A prefrontal–paraventricular thalamus circuit requires juvenile social experience to regulate adult sociability in mice

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Juvenile social isolation reduces sociability in adulthood, but the underlying neural circuit mechanisms are poorly understood. We found that, in male mice, 2 weeks of social isolation immediately following weaning leads to a failure to activate medial prefrontal cortex neurons projecting to the posterior paraventricular thalamus (mPFC→pPVT) during social exposure in adulthood. Chemogenetic or optogenetic suppression of mPFC→pPVT activity in adulthood was sufficient to induce sociability deficits without affecting anxiety-related behaviors or preference toward rewarding food. Juvenile isolation led to both reduced excitability of mPFC→pPVT neurons and increased inhibitory input drive from low-threshold-spiking somatostatin interneurons in adulthood, suggesting a circuit mechanism underlying sociability deficits. Chemogenetic or optogenetic stimulation of mPFC→pPVT neurons in adulthood could rescue the sociability deficits caused by juvenile isolation. Our study identifies a pair of specific medial prefrontal cortex excitatory and inhibitory neuron populations required for sociability that are profoundly affected by juvenile social experience.

Loneliness is becoming increasingly recognized as a serious threat to mental health. Social isolation during childhood in particular is detrimental to adult brain function and behavior across mammalian species. Children removed from a socially deprived institutional care environment by being placed into foster care at a young age show improved functional outcomes compared with those who were never placed into foster care, or those placed into foster care at a later age. In mice, juvenile social isolation (jSI), immediately following weaning for 2 weeks (21–35 d after birth: p21–p35), but not during a later period, leads to decreased sociability in adulthood, suggesting the juvenile period is a sensitive period for the establishment of adult social behavior.

A number of studies in both humans and rodents broadly implicate the medial prefrontal cortex (mPFC) as a key part of the brain networks that regulate social behavior. A recent study in mice demonstrated that subcortically projecting deep-layer mPFC neurons are particularly vulnerable to jSI and that jSI reduces their excitability and synaptic drive in adulthood. However, the subcortical targets of deep-layer mPFC projections that are vulnerable to jSI are unknown. Here, we sought to identify the specific mPFC circuitry that requires juvenile social experience to support normal sociability in adulthood.

Results

jSI impairs activation of mPFC→pPVT projection neurons upon social exposure in adulthood. To gain insight into which subcortical regions receiving projections from the mPFC are important for social behavior, we first performed immediate early gene (c-Fos) expression mapping. We showed that both the posterior part of the paraventricular nucleus of thalamus (pPVT), which relays signals to various components of the classical reward circuitry, and mPFC deep-layer neurons projecting to the pPVT were activated by exposure to a novel mouse compared with novel-object exposure (Extended Data Fig. 1).

We next used fiber photometry to examine the effect of jSI on real-time activity of mPFC→pPVT projection neurons during social exploration in adult male mice (Fig. 1a–d). Adult jSI mice displayed reduced sociability, compared with adult mice that were group-housed (GH) throughout their life, in the three-chamber test as previously reported. We next performed immediate early gene (c-Fos) expression mapping. We showed that both the posterior part of the paraventricular nucleus of thalamus (pPVT), which relays signals to various components of the classical reward circuitry, and mPFC deep-layer neurons projecting to the pPVT were activated by exposure to a novel mouse compared with novel-object exposure (Extended Data Fig. 1).

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Manipulations of mPFC→pPVT projection neuron activity bidirectionally modulate sociability in adult GH mice. To examine the extent to which activity of mPFC→pPVT projection neurons is necessary for sociability, we chemogenetically suppressed the activity of mPFC→pPVT neurons expressing inhibitory DREADD
**Fig. 1 | jSI impairs activation of mPFC→pPVT projection neurons upon social exposure in adulthood.**

**a.** Left: timeline showing weaning at p21 and subsequent 2 weeks of jSI, followed by rehousing or continued control group housing (GH), and subsequent in vivo fiber photometry calcium imaging of GCaMP6f-expressing mPFC→pPVT neurons in behaving adult mice. Right: selective viral expression of GCaMP6f in mPFC→pPVT projection neurons was achieved by injecting AAV1-DIO-GCaMP6f in mPFC and retrograde rAAV2-Cre in pPVT. **b,** Representative localization of fiber photometry (FP) optic fiber and GCaMP6f expression in mPFC. Scale bar, 500 μm. Experimental images were obtained from 42 mice, three images per mouse, with similar results obtained. **c,** During fiber photometry imaging, mice were exposed to a novel mouse or novel object (order of object and social exploration was counter-balanced). **d,** Optic fiber placement and proximity to injection sites were confirmed from all mice (GH, open circles; jSI, red circles). **e, f,** Heat maps of GCaMP6f signals (red–blue, high–low) for each of the trials (one trial per mouse) from all mice (top) and averaged traces of GCaMP6f signals from mPFC→pPVT neurons (bottom) of GH mice (e) (n = 23 biologically independent mice) and jSI mice (f) (n = 19 biologically independent mice). Right: quantification of social- or object-evoked normalized GCaMP6f signals (mean z-score of 30 s post-stimulus introduction subtracted by mean z-score of 30 s baseline) from mPFC→pPVT neurons in adult GH or jSI mice. Social exposure evoked higher responses than object exposure in GH mice (two-tailed paired t-test, t_{22} = 4.759, ****P = 0.946 × 10^{-4}) but not in jSI mice (two-tailed paired t-test, t_{18} = 0.757, P = 0.459). **g,** jSI mice showed less difference in mPFC→pPVT neuron activity between social and object exposure (S–O) than that of GH mice (two-tailed t-test, t_{40} = 2.913, **P = 0.006, n = 23 (GH), 19 (jSI) biologically independent mice). Data in **e, f** are presented as mean ± s.e.m. ACC, anterior cingulate cortex; PL, prelimbic cortex.
Fig. 2 | Chemogenetic suppression of mPFC→pPVT projection neuron activity reduces sociability in adult GH mice. a. Left: Cre-dependent iDREADD or mCherry vector and a retrograde CAV2-Cre were injected into the mPFC and pPVT, respectively, to express iDREADD in mPFC→pPVT neurons. Representative images from mPFC (middle) and pPVT (right) showing iDREADD-mCherry expression. Scale bars, 600 µm (left); 300 µm (right). Experimental images were obtained from ten mice; three images per mouse for each area were taken, with similar results obtained. b. Validation of iDREADD in mPFC→pPVT projection neuron by whole-cell patch recording in PFC slices. Left: representative trace of membrane potential before and after a bath application of CNO. Traces were obtained from seven cells from three biologically independent mice with similar results obtained. Right: quantification of membrane potential (MP) of mPFC→pPVT neurons showing reduction after CNO application (two-tailed paired t-test, n = 7 biologically independent mice). c. GH adult mice were treated with SAL or CNO (10 mg·kg⁻¹) and then underwent the three-chamber test of sociability. SAL and CNO session order was counter-balanced for each behavior test with a 7-d interval between tests. d. Viral spread validation of mice expressing iDREADD in mPFC→pPVT neurons after behavioral experiments. Gray areas represent the minimum (lighter color) and the maximum (darker color) spread of iDREADD. e. Left: CNO-treated iDREADD⁺ mice showed reduced sociability, revealed by reduced sociability scores (calculated as (social−object)/(social+object)) versus SAL (two-tailed paired t-test, t₉ = 3.548, **P = 0.006, n = 10 biologically independent mice) and disrupted behavior in the three-chamber sociability test (two-way repeated-measures (RM) ANOVA, drug (CNO or SAL) × stimulus (social or object) interaction F₁,₉₉ = 11.040, **P = 0.004; effect of drug F₁,₉₉ = 0.071, P = 0.793; effect of stimulus F₁,₉₉ = 0.731, P = 0.404; n = 10 biologically independent mice). Right: CNO-treated iDREADD⁺ mice showed no differences in motor activity and anxiety-related behaviors (open-field distance traveled, two-tailed paired t-test, t₉ = 0.183, P = 0.859, n = 10 biologically independent mice; open-field time in center, two-tailed paired t-test, t₉ = 0.843, P = 0.421, n = 10 biologically independent mice; elevated plus maze (EPM) time in open arms, two-tailed paired t-test, t₉ = 0.568, P = 0.586, n = 9 biologically independent mice). f. Left: control mCherry⁺ mice show no difference in sociability score (two-tailed paired t-test, t₉ = 0.160, P = 0.878, n = 8 biologically independent mice) and investigation time (two-way RM ANOVA, drug (CNO or SAL) × stimulus (social or object) interaction F₁,₉₉ = 1.027, P = 0.328; effect of drug F₁,₉₉ = 0.593, P = 0.454; effect of stimulus F₁,₉₉ = 8.723, P = 0.010, n = 8 biologically independent mice). Right: mCherry⁺ mice showed no difference in motor activity or anxiety-related behaviors (left, two-tailed paired t-test, t₉ = 0.599, P = 0.568, n = 8 biologically independent mice; middle, two-tailed paired t-test, t₉ = 1.881, P = 0.102, n = 8 biologically independent mice; right, two-tailed paired t-test, t₉ = 0.662, P = 0.529, n = 8 biologically independent mice). Data in b, e and f are presented as mean ± s.e.m. Il, infralimbic cortex; LHb, lateral habenula; MHB, medial habenula; NS, not significant; O, object; S, social.
Fig. 3 | Optogenetic suppression of mPFC→pPVt projection terminal activity reduces sociability in adult GH mice. a, Top: halorhodopsin NpHR3.0 AAV under the CamKII promotor was injected into mPFC and mPFC→pPVt projection terminals were optically stimulated in the pPVt using a wireless yellow LED system. Representative images of mPFC (middle) and pPVt (bottom) show selective transduction of halorhodopsin at injection areas in the mPFC and the projection target areas in the pPVt where optic fibers are located. Scale bars, 600 µm (middle); 300 µm (bottom). Experimental images were obtained from 14 mice, three images per mouse for each area, with similar results obtained. b, Mice underwent the three-chamber test of sociability with (ON) or without (OFF) light stimulation. Order of ON and OFF sessions was counter-balanced for each behavior test with a 24-h interval between tests. c, Mice with optogenetic suppression showed reduced sociability scores (two-tailed paired t-test, $t_{13} = 2.769$, *$P = 0.016$, $n = 14$ biologically independent mice) and loss of social preference in the three-chamber sociability test (two-way RM ANOVA, light (ON or OFF) x stimulus (social or object) interaction $F_{1,26} = 4.876$, *$P = 0.036$; effect of light $F_{1,26} = 1.915$, $P = 0.178$; effect of stimulus $F_{1,26} = 11.870$, $P = 0.002$). d, However, control mCherry* mice showed no difference in sociability score (two-tailed paired t-test, $t_{8} = 0.722$, $P = 0.491$, $n = 9$ biologically independent mice) and investigation time (two-way RM ANOVA, light (ON or OFF) x stimulus (social or object) interaction $F_{1,16} = 0.700$, $P = 0.415$; effect of light $F_{1,16} = 0.685$, $P = 0.420$; effect of stimulus $F_{1,16} = 16.600$, $P = 0.882 \times 10^{-3}$, $n = 9$ biologically independent mice). e, Mice underwent a three-chamber test of food preference with (ON) or without (OFF) light stimulation. ON and OFF session order was counter-balanced for each behavior test with a 24-h interval between tests. f, Left: mice with optogenetic suppression showed no difference in milkshake consumption (two-tailed paired t-test, $t_{5} = 0.143$, $P = 0.892$, $n = 6$ biologically independent mice), in food discrimination score (middle, two-tailed paired t-test, $t_{5} = 0.724$, $P = 0.491$, $n = 6$ biologically independent mice) and in investigation time (right, two-way RM ANOVA, light (ON or OFF) x stimulus (milkshake or empty) interaction $F_{1,10} = 0.183$, $P = 0.678$; effect of light $F_{1,10} = 1.478$, $P = 0.252$; effect of stimulus $F_{1,10} = 12.020$, $P = 0.006$, $n = 6$ biologically independent mice). Data in c, d and f are presented as mean ± s.e.m. E, empty; M, milkshake.
(iDREADD) (Fig. 2a–d). Suppression of mPFC→pPVt neuron activity through clozapine-N-oxide dihydrochloride (CNO) treatment in adult GH mice reduced sociability in the three-chamber test, with no impact on motor activity or measurements of anxiety-related behavior (Fig. 2e). CNO injection in mCherry-expressing control mice produced no significant differences in behavior compared with saline (SAL) injection (Fig. 2f). Chemogenetic suppression of pPVt neuron activity also reduced sociability (Extended Data Fig. 3).

To rule out the possibility that our observed results were due to inhibition of collateral projections to other brain areas, we optogenetically suppressed the activity of mPFC projection terminals in the pPVt. mPFC→pPVt projection terminals virally expressing the inhibitory opsin eNpHR3.0 under the CaMKII promotor were wirelessly illuminated by a yellow light-emitting diode (LED) over the pPVt during behavior testing (Fig. 3a,b and Extended Data Fig. 4a–e). This manipulation, which partly reduced the activity of neurons in the pPVt (Extended Data Fig. 4a–c), led to reduced sociability (Fig. 3c) without impacting anxiety-related or motor behaviors (Extended Data Fig. 4f). There were no light-induced behavioral effects in mCherry-expressing control mice (Fig. 3d and Extended Data Fig. 4g). Importantly, this manipulation did not impact natural food preference (Fig. 3e,f), suggesting that the observed sociability deficits are not a result of general impairments in natural reward-related behaviors. Collectively, these results demonstrate a causal link between mPFC→pPVt projection activity and social behavior.

To examine whether mPFC→pPVt projection activity is sufficient to increase sociability in adult GH mice, we optogenetically activated mPFC→pPVt projections virally expressing channelrhodopsin (ChR2) under the CaMKII promotor by wirelessly illuminating a blue LED implanted over the pPVt while mice performed the three-chamber test (Fig. 4a–f). We found that optogenetic activation of mPFC→pPVt projections within the social-interaction zone led to an increase in sociability, whereas activation of mPFC→pPVt projections within the object-interaction zone reduced sociability (Fig. 4e–h). Furthermore, optogenetic activation of mPFC→pPVt projections in one of two empty corral interaction zones was sufficient to increase time spent in that zone. This effect quickly disappeared after termination of light stimulation (Fig. 4i–k). Altogether, these experiments demonstrate that activation of mPFC→pPVt projections is reinforcing, and can be leveraged to increase sociability.

**Fig. 4 | Optogenetic activation of mPFC→pPVt projection terminals biases sociability in adult GH mice.** a, CaMKII-ChR2 AAV1 was injected into the mPFC and a wireless blue LED was inserted above the pPVt in GH mice. b, Left: a representative in vivo unit recording of pPVt neurons showing reliable spikes upon optogenetic stimulation (20 Hz) of mPFC projection terminals. Traces were obtained from four mice, 5–12 cells per mouse, with similar results obtained. Right: quantification of light-induced firing rates of pPVt neurons (light OFF versus light ON (15 each), two-tailed paired t-test, \( t_{\text{df}} = 160.700 \), **P = 0.001 \times 10^{-3} \), n = 86 cells from 4 biologically independent mice). c, Left: representative images of injection area of mPFC neurons expressing ChR2. Scale bar, 600 \( \mu \)m. Experimental images were obtained from ten mice, three images per mouse, with similar results obtained. Right: viral spread validation from behavior-tested mice. Gray areas represent the minimum (lighter color) and the maximum (darker color) spread of ChR2 expression in the mPFC. d, Left: representative pPVt images shows selective transduction of ChR2 below the area where optic fiber tips were inserted in the pPVt. Scale bar, 300 \( \mu \)m. Experimental images were obtained from ten mice, three images per mouse, with similar results obtained. Right: optic fiber tip location (blue line circles) was validated in all mice. e–h, GH adult mice underwent the three-chamber test of sociability with (ON) or without (OFF) light stimulation. The order of ON and OFF sessions was counter-balanced, with a 24-h interval between tests. Optogenetic stimulation was delivered to activate the mPFC→pPVt projection whenever the mouse visited the mouse interaction zone (e) or object zone (f) and was terminated immediately if the mouse exited the mouse interaction zone during the three-chamber sociability test. Order of ON and OFF sessions was counter-balanced, with a 24-h interval between tests. g, Optogenetic activation in the social zone led to increased sociability scores (two-tailed paired t-test, \( t_{\text{df}} = 4.788 \), **P = 0.990 \times 10^{-3} \), n = 10 biologically independent mice) and increased social preference (two-way RM ANOVA, light (ON or OFF) \times stimulus (social or object) interaction \( F_{\text{df}} = 12.860 \), **P = 0.002; effect of light \( F_{\text{df}} = 0.488 \), P = 0.494; effect of stimulus \( F_{\text{df}} = 94.020 \), P = 0.143 \times 10^{-1} \), n = 10 biologically independent mice). h, Optogenetic activation in the object zone led to reduced sociability scores (two-tailed paired t-test, \( t_{\text{df}} = 3.491 \), **P = 0.007, n = 10 biologically independent mice) and increased object preference (two-way RM ANOVA, light (ON or OFF) \times stimulus (social or object) interaction \( F_{\text{df}} = 15.910 \), **P = 0.861 \times 10^{-2} \); effect of light \( F_{\text{df}} = 1.227 \), P = 0.282; effect of stimulus \( F_{\text{df}} = 0.282 \), P = 0.602, n = 10 biologically independent mice). i–k, Optogenetic stimulation of mPFC→pPVt projection acutely promotes place preference. i, Adult GH mice underwent the three-chamber test without light stimulation (pre: OFF 10 min), followed by a stimulation period (stim: ON 10 min), immediately followed by another period without light stimulation (post: OFF 10 min). During the stimulation period, optogenetic stimulation was delivered to activate the mPFC→pPVt projection whenever the mouse visited the stimulation zone (S) and was terminated immediately if the mouse exited the stimulation zone. Locations of stimulation zones are counter-balanced. j, ChR2 GH mice with optogenetic stimulation showed real-time preference to the stimulation zone (S) over the nonstimulation zone (N) as indicated by increased discrimination score (calculated as (S – N)/(S + N), one-way RM ANOVA, \( F_{\text{df}} = 14.760 \), P = 0.287 \times 10^{-4} \). Tukey’s multiple comparisons test: pre vs. stim \**P = 0.309 \times 10^{-3} \), stim vs. post \**P = 0.006 \) as well as investigation time (two-way RM ANOVA, time (pre, stim or post) \times zone (stimulation zone or nonstimulation zone) interaction \( F_{\text{df}} = 7.917 \), **P = 0.001; effect of time \( F_{\text{df}} = 1.325 \), P = 0.278; effect of zone \( F_{\text{df}} = 6.500 \), P = 0.251 \times 10^{-5} \), n = 13 biologically independent mice). k, Control mCherry’ mouse showed no difference in discrimination score (one-way RM ANOVA, \( F_{\text{df}} = 0.204 \), P = 0.696, n = 6 biologically independent mice) or investigation time (two-way RM ANOVA, time (pre, stim or post) \times zone (stimulation zone or nonstimulation zone) interaction \( F_{\text{df}} = 0.134 \), P = 0.876; effect of time \( F_{\text{df}} = 0.073 \), P = 0.930; effect of zone \( F_{\text{df}} = 0.345 \), P = 0.566, n = 6 biologically independent mice). Data in b, g, h, j and k are presented as mean \pm s.e.m.
To examine the downstream synaptic targets of mPFC→pPVT projection neurons, we next performed whole-cell patch-clamp recordings from pPVT neurons combined with optogenetic activation of ChR2-expressing mPFC→pPVT neurons (Extended Data Fig. 7a). The majority of patched pPVT neurons showed evoked responses (73.3%; 22 of 30 cells for GH; 68.8%; 22 of 32 cells for CaMKII-Channelrhodopsin).
for jSI). While both groups showed comparable levels of monosynaptic excitatory connectivity (Extended Data Fig. 7b,c), jSI mice showed smaller normalized amplitude of evoked postsynaptic currents (EPSCs) compared with GH mice (Extended Data Fig. 7d), with no differences in paired pulse ratio (PPR) (Extended Data Fig. 7e). These findings suggest that jSI causes a lasting reduction in evoked synaptic transmission onto pPVT neurons. In contrast to mPFC→pPVT neurons, pPVT neurons themselves showed little vulnerability to jSI (Extended Data Fig. 5m–r).

Next, we directly examined the synaptic inputs from mPFC LTS-SST interneurons to mPFC→pPVT neurons by performing whole-cell patch-clamp recordings from Retrobead-labeled mPFC→pPVT projection neurons combined with optogenetic activation of ChR2-expressing mPFC LTS-SST interneurons (Fig. 6d). Results indicated that all patched mPFC→pPVT neurons received robust monosynaptic input from LTS-SST interneurons (Fig. 6d). The amplitude of optically evoked responses, normalized to the amplitude of the response evoked by the lowest light intensity level for each cell, was comparable between jSI and GH groups, but the PPR was reduced in jSI mice compared with GH mice (Fig. 6e,f), suggesting changes in presynaptic mechanisms of inhibitory neurotransmitter release caused by jSI. Importantly, Chrna2+ LTS-SST interneurons were previously shown to selectively target deep-layer pyramidal neurons that typically project to subcortical targets but not to callosal projection neurons or intratelencephalic neurons8; consistent with this finding, we observed no change in inhibitory drive in mPFC neurons projecting to the contralateral PFC (mPFC→cPFC) or nucleus accumbens (NAc) (mPFC→NAc) located in the upper layer in jSI mice versus GH mice (Extended Data Fig. 5).

Consistent with these results, excitatory DREADD (eDREADD)-mediated activation of mPFC LTS-SST interneurons in GH mice (which recapitulates the physiological deficit seen in jSI mice) was sufficient to reduce sociability without impacting motor activity or anxiety-related behavior (Fig. 6g–j). Collectively, these experiments demonstrate that jSI leads to enduring neurophysiological deficits in mPFC→pPVT neurons and in LTS-SST interneurons, which are likely to be the cause of the sociability deficit and reduced activation of mPFC→pPVT neurons during social exploration in jSI mice.

Fig. 5 | jSI leads to reduced intrinsic excitability and increased inhibitory input drive of mPFC→pPVT neurons in adulthood. a, mPFC→pPVT neurons were labeled with Retrobeads injected into the pPVT of mice that underwent jSI (p21–p35) or GH. Whole-cell patch-clamp recordings of mPFC→pPVT neurons were performed from mPFC slices of p21, p35 (jSI or GH) or adult (jSI or GH) mice. b–d, Assessment of intrinsic excitability of mPFC→pPVT neurons. b, Representative traces in the presence of DNQX (20μM), D-AP5 (50μM) and picrotixin (30μM) at −100-pA and 200-pA current steps. Traces were recorded from 20–23 cells from 7–9 biologically independent mice per group, with similar results obtained. c, Input–output curve in p35 and adult mice in GH (left, two-way RM ANOVA, housing × current-steps interaction F(2,126) = 1,496, P = 0.079; effect of current step F(3,252) = 339.700, P = 0.001×10−10; effect of housing F(1,126) = 0.226, P = 0.637, n = 21; effect of age F(1,126) = 9.35×10−5; n = 20, 23 cells from 7, 9 biologically independent p35 or adult mice in GH, respectively) and jSI (right, two-way RM ANOVA, housing (GH or jSI) × current-steps interaction F(2,126) = 10.790, ****P = 0.001×10−10; effect of current step F(3,252) = 494.400, P = 0.001×10−22; effect of housing F(1,126) = 41.830, P = 9.35×10−10; n = 20, 23 cells from 7, 9 biologically independent p35 or adult mice in jSI, respectively). d, At the 200-pA current step, jSI mice showed a significantly lower spike frequency compared with GH mice only after p35 during adulthood (two-way RM ANOVA, housing (GH or jSI) × age (p21, p35 or adult) interaction F(2,126) = 6.171, **P = 0.003; effect of housing F(1,126) = 4.128, P = 0.044; effect of age F(2,126) = 12.380, P = 0.132×10−3; Tukey’s multiple comparisons test: **P = 0.001 (adult; jSI versus GH), **P = 0.512×10−4 (jSI: p35 versus adult), n = 23 cells from 6 biologically independent mice (p21), n = 20 cells from 7 biologically independent jSI mice (p35), n = 21 cells from 7 biologically independent GH mice (p35), n = 23 cells from 9 biologically independent jSI mice (adult) and n = 22 cells from 7 biologically independent GH mice (adult)). Spike threshold showed no significant difference between jSI and GH (two-way RM ANOVA, housing (GH or jSI) × age (p21, p35 or adult) interaction F(2,126) = 1.447, P = 0.239; effect of housing F(1,126) = 2.625, P = 0.135; effect of age F(1,126) = 2.611, P = 0.0771). e–g, Assessment of excitatory and inhibitory drive onto mPFC→pPVT neurons. n = 23 cells from 6 biologically independent mice (p21), n = 21 cells from 7 biologically independent jSI mice (p35), n = 22 cells from 7 biologically independent GH mice (p35), n = 22 cells from 9 biologically independent jSI mice (adult) and n = 21 cells from 9 biologically independent GH mice (adult). e, Top: representative traces of sEPSCs. Traces were recorded from 21–22 cells from 7–9 mice per group, with similar results obtained. Bottom: sEPSC amplitude showed significant developmental changes but no difference between jSI and GH (frequency: two-way RM ANOVA, housing (GH or jSI) × age (p21, p35 or adult) interaction F(2,126) = 0.975, P = 0.380; effect of housing F(1,126) = 118.87, P = 0.278; effect of age F(2,126) = 3.423, P = 0.036; amplitude: two-way RM ANOVA, housing (GH or jSI) × age (p21, p35 or adult) interaction F(2,126) = 1.375, P = 0.257; effect of housing F(1,126) = 1.335, P = 0.250; effect of age F(2,126) = 20.450, P = 0.203×10−10). f, Top: representative traces of tIPSCs. Traces were recorded from 21–22 cells from 7–9 mice per group, with similar results obtained. Bottom left: tIPSC amplitude in jSI mice failed to decrease between p35 and adulthood (two-way RM ANOVA, housing (GH or jSI) × age (p21, p35 or adult) interaction F(2,126) = 6.146, **P = 0.003; effect of housing F(1,126) = 5.267, P = 0.023; effect of age F(2,126) = 3.400, P = 0.036; Tukey’s multiple comparisons test: **P = 0.826×10−4 (adult; jSI versus GH), **P = 0.001 (GH: p35 versus adult)). Bottom right: sIPSC amplitude showed significant developmental changes but no difference between jSI and GH (frequency: two-way RM ANOVA, housing (GH or jSI) × age (p21, p35 or adult) interaction F(2,126) = 1.408, P = 0.249; effect of housing F(1,126) = 2.638, P = 0.107; effect of age F(2,126) = 89.870, P = 0.001×10−20). g, The E/I ratio was significantly different between GH and jSI only after p35 in adulthood, jSI mice did not show a late developmental increase in the ratio between p35 and adulthood as GH mice did (two-way RM ANOVA, housing (GH or jSI) × age (p21, p35 or adult) interaction F(2,126) = 6.667, **P = 0.001; effect of housing F(1,126) = 8.628, P = 0.004; effect of age F(2,126) = 3.310, P = 0.040; Tukey’s multiple comparisons test: **P = 0.130×10−3 (adult; jSI versus GH), **P = 0.004 (GH: p35 versus adult)). The E/I ratio showed no significant differences between jSI and GH groups (two-way RM ANOVA, housing (GH or jSI) × age (p21, p35 or adult) interaction F(2,126) = 0.052, P = 0.949; effect of housing F(1,126) = 0.163, P = 0.687; effect of age F(2,126) = 26.770, P = 0.204×10−10). Data in c–g are presented as mean±s.e.m. D-AP5, α-(−)-2-amino-5-phosphonopentanoic acid; DNQX, 6,7-dinitroquinoxaline-2,3-dione; PC, pyramidal cell.
Activation of mPFC→pPVT neurons rescues sociability deficits in adult jSI mice. We next sought to determine whether acute restoration of mPFC→pPVT neuron activity is sufficient to ameliorate sociability deficits in adult jSI mice. We found that chemogenetic activation of mPFC→pPVT projection neurons expressing eDREADD through CNO treatment could acutely rescue sociability deficits in the adult jSI mice without impacting motor activity or anxiety-related behavior (Fig. 7a–e). CNO injection in mCherry-expressing control jSI mice produced no CNO-dependent behavioral effect (Fig. 7f).

Next, to selectively examine the contribution of mPFC→pPVT projection terminals in pPVT, we optogenetically activated mPFC projection terminals in the pPVT that virally express ChR2 by wirelessly illuminating a blue LED over the pPVT during behavior testing (Fig. 8a,b and Extended Data Fig. 9a–c). We found that this stimulation treatment acutely rescued sociability deficits in jSI mice (Fig. 8c), without impacting anxiety-related or motor behaviors (Extended Data Fig. 9d). mCherry-expressing control jSI mice did not show any light-induced behavioral effects (Fig. 8d and Extended Data Fig. 9e).

Finally, to examine whether repeated optogenetic activation of mPFC→pPVT terminals can lead to a sustained rescue of sociability deficits, jSI mice participated in two sessions where optogenetic stimulation was triggered whenever the subject investigated the mouse in the three-chamber test setting, and then underwent sociability testing on the next day without optogenetic stimulation. We found that this intervention leads to a rescue effect that is sustained 24 h after the final day of optogenetic stimulation (Fig. 8e,f and Extended Data Fig. 9f,g).

Discussion

Overall, our study identifies a pair of mPFC excitatory and inhibitory neuron populations required for normal adult sociability that are affected by social experience during the juvenile period (Fig. 8g). The finding that these deficits are not observed immediately after the isolation period but only emerge in adulthood suggests that the deficits may be caused by a mismatch between the jSI mouse’s adult social environment and adaptations resulting from experience during development. The juvenile period may be a sensitive period when social experience can shape the formation of social behavior.
window for behavioral plasticity, and, once closed, mice may not be able to adjust their social strategy. Given that the paraventricular thalamus (PVT) projects to various reward-associated areas including the NAc, the bed nucleus of the stria terminalis and the central amygdala through collateralized projections\textsuperscript{10,14}, mPFC→pPVT neurons are well positioned to provide top-down control of brain networks. Our study sheds light on a previously unrecognized role of mPFC→PVT projections in naturalistic (unconditioned) social processing in addition to their known function in Pavlovian conditioning and extinction\textsuperscript{15–18}. Among diverse subtypes of SST interneurons\textsuperscript{12}, LTS-SST cells are also well positioned to both initiate and maintain prolonged synchronous firing of subcortically projecting deep-layer pyramidal cells through slow rhythmic burst activity and rebound excitation\textsuperscript{13}. Thus, LTS-SST cells may play a pivotal role in social behavior by rhythmically activating mPFC→pPVT neurons and facilitating mPFC–pPVT synchrony. Aberrant mPFC

\begin{itemize}
  \item \textbf{a} Cre-dependent mCherry
  \item \textbf{b} Intrinsic excitability
  \item \textbf{c} Normalized eIPSC amplitude
  \item \textbf{d} Cre-dependent channelrhodopsin
  \item \textbf{e} Normalized eIPSC amplitude
  \item \textbf{f} PPR
  \item \textbf{g} Dio-eDREADD
  \item \textbf{h} c-fos
  \item \textbf{i} Gru & mCherry cells (%)
  \item \textbf{j} Three-chamber sociability test
  \item \textbf{k} Open field
  \item \textbf{l} EPM
\end{itemize}
electroencephalogram power in jSI mice during social exploration reported in a previous study may in turn reflect disrupted communication between the mPFC and PVT.

Our study adds another circuit element to the complexity of social-processing networks in the mPFC, but is consistent with recent studies demonstrating diverse activation patterns of mPFC neurons during social behavior in mice. In contrast to other mPFC projections that have been reported to reduce sociability upon activation, such as the projections to the lateral habenula (located in close proximity to the PVT), activation of mPFC→PVT projections promotes sociability in a fashion that is similar to cerebellum–ventral tegmental area projections. Future studies are warranted to reveal how different populations of mPFC projection neurons influence subcortical networks that control social behavior and are impacted by previous social experience.

Previous genetic and transcriptomic studies show that many risk genes for autism and schizophrenia are highly expressed in fetal and infant L5/6 prefrontal cortex (PFC) projection neurons. Mechanistic studies in rodents further show that function of subcortically projecting L5/6 mPFC neurons is disrupted in mouse models with risk gene disruptions. Future preclinical studies will reveal the extent to which disease risk genes impact maturation of the identified circuits. As mPFC→pPVt neurons and associated LTS-SST interneurons are sensitive to experience-dependent modulation, they are promising targets for treatments of social behavior deficits associated with psychiatric disorders that use non-invasive brain modulation techniques such as transcranial magnetic stimulation and/or transcranial direct current stimulation.

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Fig. 6 | jSI leads to increased intrinsic excitability of mPFC LTS-SST interneurons and impaired LTS-SST to mPFC→pPVt synaptic transmission in adulthood. a–c, Whole-cell patch-clamp recording from LTS-SST interneurons in mPFC slices of adult jSI or GH mice. a, mPFC LTS-SST interneurons are fluorescently labeled by injecting a Cre-dependent mCherry vector into adult Chrna2-Cre mice. Middle: representative image of Chrna2-LS-SST interneurons expressing mCherry in the mPFC. Right: colocalization of mCherry and SST immunoreactivity (green) in the mPFC. Scale bars, 150 μm (middle); 50 μm (right). Experimental images were obtained from six mice, three images per mouse, with similar results obtained. b, Assessment of intrinsic excitability of Chrna2-LS-SST interneurons in the presence of DNQX (20 μM), D-AP5 (50 μM) and picrotoxin (30 μM). Representative traces (left) at 200-pA injection recorded from mPFC LTS-SST interneurons (traces were recorded from 12–14 cells from 7 biologically independent mice per group) and (right) quantification of spike frequency (two-tailed t-test, t0.2 = 3.186, **P = 0.004, n = 12 cells from 7 biologically independent GH mice, n = 14 cells from 7 biologically independent jSI mice). c, There were no significant differences in spike threshold (two-tailed t-test, t0.2 = 0.257, P = 0.800, n = 14 cells from 7 biologically independent GH mice, n = 14 cells from 7 biologically independent jSI mice). d–f, Optogenetic interrogation of LTS-SST interneuron input onto mPFC→pPVt projection neurons. d, Cre-dependent ChR2 vector and green Retrobeads were injected into the mPFC and pPVt, respectively, to express ChR2 in LTS-SST interneurons and fluorescently label mPFC→pPVt neurons for patch-clamp recordings. Middle: a representative image showing ChR2–mCherry+ LTS-SST interneurons and Retrobeads+ mPFC→pPVt projection neurons in deep-layer mPFC. Scale bar, 50 μm. Experimental images were obtained from three mice, 16 images per mouse, with similar results obtained. Right: latency of light-evoked IPSC of mPFC→pPVt neurons indicates monosynaptic inhibitory inputs from LTS-SST interneurons but indicates no significant difference between GH and jSI mice (two-tailed t-test, t0.2 = 0.284, P = 0.778, n = 17 cells from 7 biologically independent mice for each group). e, Stimulus intensity–response amplitude curves of normalized eIPSC upon optical stimulation with a light intensity incrementally increased in a step-wise fashion (0.1 mW per mm2 per step) from the minimal stimulation level, 1. Plotted eIPSC amplitudes were normalized to the amplitude of the response evoked by the minimal stimulation intensity per cell to mitigate the impact of variations in viral expression levels. There were no significant differences in normalized eIPSC amplitude (two-way RM ANOVA, housing (GH or jSI) × light intensity step interaction F0.2,14 = 1.217, P = 0.358; effect of housing F0.2,14 = 0.872, P = 0.358; effect of light intensity step F0.2,14 = 52.460, P = 0.001 × 10−5; n = 15 cells from 7 biologically independent GH mice, n = 16 cells from 7 biologically independent jSI mice). f, Left: eIPSCs elicited by optogenetic stimulation. Representative averaged waveform showing paired-pulse facilitation in eIPSCs at a 500-ms interval. Traces were recorded from 17 cells from 7 mice per group, with similar results obtained. Right: quantification of PPR, given by second evoked amplitude/first evoked amplitude (two-tailed t-test, t0.2 = 2.220, *P = 0.034, n = 17 cells from 7 biologically independent mice for each group). g, Exhibited a decrease in eIPSC amplitude (two-way RM ANOVA, housing (GH or jSI) × synaptic transmission interaction F0.2,14 = 1.704, P = 0.208; effect of drug F0.2,14 = 0.004, P = 0.953; effect of stimulus F0.2,14 = 10.750, P = 0.004, n = 10 biologically independent mice). Right: eDREADD+ mice showed no differences in motor activity or anxiety-related behaviors when treated with SAL versus CNO (left, two-tailed paired t-test, t0.2 = 0.167, P = 0.871, n = 10 biologically independent mice; middle, two-tailed paired t-test, t0.2 = 0.332, P = 0.748, n = 10 biologically independent mice; right, two-tailed paired t-test, t0.2 = 0.434, P = 0.674, n = 10 biologically independent mice). Data in b, c, e, f, h and j are presented as mean ± s.e.m.
Fig. 7 | Chemogenetic activation of mPFC→pPVT projection neurons rescues sociability deficits in adult jSI mice. a, Left: Cre-dependent eDREADD (or mCherry) AAV8 and a retrograde rAAV2-Cre were injected into the mPFC and pPVT, respectively, to selectively express eDREADD in mPFC→pPVT neurons. Representative images of mPFC (middle) and pPVT (right) show selective transduction of eDREADD in mPFC→pPVT projection neurons. Scale bars, 600µm (middle); 300µm (right). Experimental images were obtained from 12 mice, four images per mouse for mPFC and two images per mouse for pPVT, with similar results obtained. b, Validation of eDREADD action in mPFC→pPVT projection neurons by slice whole-cell patch-clamp recordings. Representative trace (left) showing that bath application of CNO significantly increases membrane potential of mPFC→pPVT projection neuron (traces were obtained from seven cells of three biologically independent mice, with similar results obtained) and (right) quantification (two-tailed paired t-test, t = 3.682, P = 0.010, n = 7 cells from 3 biologically independent mice). c, jSI mice were treated with SAL or CNO (1mg kg⁻¹) in a counter-balanced fashion and then underwent the three-chamber test of sociability, the open-field and the EPM with a 7-d interval between tests. d, Viral spread validation of behavior-tested mice. Gray areas represent the minimum (lighter color) and the maximum (darker color) spread of eDREADD into the mPFC. e, CNO-treated eDREADD+ jSI mice show increased sociability scores versus SAL (two-tailed paired t-test, t = 3.330, *P = 0.007, n = 12 biologically independent mice) and increased social interaction (two-way RM ANOVA, drug (CNO or SAL) x stimulus (social or object) interaction F₁,₁₂ = 5.191, *P = 0.033; effect of drug F₁,₁₂ = 0.681, P = 0.418; effect of stimulus F₁,₁₂ = 6.327, P = 0.020, n = 12 biologically independent mice). eDREADD+ jSI mice showed no differences in motor activity or anxiety-related behaviors between SAL and CNO sessions (left, two-tailed paired t-test, t₀ = 0.097, P = 0.925, n = 12 biologically independent mice; middle, two-tailed paired t-test, t₁ = 1.498, P = 0.162, n = 12 biologically independent mice; right, two-tailed paired t-test, t₂ = 0.357, P = 0.728, n = 12 biologically independent mice). f, Control mCherry+ jSI mice showed no difference in sociability score (two-tailed paired t-test, t₁ = 0.393, P = 0.703, n = 10 biologically independent mice) and investigation time (two-way RM ANOVA, drug (CNO or SAL) x stimulus (social or object) interaction F₁,₁₂ = 0.307, P = 0.586; effect of drug F₁,₁₂ = 1.352, P = 0.260; effect of stimulus F₁,₁₂ = 7.914, P = 0.012, n = 10 biological independent mice). mCherry+ jSI mice showed no difference in motor activity or anxiety-related behaviors in SAL versus CNO sessions (two-tailed paired t-test, left, t₀ = 0.446, P = 0.666; middle, t₀ = 0.947, P = 0.368; right, t₁ = 1.083, P = 0.307, n = 10 biologically independent mice). Data in b, e and f are presented as mean ± s.e.m.

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Fig. 8 | Optogenetic activation of mPFC→pPVt projection terminals rescues sociability deficits in adult jSI mice. a. Top: AAV encoding ChR2 under the CamK2 promoter was injected into the mPFC and a wireless blue LED was inserted above the pPVt of adult jSI mice. Representative images of mPFC (middle) and pPVt (bottom) show selective transduction of ChR2 in injection areas in the mPFC and at projection target areas in the pPVt where optic fibers are located. Scale bars, 600μm (middle); 300μm (bottom). Experimental images were obtained from 13 mice, three images per mouse for each area, with similar results obtained. b. Adult jSI mice underwent the three-chamber test for sociability with (ON) or without (OFF) light stimulation. ON and OFF sessions were counter-balanced, with a 24-h interval between each behavior test. c. ChR2+ jSI mice receiving optogenetic stimulation showed increased sociability scores (two-tailed paired t-test, t0 = 2.284, *P = 0.041, n = 13 biologically independent jSI mice) and increased social interaction (two-way RM ANOVA, light (ON or OFF) × stimulus (social or object) interaction F1,14 = 5.064, *P = 0.034; effect of light F1,14 = 1.224, P = 0.280; effect of stimulus F1,14 = 1.865, P = 0.185, n = 13 biologically independent jSI mice). d, Control mCherry+ mice showed no difference in sociability score (two-tailed paired t-test, t1 = 0.258, P = 0.804, n = 8 biologically independent jSI mice) and investigation time (two-way RM ANOVA, light (ON or OFF) × stimulus (social or object) interaction F1,14 = 0.007, P = 0.936; effect of light F1,14 = 0.082, P = 0.779; effect of stimulus F1,14 = 0.457, P = 0.510, n = 6 biologically independent jSI mice). e. Experimental paradigm to examine whether optogenetic stimulation of mPFC→pPVt projection leads to long-term plastic changes. Baseline day (day 1): mice (12 mice) explored the three-chamber arena with novel mouse and novel object corrals for 20 min. Days 2 and 3: mice underwent the same three-chamber test as on Day 1, except mPFC→pPVt circuits were stimulated whenever test mice entered the social interaction zone surrounding a wire corral and the session was extended to 30 min. A probe test day (day 4): mice underwent the three-chamber test in the absence of optogenetic stimulation for 20 min. f, Left: sociability scores from day 1 to day 4 (first 20 min) show a significant sustained effect of light stimulation on days 2 and 3 (two-way RM ANOVA, light (ON or OFF) × day (day 1, day 2, day 3 or day 4) interaction F1,14 = 1.172, P = 0.327; effect of light F1,12 = 45.940, ****P = 0.833×10−4; effect of day F0.044,14,12 = 1.012, P = 0.373, n = 12 biologically independent jSI mice). Right: on day 4, recovery of sociability persisted in the absence of light stimulation (two-tailed paired t-test, t0 = 2.654, *P = 0.022, n = 12 biologically independent jSI mice). Data in c, d and f are presented as mean ± s.e.m. g, Summary scheme: activation of mPFC→pPVt projection neurons is essential for normal sociability in adult GH mice. However, these neurons show decreased intrinsic excitability and an increased inhibitory input drive from mPFC LTS-SSt interneurons when deprived of juvenile social experience, a manipulation that leads to decreased social interaction in adulthood. Decreased social interaction can be induced in GH animals by inhibiting mPFC→pPVt projection or activating mPFC LTS-SSt interneurons in adulthood, and sociability of jSI mice can be rescued by increasing PFC→pPVt projection neuron activity.

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Methods

Animals. Male C57Bl/6 wild-type mice, Chrna2-Cre mice (Tg(Chrna2-cre)1Kldr), PV-Cre mice (stock no. 017320, Jackson Laboratory) and SST-Cre mice (stock no. 013044, Jackson Laboratory) crossed with Cre-dependent eGFP-L10a mice (Ribo-6GF no. 027507, Jackson Laboratory) were used. Animals were group housed in standard laboratory cages in a temperature (22°C) and humidity (30–70%)-controlled vivarium with a 12:12 light/dark cycle. Food and water were provided ad libitum throughout the experiment. Viral injections were performed when mice were 6–7 weeks old for behavioral experiments, and were performed when mice were 9–10 weeks old for electrophysiological experiments. Behavior experiments took place when mice were 10–15 weeks old. For SfL, wild-type C57Bl6 mice (Charles River Laboratories, shipped at p14 for experiments comparing SfL versus GH) and Chrna2-Cre mice (behavioral and electrophysiological experiments) were isolated from weaning (p21) for 2 weeks. Mice were regrouped with age-, sex- and strain-matched males at p53 (5–5 mice per cage), cohoused for more than 1 month before behavior testing. For adulthood testing mice were 10–15 weeks old. Procedures were performed in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals and were approved by the Icahn School of Medicine at Mount Sinai Institutional Animal Care and Use Committee.

Stereotaxic surgery. Mice were anesthetized with isoflurane and head-fixed in a mouse stereotaxic apparatus (Narishige). For chemogenetic and optogenetic studies, bilateral viral injections were made at 6–7 weeks old in the following stereotaxic coordinates of the mPFC: (1) anteroposterior (AP) +2.3 mm, mediolateral (ML) ±0.4 mm and dorsoventral (DV) −1.3 mm and 1.8 mm; (2) AP +1.7 mm, ML ±0.4 mm, and DV −1.7 mm and −1.3 mm; (3) AP +1.3 mm, ML ±0.4 mm, and DV −1.3 mm and −1.0 mm. Midline injections were made at a single location for pPVT: AP −1.8 mm, ML 0 mm, DV −2.8 mm. Bilateral injections were made at one location per hemisphere for NAc: AP +1.45 mm, ML ±0.8 mm, DV −4.5 mm. Unilateral injections were made at six locations per hemisphere for the central PFC: (1) AP +2.5 mm, ML ±0.4 mm, and DV −1.3 mm and −1.0 mm; (2) AP ±1.7 mm, ML ±0.4 mm, and DV −1.7 mm and −1.3 mm; (3) AP ±1.3 mm, ML ±0.4 mm, and DV −1.3 mm and −1.0 mm. Each injection contained 400 nl of AAV1-EF1-dfox-hChR2-mCherry-WPRE-hGH (DIO-hChR2-mCherry) (Addgene), AAV1-CamKIIa-eNpHR3.0-FyFP (halkorhodopsin 3.0) (Addgene), AAV8-k SYN-DIO-hM4D-mCherry (iDREADD) (Addgene), AAV8-hSyn-DIO-55Q-mCherry (eDREADD) (Addgene), AAV8-hSyn-DIO-chR2-mCherry (Addgene), AAV1-CamKIIa-eNpHR3.0-FyFP (halkorhodopsin 3.0) (Addgene), AAV8-k SYN-DIO-hM4D-mCherry (iDREADD) (Addgene), AAV8-hSyn-DIO-hM4D-mCherry (eDREADD) (Addgene), AAV8-CamKII-GFP-Cre (CamKII-Cre) (Addgene), CAV-Cre (Cre-Cav) (Montpellier) and/or rAAV2-CAG-Cre-WPRE (Boston Children's Hospital). All viruses were infused at a rate of 600 nl/min using a microinjector and glass pipette. For fiber photometry surgeries, a unilateral injection was made at AP +1.7, ML −0.4 mm and DV −1.7 mm with 500 nl of AAV1-Syn-FLEX-GCAmp6f-WPRE-SV40 (Flex-GCaMP6f) (Addgene). A 1.6 mm in length fiber-optic cannula with a 0.48 numerical aperture and a 400-μm core diameter (Doric Lenses) was implanted over the desired recording site (AP +1.7, ML −0.4 mm and DV −1.3 mm) using Metabond and dental cement. For wireless optogenetic surgeries, a 1-mm-diameter hole was drilled at a single location for implantation over the pPVT and, a 2.6-mm-length and 500-μm-diameter LED fiber optic fiber (Amuzu) was then implanted above the pPVT at AP −1.8 mm, ML ±0.2 mm, −2.8 mm using Metabond and dental cement. If retrobeads (Lumafluor) were injected with an injection rate of 60 nl min−1 for the viral spread validation of mice used for photometry surgeries, a unilateral injection was made at AP −1.8 mm, ML 0 mm, DV −2.8 mm. Midline injections were made at a single location for pPVT: AP −1.8 mm, ML 0 mm, DV −2.8 mm. Bilateral injections were made at one location per hemisphere for NAc: AP +1.45 mm, ML ±0.8 mm, DV −4.5 mm. Unilateral injections were made at six locations per hemisphere for the central PFC: (1) AP +2.5 mm, ML ±0.4 mm, and DV −1.3 mm and −1.0 mm; (2) AP ±1.7 mm, ML ±0.4 mm, and DV −1.7 mm and −1.3 mm; (3) AP ±1.3 mm, ML ±0.4 mm, and DV −1.3 mm and −1.0 mm. Each injection contained 400 nl of AAV1-EF1-dfox-hChR2-mCherry-WPRE-hGH (DIO-hChR2-mCherry) (Addgene), AAV1-CamKIIa-eNpHR3.0-FyFP (halkorhodopsin 3.0) (Addgene), AAV8-k SYN-DIO-hM4D-mCherry (iDREADD) (Addgene), AAV8-hSyn-DIO-55Q-mCherry (eDREADD) (Addgene), AAV8-hSyn-DIO-chR2-mCherry (Addgene), AAV1-CamKII-GFP-Cre (CamKII-Cre) (Addgene), CAV-Cre (Cre-Cav) (Montpellier) and/or rAAV2-CAG-Cre-WPRE (Boston Children's Hospital). All viruses were infused at a rate of 600 nl/min using a microinjector and glass pipette. For fiber photometry surgeries, a unilateral injection was made at AP +1.7, ML −0.4 mm and DV −1.7 mm with 500 nl of AAV1-Syn-FLEX-GCAmp6f-WPRE-SV40 (Flex-GCaMP6f) (Addgene). A 1.6 mm in length fiber-optic cannula with a 0.48 numerical aperture and a 400-μm core diameter (Doric Lenses) was implanted over the desired recording site (AP +1.7, ML −0.4 mm and DV −1.3 mm) using Metabond and dental cement. For wireless optogenetic surgeries, a 1-mm-diameter hole was drilled at a single location for implantation over the pPVT and, a 2.6-mm-length and 500-μm-diameter LED fiber optic fiber (Amuzu) was then implanted above the pPVT at AP −1.8 mm, ML ±0.2 mm, −2.8 mm using Metabond and dental cement. For retrobeads (Lumafluor) injection to allow for sufficient viral expression. During recording sessions, two excitation LEDs (Thorlabs) reflected off dichroic mirrors to record GCaMP6f specific signal (465 nm) and nonspecific autofluorescence-related signals as a control (405 nm). A fiber-optic patch cord (Doric Lenses, MFP_400/304/0.48_1.3) was attached to the implanted fiber-optic cannula with cubic zirconia sheathes. The fiber-optic cable was coupled to two LEDs, which pass light through a GFP (470 nm) or violet (405 nm) excitation filter (Thorlabs), and dichroic mirrors. Emitted light passed through the fiber-optic cable is passed through the dichroic mirror and emission filters, and through a 0.50 NA microscope lens (62–561, Edmund Optics), and then focused and projected onto a photodetector (Model 2151 Femtowatt Photoreceiver). Real-time signal processors (RX8 and RZ5P, Tucker-Davis Technologies) and software (OpenEx v.2.20 for RX8 and Synavex v.9 for RZ5P, Tucker-Davis Technologies) were used to sinusoidally modulate each LED’s output at different frequencies to un-mix signals from each LED. Signals were collected at a sampling frequency of 381 Hz (RX8) or 1,018 Hz (RZ5P).

Behavior. Before behavior testing, mice were habituated to the fiber-optic cable for 30 min each day for 3 d. For analysis of activity during social exploration and object exploration, mice were first habituated to arena for 3 min, and then a 5-min baseline was recorded for each mouse in a novel open-field arena (43×43×33 cm) before each exploration testing. Following baseline, we placed a novel object or a novel age-, sex- and strain-matched mouse in the arena (counter-balanced between object and social) for 5 min.

Analysis. Analysis was performed with custom scripts used previously11 with python v.2.7 (Python Software Foundation). Each channel was first normalized by subtracting the median value for the entire recording period for both the signal (405 nm) and the noise (465 nm) channels. The 405 nm channel was then normalized using a Savitzky–Golay filter to remove high-frequency changes in the 405 channel, and then subtracted from the 465 channel. This served to mitigate movement-related signals and fluorescence bleaching. The signal was then z-scored using the 30-s baseline period directly before introducing the mouse or an object. To compare social versus object mPFC→pPVT projection neuronal activity, we compared mean z score during baseline periods (30 s before introducing a stimulus) with the 30 s after introducing either the mouse or the object.

Behavior testing. For behavior testing with iDREADD and eDREADD manipulations, clozapine-N-oxide dibydrochloride (CNO; Tocris Bioscience) was injected intraperitoneally into intrasplenically microinjected iDREADD (iDREADD experiments) or 1 mg/kg (eDREADD experiments) dose 30 min before behavior testing. Mice received CNO and SAL in a counter-balanced fashion with 1 week of wash-out time between treatments. Mice were excluded from behavior tests if they demonstrated significant alterations in motor responses due to intraperitoneal procedures.

For behavior testing with wireless optogenetic modulation, before behavior testing, mice were habituated to either the three-chamber or the open field for one 20-min habituation phase while wearing a dummy version of the receiver (Amuzu, Teleopto wireless optogenetics system). For the continuous stimulation/inhibition experiments, during three-chamber tests, elevated plus maze and open-field tests, a 470-nm blue light cycle of 470-nm blue light or control stimulation was triggered using a signal generator (Rigol Technologies) during the entire ‘ON’ testing session. All experiments were counter-balanced with respect to whether mice received optogenetic stimulation/inhibition on the first day of testing, or the first day of testing, and there was a 24-h interval between ON and OFF sessions. In some optogenetic activation experiments (Fig. 4c–k and
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Three-chamber test. Testing was conducted in the sociability cage (Noldus): a three-chambered rectangle with clear acrylic walls and a white matte bottom. Mice were habituated to the center chamber for 10 min, followed by 10 min of habituation to the full chamber 1 d before testing. On the day of testing, mice were placed in the center chamber for 5 min, followed by 5 min of exploration of the full chamber with empty wire corrals. To test sociability, an age, sex- and strain-matched mouse was placed under a wire corral in the ‘social chamber’ and a novel object was placed under the corral in the ‘object chamber.’ The subject mouse was allowed to freely investigate for a 10-min test phase. Behavior was recorded and scored by Ethovision v.9.14 (Noldus). Social interaction time or object interaction time was defined as the cumulative amount of time that the mouse’s nose was detected by Ethovision) was detected in the interaction zone, which is a circular zone surrounding the corral containing the mouse or object. For the experiment where mPFC→pPVT neurons were repeatedly stimulated to induce a long-lasting rescue of jSI social deficits, mice explored the three-chambered arena with either mouse or novel object corral for 20 min on baseline day (day 1), followed by days 2 and 3, when mice explored either the same mouse or object in chambers for 30 min while receiving light stimulation when their nose entered the social interaction zone surrounding a corral with a mouse. On day 4 (probe test day), mice explored the arenas with the same mouse or object in the absence of optogenetic stimulation for 20 min. For the real-time preference three-chamber test using mice expressing fluorescently labeled neurons in pPVT projection neurons to mPFC, mice were habituated in the center chamber for 5 min and then were allowed to explore the entire apparatus (without stimuli present) for 5 min. Additionally, mice were habituated to the milkshake reward and the testing apparatus by completing a ‘dry run’ of the testing procedure (including both habituation and milkshake preference test phases) on the day before the first day of testing. On this day, mice were also given access to a milkshake-filled basin and an empty basin in their home cage for roughly 4 h.

Elevated plus maze. The elevated plus maze is a behavioral assay that is frequently used to measure anxiety-related and exploratory behavior by comparing time spent in enclosed arms compared with open arms in a plus-shaped maze with four square-shaped arms that are elevated above the ground. Mice were placed in the center chamber and allowed to freely explore for 8 min. Behavior was recorded and scored by Ethovision v.9 and v.14.

Open field. To assess anxiety-related and locomotor behaviors, we used a square acrylic arena (43 × 43 × 33 cm) equipped with a panel of 16 horizontal infrared emitters, two identical empty corrals without light stimulation (10 min), and a novel object corral for 20 min on baseline day (day 1), followed by days 2 and 3, when mice explored either the same mouse or object in chambers for 30 min while receiving light stimulation when their nose entered the social interaction zone surrounding a corral with a mouse. On day 4 (probe test day), mice explored the arenas with the same mouse or object in the absence of optogenetic stimulation for 20 min. For the real-time preference three-chamber test using mice expressing fluorescently labeled neurons in pPVT projection neurons to mPFC, mice were habituated in the center chamber for 5 min and then were allowed to explore the entire apparatus (without stimuli present) for 5 min. Additionally, mice were habituated to the milkshake reward and the testing apparatus by completing a ‘dry run’ of the testing procedure (including both habituation and milkshake preference test phases) on the day before the first day of testing. On this day, mice were also given access to a milkshake-filled basin and an empty basin in their home cage for roughly 4 h.

Milkshake three-chamber test. Testing was conducted in a custom-designed three-chambered apparatus (61 × 40.5 × 23.5 cm3) with white matte walls and floor. A small cylindrical aluminum basin (approximately 5 cm in diameter and 1.5 cm in height) filled with milkshake was placed in one of the side chambers and an identical empty basin was placed in the opposite chamber. To test for milkshake preference, mice were allowed to freely investigate the apparatus with the option to consume milkshake for 10 min. Milkshake consisted of 15% chocolate syrup (The Hershey Company), 15% sweetened condensed milk (Eagle Family Foods Group) and 79% water by volume. Behavior was recorded and scored via Ethovision v.14 (Noldus) or ANY-Maze v.6.06 (Stoelting), with ‘milkshake interaction time’ or ‘empty basin interaction time’ defined as the cumulative amount of time when the mouse’s nose-point was within an interaction zone circumscribed about each stimulus (approximately 10 cm in diameter). During testing, mice were habituated in the center chamber for 5 min and then were allowed to explore the entire apparatus (without stimuli present) for 5 min. Additionally, mice were habituated to the milkshake reward and the testing apparatus by completing a ‘dry run’ of the testing procedure (including both habituation and milkshake preference test phases) on the day before the first day of testing. On this day, mice were also given access to a milkshake-filled basin and an empty basin in their home cage for roughly 4 h.

Social versus object exploration for c-Fos immunohistochemistry. Male C57Bl/6 wild-type mice (4–8 months) were isolated for 24 h before testing. Behavior was conducted in the three-chambered apparatus. The object exploration group was exposed to a novel object only, and the social exploration group was exposed to a novel mouse only. Mice were perfused 90 min after completing the social/object exploration phase.

Patch-clamp recording. Animals were decapitated under isoflurane anesthesia. Brains were quickly removed and transferred into ice-cold (0–4°C) artificial cerebrospinal fluid (ACSF) of the following composition (in mM): 210.3 sucrose, 11 glucose, 2.5 KCl, 1 NaH2PO4, 26.2 NaHCO3, 0.5 CaCl2 and 4 MgCl2. Acute coronal slices (300 μm) containing the mPFC or pPVT were sectioned using a VT1200S vibratome (Leica Microsystems). Slices were allowed to recover for 40 min at room temperature in the same solution, but with reduced sucrose (170 mM) and addition of NaCl (10 mM). Following recoveries were maintained at room temperature in standard ACSF composed of the following (in mM): 119 NaCl, 2.5 KCl, 1 NaH2PO4, 26.2 NaHCO3, 11 glucose, 2 CaCl2, and 2 MgCl2. Patch-clamp recordings were performed from fluorescently labeled neurons in deep-layer mPFC (including anterior cingulate cortex and prelimbic cortex at AP 2.3 mm and 1.3 mm) using borosilicate glass electrodes (3–5 MΩ). Whole-cell voltage-clamp recordings were obtained with the internal solution containing (in mM): 120 Cs-methanesulfonate, 10 HEPES, 0.5 EGTA, 8 NaCl, 4 Mg-ATP, 1 P-1Q, 10 Na-phosphocreatine and 0.4 Na-GTP. Current-clamp recordings were obtained with the internal solution containing (in mM): 127.5 K-methanesulfonate, 10 HEPES, 5 KCl, 5 Na-phosphocreatine, 2 MgCl2, 2 Mg-ATP, 0.5 TTX or 0.3 Na-GTP (DEAE-Dextran). DEAE-Dextran and ACSF. Miniature EPSCs and IPSCs were recorded in the presence of tetrodotoxin (TTX) (1 μM, Abcam) in the bath solution. Spontaneous and miniature EPSCs and IPSCs were separated by holding the neuron at the reversal potential for excitatory or inhibitory post synaptic currents, allowing for isolation of EPSCs and IPSCs at −60 mV and 0 mV, respectively.

White light was delivered by a 100-W long-arc tungsten halogen lamp. Optogenetic stimulation for 20 min. For the real-time preference three-chamber test, mice were allowed to freely investigate the apparatus with the option to consume milkshake for 10 min. During the stimulation session, optogenetic stimulation was delivered whenever the mouse visited the stimulation zone surrounding a corral with a mouse entered the stimulation zone. The stimulation zone was counter-balanced.

To validate modulation of mPFC→pPVT projection neurons with eNhPr3.0, iDREADD or eDREADD, whole-cell recordings in gap-free mode were obtained with borosilicate glass electrodes (5–8 MΩ) filled with a current-clamp internal solution (in mM: 127.5 K-methanesulfonate, 10 HEPES, 5 KCl, 5 Na-phosphocreatine, 2 MgCl2, 2 Mg-ATP, 0.6 EGTA and 0 Na-GTP). Fluorescently labeled cells were visualized on an upright microscope equipped for both digital image correlation (DIC) and fluorescence visualization. Cells were held at −55 mV to avoid potassium reversal. For chemogenetic, 10 μM CNO was bath-applied to a cell until 30 min of baseline (10 min) had elapsed. Baseline potential was compared to baseline and 5 min after CNO application. For optogenetics, frequency of spikes induced by current injection in neurons expressing eNhPr3.0 were recorded before during, and after yellow light stimulation using TTL-pulsed microscope objective-coupled LEDs (450 nm, Prizmatix). At the beginning of each experiment, an input–output curve was expressed as a stimulus intensity–response amplitude curve was established for each neuron independently by incrementally increasing light intensity in a step-wise fashion (0.1 mW/mm2 per step), from the minimum stimulation level 1, to determine the intensity of the stimulation that evoked postsynaptic current reliably at the plateau of the curve and to ensure activation of all ChR2-expressing terminals within the field of view. The power of light stimuli was quantified using an optical power meter (Thorlabs). For PPR experiments, we set the intensity of the stimulus at the plateau level when additional increase in the light intensity did not affect the amplitude of the light-evoked responses. Short-term dynamics were tested with twin pulses separated by 500 ms. Stimuli were given every 20 s and at least 15 responses were averaged at each interpulse interval. Paired-pulse recordings were conducted in standard ACSF. To limit the impact of disynaptic excitation, PPR was determined as a ratio of the amplitude of EPSC2 to the amplitude of EPSC1. To isolate monosynaptic connections, eIPSCs were recorded in the presence of TTX (1 μM), 4-aminopyridine (4-AP) (100 μM) and picrotoxin (100 μM).

In vivo electrophysiology. Sixteen-channel silicon optoelectrodes with 177-μm2 recording sites (NeuroNexus Technologies) spaced 50 μm apart were used to record neuronal activity in the pPVT. All in vivo recordings were acquired using the Omniplex A system (Plexon). Spike signals were filtered at a bandpass of 300 Hz to 8kHz. Sorting of single units was carried out using principal component analysis (Plexon Offline Sorter v.3.2.2 (Plexon). At 3 weeks after virus injection, in vivo electrophysiological experiments with optogenetic stimulation were performed under 0.5–1% isoflurane anesthesia. Blue light illumination (wavelength 473 nm, 2.5 ms duration, 20 Hz) or yellow light illumination (wavelength 565 nm, continuous stimulation) was delivered using an optic fiber (diameter 105 μm) coupled to the extracellular recording electrode, which was inserted into the brain.
pPVT based on stereotaxic coordinates. The power at the fiber-optic tip was approximately 10 mW (blue light) and 4.5 mW (yellow light).

**Statistics.** Statistical analyses were performed using Prism v.8 (Graphpad). No statistical methods were used to predetermine sample sizes but our sample sizes are similar to those reported in previous publications\(^{11,28}\). Data collection and analysis were not performed blind to the conditions of the experiments. Subjects (mice) were randomly assigned into study groups. For all studies, independent animals were used as replicates. Replication studies confirmed our results in all experiments. Data distribution was assumed to be normal but this was not formally tested. Statistical analyses for fiber photometry were conducted using parametric tests on z-scored data. Analyses comparing multiple time points were conducted with paired \(t\)-tests. Behavioral tests in DREADD and optogenetic experiments were analyzed using two-way analysis of variance (ANOVA) or paired \(t\)-tests, as indicated. For patch-clamp physiology, statistical analyses were conducted using \(t\)-tests or two-way ANOVAs. All statistical tests were two-tailed. Bar graphs and photometry averaged traces are presented as the mean and error bars represent the s.e.m.

**Reporting Summary.** Further information on research design is available in the Nature Research Reporting Summary linked to this article.

**Data availability**
Source data are provided with this paper. The remaining relevant data are available from the corresponding author on reasonable request.

**Code availability**
Codes for fiber photometry analysis are available from the authors upon reasonable request.

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**Author contributions**
K.Y. and H.M. designed and analyzed experiments and wrote the manuscript with inputs from all authors. K.Y. performed most experiments, including surgeries, slice electrophysiology and behavior experiments, in part assisted by L.K.B., Y.G., K.J.N. and M.S. M.B.L. performed a part of behavioral experiments. D.K. performed the in vivo electrophysiology experiment. M.E.E and S.J.R. assisted with fiber photometry experiments and analysis. S.I. and K.C. assisted with viral validation and immunohistochemistry. S.A. supervised L.K.B. K.K. contributed to experiments and analysis with Chrna2-Cre mice.

**Competing interests**
The authors declare no competing interests.

**Additional information**
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Extended Data Fig. 1 | c-Fos mapping of cortical and sub-cortical regions upon social exposure. a, (left) Mice were exposed in a 3-chamber apparatus for 10 min to either a novel mouse (social:S) under a wire corral, a novel object (object:O) under a wire corral, or kept in their home cage (HC), and then perfused 90 min after the end of the exposure. Brains were then stained for c-Fos, a marker of neuronal activity. (middle) Among many brain areas, including several areas that are known to be involved in social behavior, the posterior PVt (pPVt) showed significant c-Fos induction in social groups compared with both object and homecage groups. (one-way ANOVA, $F_{2,9} = 34.020, P = 0.636 \times 10^{-4}$ followed by a Tukey’s post hoc test: pPVt: social vs object: **$P = 0.003$, social vs home cage: ****$P = 0.468 \times 10^{-4}$, $n = 4$ biologically independent mice each) *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. (right) Representative c-Fos staining images from pPVt. Scale bar: 200um. Experimental images were obtained from each 12 mice, a few images per mouse, with similar results obtained. b, (upper left) mPFC→pPVt projection neurons were labeled by retrobeads injected into the pPVt. representative images showing (right) beads in layer 5/6 mPFC→pPVt neurons (scale bar: 100um), and (bottom left) beads at injection site in pPVt (scale bar: 200um). Experimental images were obtained from 16 mice, a few images per mice, with similar results obtained. c, (left) Representative images showing preferential c-Fos induction in mPFC→pPVt neurons by social exposure (Scale bar: 50um, experimental images were obtained from 16 mice, a few images per mice, with similar results obtained), and (right) quantification (one-way ANOVA, $F_{2,13} = 19.750, P = 0.115 \times 10^{-3}$, followed by a Tukey’s post hoc test, Social vs object $P = 0.056$, Object vs home cage **$P = 0.009$, Social vs home cage ****$P = 0.789 \times 10^{-4}$, $n = 5$ biologically independent social exposed mice, $n = 5$ biologically independent object exposed mice, $n = 6$ biologically independent home caged mice). PL: prelimbic cortex, IL: infralimbic cortex, cg1/2: cingulate cortex1/2, Pir: Piriform cortex, aPVT: anterior paraventricular thalamus, pPVT: posterior paraventricular thalamus, MD: medial dorsal thalamus, Nac: nucleus accumbens, BLA: basolateral amygdala, LA: lateral amygdala, CeA: central amygdala, dPAG: dorsal periaqueductal gray, PVN: paraventricular nucleus of hypothalamus. Data in a, c are presented as mean±s.e.m.
**Extended Data Fig. 2 | Juvenile social isolation leads to long lasting reduction of sociability in adult mice.**

**a.** Timeline showing weaning at p21 and subsequent 2 weeks of juvenile social isolation (jSI), followed by re-housing or control group housing (GH). jSI mice showed reduced sociability scores vs GH mice in a 3 chamber sociability test, in which a mouse chooses between a social target and an object, and time spent investigating both is measured and compared (two tailed t-test, $t_{36} = 2.154$, $P = 0.038$, $n = 20$ biologically independent GH mice, $n = 18$ biologically independent jSI mice) and reduced social interaction (two-way RM ANOVA, housing (GH/jSI) × stimulus (social/object) interaction $F_{1,36} = 7.042$, $P = 0.012$, effect of housing $F_{1,36} = 1.117$, $P = 0.298$, effect of stimulus $F_{1,36} = 14.860$, $P = 0.460 \times 10^{-3}$, $n = 20$ biologically independent GH mice, $n = 18$ biologically independent jSI mice). jSI mice showed no difference in distance traveled during the open field test (two tailed t-test, $t_{36} = 0.939$, $P = 0.354$, $n = 20$ biologically independent GH mice, $n = 18$ biologically independent jSI mice), suggesting normal motor activity. While jSI mice showed reduced time in center during open field test (two tailed t-test, $t_{36} = 2.054$, $P = 0.047$, $n = 20$ biologically independent GH mice, $n = 18$ biologically independent jSI mice), they showed no difference in an independent anxiety task (elevated plus maze (EPM)) two tailed t-test, $t_{38} = 0.926$, $P = 0.360$, $n = 20$ biologically independent GH mice, $n = 18$ biologically independent jSI mice). Data in **b, c** are presented as mean ± s.e.m.
Extended Data Fig. 3 | Chemogenetic suppression of pPVt neuron activity reduces sociability in adult group-housed mice. 

**a**, (left) AAV8-DIO-iDREADD (or mCherry) was injected together with AAV1-CaMKII-Cre in the pPVt. (right) A representative image shows selective transduction at injection areas of pPVt. Scale bar: 300 μm. Experimental images were obtained from 12 mice, three images per mouse, with similar results obtained. **b**, Validation of iDREADD action in pPVt neurons by slice whole-cell patch clamp recording. (left) A representative trace shows that bath application of CNO significantly decreases membrane potential of pPVt neurons. Traces were recorded from 7 cells from 3 biologically independent mice, with similar results obtained. (Right) Quantification shows a reduction in membrane potential after CNO application (two-tailed paired t-test, t\(_6\)=4.177, **P=0.006, n=7 cells from 3 biologically independent mice). **c**, Mice were treated with saline (SAL) or CNO (10 mg/kg) and then underwent the 3 chamber test of sociability. For CNO and SAL injections, order is counter-balanced. **d**, Viral spread validation at injection areas of pPVt from post-behavioral testing mice. Gray areas represent the minimum (lighter colour) and the maximum (darker colour) spread of iDREADD into the pPVt. **e**, (left) CNO-treated iDREADD+ mice showed reduced sociability, revealed by reduced sociability scores vs. SAL (two-tailed paired t-test, t\(_{11}\)=2.257, *P=0.045, n=12 biologically independent mice), and disrupted behavior in 3 chamber sociability task (two-way RM ANOVA, housing (GH/jSI) × stimulus (social/object) interaction F\(_{12,22}\)=4.894, *P=0.038, effect of drug F\(_{1,22}\)=0.032, P=0.859, effect of stimulus F\(_{12,22}\)=0.109, P=0.745, n=12 biologically independent mice). (right) iDREADD+ mice showed no differences in motor activity or anxiety-related behaviors (Left; two-tailed paired t-test, t\(_{11}\)=0.688, P=0.506, n=12 biologically independent mice Middle; two-tailed paired t-test, t\(_{11}\)=1.604, P=0.137, n=12 biologically independent mice, Right; two-tailed paired t-test, t\(_{11}\)=1.096, P=0.299, n=11 biologically independent mice) as a result of CNO vs. SAL treatment. **f**, (left) Control mCherry+ mice showed no difference in sociability score (two-tailed paired t-test, t\(_{7}\)=1.459, P=0.188, n=8 biologically independent mice) or investigation time (two-way RM ANOVA, housing (GH/jSI) × stimulus (social/object) interaction F\(_{12,14}\)=0.352, P=0.563, effect of drug F\(_{1,14}\)=0.024, P=0.880, effect of stimulus F\(_{12,14}\)=8.630, P=0.011, n=8 biologically independent mice) as a result of CNO vs. SAL treatment. (right) Control mCherry+ mice showed no difference in motor activity or anxiety-related behaviors (Left; two-tailed paired t-test, t\(_{7}\)=0.981, P=0.359, n=8 biologically independent mice, Middle; two-tailed paired t-test, t\(_{7}\)=0.317, P=0.761, n=8 biologically independent mice Right; two-tailed paired t-test, t\(_{7}\)=0.662, P=0.529, n=8 biologically independent mice). Data in **b**, **e**, **f** are presented as mean±s.e.m.
Extended Data Fig. 4 | Optogenetic suppression of mPFC→pPVT projection terminals does not change motor activity or anxiety-related behaviors in group-housed mice. a, (left) Halorhodopsin NpHR3.0 AAV under CamKII promotor was injected into mPFC and mPFC→pPVT projection terminals were illuminated at the pPVT using a wireless LED system for behavioral testing. (middle/right) Validation of optogenetic suppression by patch-clamp recording from halorhodopsin NpHR3-expressing mPFC→pPVT projection neurons. (middle) Representative trace showing decreased action potentials of mPFC→pPVT projection neurons upon optogenetic stimulation (traces were recorded from 7 cells from 3 biologically independent mice, with similar results obtained), and (right) quantification (one-way RM ANOVA, \( F_{13,211,932} = 91.940, P = 0.167 \times 10^{-6} \), Tukey’s multiple comparisons test: 1st OFF vs ON, ***\( P = 0.144 \times 10^{-6} \), ON vs 2nd OFF, ****\( P = 0.328 \times 10^{-6} \), \( n = 7 \) cells from 3 biologically independent mice.). b, c, In vivo validation of optogenetic suppression of mPFC→pPVT projection terminals. b, Representative in vivo recordings of pPVT neurons showing a significant decrease (top), increase (middle), or no change (bottom) in spike activity upon yellow light delivery over mPFC→pPVT projection terminals expressing halorhodopsin NpHR3 in pPVT. Experimental traces were obtained from 4 mice, 13-16 cells per mouse, with similar results obtained. c, Distribution of pPVT neurons showing light-induced decreased firing (11 out of 57 cells from 4 biologically independent mice, 19%), increased firing (9 out of 57 cells from 4 biologically independent mice, 16%), or no change (37 out of 57 cells from 4 biologically independent mice, 65%). Effect of light stimulation for each unit was quantified by comparing the firing rates between light off period and light on period (5 s each) of 6 sessions per cell through paired t-test. d, Viral spread validation of NpHR3-expression from post-behavior testing mice at injection areas. Gray areas represent the minimum (lighter colour) and the maximum (darker colour) spread of NpHR3-expression in the mPFC. e, Optic fiber location (yellow line circles) was validated in all mice. Experimental images were obtained from 14 mice, three images per mouse, with similar results obtained. f, Mice underwent open field testing, and elevated plus maze (EPM) with (ON) or without (OFF) light stimulation. ON and OFF session order was counter-balanced for each behavior test with a 24-hour interval between tests. Mice with optogenetic suppression showed no differences in motor activity or anxiety-related behaviors between ON and OFF sessions (Left; two tailed paired t-test, \( t_{13} = 0.747, P = 0.469 \), \( n = 14 \) biologically independent mice). Middle; two tailed paired t-test, \( t_{13} = 0.455, P = 0.657 \), \( n = 14 \) biologically independent mice. Right; two tailed paired t-test, \( t_{13} = 1.028, P = 0.323 \), \( n = 14 \) biologically independent mice). g, Control mCherry+ mice showed no difference in motor activity or anxiety-related behaviors between light ON and OFF sessions (Left; two tailed paired t-test, \( t_{13} = 0.528, P = 0.612 \), \( n = 9 \) biologically independent mice, Right; two tailed paired t-test, \( t_{13} = 0.147, P = 0.987 \), \( n = 9 \) biologically independent mice). Data in a, f, g are presented as mean ± s.e.m.
Extended Data Fig. 5 | See next page for caption.
Extended Data Fig. 5 | Juvenile social isolation does not change excitability, nor E/I input ratio of mPFC → NAc neurons, mPFC → cPFC neurons, or pPVT neurons in adulthood. a–f, Whole-cell patch clamp recording from mPFC→NAc neurons in adult jSI or GH mice. b, c, Assessment of intrinsic excitability of PFC→NAc neurons in the presence of DNQX, D-AP5, and picrotoxin. b, (Left) Input-output curves showed no differences in spike frequency between jSI and GH mice (two-way RM ANOVA, housing (GH/jSI) x current step interaction $F_{17,391} = 0.388, P = 0.992$, effect of housing $F_{1,21} = 1.103, P = 0.302$, effect of current step $F_{2,427, 71,50} = 161.200, P = 0.001 \times 10^{-14}, n = 15$ cells from 8 biologically independent GH mice, $n = 23$ cells from 8 biologically independent jSI mice). (right) No significant differences in spike frequency at 200pA between jSI and GH (two tailed t-test, $t_{18} = 0.399, P = 0.692, n = 15$ cells from 8 biologically independent jSI mice, $n = 23$ cells from 8 biologically independent GH mice, $n = 23$ cells from 8 biologically independent jSI mice). No significant differences in (d) sEPSC frequency (two tailed t-test, $t_{26} = 0.308, P = 0.760, n = 23$ cells from 8 biologically independent jSI mice), sEPSC amplitude (two tailed t-test, $t_{18} = 0.305, P = 0.762, n = 27$ cells from 8 biologically independent GH mice, $n = 23$ cells from 8 biologically independent jSI mice), or (e) sIPSC amplitude (two tailed t-test, $t_{18} = 0.384, P = 0.277, n = 27$ cells from 8 biologically independent jSI mice, $n = 23$ cells from 8 biologically independent GH mice, $n = 23$ cells from 8 biologically independent jSI mice). No significant differences in (f) sEPSC/sIPSC frequency ratio (two tailed t-test, $t_{18} = 2.136, P = 0.026, n = 20$ cells from 8 biologically independent GH mice, $n = 21$ cells from 9 biologically independent jSI mice), or sEPSC/sIPSC amplitude (two tailed t-test, $t_{18} = 1.804, P = 0.079, n = 20$ cells from 8 biologically independent GH mice, $n = 21$ cells from 9 biologically independent jSI mice) between jSI and GH. Data in b–f are presented as mean ± s.e.m.
Extended Data Fig. 6 | mEPSC and mIPSC of mPFC→pPVt neurons, mPFC→NAc neurons, and mPFC→cPFC neurons. a–c, Whole-cell slice patch clamp recording from mPFC→pPVt neurons in adult jSI mice (20 cells from 6 biologically independent mice) or GH mice (19 cells from 5 biologically independent mice). b, mEPSC frequency was significantly lower in jSI mice compared to GH mice (two tailed t-test, t_{37} = 2.730, **P = 0.964 × 10^{-2}) but there were no significant differences in mEPSC amplitude (two tailed t-test, t_{37} = 1.150, P = 0.258). c, There were no significant differences in mIPSC frequency (two tailed t-test, t_{37} = 0.101, P = 0.920) or mIPSC amplitude (two tailed t-test, t_{37} = 0.303, P = 0.764) between jSI and GH. d–f, Whole-cell patch clamp recording from mPFC→NAc neurons in adult jSI mice (n = 20 cells from 6 biologically independent mice) or GH mice (n = 20 cells from 6 biologically independent mice). e, There were no significant differences in mEPSC frequency (two tailed t-test, t_{38} = 0.002, P = 0.998) or mEPSC amplitude (two tailed t-test, t_{38} = 0.495, P = 0.624) between jSI and GH. f, There were no significant differences in mIPSC frequency (two tailed t-test, t_{38} = 0.066, P = 0.948) or mIPSC amplitude (two tailed t-test, t_{38} = 0.455, P = 0.652) between jSI and GH. g–i, Whole-cell patch clamp recording from mPFC→cPFC neurons in adult jSI mice (n = 19 cells from 5 biologically independent mice) or GH mice (n = 18 cells from 5 biologically independent mice). h, There were no significant differences in mEPSC frequency (two tailed t-test, t_{35} = 1.559, P = 0.128), or mEPSC amplitude (two tailed t-test, t_{35} = 1.275, P = 0.211) between jSI and GH. i, There were no significant differences in mIPSC frequency (two tailed t-test, t_{35} = 0.247, P = 0.807) or mIPSC amplitude (two tailed t-test, t_{35} = 1.579, P = 0.123) between jSI and GH. Data in b, c, e, f, h, i are presented as mean ± s.e.m.
**Extended Data Fig. 7** | Optogenetic interrogation of mPFC→pPVT projection inputs onto pPVT neurons. 

a. ChR2-encoding AAV1 was injected into the mPFC to express ChR2 in mPFC neurons. Whole cell patch-clamp recordings were performed while optogenetically activating mPFC→pPVT projection terminals in pPVT slices. 

b. Excitatory connectivity was assessed by normalized postsynaptic currents (PSCs) recorded at -70 mV from pPVT neurons before and after application of tetrodotoxin (TTX; 1 µM) with 4-aminopyridine (4-AP; 100 µM). A majority of pPVT neurons received a monosynaptic input from mPFC. There was no difference in mono/polysynaptic ratio (two tailed t-test, t\(_{13}\) = 0.349, P = 0.733, n = 8 cells from 5 biologically independent GH mice, n = 7 cells from 5 biologically independent jSI mice). 

c. (upper) Representative traces showing that optogenetic activation of mPFC→pPVT axons was blocked by DNQX (20 µM). pPVT neurons were clamped at -70 mV while optogenetically stimulating mPFC→pPVT axons before and after bath application of DNQX. Traces are recorded from 3 cells from 2 biologically independent mice, with similar results obtained. (bottom) Averaged amplitude decreases after application of DNQX (two tailed t-test, t\(_{2}\) = 17.790, **P = 0.003, n = 3 cells from 2 biologically independent mice). 

d. (left) Representative eEPSC of pPVT neurons upon optogenetic activation of mPFC→pPVT axons in GH and jSI mice through gradually changing the intensity. Traces were recorded from 17 cells from 7 biologically independent mouse per group, with similar results obtained. (right) Intensity–amplitude curves showing the relationship between stimulus intensity and normalized eEPSC amplitude. Normalized eEPSC amplitude was lower in jSI mice than GH mice (two-way RM ANOVA, housing (GH/jSI) × current step interaction F\(_{4,25}\) = 3.740, **P = 0.003, effect of housing F\(_{1,20}\) = 4.173, P = 0.048, effect of current step F\(_{4,225}\) = 25.830, P = 0.174x10\(^{-5}\), n = 17 cells from 7 biologically independent GH mice, n = 17 cells from 7 biologically independent jSI mice). 

e. There were no significant differences in PPR at a 500-ms interval (two tailed t-test, t\(_{25}\) = 1.551, P = 0.134, n = 17 cells from 7 biologically independent GH mice, n = 21 cells from 8 biologically independent jSI mice). Data in c, d, e are presented as mean ± s.e.m.
Extended Data Fig. 8 | Juvenile social isolation increases excitability of mPFC low-threshold spiking (LTS)-SST interneurons in adulthood. a, Whole-cell patch clamp recording from mPFC SST interneurons in adult jSI or GH SST-GFP mice (SST-Cre mice crossed with Cre-dependent eGFP-L10a mice). b, Classification of SST cells based on firing patterns. SST cells were classified into 3 sub-types (low-threshold spike: LtS, quasi-fast spiking: QFS, adapting: AD) in L5/6 and mainly AD type in L2/3. c, % of sub-type of SST interneurons in L5/6 and L2/3 in GH and jSI mice. d, Assessment of intrinsic excitability of SST-LtS interneurons in L5/6 in the presence of DNQX, D-AP5, and picrotoxin. traces were recorded from 14-15 cells from 4 biologically independent mice per group, with similar results obtained. (left) Representative traces at 100 pA injection recorded from SST-LtS cells. jSI group shows reduced spike frequency at 200pA and -100pA (two tailed t-test, \(t_{27} = 3.097\), **\(P = 0.005\), \(n = 14\) cells from 4 biologically independent GH mice, \(n = 15\) cells from 4 biologically independent jSI mice). e, SST-QFS type show comparable excitability between GH and jSI at 100pA (two tailed t-test, \(t_{47} = 1.614\), \(P = 0.113\), \(n = 23\) cells from 6 biologically independent GH mice, \(n = 26\) cells from 6 biologically independent jSI mice). f, g, The jSI group shows decreased excitability in (f) L5/6 SST-AD type at 100pA (two tailed t-test, \(t_{35} = 2.905\), **\(P = 0.006\), \(n = 17\) cells from 6 biologically independent GH mice, \(n = 20\) cells from 6 biologically independent jSI mice), and (g) L2/3 SST-AD type at 100pA (two tailed t-test, \(t_{30} = 2.186\), *\(P = 0.037\), \(n = 24\) cells from 6 biologically independent GH mice, \(n = 18\) cells from 6 biologically independent jSI mice). Data in d-g are presented as mean ± s.e.m.
Extended Data Fig. 9 | Optogenetic stimulation of mPFC→pPVT projection terminals does not change motor activity or anxiety-related behaviors in adult jSI mice. a, CaMKII-Chr2 AAV1 was injected into the mPFC and a wireless blue LED was inserted above the pPVT in jSI mice. b, jSI mice underwent testing in the open field and elevated plus maze (EPM) with (ON) or without (OFF) light stimulation (20 Hz) (c) (left) Viral spread validation from behavior-tested mice. Gray areas represent the minimum (lighter colour) and the maximum (darker colour) spread of Chr2 expression into the mPFC. (right) Optic fiber tip location (blue line circles) was validated in all mice. Experimental images were obtained from 13 mice, three images per mouse for both mPFC and pPVT, with similar results obtained. d, Chr2+ jSI mice showed no differences in motor activity or anxiety-related behaviors between ON and OFF sessions (Left; two tailed paired t-test, \( t_{12} = 0.346, P = 0.735, n = 13 \) biologically independent jSI mice, Middle; two tailed paired t-test, \( t_{12} = 0.431, P = 0.674, n = 13 \) biologically independent jSI mice, Right; two tailed paired t-test, \( t_{12} = 1.364, P = 0.198, n = 13 \) biologically independent jSI mice). e, Control mCherry+ jSI mice showed no difference in motor activity or anxiety-related behaviors between ON and OFF sessions (Left; two tailed paired t-test, Left; \( t_{7} = 0.970, P = 0.365, n = 8 \) biologically independent jSI mice, Middle; \( t_{7} = 0.183, P = 0.860, n = 8 \) biologically independent jSI mice Right; \( t_{7} = 0.083, P = 0.936, n = 8 \) biologically independent jSI mice). f, g, Investigation time of each stimulus during the first 20 mins of 3 chamber testing from Day 1 to Day 4 of ON group (f) and OFF group (g) during the repeated optogenetic stimulation study in Fig. 8e, f. Data in d-g are presented as mean ± s.e.m.
Reporting Summary

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For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

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- For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
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- Estimates of effect sizes (e.g. Cohen’s d, Pearson’s r), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection

- Ethovision v. 9, 14 [Noldus]
- real-time signal processor (RX8, and RZSP, Tucker-Davis Technologies) with software (OpenEx version 2.20 and Synapse version 90, Tucker-Davis Technologies)
- Omniplex system A [Plexon]
- Multiclamp 700B (Axon Instruments)
- pClamp 10 v. 10.6.2.2 (Molecular Devices)
- LSM780 confocal microscopy (Zeiss)
- ANY-Maze v. 6.06 (Stoelting Co.)
- Fusion v. 4 (Omnitech Electronics)

Data analysis

- Prism v. 8 [Graphpad]
- Python v. 2.7 (Python Software Foundation) and scripts previously established and used in a published study (Bicks et al Nature Communications) for fiber photometry data analysis.
- MiniAnalysis v. 6.0.7 (Synaptosoft)
- Signal 4 v.4 (Cambridge Electronic Design)
- Offline sorter v. 3.2.2 (Plexon)
- ImageJ v. 1.52j (National Institutes of Health [NIH])

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Policy information about availability of data
All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:
- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

The source data underlying Figs. 1–8 and Extended Data Figs. 1–9 are provided as source data files. The rest of relevant data are available from the corresponding author on reasonable request.

Field-specific reporting

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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size  No statistical methods were used to pre-determine sample sizes but our sample sizes are similar to those reported in previous publications (e.g. Bicks et al., 2020, Carta et al 2019)

Data exclusions  Mice were excluded from the study if their viral expression could not be validated or when there was equipment failure during behavior, or if they demonstrated significant alterations in motor responses due to i.p. procedures.

Replication  For all studies, independent animals were used as replicates. Replication studies confirmed our results in all experiments.

Randomization  We randomly assigned mice into study groups

Blinding  Data collection and analysis were not performed in a blinded fashion due to the nature of the experiments.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems  n/a  Involved in the study
☐ Antibodies
☐ Eukaryotic cell lines
☐ Palaeontology and archaeology
☐ Animals and other organisms
☐ Human research participants
☐ Clinical data
☐ Dual use research of concern

Methods  n/a  Involved in the study
☐ ChIP-seq
☐ Flow cytometry
☐ MRI-based neuroimaging

Antibodies

Antibodies used
- Rabbit anti-cFos antibody (Synaptic Systems #226003, 1:5000)
- Rabbit anti-SST antibody (Peninsula Laboratories #T-4103.0050; 1:1000)
- Alexa 568 goat anti-rabbit IgG (H+L) (Life Technologies; #A-11036; 1:500)
- Alexa 647 goat anti-rabbit IgG (H+L) (Life Technologies; #A-21245, 1:500)

Validation
- Data sheet and website of Synaptic systems describes that anti-c-fos antibody was validated to react specifically with mouse c-fos, and has been used and cited by over 40 papers.
- Peninsula laboratories do not provide information about the validation of Anti-somatostatin antibody, but we confirmed colocalization with other anti-SST Ab from other company (rat-anti-SST antibody from Millipore #MAB354) and are used in published
Life technologies validated the specificity of Alex 568 goat anti-rabbit IgG (H+L) and Alexa 647 goat anti-rabbit IgG (H+L) (Life Technologies; # A-21245, 1:500) for rabbit immunoglobulins based on their datasheet. To minimize cross-reactivity, these secondary antibodies have been affinity purified and crossadsorbed against bovine IgG, goat IgG, mouse IgG, rat IgG, and human IgG. Cross-adsorption or pre-adsorption is a purification step to increase specificity of the antibody resulting in higher sensitivity and less background staining. The secondary antibody solution is passed through a column matrix containing immobilized serum proteins from potentially cross-reactive species. Only the nonspecific-binding secondary antibodies are captured in the column, and the highly specific secondaries flow through. The benefits of this extra step are apparent in multiplexing/multicolor-staining experiments (e.g., flow cytometry) where there is potential cross-reactivity with other primary antibodies or in tissue/cell fluorescent staining experiments where there may be the presence of endogenous immunoglobulins.

**Animals and other organisms**

Policy information about studies involving animals: ARRIVE guidelines recommended for reporting animal research

| Laboratory animals | Male C57Bl/6 wildtype mice, male Chrna2-Cre mice (Tg(Chrna2-cre)1Kldr), male PV-cre mice (stock number 017320, Jackson Laboratory), male SST-cre mice (stock number 013044, Jackson Laboratory) crossed with Cre-dependent eGFP-L10a mice (Ribo-GFP #024750, Jackson Laboratory) were used. Only male mice were used for experiments. To breed experimental mice, both sex were used for all mouse lines. Animals were group housed in standard laboratory cages in a temperature (72 degrees Fahrenheit)- and humidity (30-70%)- controlled vivarium with a 12:12 light/dark cycle. Food and water were provided ad libitum throughout the experiment. Viral injections were performed when mice were 6-7 weeks old for behavioral experiments, and were performed when mice were 9-10 weeks old for electrophysiological experiments. Behavior experiments took place when mice were 10-15 weeks old. For juvenile social isolation (jSI), wild type C57Bl6 mice (Charles River Laboratories, shipped at p14 for experiments comparing jSI vs GH) and Chrna2-Cre mice (behavioral and electrophysiological experiments) were isolated from weaning (p21) for two weeks. Mice were re-grouped with age- sex- and strain- matched males at p35 (3-5 mice per cage), co-housed for more than 1 month and then behaviorally tested in adulthood when mice were 10-15 weeks old. Slice recordings were performed at p21, p35, and adult 10-15 weeks. |
| Wild animals | No wild animals were used in the study. |
| Field-collected samples | No field collected samples were used in the study. |
| Ethics oversight | All animal protocols were approved by IACUC at the Icahn School of Medicine at Mount Sinai. |

Note that full information on the approval of the study protocol must also be provided in the manuscript.