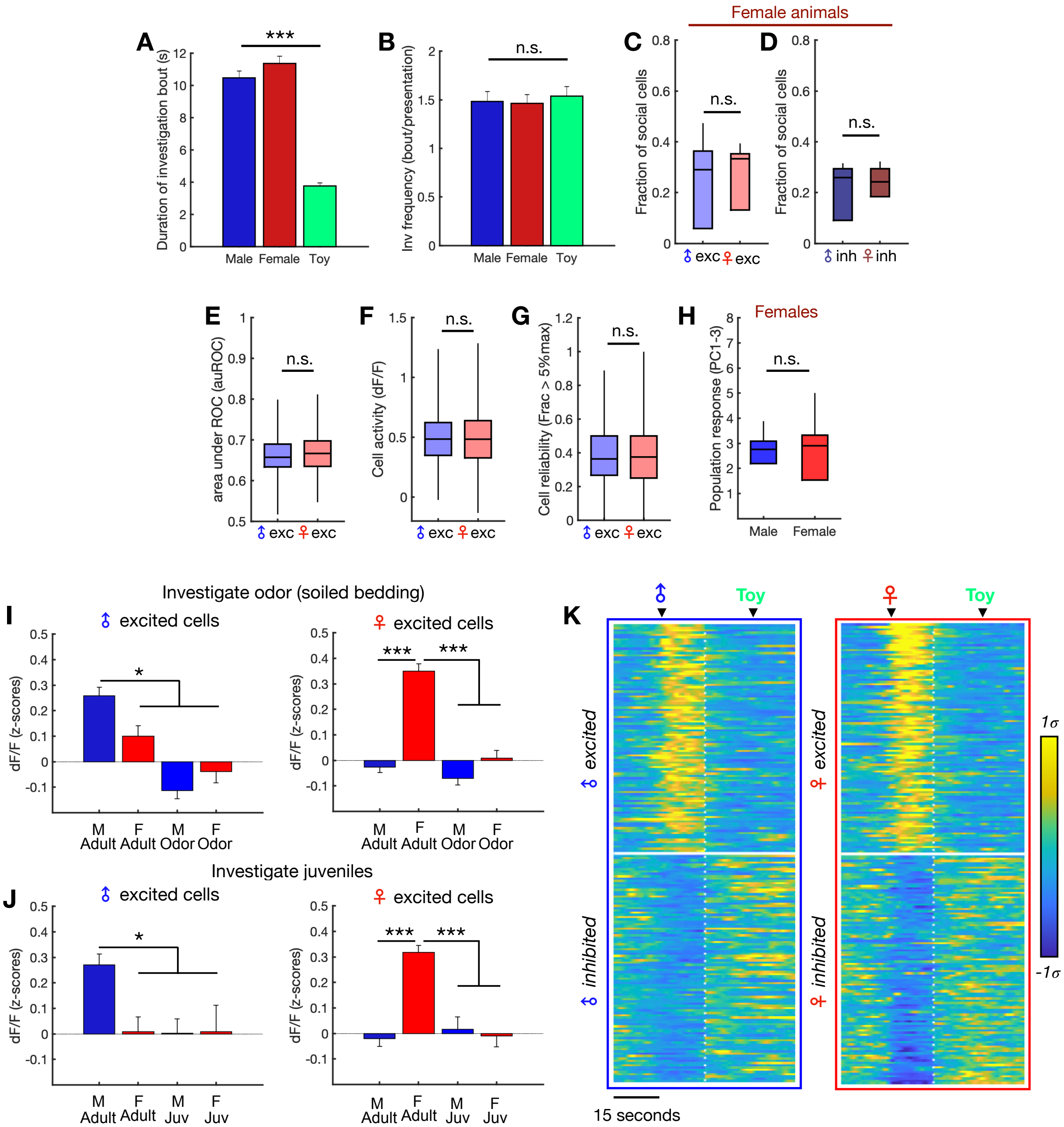

Figure S1. Analysis of social investigation and firing properties of male and female cells, Related to Figure 1

(A) Comparison of the duration of male, female, and toy investigation bouts in the home cage social investigation assay (mean ± SEM, $p = 1.4e-47$).

(B) Comparison of the frequency of male, female, and toy investigation bouts. (mean ± SEM, $p = 0.89$).

(C-D) Fractions of male- and female-excited (C) or suppressed (D) cells among socially responsive neurons recorded from female animals (C, $p = 1$; D, $p = 0.89$). Mixed cells responding to more than one category constituted 5.31 ± 1.96% (mean ± SEM) of socially responsive cells.

(E) Distributions of auROC (area under ROC) values for male- and female-excited cells ($p = 0.202$)

(F) Response strength of cells during social investigation computed as the average Z-scored ΔF/F activity over all male or female investigation bouts ($p = 0.84$).

(G) Response reliability of cells during social investigation computed as the fraction of bouts where the change in activity exceeds 5% of maximum ($p = 0.90$).

(H) Comparison of the population response amplitude during male vs. female interaction in female animals. Population responses (projected to PC1-3) are measured over the first 10 seconds of interaction and averaged across bouts within each animal ($p = 0.8413$).

(I) Activity of male-excited and female-excited cells during investigation of adults or odors (using soiled bedding from male and female conspecifics) (mean ± SEM).

(J) Activity of male-excited and female-excited cells during investigation of male or female adult or juvenile conspecifics (mean ± SEM).

(K) Average responses for male and female neurons centered around onset of stimulus (male or female) and toy. The top 100 neurons, sorted using rank-ordered auROC values, are shown for each cell type.

In (A-B), one-way ANOVA; (C-D, H) Mann-Whitney U test, $n = 5$ animals; (E-G) Mann-Whitney U test, $n = 228$ male cells and 331 female cells; (I-J) Tukey's range test, $n = 24$ male cells and $n = 51$ female cells (I), $n = 39$ male cells and $n = 87$ female cells (J).

***$p < 0.001$, *$p < 0.05$, n.s. = not significant.

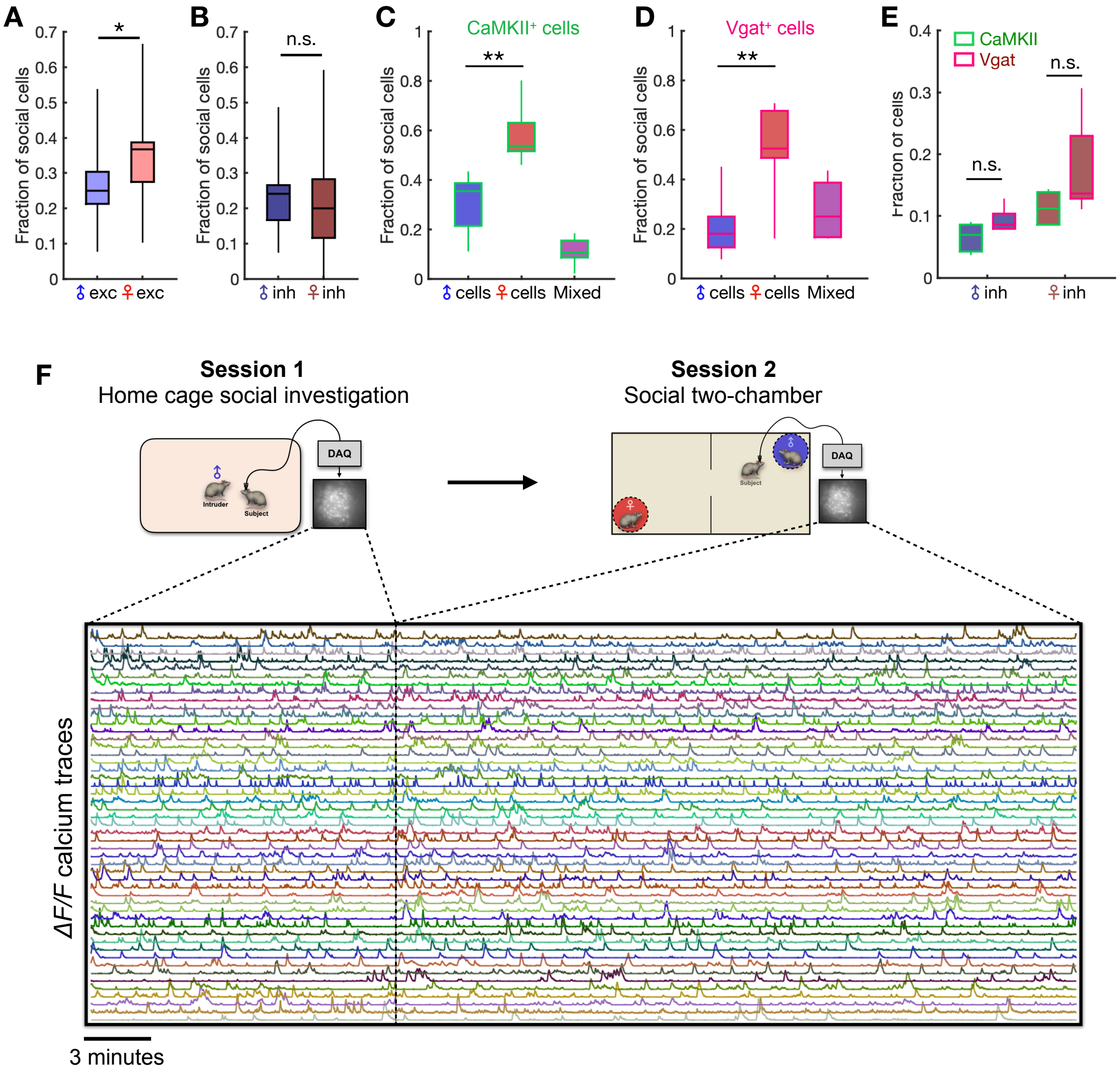

Figure S2. Analysis of sex-encoding cells with equalized behavior sampling, Related to Figures 1 and 3

(A-B) Fractions of male- and female-excited (A) or suppressed (B) cells computed using ROC analysis where calcium traces and behavior vectors have been normalized to equalize representation of male and female events (see Methods). This controls for differences that may be due to unequal sampling of behavior variables (A, $p = 0.012$; B, $p = 0.42$).

(C-D) Fractions of male and female neurons in the CaMKII+ or Vgat+ population computed using ROC analysis after equalizing representation of male and female bouts (A, 0.0012; C, $p = 0.009$).

(E) Fractions of male- and female-inhibited cells within the CaMKII+ and GABAergic populations ($p = 0.68$ (male-inhibited), $p = 0.096$ (female-inhibited)).

(F) Schematic showing sequential home cage social investigation and two-chamber experiments. The microscope is not removed between sessions, and fluorescence videos from each session are concatenated and processed together so that the same exact neurons are identified across sessions. Image shows 50 example neurons recorded from one animal across both sessions. Example fluorescence images for illustration only.

In (A-B) Mann-Whitney U test, n = 23 animals; (C-E) One-way ANOVA followed by Tukey's range test, n = 6 animals per group.

**$p < 0.01$, *$p < 0.05$, n.s. = not significant.

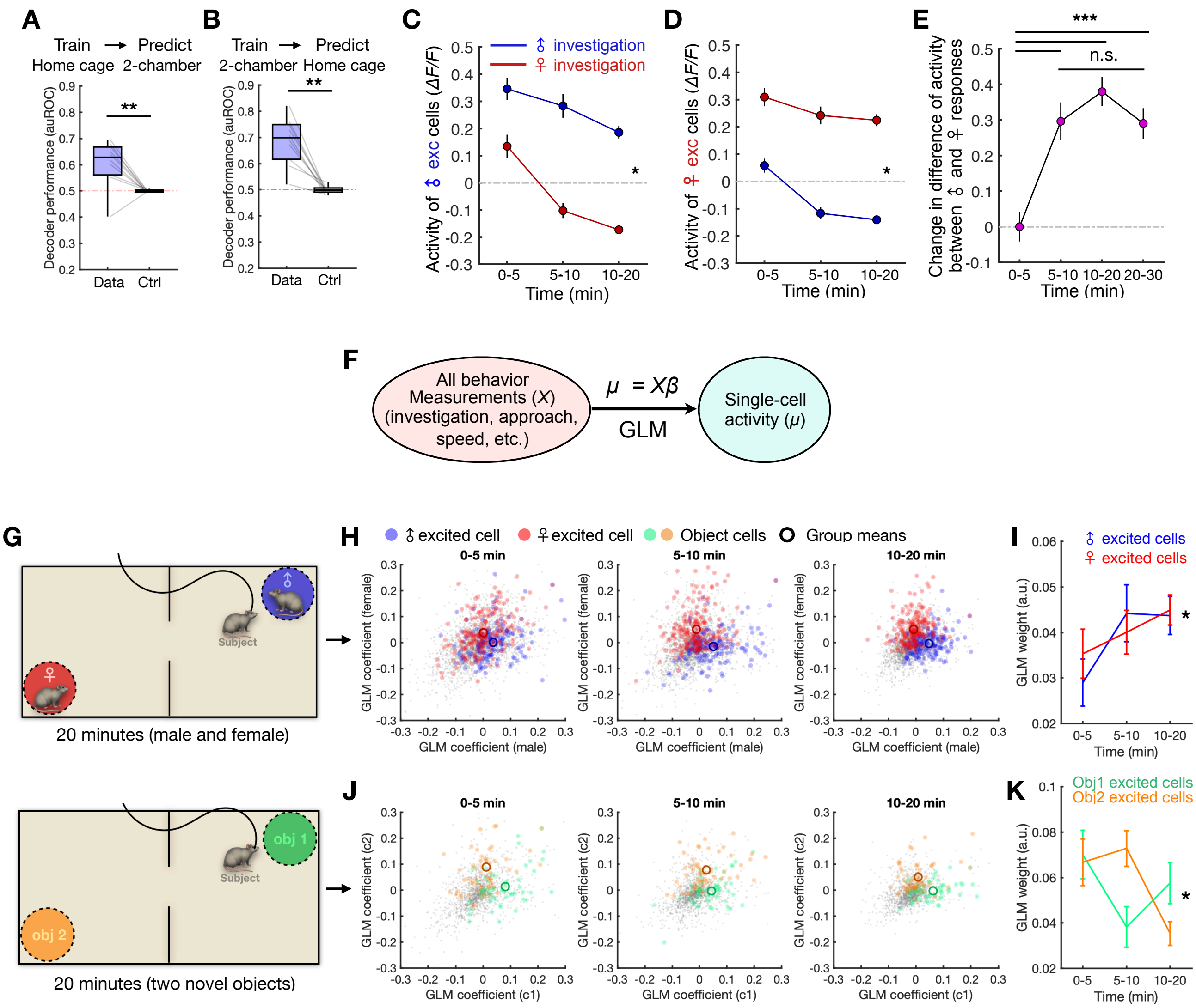

Figure S3. Encoding of social information is strengthened over time, Related to Figure 4

(A) Performance of Fisher's linear discriminant (FLD) decoders trained on calcium data from the home cage session to classify male vs. female investigation using population activity in the two-chamber session. Performance is compared with null models constructed using time-permuted calcium traces (p = 0.0098).

(B) Performance of decoders constructed using data from the two-chamber session to predict the sex identity of interaction events in the home cage, compared with performance of null models as in (A) (p = 0.002).

(C-D) Mean activity of male-excited or female-excited neurons during male and female investigation events over different time epochs during the two-chamber experiment (mean ± SEM, p = 0.021 (C), p = 0.048 (D), time/sex interaction).

(E) Change in differential activity of male and female cells evoked by male vs. female investigation during different time epochs in the two-chamber (mean ± SEM). Stimulus-evoked activity in each epoch is normalized to overall population activity.

(F) Generalized linear models are constructed to model single-neuron activity using behavior variables including male and female investigation (see Methods). Model weights are analyzed to measure cell tuning to different variables over time.

(G) Schematic showing two-chamber sessions with either male and female stimuli (top) or novel objects (bottom).

(H) Weights from generalized linear models (GLM) fit to model single-neuron calcium activity as a function of task parameters in the two-chamber assay (see Methods). Plots show weights fit to male and female investigation for male- (blue) and female-excited (red) cells during different epochs of the session.

(I) GLM weights fit to tuned stimuli in male and female cells over different epochs of the experiment (p = 0.032, time factor).

(J) GLM weights fit to model single neuron activity as in (H) from two-chamber sessions with no social stimuli. Colored dots show weights fit to investigation of each of the novel objects for cells that are significantly active during novel object investigation.

(K) GLM weights fit to model object-excited cells during investigation of tuned stimulus. Weights do not increase over time, as is seen with male and female cells. (mean ± SEM, p = 0.052, time factor)

In (A-B), Wilcoxon signed-rank test, n = 10 animals; (C-D) Two-way ANOVA, n = 125 cells (C), n = 166 cells (D); (E) One-way ANOVA followed by Tukey's range test, n = 291 cells; (I, K) Two-way ANOVA.

***p < 0.001, *p < 0.05, n.s. = not significant.

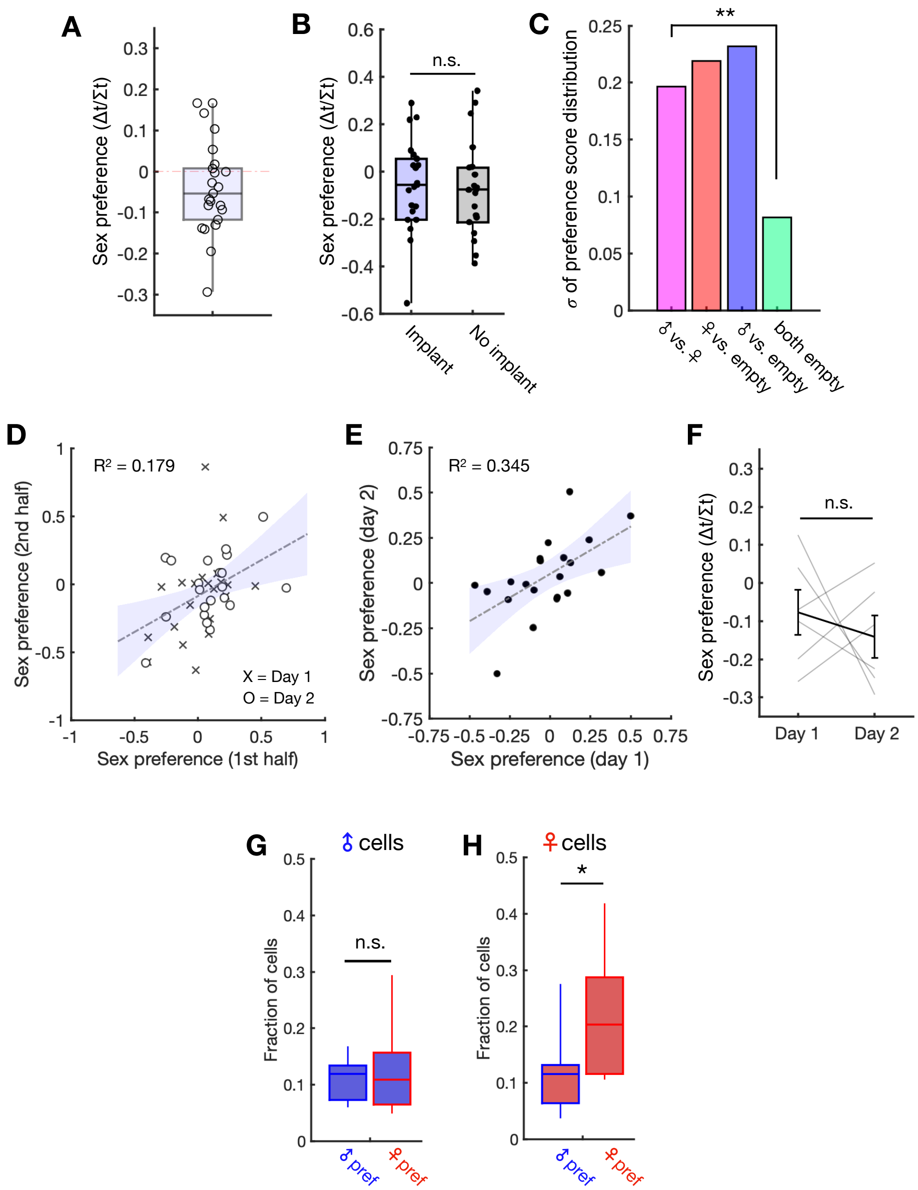

Figure S4. Analysis of sex preference behavior in the two-chamber, Related to Figure 5

(A) Distribution of sex preference scores in the home cage assay [(T♂-T♀)/(T♂+T♀)].

(B) Distribution of sex preferences scores in the two-chamber assay for animals that were implanted and wearing a microendoscope and animals that were not (Mann-Whitney U test, $p = 0.5629$).

(C) Variability (standard deviation) in preference scores for animals in different two-chamber conditions. The natural variability in sex preference during the male/female two-chamber (n = 21 animals) is similar to the variability in social preference elicited by male (n = 13 animals) or female (n = 13 animals) conspecifics compared to an empty cup, but higher than when social stimuli are not present (n = 15 animals) ($p = 0.0016$).

(D) Linear correlation between sex preference scores calculated over two halves of the two-chamber. Data is pooled across two days of testing ($R^2 = 0.179$, $p = 0.0052$).

(E) Linear correlation between sex preference scores across two days of testing with novel stimulus animals (95% confidence interval, $R^2 = 0.345$, $p = 0.0051$).

(F) Sex preference scores for animals in a two-chamber assay before and after sexual experience with female conspecifics (Mann-Whitney U test, $p = 0.69$).

(G-H) Fractions of male (G) and female (H) cells in male- and female-preferring animals (top and bottom 30%) computed using ROC analysis after equalizing representation of male and female bouts (G, $p = 0.5$; H, $p = 0.0036$).

In (B), Mann-Whitney U test, n = 21 animals per condition; (C) two-sample F-test; (D-E) Linear regression, n = 42 sessions (D), n = 21 animals (E); (F) Wilcoxon signed-rank test, n = 6 animals; (G-H) One-sided Mann-Whitney U test, n = 7 animals per group.

**$p < 0.01$, *$p < 0.05$, n.s. = not significant.

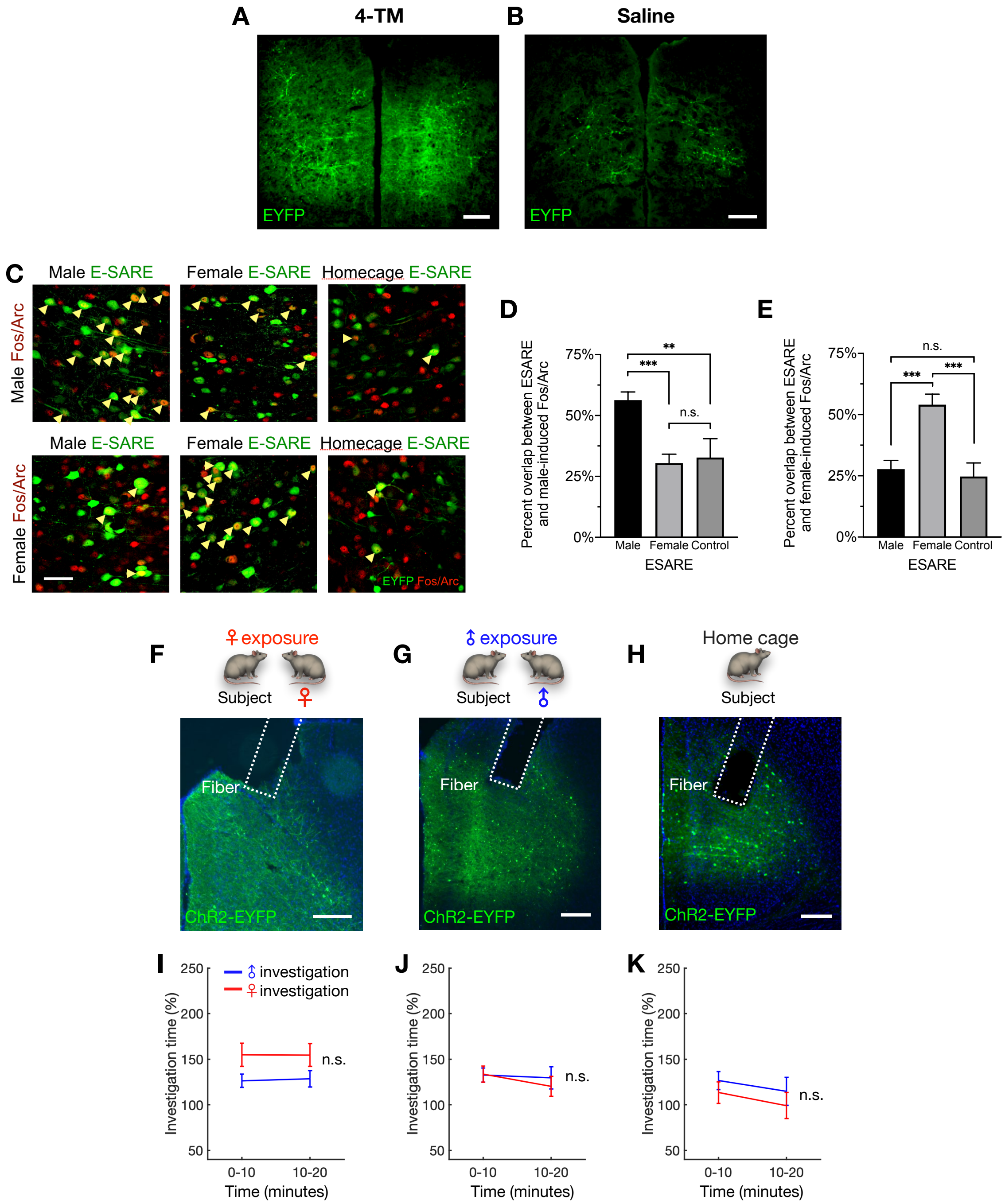

Figure S5. Histological analysis of activity-dependent labeling and optogenetics controls, Related to Figure 6

(A) Expression of ChR2-YFP in dmPFC neurons in an animal that was exposed to male conspecifics and received injection of tamoxifen.

(B) Expression of ChR2-YFP in an animal that was exposed to male conspecifics and received a saline injection.

(C) Representative fluorescent images showing overlap (yellow) between cells expressing Fos/Arc (red) after exposure to male (top row) or female (bottom row) conspecifics, and cells tagged by E-SARE (green) weeks prior when exposed to male (left) conspecifics, female (middle) conspecifics, or home cage (right). E-SARE-Cre labels 298 cells/mm^2 for male exposure and 323 cells/mm^2 for female exposure, and there is no significant difference between them ($p = 0.46$).

(D) Quantification of percentage overlap of male-induced Fos/Arc activated cells with E-SARE labeled cells (mean ± SEM, $p < 0.0001$, E-SARE x Fos/Arc interaction).

(E) Quantification of percentage overlap of female-exposed Fos/Arc activated cells with E-SARE labeled cells (mean ± SEM, $p < 0.0001$, E-SARE x Fos/Arc interaction).

(F) Fiber placement above dmPFC and expression of ChR2 in female cells.

(G) Fiber placement and expression of ChR2 in male cells.

(H) Fiber placement and expression of ChR2 in non-social dmPFC neurons.

(I) Time spent investigating (%) male and female in the two-chamber assay for the cohort of female-exposed animals in separate sham sessions where the optic fiber was attached but no light was delivered (mean ± SEM, $p = 0.82$ time/sex interaction).

(J) Time spent investigating male and female for the male-exposed cohort in control sessions with fiber attachment and no light (mean ± SEM, $p = 0.47$, time x sex interaction).

(K) Time spent investigating male and female for the home cage control cohort in sessions with fiber attachment and no light (mean ± SEM, $p = 0.93$ time, x sex interaction).

In (D-E) two-way ANOVA followed by Sidak's multiple comparison test, $n \geq 10$ sections from four independently injected hemispheres per condition (for all six conditions); (I-K), two-way repeated measures ANOVA, n = 8 (I), 14 (J), and 9 (K) animals.

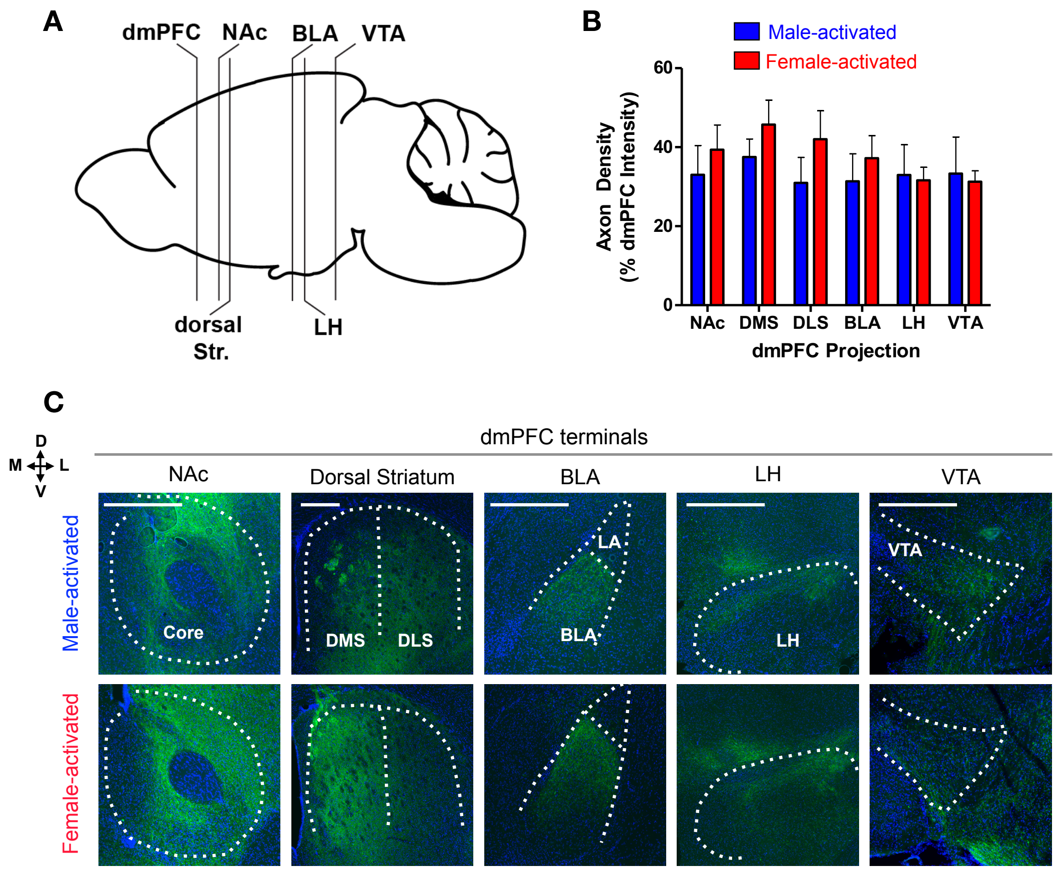

Figure S6. Axonal projections of male- and female-activated neurons, Related to Figure 6

(A) Sagittal view showing positions of brain regions imaged to quantify fluorescence intensity of axonal projections expressing ChR2-EYFP.

(B) Quantification of fluorescent pixels per ROI for each condition ($p = 0.20$ region x cell interaction). The axon density (expressed as % dmPFC Intensity) represents the fraction of fluorescent pixels normalized to dmPFC intensity within each mouse.

(C) Representative fluorescent images containing images of axon terminals in nucleus accumbens (NAc), dorsal striatum (dorsomedial: DMS and dorsolateral: DLS), basolateral amygdala (BLA), lateral hypothalamus (LH), and ventral tegmental area (VTA) from male-activated (top row) or female-activated (bottom row) mice. Scale bars represent 500 um in all images.

Scale bar = 200 μm.

Table S1. Summary statistics for ANOVA analysis, Related to STAR Methods

Figure	Sample Size	Analysis	Statistical Test Values
3E	N = 6 animals (CaMKII cells)	One-way ANOVA	F(2,15) = 41.01, P = 2.305e-7
3F	N = 6 animals (GABAergic cells)	One-way ANOVA	F(2,15) = 9.8, P = 0.0019
3I	N = 6 animals (GABAergic cells) N = 6 animals (CaMKII cells)	Two-way ANOVA Factor 1 – cell type Factor 2 – sex-encoding identity	Factor 1 (encoding identity): F(1,20) = 8.56, P = 0.0084 Factor 2 (cell type): F(1,20) = 20.82, P = 0.0002 Factor 1 x Factor 2 interaction: F(1,20) = 0.9, P = 0.7718
4H	N = 291 social cells	One-way ANOVA	F(2,870) = 19.25, P = 6.6e-9
4I	N = 115 object cells	One-way ANOVA	F(2,342) = 0.14, P = 0.8662
6C-D	N = 8 animals (female exposed E-SARE)	Two-way repeated measures ANOVA Factor 1 – time Factor 2 – stimulus identity (male/female)	Factor 1 (time): F(2,28) = 3.376, P = 0.048 Factor 2 (stimulus identity): F(1,14) = 3.376, P = 0.088 Factor 1 x Factor 2 interaction: F(2,28) = 6.748, P = 0.004
6F-G	N = 14 animals (male exposed E-SARE)	Two-way repeated measures ANOVA Factor 1 – time Factor 2 – stimulus identity (male/female)	Factor 1 (time): F(2,52) = 1.908, P = 0.158 Factor 2 (stimulus identity): F(1,26) = 0.091, P = 0.765 Factor 1 x Factor 2 interaction: F(2,52) = 3.653, P = 0.032
6I-J	N = 9 animals (home cage exposed E-SARE)	Two-way repeated measures ANOVA Factor 1 – time Factor 2 – stimulus identity (male/female)	Factor 1 (time): F(2,32) = 0.464, P = 0.633 Factor 2 (stimulus identity): F(1,16) = 0.928, P = 0.406 Factor 1 x Factor 2 interaction: F(2,32) = 0.085, P = 0.775
S1A	N = 273 male investigation bouts N = 269 female investigation bouts N = 283 object investigation bouts	One-way ANOVA	F(2,822) = 123.37, P = 1.404e-47
S1B	N = 23 animals	One-way ANOVA	F(2,66) = 0.14, P = 0.8692
S1I	N = 39 male-excited cells	One-way ANOVA	F(3,152) = 18.55, P = 2.629e-10
S1I	N = 87 female-excited cells	One-way ANOVA	F(3,344) = 52.22, P = 7.756e-28
S1J	N = 24 male-excited cells	One-way ANOVA	F(3,92) = 3.69, P = 0.0146
S1J	N = 51 female-excited cells	One-way ANOVA	F(3,200) = 17.73, P = 3.047e-10
S2C	N = 6 animals (CaMKII cells)	One-way ANOVA	F(2,15) = 30.62, P = 5.059e-6
S2D	N = 6 animals (GABAergic cells)	One-way ANOVA	F(2,15) = 6.7, P = 0.0083
S2E	N = 6 animals (GABAergic cells) N = 6 animals (CaMKII cells)	Two-way ANOVA Factor 1 – cell type Factor 2 – sex-encoding identity	Factor 1 (encoding identity): F(1,20) = 13.05, P = 0.0017 Factor 2 (cell type): F(1,20) = 6.41, P = 0.0198 Factor 1 x Factor 2 interaction: F(1,20) = 0.91, P = 0.3519
S3C	N = 125 male-excited cells	Two-way ANOVA Factor 1 – time Factor 2 – stimulus identity (male/female)	Factor 1 (time): F(2,744) = 24.67, P = 4.238e-11 Factor 2 (stimulus identity): F(1,744) = 133.95, P = 1.336e-28 Factor 1 x Factor 2 interaction: F(2,744) = 3.89, P = 0.0209

S3D	N = 166 female-excited cells	Two-way ANOVA Factor 1 – time Factor 2 – stimulus identity (male/female)	Factor 1 (time): $F(2,990) = 17.49$, $P = 3.4387e-8$ Factor 2 (stimulus identity): $F(1,990) = 236.17$, $P = 5.8476e-48$ Factor 1 x Factor 2 interaction: $F(2,990) = 3.05$, $P = 0.048$
S3E	N = 291 social cells	One-way ANOVA	$F(3,1160) = 13.75$, $P = 8.15e-9$
S3I	N = (145, 147, 150) male-excited cells (t1, t2, t3) N = (192, 185, 188) female-excited cells (t1, t2, t3)	Two-way ANOVA Factor 1 – time Factor 2 – encoding identity (male/female)	Factor 1 (time): $F(2,1001) = 3.46$, $P = 0.0319$ Factor 2 (encoding identity): $F(1,1001) = 0.08$, $P = 0.7771$ Factor 1 x Factor 2 interaction: $F(2,1001) = 0.56$, $P = 0.5714$
S3K	N = (53, 54, 54) object 1-excited cells (t1, t2, t3) N = (56, 59, 55) object 2-excited cells (t1, t2, t3)	Two-way ANOVA Factor 1 – time Factor 2 – encoding identity (obj1/obj2)	Factor 1 (time): $F(2,325) = 3.09$, $P = 0.047$ Factor 2 (encoding identity): $F(1,325) = 0.18$, $P = 0.6756$ Factor 1 x Factor 2 interaction: $F(2,325) = 5.43$, $P = 0.0048$
S5D-E	Figure S5D N = 20 hemisections (Male E-SARE & Male Fos/Arc) N = 16 hemisections (Female E-SARE & Male Fos/Arc) N = 10 hemisections (Nonsocial E-SARE & Male Fos/Arc) Figure S5E N = 21 hemisections (Male E-SARE & Female Fos/Arc) N = 17 hemisections (Female E-SARE & Female Fos/Arc) N = 12 hemisections (Nonsocial E-SARE & Female Fos/Arc)	Two-way ANOVA Factor 1 – Fos/Arc stimulus (male/female) Factor 2 – E-SARE stimulus (male/female/nonsocial)	Factor 1 (Fos/Arc stimulus): $F(1,90) = 1.407$, $P = 0.2387$ Factor 2 (E-SARE stimulus): $F(2,90) = 4.987$, $P = 0.0088$ Factor 1 x Factor 2 interaction: $F(2,90) = 20.53$, $P < 0.0001$
S5I	N = 8 animals (female exposed E-SARE)	Two-way repeated measures ANOVA Factor 1 – time Factor 2 – stimulus identity (male/female)	Factor 1 (time): $F(1,14) = 0.036$, $p = 0.850$ Factor 2 (stimulus identity): $F(1,14) = 3.745$, $p = 0.073$ Factor 1 x Factor 2 interaction: $F(1,14) = 0.055$, $p = 0.817$
S5J	N = 14 animals (male exposed E-SARE)	Two-way repeated measures ANOVA Factor 1 – time Factor 2 – stimulus identity (male/female)	Factor 1 (time): $F(1,26) = 1.33$, $p = 0.259$ Factor 2 (stimulus identity): $F(1,26) = 0.117$, $p = 0.735$ Factor 1 x Factor 2 interaction: $F(1,26) = 0.529$, $p = 0.473$
S5K	N = 9 animals (home cage exposed E-SARE)	Two-way repeated measures ANOVA Factor 1 – time Factor 2 – stimulus identity (male/female)	Factor 1 (time): $F(1,16) = 1.127$, $p = 0.304$ Factor 2 (stimulus identity): $F(1,16) = 1.129$, $p = 0.304$ Factor 1 x Factor 2 interaction: $F(1,16) = 0.009$, $p = 0.925$
S6B	N = 4 animals (male E-SARE) N = 4 animals (female E-SARE)	Two-way ANOVA Factor 1 – brain region Factor 2 – cell type (male/female-encoding)	Factor 1 (brain region): $F(5,30) = 2.79$, $P = 0.0348$ Factor 2 (cell type): $F(1,6) = 0.32$, $P = 0.5894$ Factor 1 x Factor 2 interaction: $F(5,30) = 1.579$, $P = 0.1961$