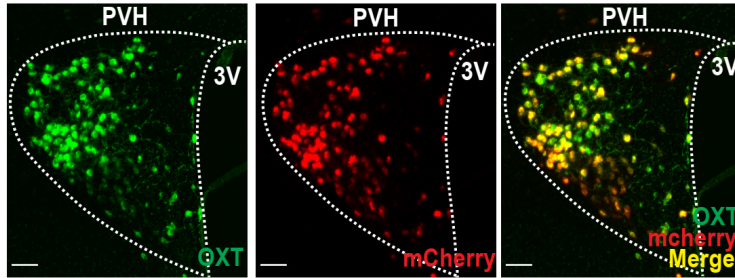**Title**

Oxytocin activity in the Paraventricular and Supramammillary Nuclei of the Hypothalamus is Essential for Social Recognition Memory in Rats
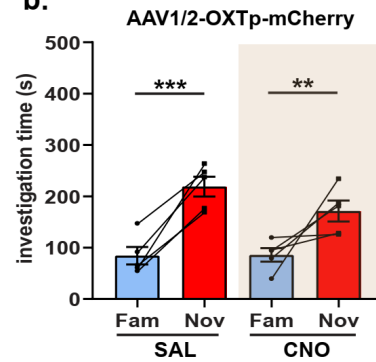
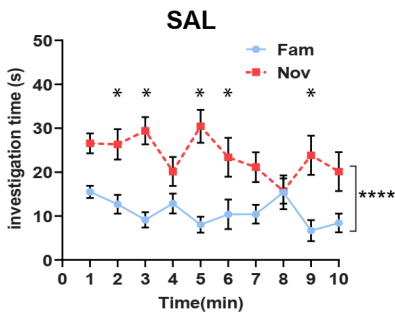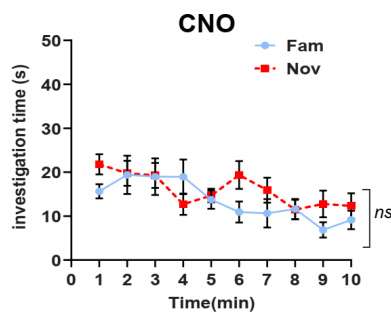# SUPPLEMENTAL INFORMATION

Figure 1. Supplemental Data
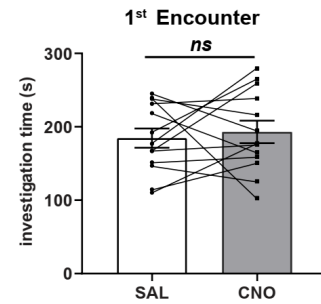
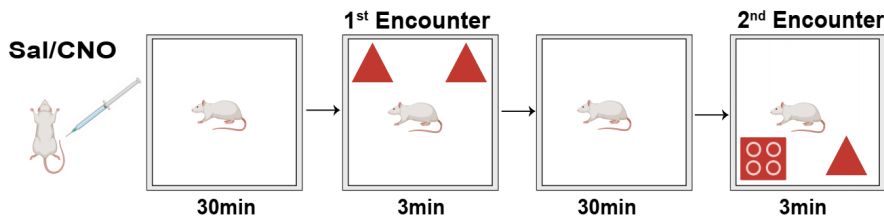**a.**

b.

c. SAL

d. CNO

e. 1st Encounter

f.

g.

h. 1st Encounter

i.

Fig 2. Supplemental data

a.

SAL

b.

CNO

c.

1ˢᵗ encounter

Figure 3. Supplemental data

a.

b. SAL

c. OXTR anta

d. 1st encounter
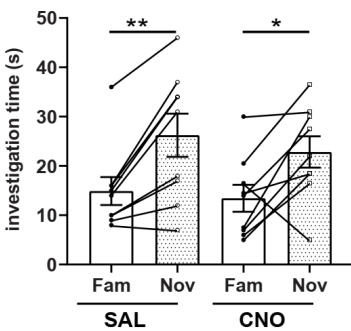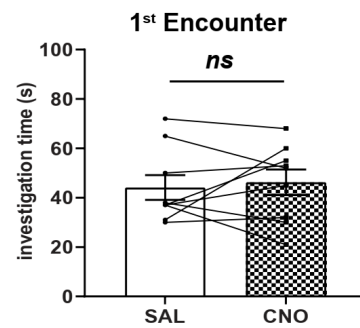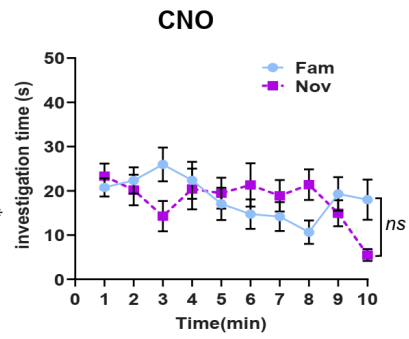
e.

f.

g.

Figure Legends

Figure 1 (Supplemental data)

a. A representative image showing overlap between AAV1/2-OXTp-mCherry (control) expression and OXT neurons in the PVH. **b.** Total investigation time of the Nov vs. Fam stimuli during the 2nd encounter in rats injected with the AAV1/2-OXTp-mCherry virus. Both SAL and CNO treated rat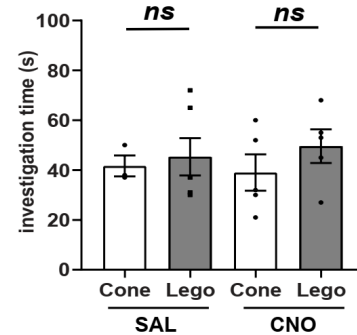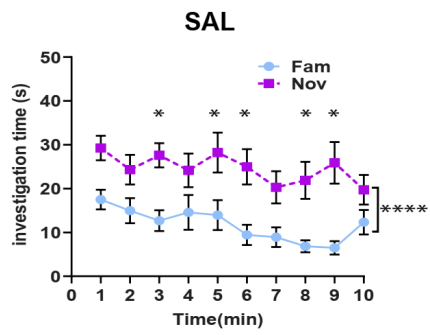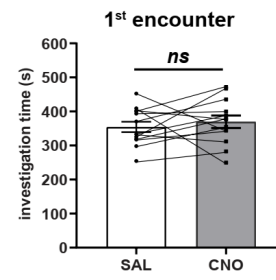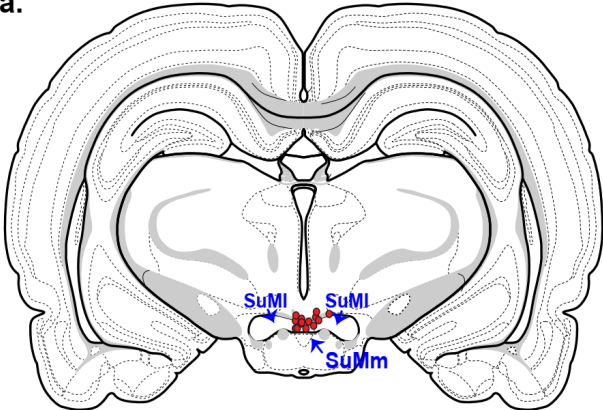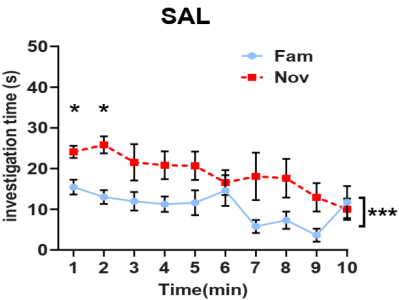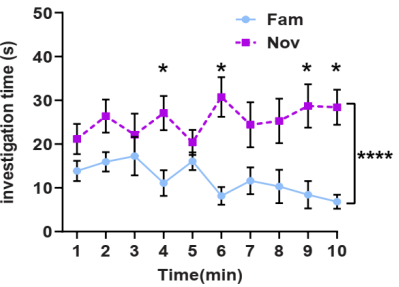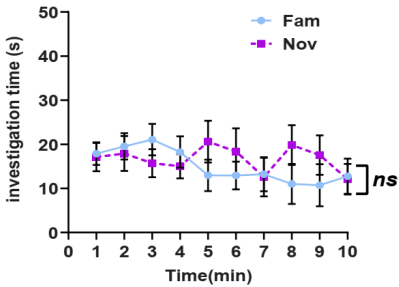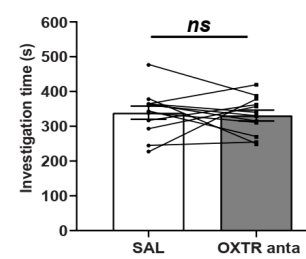s showed a significant preference for the Nov over the Fam social stimuli (two-way repeated measures (RM) ANOVA, social preference (Fam v Nov) x treatment (SAL v CNO) interaction ($F_{1,8}$ = 1.311, P=0.28, n=5), effect of social preference ($F_{1,8}$ = 75.01, **P<0.0001) effect of treatment ($F_{1,8}$ = 1.15, P=0.31), post-hoc, Sidak multiple comparison test, SAL (Fam v Nov, ***P=0.0001), CNO (Fam v Nov, **P=0.006). **c.** Investigation time of Nov vs Fam stimuli across time following SAL or CNO injection during short-term SRM. SAL injected group show consistent preference for Nov over Fam stimuli across time (two-way RM ANOVA, time x social preference (Fam v Nov) ($F_{9,234}$ = 2.36, *P=0.01$), effect of social preference, $F_{1,26}$ = 44.71, ****P<0.0001$), effect of time ($F_{9,234}$ = 1.2, P=0.29). Post-hoc Sidak multiple comparison test revealed a significant difference in investigation time between Fam and Nov at several time points (T2, *P=0.01, T3, ****P<0.0001, T5 ****P<0.0001, T6, *P=0.03, T9 ***P=0.01). **d.** There was no clear preference for Fam or Nov stimuli across time following CNO injection (two-way RM ANOVA, time x social preference ($F_{9,234}$ = 1.500, P=0.148, ns), effect of social preference (Nov v Fam, $F_{1,26}$ = 1.178, P=0.28, ns). **e.** Total investigation time of social stimuli during the 1st encounter. There was no significant difference in the total investigation time between SAL and CNO treatment groups during the 1st encounter (two-tailed paired student's t-test, t_{12}=0.45, P=0.65, ns). **f.** A Schematic of the short-term novel object recognition paradigm. Saline or CNO was injected 30min prior the 1st encounter **g.** Total investigation time of the Nov vs. Fam stimuli during the 2nd encounter. SAL and CNO injected rats showed significant preference for the Nov vs. Fam object (two-way RM ANOVA, treatment x social preference (Fam v Nov) ($F_{1,14}$ = 0.01, P=0.91, n=8), effect of object preference, $F_{1,14}$ = 11.27,

P<0.004), effect of treatment ($F_{1,14}$ = 0.23, *P*=0.63). Post-hoc Sidak multiple comparison test revealed a significant difference in investigation time between Fam and Nov object in both SAL and CNO treated groups SAL (Fam obj v Nov obj), *P*=0.01), CNO (Fam obj v Nov obj, *P*=0.014). **h. Investigation time of the object stimuli during the 1st encounter. There was no significant difference in the investigation time during the 1st encounter between SAL and CNO injected groups (two-tailed paired student's *t*-test, t_7=0.52, *P*=0.61, *ns*). **i.** There was no innate preference for either of the two kinds of objects (cone v lego) following SAL or CNO injection (two-tailed unpaired t-test, lego v cone, SAL, t_7=0.32, *P*=0.75, *ns,* Lego v Cone, CNO, t_8 = 1.07, *P*=0.31, ns). SAL, Saline, CNO, Clozapine N Oxide.

Figure 2 (Supplemental data)

a. Investigation time of the Nov vs. Fam stimuli across time following SAL or CNO injection during long-term SRM**.** SAL injected rats showed a clear preference for the Nov over the Fam stimuli across time, (two-way RM ANOVA, time x social preference ($F_{9, 234}$ = 0.7475, *P*=0.665, *ns*) effect of time, ($F_{9, 234}$ = 2.038, *P*=0.036) effect of social preference ($F_{1, 26}$ = 28.94, ****P*<0.0001), Post-hoc sidak multiple comparison test revealed a significant difference in investigation time between Fam and Nov at several time points in the SAL injected group (T3, *P*=0.013, T5, *P*=0.022, T6 *P*=0.008, T8 *P*=0.012, T9 *P*=0.0004). **c.** The same animals showed no clear preference for the Fam over the Nov stimuli across time following CNO injection, (time x social preference ($F_{9, 234}$ = 3.240, ***P*=0.001), effect of social preference ($F_{1, 26}$ = 0.03546, P=0.852, *ns*), effect of time ($F_{9, 234}$ = 2.236, *P*=0.020). **b.** Investigation time of the social stimuli during the 1st encounter. There were no significant differences between SAL and CNO injection groups during the 1st encounter (two-tailed paired student's *t*-test, t_{12}=0.67, *P*=0.51, *ns*). SAL, saline, CNO, Clozapine N Oxide.

Figure 3 (Supplemental data)

a. A modified Swanson rat atlas image (level 34) with red circles highlighting tip of infusion cannula. Each dot represents one animal from the cohort. **b.** Investigation time for Fam vs. Nov stimuli across time following SAL or OXTR anta infusion during short-term SRM. SAL infused group showed a clear preference for Nov over Fam stimuli across time (two-way RM ANOVA, time x social preference (Fam v Nov) ($F_{9,162}$ = 1.13, P=0.34, *ns*), effect of social preference, $F_{1,18}$ = 21.08, ****P*<0.0002), effect of time ($F_{5.5.,99.1}$ = 2.63, **P*=0.02). Post-hoc Sidak multiple comparison test revealed a significant difference in investigation time between Fam and Nov at several time points (T1, *P=0.02, T2, **P=0.006). **c.** The same animals showed no clear preference for the Fam over the Nov stimuli across time following OXTR anta infusion (two-way RM ANOVA, time x social preference ($F_{9,162}$ = 0.267, P=0.98, *ns*), effect of time ($F_{3.7,67.76}$ = 2.567, **P*=0.049) effect of social preference (Nov v Fam, $F_{1,18}$ = 0.701, P=0.41, *ns*). **d.** Investigation time of the social stimuli during the 1st encounter. There was no significant difference between SAL and OXTR anta group during the 1st encounter (two-tailed student's *t* test, t_6=1.3, P=0.2, *ns*). **e.** Investigation time for Fam vs. Nov stimuli across time following SAL or OXTR anta infusion during long-term SRM. (two-way RM ANOVA, time x social preference (Fam v Nov) ($F_{9,198}$ = 1.936, **P*=0.048), effect of social preference, $F_{1,22}$ = 31.44, *****P*<0.0001), effect of time ($F_{5.36,121.8}$=0.22, P=0.96, *ns*). Post-hoc Sidak multiple comparison test revealed a significant difference in investigation time between Fam and Nov at several time points (T4, *P=0.037, T6, **P=0.003, T9 *P=0.02, T10, **P=0.001). **f.** The same animals showed no clear preference for the Fam over the Nov stimuli across time following OXTR anta infusion(two-way RM ANOVA, time x social preference ($F_{9,198}$ = 0.9, P=0.52, *ns*), effect of time ($F_{6.41,141.1}$ = 0.67, P=0.68, *ns*) effect of social preference (Nov v Fam, $F_{1,22}$ = 0.52, P=0.47, *ns*). **g.** Investigation time of the social stimuli during the 1st encounter. There was no significant difference between SAL and OXTR anta infused group

during the 1st encounter on the long-term SRM (two-tailed student's t test, $t11=0.36$, $P=0.72$, *ns.*

SAL, Saline, OXTR anta, OXTR antagonist.