SUPPLEMENTARY INFORMATION



Supplementary Figure 1. Demonstrator fear conditioning. (a) Schematic timeline of demonstrator testing for explicit fear (black), compared with social fear (gray). (b) (left) Demonstrators (n = 10) express fear-potentiated startle (FPS) to contextual cues, which is not correlated (right) with observer freezing during conditioning (Pearson $R^2 = 0.294$, p = 0.105). (c) (left) Demonstrators express significantly elevated cued FPS at post-test (two-tailed student's t test: t(9) = 3.52, p = 0.007) which shows (right) a near-significant correlation with observer freezing during conditioning (Pearson $R^2 = 0.294$, p = 0.105). Error bars represent s.e.m. ** p < 0.01.



Supplementary Figure 2. Validation of automated freezing behavior. Automated averaged freezing counts and manual scoring of total freezing behavior are significantly correlated using a (**a**) <0.3AU locomotor activity threshold (Pearson $R^2 = 0.65$; p < 0.0001), compared to a (**b**) <0.2AU threshold (Pearson $R^2 = 0.34$; p = 0.009). Data represents DREADD animals (n = 19; see Fig 4).



Supplementary Figure 3. Non-specific observer freezing behavior in control conditioning experiments. (a) Non-specific freezing behavior of familiar (black, n = 13) and unfamiliar (gray, n = 13) male and familiar (black, n = 10) and unfamiliar (gray, n = 10) female observer mice during (left) noDem (ANOVA: sex effect $F_{1,45} = 9.29$, p = 0.004; familiarity effect $F_{1,45} = 0.166$, p = 0.685; sex x familiarity interaction $F_{3,45} = 0.990$, p = 0.325) and (**right**) noShock (ANOVA: sex effect $F_{1,45} = 2.04$, p = 0.160; familiarity effect $F_{1,45} = 0.49$, p = 0.487; interaction effect $F_{3,45} = 1.07$, p = 0.307) control experiments. (b) Non-specific freezing behavior of male observer mice following acute intranasal oxytocin (blue, n = 12) or saline (gray, n = 12) during noDem (two-tailed student's t test: t(23) = 0.88, p = 0.388) and noShock (twotailed student's t test: t(23) = 1.53, p = 0.152) control experiments. (c) Non-specific freezing behavior of male observer mice following chronic intranasal oxytocin (light blue, n = 11) or saline (gray, n = 11) during noDem (two-tailed student's t test: t(21) = 1.16, p = 0.261) and noShock (two-tailed student's t test: t(21) = 1.16, p = 0.261) and noShock (two-tailed student's t test: t(21) = 1.16, p = 0.261) and noShock (two-tailed student's t test: t(21) = 1.16, p = 0.261) and noShock (two-tailed student's t test: t(21) = 1.16, p = 0.261) and noShock (two-tailed student's t test: t(21) = 1.16, p = 0.261) and noShock (two-tailed student's t test: t(21) = 1.16, p = 0.261) and noShock (two-tailed student's t test: t(21) = 1.16, p = 0.261) and noShock (two-tailed student's t test: t(21) = 1.16, p = 0.261) and noShock (two-tailed student's t test: t(21) = 1.16, p = 0.261) and noShock (two-tailed student's t test: t(21) = 1.16, p = 0.261) and noShock (two-tailed student's t test: t(21) = 1.16, p = 0.261) and noShock (two-tailed student's t test: t(21) = 1.16, p = 0.261) and noShock (two-tailed student's t test: t(21) = 1.16, p = 0.261) and noShock (two-tailed student's t test: t(21) = 1.16, p = 0.261) and noShock (two-tailed student's t test: t(21) = 1.16, p = 0.261) and noShock (two-tailed student's t test: t(21) = 1.16, p = 0.261) and noShock (two-tailed student's t test: t(21) = 1.16, p = 0.261) and noShock (two-tailed student's t test: t(21) = 1.16, p = 0.261) and noShock (two-tailed student's t test: t(21) = 1.16, p = 0.261, p = 0.1.07, p = 0.296) control experiments. (d) Non-specific freezing behavior of male observer mice following saline (I.P., black, n = 12), or 5mg/kg (I.P., pink, n = 12) or 10mg/kg (I.P., red, n = 12) OXTA during noDem (ANOVA: $F_{2.35} = 0.65$, p = 0.529) and noShock (ANOVA: $F_{2.35} = 0.26$, p = 0.768) control experiments. (e) Non-specific freezing behavior of male (Oxt-Cre/+) observer mice expressing rM3D(Gs) following CNO (I.P., green, n = 10) or saline (I.P., gray, n = 9) or expressing GFP following CNO (I.P., white, n = 10) during noDem (ANOVA: $F_{2,28} = 0.02$, p = 0.981) and noShock (ANOVA: $F_{2,28} = 5.18$, p = 0.02, p = 0.981) and noShock (ANOVA: $F_{2,28} = 5.18$, p = 0.02, p = 0.981) and noShock (ANOVA: $F_{2,28} = 5.18$, p = 0.02, p = 0.981) and noShock (ANOVA: $F_{2,28} = 5.18$, p = 0.02, p = 0.981) and noShock (ANOVA: $F_{2,28} = 5.18$, p = 0.02, p = 0.981) and noShock (ANOVA: $F_{2,28} = 5.18$, p = 0.02, p = 0.981) and noShock (ANOVA: $F_{2,28} = 5.18$, p = 0.02, p = 0.981) and noShock (ANOVA: $F_{2,28} = 5.18$, p = 0.02, p = 0.981) and noShock (ANOVA: $F_{2,28} = 5.18$, p = 0.02, p = 0.981) and noShock (ANOVA: $F_{2,28} = 5.18$, p = 0.02, p = 0.981) and noShock (ANOVA: $F_{2,28} = 5.18$, p = 0.02, p = 0.981) and P = 0.9810.013) control experiments. Error bars represent s.e.m. * p < 0.05; ** p < 0.01.



Supplementary Figure 4. Relationship between observer and demonstrator freezing behavior. (a) Observer freezing was correlated with the degree of freezing expressed by the demonstrator in familiar pairs (black, n = 10; Pearson $R^2 = 0.455$, p = 0.032), but not in unfamiliar pairs (gray, n = 10; Pearson $R^2 = 0.264$, p = 0.129). (b) Familiar and unfamiliar demonstrators exhibited equivalent freezing behavior during conditioning (two-tailed student's t test: t(19) = 0.007, p = 0.997).



Supplementary Figure 5. Demonstrator vocalizations. Female demonstrators produced more vocalizations than males (ANOVA: effect of sex $F_{1,43}$ = 6.37, p = 0.016). Error bars represent s.e.m. ** p < 0.01.



Supplementary Figure 6. Increased locomotor activity across conditioning days in familiar male observers. Averaged locomotor activity during acclimation of Cond1 was significantly lower in familiar male observers (two-tailed student's t test: t(25) = 3.08, p = 0.008) compared to unfamiliar observers (see also Fig. 1e) and increased significantly across subsequent conditioning days (repeated measure ANOVA: $F_{5,77} = 3.86$, p = 0.004; effect of familiarity $F_{1,77} = 5.96$, p = 0.017; effect of day $F_{2,77} = 4.28$, p = 0.018; familiarity x day interaction $F_{2,77} = 5.36$, p = 0.007). Error bars represent s.e.m. ** p < 0.01.



Supplementary Figure 7. Freezing behavior in familiar male mice administered acute intranasal oxytocin. Compared to saline-treated controls (black, n = 5), familiar male observers treated with acute intranasal oxytocin (20ug/kg; blue, n = 5) thirty minutes prior to demonstrator conditioning showed equivalent freezing (two-tailed student's t test: t(9) = 0.86, p = 0.41). Error bars represent s.e.m.



Supplementary Figure 8. Pavlovian fear behaviors of mice following acute intranasal oxytocin. Compared to saline-treated controls (gray, n = 11), male mice treated with acute intranasal oxytocin (20ug/kg; blue, n = 12) thirty minutes prior to Pavlovian conditioning showed equivalent (**a**) acquisition (**left**, across trials; **right**, averaged) (two-tailed student's t test: t(22) = 0.179, p = 0.859), and (**b**) expression of contextual (two-tailed student's t test: t(22) = 0.401) and (**c**) cued fear-potentiated startle (post; two-tailed student's t test: t(22) = 0.03, p = 0.975). Error bars represent s.e.m.



Supplementary Figure 9. Demonstrator vocalizations in acute and chronic intranasal oxytocin experiments. Demonstrators paired with observer mice administered intranasal oxytocin (acute and chronic; blue; n = 23) produced equivalent vocalization durations, compared to demonstrators paired with saline control mice (gray; n = 23) (ANOVA: $F_{3,68} = 0.013$, p = 0.908). Error bars represent s.e.m.



Supplementary Figure 10. Startle reactivity and Pavlovian fear acquisition following chronic intranasal oxytocin. (a) Startle reactivity was significantly elevated in mice administered chronic intranasal OXT (light blue, n = 11) versus saline controls (gray, n = 11) (excluding baseline; ANOVA: $F_{9,109} = 10.578$, p < 0.0001; effect of treatment, $F_{1,109} = 8.98$, p = 0.004; effect of level, $F_{4,109} = 19.34$, p < 0.0001; treatment x level interaction $F_{9,109} = 1.05$, p = 0.387). See Fig 3 for testing schedule. (b) Averaged freezing during acquisition of Pavlovian fear was equivalent between mice administered chronic intranasal oxytocin (light blue, n = 7) or saline (gray, n = 6) (two-tailed student's t test: t(12) = 0.134, p = 0.896). Error bars represent s.e.m. ** p < 0.01.



Supplementary Figure 11. Confirmation of qRT-PCR amplicons. Original gel blot for qRT-PCR amplicons of the Gapdh (110bps, Lane 2) and Oxtr (135bps, Lane 3) transcripts at expected sizes. DNA base pair ladder is shown in Lane 1.



Supplementary Figure 12. Pavlovian fear acquisition and correlations with social fear following systemic oxytocin antagonist. (a) OXTA (5mg/kg, n = 9; 10mg/kg, n = 12; thirty minutes prior) did not disrupt direct acquisition of Pavlovian fear, compared to saline-treated familiar mice (n = 10) (ANOVA: $F_{2,30} = 0.506$, p = 0.608) (b) Saline-treated observer mice displayed a significant correlation between socially and directly acquired fear (upper panel; Pearson $R^2 = 0.434$, p = 0.038), whereas this relationship was absent in mice treated with 5mg/kg (middle panel; Pearson $R^2 = 0.035$, p = 0.606) or 10mg/kg (bottom panel; Pearson $R^2 = 0.194$) OXTA. Error bars represent s.e.m.



Supplementary Figure 13. Demonstrator vocalizations in chemogenetic experiments.

Demonstrators elicited equivalent vocalization durations for observer mice expressing rM3D(Gs) administered saline (gray; n = 9) or CNO (3mg/kg, I.P.; green; n = 10), and observer mice not expressing rM3D(Gs) administered CNO (white; n = 10) (ANOVA: $F_{2,29} = 0.215$, p = 0.807). Error bars represent s.e.m.



Supplementary Figure 14. Pavlovian fear behaviors of chemogenetic mice. In observer mice expressing the rM3D(Gs) receptor, saline (gray, n = 7) and CNO (3mg/kg, I.P.; green, n = 8) treatment thirty minutes prior to conditioning produced similar (**a**) acquisition (**left**, across trials; **right**, averaged) (two-tailed student's t test: t(14) = 1.380, p = 0.190), and expression of (**b**) contextual (two-tailed student's t test: t(14) = 0.362) and (**c**) cue-specific fear (post; two-tailed student's t test: t(14) = 0.429, p = 0.675). Error bars represent s.e.m.

	Fam/	Unfam/	Fam/	Unfam/	Unfam/	Unfam/	Unfam/	Unfam/	Unfam/	Unfam/	Fam/	Fam/	Fam/
	Male	Male	Female	Female	Acute	Acute	Chronic	Chronic	CNO	SAL	SAL	5mg/kg	10mg/kg
					ΟΧΤ	SAL	ΟΧΤ	SAL				ΟΧΤΑ	ΟΧΤΑ
Average	8.00	8.15	8.30	7.60	*	*	7.63	5.66	7.80	7.70	7.50	7.66	7.90
S.E.	0.73	0.37	0.33	0.58	*	*	0.43	0.92	0.53	0.58	0.43	0.16	0.58
Sig?	n.s.		n.s.		n.s.		n.s.		n.s.		n.s.		

Supplementary Table 1. Litter sizes for experimental groups.

* Data not available.

Supplementary Movie 1. Freezing behavior of a familiar observer male mouse (bottom) in response to the conditioning of a demonstrator conspecific (top).

Supplementary Movie 2. Escape behavior of a familiar observer male mouse in response to the conditioning of a demonstrator conspecific (during Cond(open))