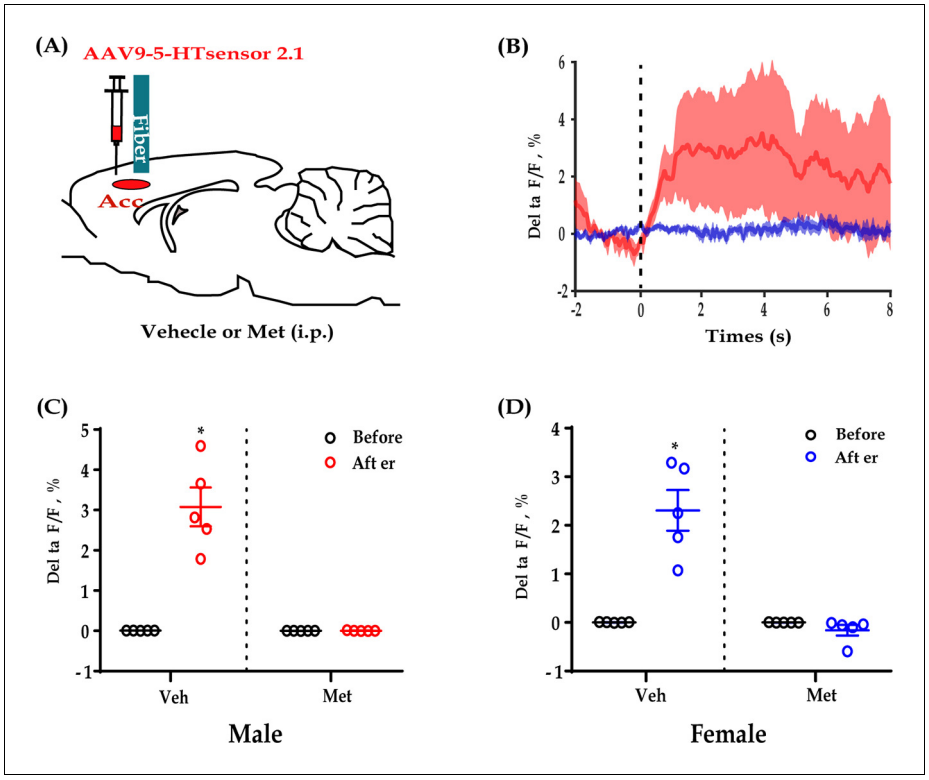

Figure 5—figure supplement 2. 5-HT sensor fluorescence changes during allogrooming could be blocked by Met. (**A**) Schematic diagrams. (**B**) Combined individual 5-HT sensor fluorescence traces during allogrooming in response to Met (blue) or Veh (red). (**C**) Quantification of 5-HT sensor fluorescence signals during allogrooming in response to Met or Veh in male subjects (time: $F_{(1,8)}$ = 40.556, p<0.001, $BF_{(incl)}$ = 12.206; group: F(1,8) = 40.808, p<0.001, $BF_{(incl)}$ = 3.090; time × group: $F_{(1,8)}$ = 40.427, p<0.001, $BF_{(incl)}$ = 1289.130; post-hoc comparisons: Veh-after vs Veh-before/Met-before/Met-after, all p<0.001; Veh-before vs Met-before vs Met-after, all p>0.05). (**D**) Quantification of 5-HT sensor fluorescence signals during allogrooming in response to Met or Veh in female subjects (time: $F_{(1,8)}$ = 24.096, p = 0.001, $BF_{(incl)}$ = 5.265; group: $F_{(1,8)}$ = 32.819, p<0.001 $BF_{(incl)}$ = 2.818; time × group: $F_{(1,8)}$ = 31.760, $BF_{(incl)}$ = 477.495; post-hoc comparisons (Tukey): Veh-after vs Veh-before/Met-before/Met-after, all p<0.001; Veh-before vs Met-before vs Met-after, all p>0.05). *N* = 6 in each group. Two-way repeated measures ANOVA and two-way Bayesian repeated measures ANOVA. Post-hoc comparisons: Tukey. Data are presented as mean ± SE, **p<0.01. For raw data, please refer to ***Figure 5—figure supplement 2—source data 1***. ANOVA: analysis of variance; 5-HT: serotonin; Met: metergoline, a 5-HT receptor antagonist; Veh: vehicle.

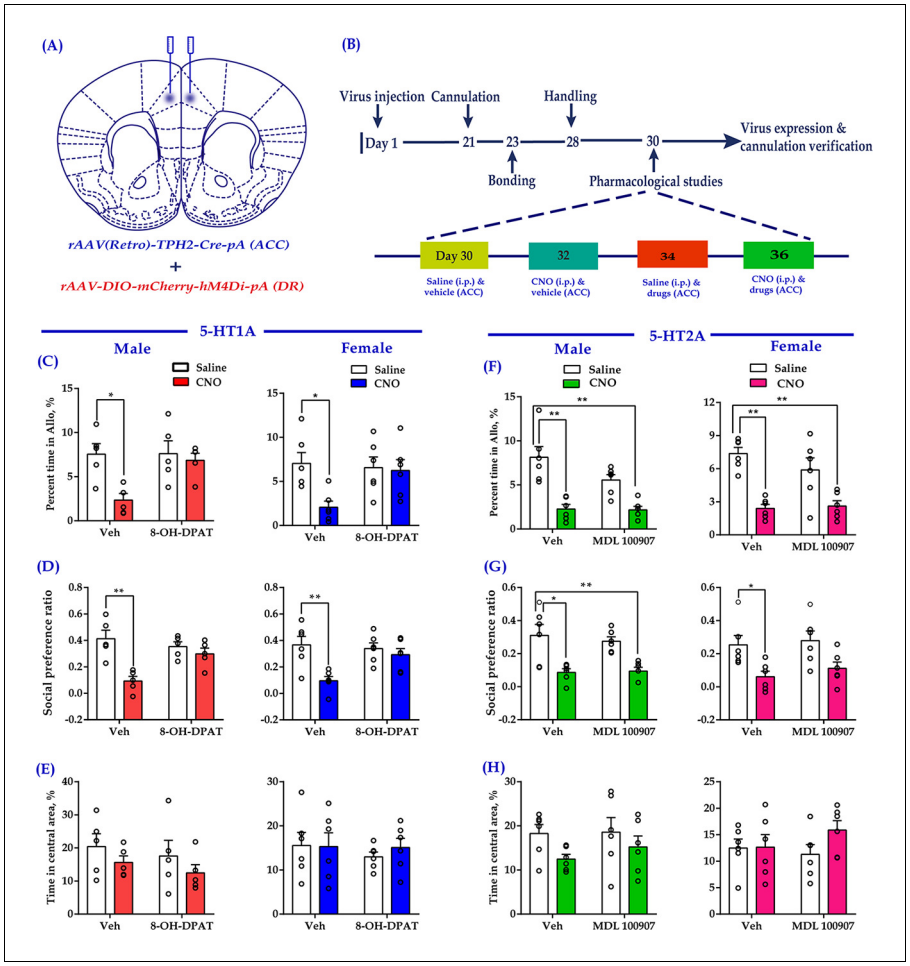

Figure 6. Intra-ACC injection of 8-OH-DPAT reduced sociability deficits induced by chemogenetic inhibition of DR 5-HT neurons in the DR→ACC neural circuit. (**A**) Schematic representation of ACC infusion sites and virus strategy. (**B**) Timeline of experimental design. (**C**) Effect of a 5-HT1AR agonist 8-OH-DPAT on allogrooming time in the consolation test (n = 5 for male, n = 6 for female; male: treatment 1: $F_{(1,16)}$ = 8.230, p = 0.011, $BF_{(incl)}$ = 3.005; treatment 2: $F_{(1, 16)}$ = 4.926, p = 0.041, $BF_{(incl)}$ = 1.380; treatment 1 × treatment 2: $F_{(1,16)}$ = 4.680, p = 0.046, $BF_{(incl)}$ = 1.737; post-hoc comparisons: Veh + Saline vs Veh + CNO, p = 0.013; Veh + Saline vs 8-OH-DPAT+CNO, p = 0.967; female: treatment 1: $F_{(1, 20)}$ = 5.632, p = 0.028, $BF_{(incl)}$ = 1.911; treatment 2: $F_{(1, 20)}$ = 2.746, p = 0.113, $BF_{(incl)}$ = 0.831; treatment 1 × treatment 2: $F_{(1, 20)}$ = 4.320, p = 0.051, $BF_{(incl)}$ = 1.714; post-hoc comparisons: Veh + Saline vs Veh + CNO, p = 0.024; Veh + Saline vs 8-OH-DPAT+CNO, p = 0.957). (**D**) Effect of 8-OH-DPAT on social preference ratio in the three-chamber test (n = 5 for male, n = 6 for female; male: treatment 1: $F_{(1,16)}$ = 12.622, p = 0.003, $BF_{(incl)}$ = 3.750; treatment 2: $F_{(1,16)}$ = 4.993, p = 0.040, $BF_{(incl)}$ = 0.987; treatment 1 × treatment 2: $F_{(1,16)}$ = 12.622, p = 0.003, $BF_{(incl)}$ = 10.906; post-hoc comparisons: Veh + Saline vs Veh + CNO, p < 0.001; Veh + Saline vs 8-OH-DPAT+CNO, p = 0.788; female: treatment 1: $F_{(1, 20)}$ = 10.820, p = 0.004, $BF_{(incl)}$=6.321; treatment 2: $F_{(1, 20)}$ = 3.075, p = 0.095, $BF_{(incl)}$ = 0.858; treatment 1 × treatment 2: $F_{(1, 20)}$ = 5.437, p = 0.030, $BF_{(incl)}$ = 2.347; post-hoc comparisons: Veh + Saline vs Veh + CNO, p = 0.004; Veh + Saline vs 8-OH-DPAT + CNO, p = 0.702). (**E**) Effect of 8-OH-DPAT on time spent in the central area in the open-field test (n = 5 for male, n = 6 for female; male: treatment 1: $F_{(1,16)}$ = 2.063, p = 0.170, $BF_{(incl)}$ = 0.854; treatment 2: $F_{(1,16)}$ = 0.759, p = 0.396, $BF_{(incl)}$ = 0.523; treatment 1 × treatment 2: $F_{(1,16)}$ = 0.003, p = 0.960, $BF_{(incl)}$ = 0.498; female: treatment 1: $F_{(1, 20)}$ = 0.144, p = 0.708, $BF_{(incl)}$ = 0.393; treatment 2: $F_{(1, 20)}$ = 0.324, p = 0.575, $BF_{(incl)}$ = 0.422; treatment 1 × treatment 2: $F_{(1, 20)}$ = 0.227, p = 0.639, $BF_{(incl)}$ = 0.494). (**F**) Effect of a 5-HT2AR antagonist (MDL 100907) on allogrooming time in the consolation test (n = 6 in each group; male: treatment 1: $F_{(1, 20)}$ = 37.118, p < 0.001, $BF_{(incl)}$ = 1591.039; treatment 2: $F_{(1, 20)}$ = 3.094, p = 0.094, $BF_{(incl)}$ = 0.940; treatment 1 × treatment 2: $F_{(1, 20)}$ = 2.697, p = 0.116, $BF_{(incl)}$ = 1.101; post-hoc comparisons: Veh + Saline vs Veh + CNO, p < 0.001; Veh + Saline vs MDL 100907 + CNO, p<0.001; female: treatment 1: $F_{(1, 20)}$ = 36.168, p<0.001, $BF_{(incl)}$ = 2649.973; treatment 2: $F_{(1, 20)}$ = 0.860, p = 0.365, $BF_{(incl)}$ = 0.484; treatment 1 × treatment 2: $F_{(1, 20)}$ = 1.554, p = 0.227, $BF_{(incl)}$ = 0.780; post-hoc comparisons: Veh + Saline vs Veh + CNO, p<0.001; Veh + Saline vs MDL 100907 + CNO, p < 0.001). (**G**)

Figure 6 continued on next page

Figure 6 continued

Effect of MDL 100907 on social preference ratio in the three-chamber test (n = 6 in each group; male: treatment 1: $F_{(1, 20)}$ = 26.890, p<0.001, $BF_{(incl)}$ = 771.577; treatment 2: $F_{(1, 20)}$ = 0.124, p = 0.729, $BF_{(incl)}$ = 0.386; treatment 1 × treatment 2: $F_{(1, 20)}$ = 0.316, p = 0.580, $BF_{(incl)}$ = 0.496; post-hoc comparisons: Veh + Saline vs Veh + CNO, p = 0.003; Veh + Saline vs MDL 100907 + CNO, p = 0.004; female: treatment 1: $F_{(1, 20)}$ = 13.991, p = 0.001, $BF_{(incl)}$ = 34.866; treatment 2: $F_{(1, 20)}$ = 0.620, p = 0.440, $BF_{(incl)}$ = 0.443; treatment 1 × treatment 2: $F_{(1, 20)}$ = 0.071, p = 0.793, $BF_{(incl)}$ = 0.500; post-hoc comparisons: Veh + Saline vs Veh + CNO, p = 0.047; Veh + Saline vs MDL 100907 + CNO, p = 0.191). (**H**) Effect of MDL 100907 on time spent in the central area in the open-field test (n = 6 in each group; male: treatment 1: $F_{(1, 20)}$ = 3.702, p = 0.069, $BF_{(incl)}$ = 1.469; treatment 2: $F_{(1, 20)}$ = 0.415, p = 0.527, $BF_{(incl)}$ = 0.429; treatment 1 × treatment 2: $F_{(1, 20)}$ = 0.270, p = 0.609, $BF_{(incl)}$ = 0.520; female: treatment 1: $F_{(1, 20)}$ = 1.506, p = 0.234, $BF_{(incl)}$ = 0.655; treatment 2: $F_{(1, 20)}$ = 0.274, p = 0.606, $BF_{(incl)}$ = 0.417; treatment 1 × treatment 2: $F_{(1, 20)}$ = 1.305, p = 0.267, $BF_{(incl)}$ = 0.698). Two-way ANOVA along with two-way Bayesian ANOVA. Error bars are ± SEM. *p < 0.05, **p < 0.01. For raw data in this figure, please refer to ***Figure 6—source data 1***. Anta: antangonist; ACC: anterior cingulate cortex; ANOVA: analysis of variance; DR: dorsal raphe nucleus; 5-HT: serotonin.

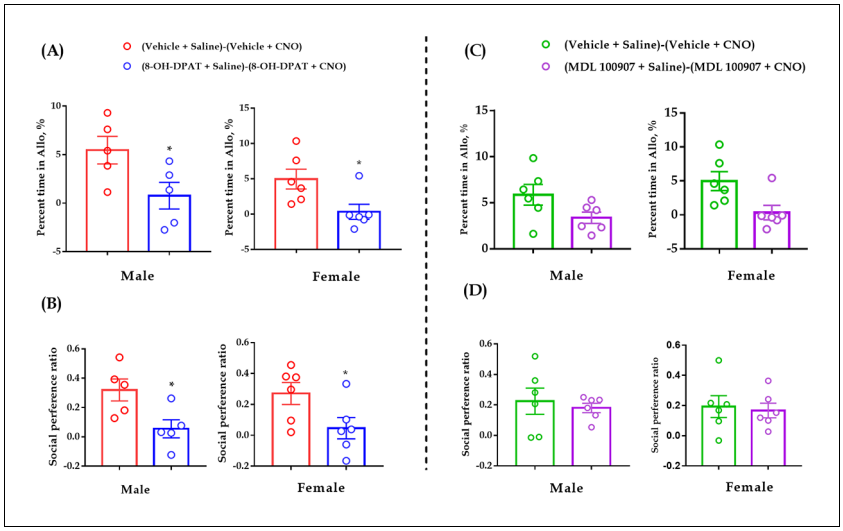

Figure 6—figure supplement 1. Comparisons of 'Vehicle + Saline - Vehicle + CNO' vs '8-OH-DPAT + Saline - 8-OH-DPAT + CNO' (A and B) and 'Vehicle + Saline - Vehicle + CNO' vs 'MDL 100907 + Saline MDL 100907 + CNO' (C and D). (**A**) Male: *N* = 5 in each group. (Vehicle + Saline)-(Vehicle + CNO) vs (8-OH-DPAT+Saline)-(8-OH-DPAT +CNO): $t_{(8)}$ = 2.375, p = 0.045, BF_{+0} = 3.750 with median posterior δ = 0.999, 95% CI = [0.091 to 2.493]. Female: *N* = 6 in each group. (Vehicle + Saline)-(Vehicle + CNO) vs (8-OH-DPAT+Saline)-(8-OH-DPAT+CNO): $t_{(10)}$ = 2.639, p = 0.025, BF_{+0} = 5.482 with median posterior δ = 1.075, 95% CI = [0.123 to 2.453]. (**B**) Male: *N* = 5 in each group. (Vehicle + Saline)-(Vehicle + CNO) vs (8-OH-DPAT+Saline)-(8-OH-DPAT+CNO): $t_{(8)}$ = 2.727, p = 0.026, BF_{+0} = 5.368 with median posterior δ = 1.174, 95% CI = [0.123 to 2.767]. Female: *N* = 6 in each group. (Vehicle + Saline)-(Vehicle + CNO) vs (8-OH-DPAT+Saline)-(8-OH-DPAT+CNO): $t_{(10)}$ = 2.273, p = 0.046, BF_{+0} = 3.609 with median posterior δ = 0.906, 95% CI = [0.087 to 2.203]. (**C**) Male: *N* = 6 in each group. (Vehicle + Saline)-(Vehicle + CNO) vs (MDL 100907 + Saline)-(MDL 100907 + CNO): $t_{(10)}$ = 1.935, p = 0.082, BF_{+0} = 2.487 with median posterior δ = 0.766, 95% CI = [0.063 to 1.981]. Female: *N* = 6 in each group. (Vehicle + Saline)-(Vehicle + CNO) vs (MDL 100907 + Saline)-(MDL 100907 + CNO): $t_{(10)}$ = 1.092, p = 0.300, BF_{+0} = 1.076 with median posterior δ = 0.491, 95% CI = [0.029 to 1.492]. (**D**) Male: *N* = 6 in each group. (Vehicle + Saline)-(Vehicle + CNO) vs (MDL 100907 + Saline)-(MDL 100907 + CNO): $t_{(10)}$ = 0.481, p = 0.641, BF_{+0} = 0.648 with median posterior δ = 0.357, 95% CI = [0.018 to 1.210]. Female: (Vehicle + Saline)-(Vehicle + CNO) vs (MDL 100907 + Saline)-(MDL 100907 + CNO): $t_{(10)}$ = 0.295, p = 0.774, BF_{+0} = 0.567 with median posterior δ = 0.325, 95% CI = [0.016 to 1.138]. Independent samples *t*-test. Data are presented as mean ± SE, *p < 0.05. For raw data, please refer to ***Figure 6—figure supplement 1—source data 1***.

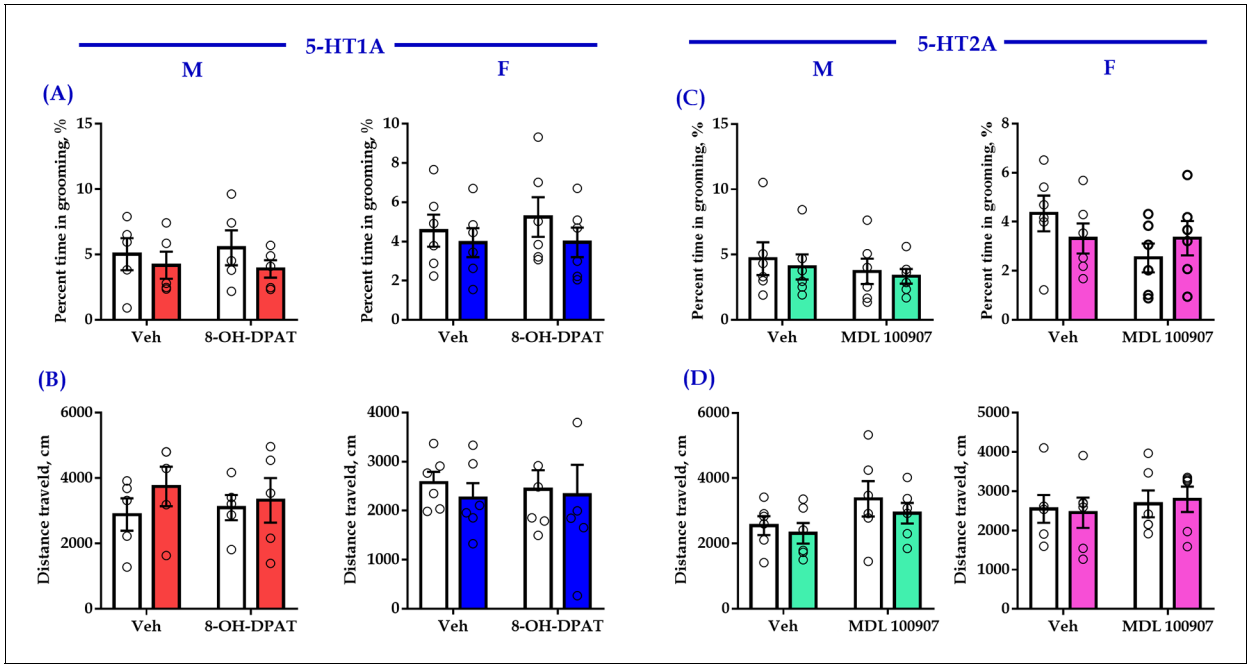

Figure 6—figure supplement 2. Chemogenetic inhibition of DR 5-HT neuron in the DR-ACC neural circuit along with intra-ACC. Injection of 5-HT1AR agonist (8-OH-DPAT) or 5-HT2AR antagonist (MDL 100907) had no significant effect on control behaviors of selfgrooming (**A, C**) and distance traveled in the open-field test (**B, D**). (**A**) Male: *N* = 5 in each group. Treatment 1: $F_{(1,16)}$ = 1.266, p = 0.277, $BF_{(incl)}$ = 0.642; treatment 2: $F_{(1,16)}$ = 0.009, p = 0.926, $BF_{(incl)}$ = 0.393; treatment 1 × treatment 2: $F_{(1,16)}$ = 0.126, p = 0.728, $BF_{(incl)}$=0.535. Female: *N* = 6 in each group. Treatment 1: $F_{(1,20)}$ = 1.292, p = 0.269, $BF_{(incl)}$ = 0.616; treatment 2: $F_{(1,20)}$ = 0.184, p = 0.673, $BF_{(incl)}$ = 0.399; treatment 1 × treatment 2: $F_{(1,20)}$ = 0.165, p = 0.689, $BF_{(incl)}$ = 0.502. (**B**) Male: *N* = 5 in each group. Treatment 1: $F_{(1,16)}$ = 0.964, p = 0.341, $BF_{(incl)}$ = 0.580; treatment 2: $F_{(1,16)}$ = 0.037, p = 0.849, $BF_{(incl)}$ = 0.407; treatment 1 × treatment 2: $F_{(1,16)}$ = 0.329, p = 0.574, $BF_{(incl)}$ = 0.543. Female: *N* = 6 in each group. Treatment 1: $F_{(1,20)}$ = 0.121, p = 0.732, $BF_{(incl)}$ = 0.391; treatment 2: $F_{(1,20)}$ = 0.017, p = 0.897, $BF_{(incl)}$ = 0.375; treatment 1 × treatment 2: $F_{(1,20)}$ = 0.238, p = 0.631, $BF_{(incl)}$ = 0.498. (**C**) Male: *N* = 6 in each group. Treatment 1: $F_{(1,20)}$ = 0.272, p = 0.608, $BF_{(incl)}$ = 0.416; treatment 2: $F_{(1,20)}$ = 0.758, p = 0.394, $BF_{(incl)}$ = 0.504; treatment 1 × treatment 2: $F_{(1,20)}$ = 0.017, p = 0.897, $BF_{(incl)}$ = 0.444. Female: *N* = 6 in each group. Treatment 1: $F_{(1,20)}$ = 0.026, p = 0.874, $BF_{(incl)}$ = 0.377; treatment 2: $F_{(1,20)}$ = 1.861, p = 0.188, $BF_{(incl)}$ = 0.728; treatment 1 × treatment 2: $F_{(1,20)}$ = 1.904, p = 0.183, $BF_{(incl)}$ = 0.855. (**D**) Male: *N* = 6 in each group. Treatment 1: $F_{(1,20)}$ = 0.794, p = 0.384, $BF_{(incl)}$=0.502; treatment 2: $F_{(1,20)}$ = 3.562, p = 0.074, $BF_{(incl)}$ = 1.404; treatment 1 × treatment 2: $F_{(1,20)}$ = 0.075, p = 0.788, $BF_{(incl)}$=0.477. Female: *N* = 6 in each group. Treatment 1: $F_{(1,20)}$ = 6.695e−4, p = 0.980, $BF_{(incl)}$ = 0.375; treatment 2: $F_{(1,20)}$ = 0.453, p = 0.508, $BF_{(incl)}$ = 0.449; treatment 1 × treatment 2: $F_{(1,20)}$ = 0.089, p = 0.768, $BF_{(incl)}$ = 0.462. Data are presented as mean ± SE. Two-way ANOVA and two-way Bayesian ANOVA. Post-hoc comparisons: Tukey. For raw data, please refer to ***Figure 6—figure supplement 2—source data 1***. ACC: anterior cingulate cortex; DR: dorsal raphe nucleus; M: male; F: female; 5-HT, serotonin.