



# Corticotropin-releasing hormone signaling from prefrontal cortex to lateral septum supports social novelty preference

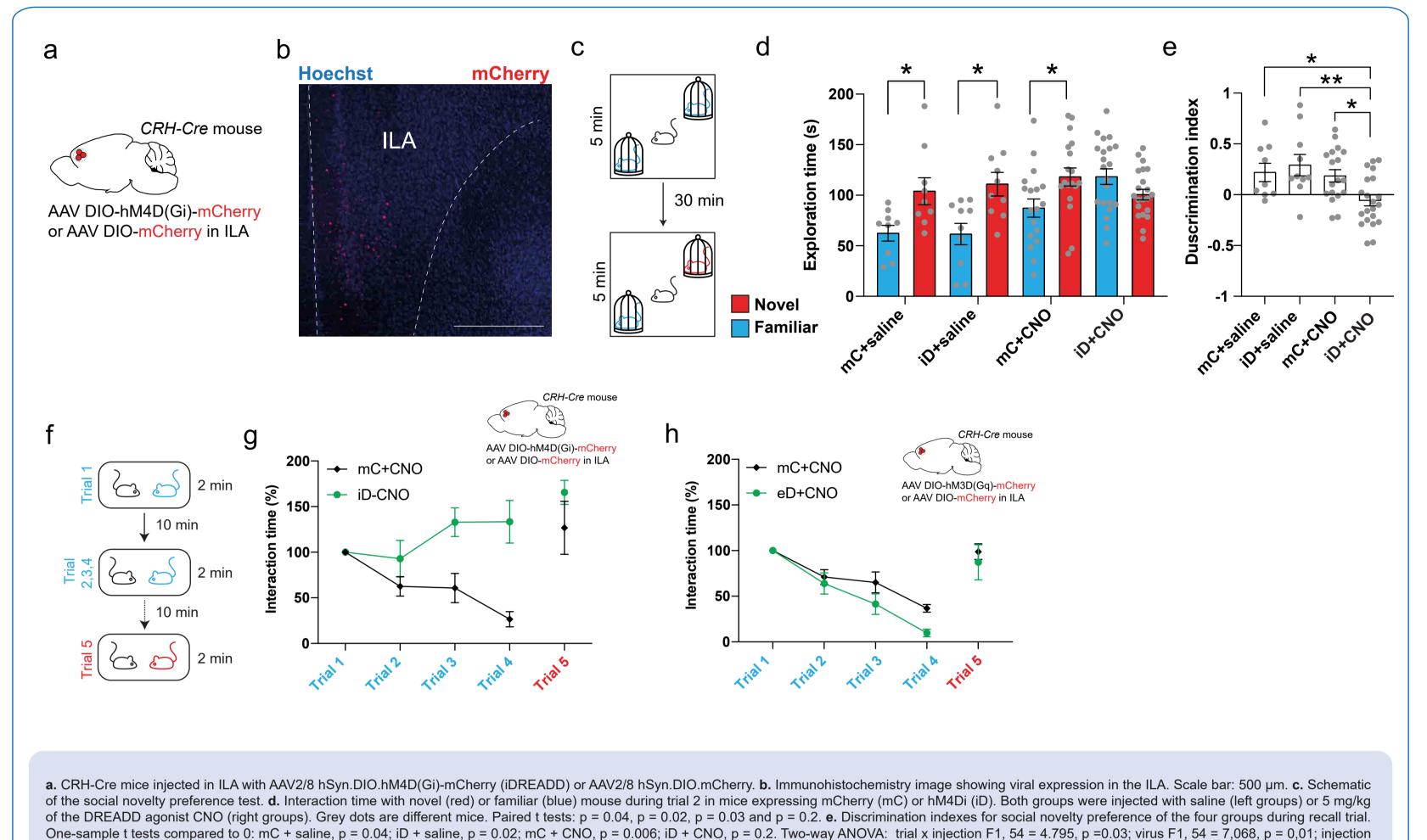
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Social preference, the decision to interact with one member of the same species over another, is a key feature of optimizing social interactions.

At present, it is unclear which neuronal circuits guide social preferences and whether such circuits promote social interactions with the preferred individuals or suppress interactions with the non-preferred ones.

Here, we identify a population of inhibitory neurons in ILA that express the neuropeptide corticotropin-releasing hormone (CRH) and project to the rostro-dorsal region of LS (rdLS). Release of CRH from ILA in rdLS during interactions with familiar mice disinhibits rdLS neurons, thereby suppressing interactions with familiar mice and contributing to social novelty preference. We further demonstrate how the maturation of CRH expression during the first two post-natal weeks enables the developmental shift from a preference for littermates in juveniles to a preference for novel mice in adults.

#### Silencing ILACRH cells impairs social novelty preference (SNP) and familiarization.



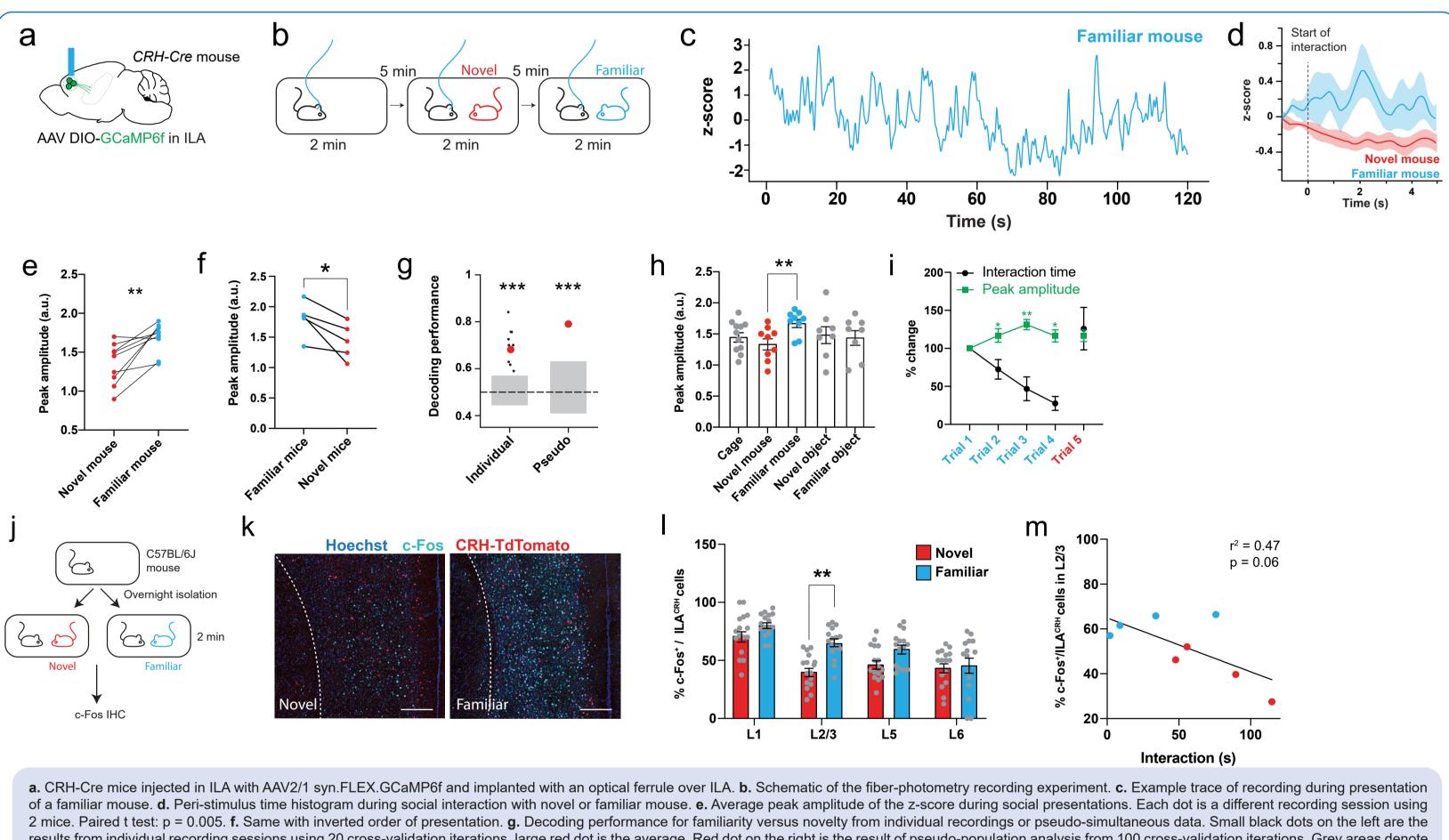
F1, 54 = 1,535, p = 0.2. Tukey's multiple comparisons tests compared to the iD + CNO group: mC + saline, p = 0.04; iD + saline, p = 0.008; mC + CNO, p = 0.04. f. Schematic of the repetitive social presentation test. g. Norma-

lized interaction times during social presentations (inhibitory DREADD-expressing mice and controls injected with CNO). 8 mice per group. Two-way ANOVA: trial x virus F(12,139) = 2.09, p = 0.02; trial F4,139 = 17.21, p <

0.0001; virus F3,139 = 15.76, p < 0.0001. h. Normalized interaction times during repetitive social presentation test in CRH-Cre mice injected in ILA with AAV5 hSyn.DIO.hM3D(Gq)-mCherry (excitatory DREADD) or with AAV5

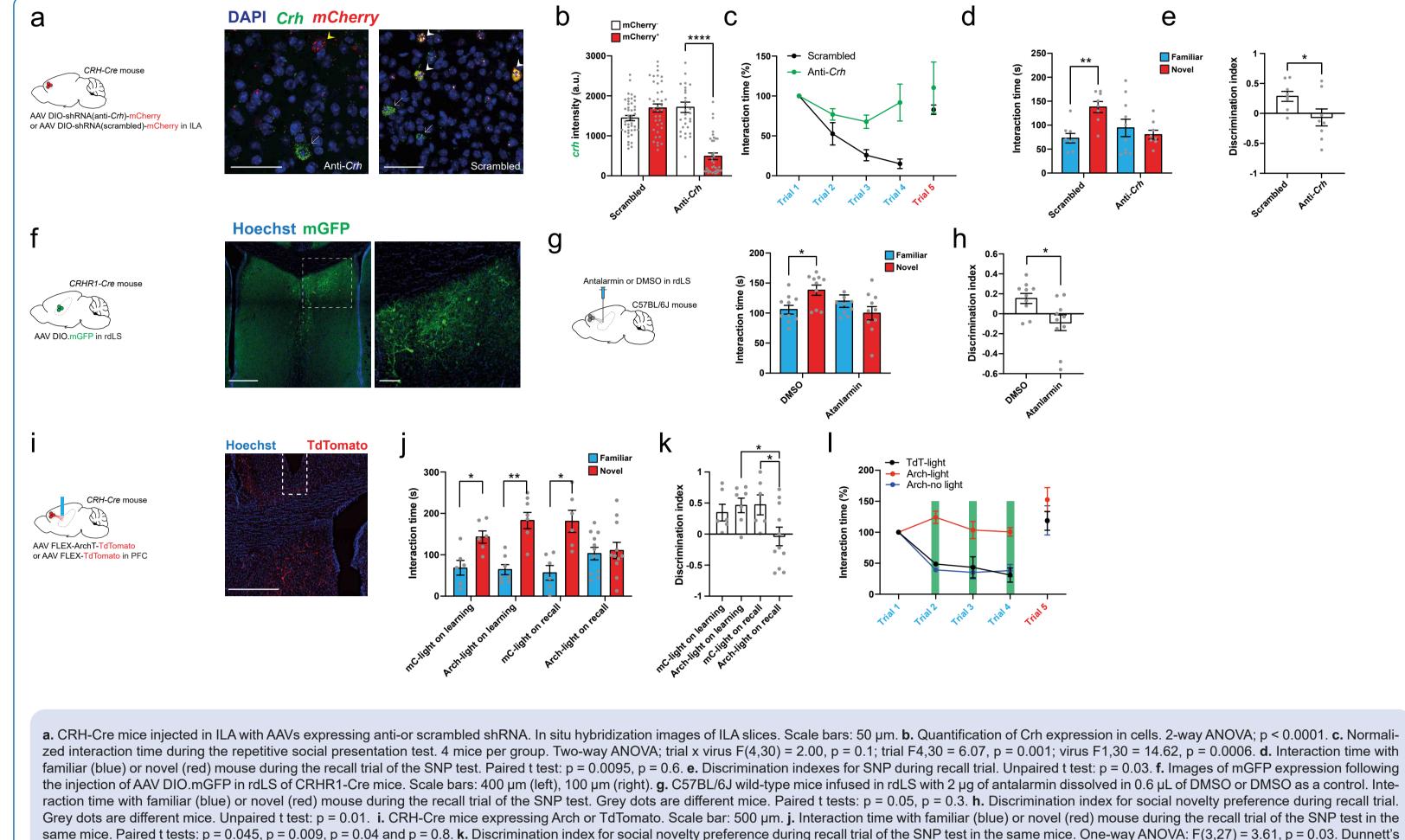
hSyn.DIO.mCherry as a control. 8 mice per group. Two-way ANOVA: trial x virus F(12,140) = 0.96, p = 0.5; trial F4,140 = 34.21, p < 0.0001; virus F(3,140) = 3.01, p = 0.03.

ILACRH cells respond to social familiarity.



2 mice. Paired t test: p = 0.005. **f.** Same with inverted order of presentation. **g.** Decoding performance for familiarity versus novelty from individual recordings or pseudo-simultaneous data. Small black dots on the left are the results from individual recording sessions using 20 cross-validation iterations, large red dot is the average. Red dot on the right is the result of pseudo-population analysis from 100 cross-validation iterations. Grey areas denote chance level computed using permutation tests (2.5 - 97.5 percentiles in distribution of shuffled decoding performances). **h.** Average peak amplitudes during each type of presentation. Kruskal-Wallis test: F4,36 = 4.991, p = 0.07. Multiple comparisons tests: cage vs. familiar mouse, p = 0.3; novel vs. familiar mouse, p = 0.3; familiar mouse, p = 0.3;

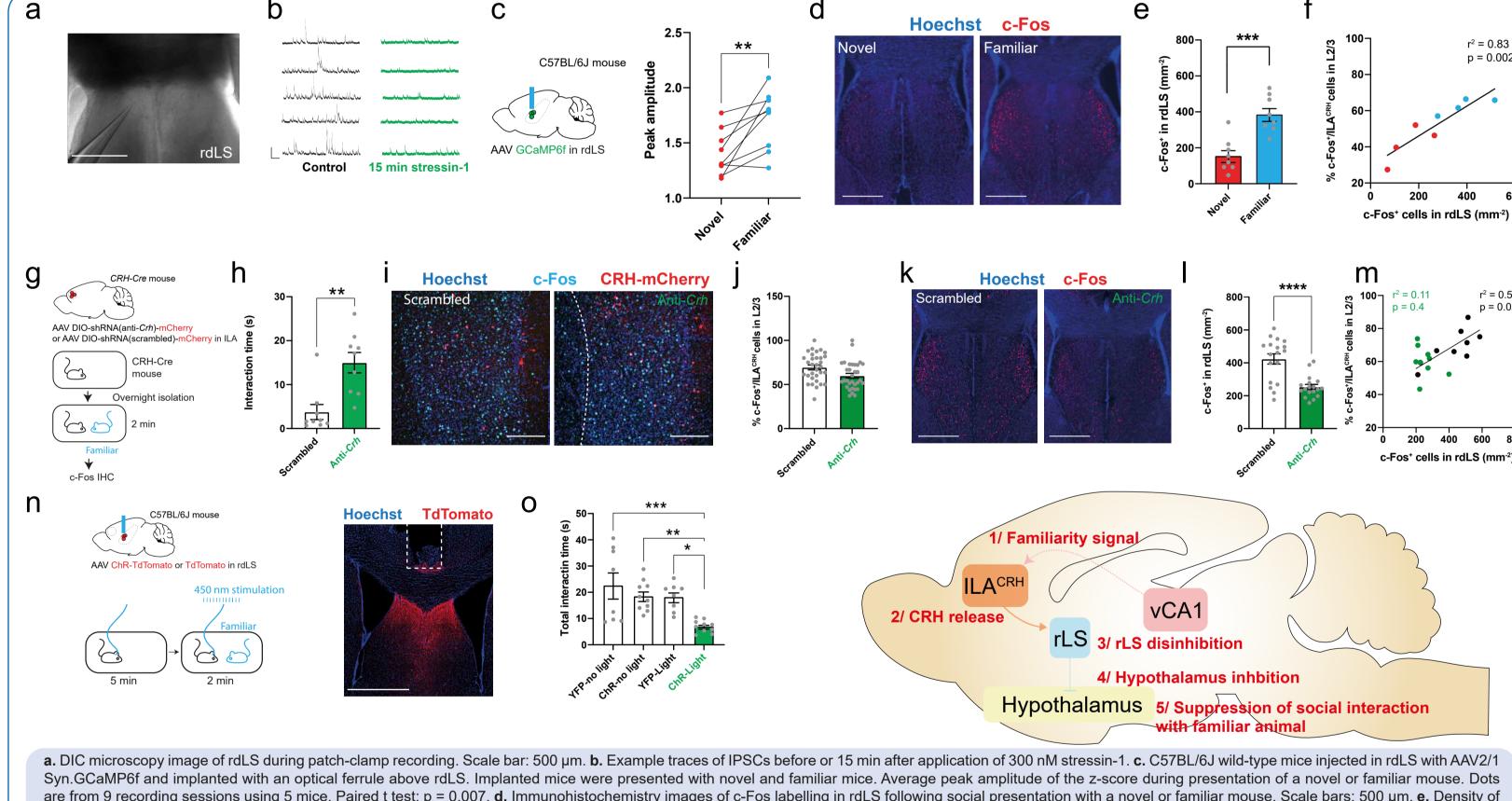
# CRH release from ILA in rdLS suppresses social interactions with familiar mice and supports social novelty preference.



multiple comparisons tests: mC + light on learning vs. Arch + light on recall, p = 0.1; Arch + light on recall, p = 0.03; mC + light on recall vs. Arch + light on recall, p = 0.04. m. Normalized interaction

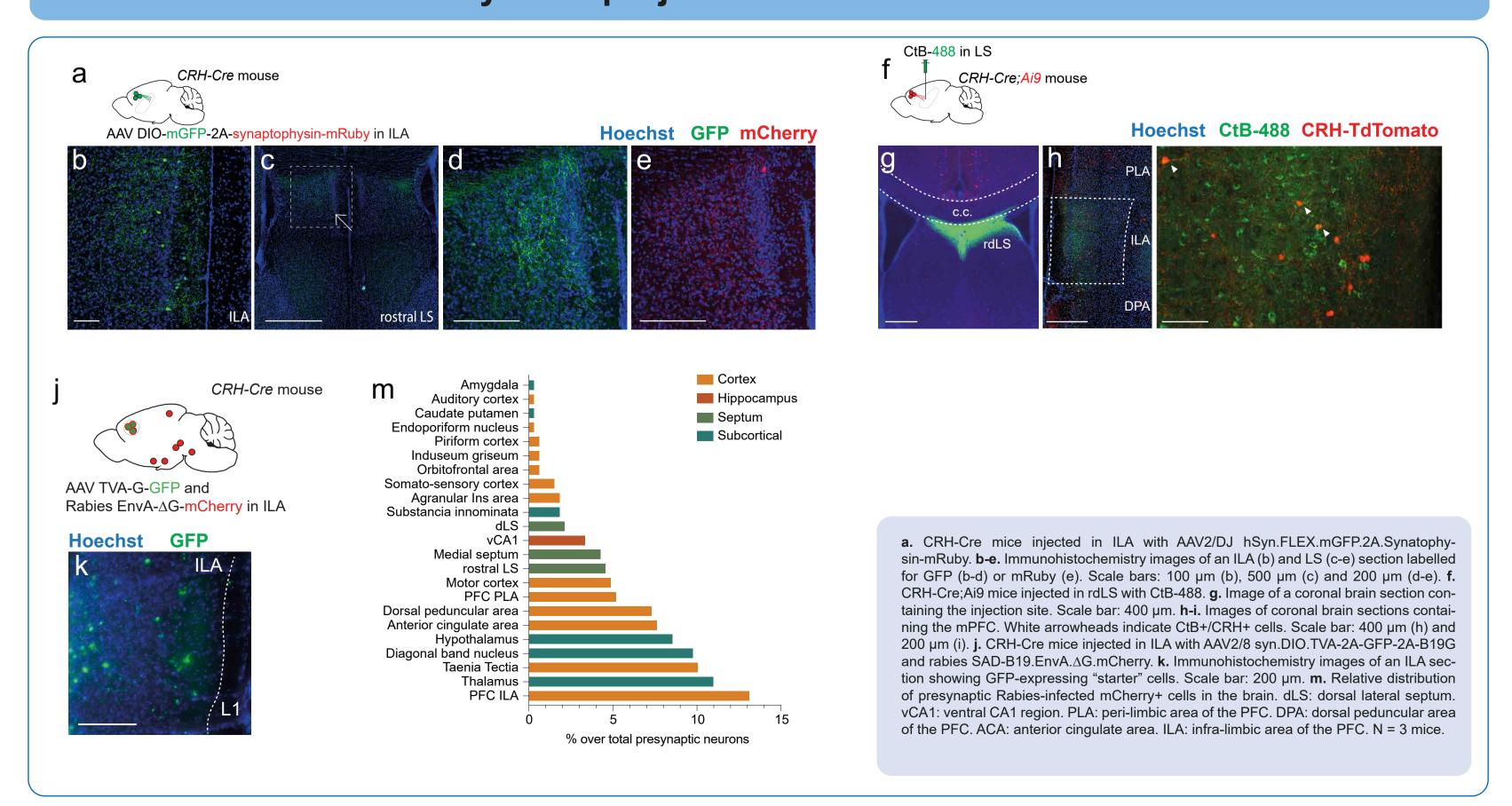
time during the repetitive social presentation test in the same mice (4 mice and 3 mice respectively). Two-way ANOVA: trial x group F8,40 = 2.50, p = 0.03; trial F(4,40) = 19.06, p < 0.0001; group F(2,40) = 27.95, p < 0.0001.

# CRH signaling from ILA in rdLS suppresses social interactions with familiar mice through rdLS disinhibition.

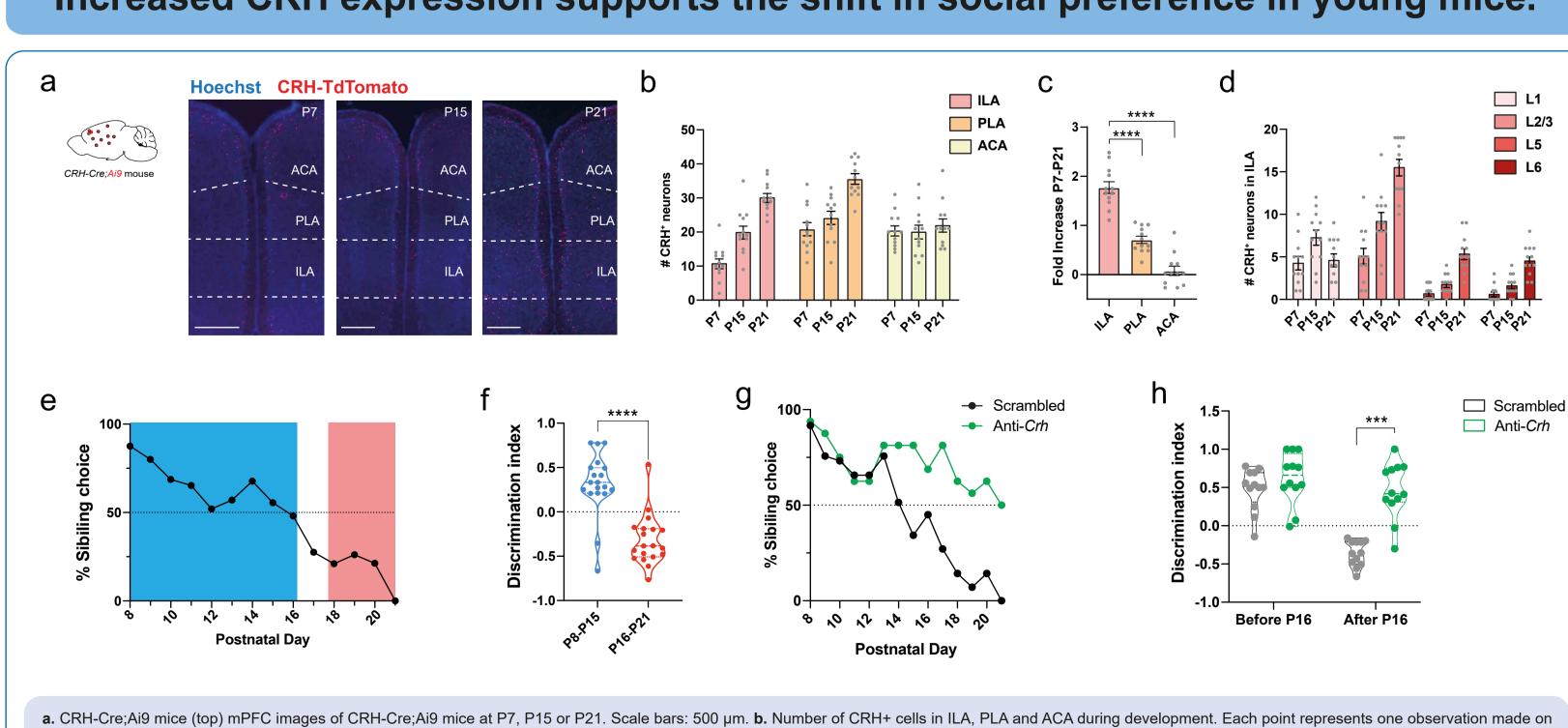


Syn.GCaMP6f and implanted with an optical ferrule above rdLS. Implanted mice were presented with novel and familiar mice. Average peak amplitude of the z-score during presentation of a novel or familiar mouse. Dots are from 9 recording sessions using 5 mice. Paired t test: p = 0.007. **d.** Immunohistochemistry images of c-Fos labelling in rdLS following social presentation with a novel or familiar mouse. Scale bars: 500  $\mu$ m. **e.** Density of rdLS cells positive for c-Fos. We made one observation on each side of a rLS section. 4 mice per group. Unpaired t test, p = 0.0003. **f.** Percentage of layer 2/3 ILACRH cells positive for c-Fos (cf. Fig. 2s) vs. density of rdLS cells positive for c-Fos following social interaction. Each point represents a mouse. **g.** CRH-Cre mice expressing shRNA against Crh or a control presented with a familiar mouse for 2 min before being processed for immunohistochemistry. **h.** Duration of interaction during familiar presentation. Each point is one mouse. Unpaired t test, p = 0.001. **i,k.** Immunohistochemistry images of c-Fos labelling in ILA (i) and rdLS (k). Scale bars: 300  $\mu$ m. **j.** Percentage of layer 2/3 ILACRH cells positive for c-Fos in layer 2/3 of ILA. Each point corresponds to each side of 2 sections. 9 mice per group. **l.** Density of rdLS cells positive for c-Fos. We made one observation on each side of a rLS section. 9 mice per group. Unpaired t test, p < 0.0001. **m.** Percentage of layer 2/3 ILACRH cells positive for c-Fos vs. density of rdLS cells positive for c-Fos following social interaction with a familiar mouse. Each point represents one mouse. **n.** C57BL/6J wild-type mice were injected with AA2/2 hSyn1.hChR2(H134R)-mCherry or AA2/2 hSyn1.mCherry as control and an optical fiber was implanted above the injection site. Mice were then presented to a familiar mouse for 2 min meanwhile 450 nm light was applied (20 Hz, 1 ms). Mice were also run without light as additional controls. Scale bar: 1 mm. **o.** Total interaction time with familiar mouse. Each point represen

### CRH cells from the ILA layer 2/3 project to rostral LS.



## Increased CRH expression supports the shift in social preference in young mice.



**a.** CRH-Cre;Ai9 mice (top) mPFC images of CRH-Cre;Ai9 mice at P7, P15 or P21. Scale bars:  $500 \, \mu m$ . **b.** Number of CRH+ cells in ILA, PLA and ACA during development. Each point represents one observation made on each side of 2 section, 3 mice per group. **c.** Fold-increase of CHR+ cells between P7 and P21. P21 values compared to the average P7 value. One-way ANOVA: F2,33 = 75.68, p < 0.0001. Dunnett's multiple comparisons tests: ILA vs. PLA, p < 0.0001 and ILA vs. ACA, p < 0.0001. **d.** Number of CRH+ cells per ILA layers during development. Each point represents one observation made on each side of 2 section, 3 mice per group. **e.** Percentage of familiar choice during development, 19 mice. **f.** Discrimination index for familiar kin before and after postnatal day 16. Each point represents a mouse, 19 mice. Unpaired t test, p < 0.001. **g.** Percentage of familiar choice during development in CRH-Cre mice injected in ILA with AAV2/9 CMV-DIO-(mCherry-U6)-shRNA(anti-Crh) to downregulate Crh or control AAV2/9 CMV-DIO-(mCherry-U6)-shRNA(scrambled). 12 pups per group. Chi-square test: p < 0.0001. **h.** Discrimination index for familiar kin before and after postnatal day 16. Each point represents a mouse, 12 pups per group. Unpaired t tests: p = 0.3 and p < 0.0001. For the entire figure, bar graphs represent mean  $\pm$  S.E.M.







