Unexpected role of interferon-γ in regulating neuronal connectivity and social behaviour

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Immune dysfunction is commonly associated with several neurological and mental disorders. Although the mechanisms by which peripheral immunity may influence neuronal function are largely unknown, recent findings implicate meningeal immunity influencing behaviour, such as spatial learning and memory1. Here we show that meningeal immunity is also critical for social behaviour; mice deficient in adaptive immunity exhibit social deficits and hyper-connectivity of fronto-cortical brain regions. Associations between rodent transcriptomes from brain and cellular transcripts in response to T-cell-derived cytokines suggest a strong interaction between social behaviour and interferon-γ (IFN-γ)-driven responses. Concordantly, we demonstrate that inhibitory neurons respond to IFN-γ and increase GABAergic (γ-aminobutyric-acid) currents in projection neurons, suggesting that IFN-γ is a molecular link between meningeal immunity and neural circuits recruited for social behaviour. Meta-analysis of the transcriptomes of a range of organisms reveals that rodents, fish, and flies elevate IFN-γ/JAK-STAT-dependent gene signatures in a social context, suggesting that the IFN-γ signalling pathway could mediate a co-evolutionary link between social/aggregation behaviour and an efficient anti-pathogen response. This study implicates adaptive immune dysfunction, in particular IFN-γ, in disorders characterized by social dysfunction and suggests a co-evolutionary link between social behaviour and an anti-pathogen immune response driven by IFN-γ signalling.

Social behaviour is beneficial for many processes critical to the survival of an organism, including foraging, protection, breeding, and, for higher-order species, mental health23. Social dysfunction manifests in several neurological and mental disorders such as autism spectrum disorder, frontotemporal dementia, and schizophrenia, among others4. Likewise, imbalance of cytokines, a disparity of T-cell subsets, and overall immune dysfunction is often associated with the above-mentioned disorders5,7. However, the fundamental mechanism(s) by which dysfunctional immunity may interfere with neural circuits and contribute to behavioural deficits remain unclear.

To test whether adaptive immunity is necessary for normal social behaviour, we tested SCID mice (deficient in adaptive immunity) using the three-chamber sociability assay9 (Extended Data Fig. 1a). This assay quantifies the preference of a mouse for investigating a novel mouse versus an object, and has been used to identify deficits in multiple mouse models of disorders that present with social dysfunction7. Unlike wild-type mice, SCID mice lacked social preference for a mouse over an object (Fig. 1a). Importantly, SCID mice did not show anxiety, motor, or olfactory deficits (Extended Data Fig. 1b–j). We confirmed that SCID mice have social deficits by analysing social interactions in a home cage (Extended Data Fig. 1k). To test whether social deficits were reversible, we repopulated 4-week-old SCID mice with wild-type lymphocytes (Extended Data Fig. 11–n) and measured social behaviour 4 weeks after transfer. SCID mice repopulated with lymphocytes, unlike those injected with the vehicle, showed social preference indistinguishable from wild-type mice (Fig. 1b).

Recent clinical findings indicate that disturbed circuit homeostasis, resulting in hyper-connectivity, is a feature of children with autism spectrum disorder10. Imaging studies using task-free resting-state fMRI (rsfMRI), revealed hyper-connectivity among frontal cortical nodes in patients with autism spectrum disorder11. Disturbances in resting state connectivity are also observed in mice with social deficits12. rsfMRI is an unbiased technique used to assess synchrony between brain regions over time by comparing spontaneous fluctuations in blood oxygenation level-dependent (BOLD) signals13. To assess the influence of adaptive immunity on functional connectivity, we analysed resting-state BOLD signals from wild-type and SCID mice (Extended Data Fig. 2a). SCID mice exhibited hyper-connectivity between multiple frontal and insular regions (Fig. 1c, d, Extended Data Fig. 2b and Supplementary Table 1) implicated in social behaviour and autism spectrum disorder. Notably, repopulating SCID mice with lymphocytes rescued aberrant hyper-connectivity observed in vehicle-treated SCID controls (Fig. 1c, d and Extended Data Fig. 2b). Interestingly, other functionally connected regions, not directly implicated in social function, such as interhemispheric connectivity between motor and somatosensory cortex, were not affected by a deficiency in adaptive immunity (Supplementary Table 1). Using another approach to analyse neuronal activation in a task-based system, we demonstrated that SCID mice exposed to a social stimulus exhibited hyper-responsiveness in the prefrontal cortex (PFC; increased number of c-fos+ cells in PFC; Fig. 1e, f) but not the hippocampus (Extended Data Fig. 2c).

We previously demonstrated that T cells influence learning behaviour and exert their beneficial effects presumably from the meninges1,14. To address the role of meningeal T cells in social behaviour, we decreased the extravasation of T cells into the meninges of wild-type mice using antibodies against VLA4 (ref. 15), an integrin expressed on T cells (among other immune cells) required for CNS homing. Partial elimination of T cells from meninges (Extended Data Fig. 3) was sufficient to cause a loss in social preference (Fig. 1g). Despite their proximity to the brain, meningeal T cells do not enter the brain parenchyma, suggesting their effect is mediated by soluble factors. To

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identify which T-cell-mediated pathways are involved in regulating social behaviour, we used gene set enrichment analysis (GSEA) to search for T-cell-mediated response signatures (IFN-γ, IL-4/IL-13, IL-17, IL-10, TGF-β) in 41 transcriptomes from mouse and rat brain cortices. GSEA assesses whether the expression of a previously defined group of related genes is enriched in one biological state. In this case, Correlation matrix from the three-chamber test (a, b, g) were analysed by applying a two-way ANOVA for social behaviour and genotype/treatment, followed by Sidak’s post hoc test. Bars, mean times investigating ± s.e.m. Antidepress., antidepressants; anticonvuls., anticonvulsants; antipsy., antipsychotics.

f, Elevated numbers of c-fos+ cells in the prefrontal and orbital cortices of SCID compared with wild-type mice (n = 9–10 mice per group; **P < 0.01, Sidak’s post hoc test; pooled two independent experiments). g, Acute partial depletion of meningeal T cells caused social deficits (n = 12, 13 mice per group; ANOVA for interaction (F(2,33) = 7.900, P < 0.01; **P < 0.001, Sidak’s post hoc test; pooled two independent experiments). h, Circos plot showing the connectivity map derived from the pairwise comparison of transcriptome data sets. The representations of IFN-γ, IL-4/IL-13, IL-17, and IL-10/ TGF-β data set connectivity are shown in orange, green, blue, and purple, respectively. Each line represents a pairwise data set overlap, which was determined using GSEA analysis and filtered by P < 0.05 and normalized enrichment score (NES) > 1.5. See Extended Data Fig. 5 for labels. Data from the three-chamber test (a, b, g) were analysed by applying a two-way ANOVA for social behaviour and genotype/treatment, followed by Sidak’s post hoc test. Bars, mean times investigating ± s.e.m. Antidepress., antidepressants; anticonvuls., anticonvulsants; antipsy., antipsychotics.

Meningeal T-cell compartment is necessary for supporting neuronal connectivity and social behaviour. a, Wild-type mice exhibit social preference absent in SCID mice (ANOVA for genotype (F(2,30) = 6.370, P = 0.0181; n = 14 mice per group; **P < 0.01, Sidak’s post hoc test; pooled two independent experiments). b, Repopulating the adaptive immune system in SCID mice restored normal social behaviour (n = 17, 16, 15 mice per group; ANOVA for genotype (F(2,34) = 8.282, P = 0.0009 and interaction (F(2,68) = 9.146, P = 0.0005; **P < 0.001; **P < 0.01, Sidak’s post hoc test; pooled three independent experiments). c, Correlation matrices from wild-type, SCID, and repopulated (Repop.) SCID mice were generated by rsfMRI. L, left; R, right; FrA, frontal association area; PrL, prelimbic cortex; Ins, insula; OrbC, orbital cortex. d, Correlation values from rsfMRI. Box and whisker plots extend to the 25th and 75th percentiles with the centre-line showing the mean. Whiskers represent the minimum and maximum data points (n = 8, 9, 4 mice per group; ANOVA < 0.05; *P < 0.05, Sidak’s post hoc test; pooled two independent experiments). e, Immunohistochemistry of c-fos in the PFC.

A substantial number of meningeal T cells are capable of expressing IFN-γ (41.95% ± 6.34 of TCR+ cells; Extended Data Fig. 5a) and recent work has proposed a role for IFN-γ in T-cell trafficking into meningeal spaces26. To assess the potential role of IFN-γ in mediating the influence of T cells on social behaviour, we first examined the social behaviour of IFN-γ-deficient mice and determined that they had social deficits (Fig. 2a). Importantly, IFN-γ-deficient mice did not show anxiety or motor deficits (Extended Data Fig. 5b–g). Similar to SCID mice, IFN-γ-deficient mice also exhibited aberrant hyper-connectivity in fronto-cortical/insular regions (Fig. 2b, c and Supplementary Table 1). Although repopulating SCID mice with lymphocytes from wild-type mice restored a social preference, repopulating SCID mice with lymphocytes from Ifng−/− mice did not have such an effect (Extended Data Fig. 6a). Remarkably, a single injection of recombinant IFN-γ into the cerebrospinal fluid (CSF) of Ifng−/− mice was sufficient to restore their social preference when tested 24 h after injection (Fig. 2d) and reduce overall hyper-connectivity in the PFC (Extended Data Fig. 6b). To further validate a role for IFN-γ signalling in social behaviour, we tested mice deficient for the IFN-γ receptor (Ifngr1−/− mice) and found that they had a similar social deficit as observed in Ifng−/− mice (Extended Data Fig. 6c), which, as expected, was not rescued by injecting recombinant IFN-γ into the CSF (Extended Data Fig. 6d). On the basis of our previous demonstration of a role for IL-4 produced by meningeal T cells in spatial learning behaviour1, we assessed whether deficiency in IL-4 would also result in social deficits. IL-4-deficient mice did not demonstrate social deficits; in fact, they spent more time investigating a novel mouse than a novel object compared with wild-type mice (Extended Data Fig. 6e).
To determine which cell types in the brain respond to IFN-γ, we analysed mouse PFC for the expression of IFN-γ receptor subunits 1 and 2 and found that both neurons and microglia express mRNA and protein for R1 and R2 subunits of the IFN-γ receptor (Fig. 2e, f and Extended Data Fig. 7). Microglia are CNS-resident macrophages and are known to express the IFN-γ receptor. However, genetically deleting STAT1, the signalling molecule downstream of the IFN-γ receptor, from microglia (and other cells of myeloid origin), did not disturb normal social preference (Extended Data Fig. 8). These results led us to focus on neuronal responses to IFN-γ as they relate to social behaviour.

To assess a role for IFN-γ in neuronal signalling, we deleted Ifngr1 in PFC neurons via adeno-associated virus (AAV) delivery of Cre recombinase under the Synapsin I promoter (Extended Data Fig. 9). Attenuating IFN-γ signalling in PFC neurons was sufficient to alter mouse behaviour in a three-chamber social task and result in a lack of social preference (Fig. 2g), reinforcing the importance of IFN-γ signalling on neurons for social behaviour. Injecting recombinant IFN-γ into the CSF activated layer I neurons in mice as assessed by c-fos immunoreactivity (Extended Data Fig. 10a, b). These neurons are almost entirely inhibitory18, suggesting that IFN-γ may drive regional inhibition of circuits by directly activating layer I inhibitory neurons located in close proximity to the brain surface and CSF. To investigate mechanisms downstream of the IFN-γ receptor, we used Vgatcre::Stat1fl/fl mice. Deletion of STAT1 from GABAergic inhibitory neurons was sufficient to induce deficits in social behaviour (Fig. 2h), suggesting that the IFN-γ may be signalling through inhibitory neurons.

To directly assess if IFN-γ can drive inhibitory tone in the PFC, we measured inhibitory currents in layer II/III pyramidal cells from acutely prepared brain slices from wild-type mice. In addition to receiving phasic inhibitory synaptic input, these cells are also held under a tonic GABAergic current that serves to hyperpolarize their resting membrane potential (Fig. 2i). Tonic GABAergic currents are extrasynaptic and can yield long-lasting network inhibition19. We observed that IFN-γ augmented tonic current (Fig. 2i, j and Extended Data Fig. 10c, d), suggestive of elevated levels of ambient GABA during application of IFN-γ. Deleting the IFN-γ receptor from inhibitory neurons (Vgatcre::Ifngr1fl/fl mice) prevented IFN-γ from augmenting tonic inhibitory current (Extended Data Fig. 10e). Given that IFN-γ promotes inhibitory tone, we tested whether IFN-γ could prevent aberrant neural discharges by injecting IFN-γ into the CSF and then chemically inducing seizures with the GABA type A receptor antagonist pentylentetrazole (PTZ). Mice injected with IFN-γ were less prone to seizures than controls (Fig. 2j).
Since low-aggressive flies upregulate genes in the JAK/STAT pathway with an interaction between social behaviour and the anti-pathogen signature is upregulated in aggregated organisms. This is consistent with the observation that immune response programs (Supplementary Tables 3–7). Zebrafish and domesticated mice with diazepam to augment GABAergic transmission20. It is intriguing that IFN-γ delayed seizure onset and lowered seizure severity (Fig. 2k). Further, to test whether overcoming causes social deficits in IFN-γ-deficient mice, we treated Ifng−/− mice with diazepam to augment GABAergic transmission20. Diazepam successfully rescued social behaviour of Ifng−/− mice, similar to the effect observed with recombinant IFN-γ treatment (Fig. 2l), suggesting that social deficits, caused by a deficiency in IFN-γ, may arise from inadequate control of GABAergic inhibition by IFN-γ.

It is intriguing that IFN-γ, predominately thought of as an anti-pathogen cytokine, can play such a profound role in maintaining proper social function. Since social behaviour is crucial for the survival of a species and aggregation increases the likelihood of spreading pathogens, we hypothesized that there was co-evolutionary pressure to increase an anti-pathogen response as sociability increased, and that the IFN-γ pathway may have influenced this co-evolution. To test this hypothesis, we analysed metadata of publically available transcriptomes from multiple organisms including the rat, mouse, zebrafish, and fruit fly. Using GSEA, we determined that transcripts from social rodents (acutely group-housed) are enriched for an IFN-γ responsive gene signature (Fig. 3a, b and Supplementary Tables 3–7). Conversely, rodents that experienced social isolation demonstrated a dramatic loss of the IFN-γ responsive gene signature (Supplementary Tables 3–7). Zebrafish and flies showed a similar association between anti-pathogen and social responses (Fig. 3c, d). We observed that immune response programs were highly enriched in the brain transcriptomes of flies selected for low aggressiveness traits (a physiological correlate for socially experienced flies21; Fig. 3d and Supplementary Tables 3–7). We next analysed the promoters of these highly upregulated social genes and found them to be enriched for STAT1 transcription factor binding motifs (Fig. 3a–d). These data suggest that, even in the absence of infection, an IFN-γ gene signature is upregulated in aggregated organisms. This is consistent with an interaction between social behaviour and the anti-pathogen response, a dynamic that could be mediated by the IFN-γ pathway. Since low-aggressive flies upregulate genes in the JAK/STAT pathway (canonical downstream of IFN-γ receptors in higher species), yet lack IFN-γ or T cells, it is intriguing to speculate that T-cell-derived IFN-γ may have evolved in higher species to more efficiently regulate an anti-pathogen response during increased aggregation of individuals.

Our results reveal a novel role for meningeal immunity in regulating neural activity and social behaviour through IFN-γ. The role of immune molecules has been previously shown to control brain development and function22–26. A role for cytokines in influencing behaviour has been proposed, primarily in the context of sickness behaviour and pain27,28. These examples, however, have predominately focused on peripheral nerves and non-neuronal targets. Here we show that CNS neurons directly respond to IFN-γ derived from meningeal T cells to elevate tonic GABAergic inhibition and prevent aberrant hyper-excitability in the PFC. These data suggest that social deficits in numerous neurological and psychiatric disorders may result from impaired circuitry homeostasis derived from dysfunctional immunity. On the basis of our findings, it is also plausible that subtle homeostatic changes in meningeal immunity may also contribute to modulating neuronal circuits that are responsible for our everyday behaviours and personality. Given this communication between immunity and neuronal circuits29, it is intriguing to hypothesize that these pathways might be vulnerable to manipulation by fast-evolving pathogens. Improved comprehension of these pathways could improve our understanding of the aetiology of neurodevelopmental and neuropsychiatric disorders associated with maternal infections and/or aberrant inflammation and may result in the development of new therapeutic targets.

**Online Content** Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

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Author Contributions A.J.F. and J.K. designed and performed experiments and wrote the manuscript. Y.X. and V.L. provided intellectual contributions and analysed all transcriptome data. S.D.T., Z.W., S.N.P., and H.C. analysed RNA-seq data. N.T. and C.C.O. analysed BOLD data. R.L.M., W.B., I.S., S.P.G., M.M.S. and M.P.B. provided intellectual contributions and assisted with experimental procedures. M.P.B. performed all electrophysiological experiments. K.S.L. critically reviewed the manuscript.

Author Information RNA-seq data have been deposited in the Gene Expression Omnibus under accession number GSE81783. Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to A.J.F. (afiliano@virginia.edu), V.L. (Vladimir.Litvak@umassmed.edu) or J.K. (kipnis@virginia.edu).

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METHODS

Mice. All mice (C57BL/6) were either bred in-house or purchased from the Jackson Laboratory. For each individual experiment, the control mice were obtained from the same institution as test mice. In the case that mice were purchased, they were maintained for at least 1 week to habituate before manipulation/experimentation. When possible, mice used for experiments were littermates. These include all electrophysiology experiments, all cohorts using Stat1fffl mice, experiments analysing inducible nNOS expression, and experiments using male/female pairing to test for differences between regions of interest. If a connection strength was above the threshold, it was kept as an edge in the network, otherwise it was discarded. When comparing networks from multiple sample groups, the threshold was determined by calculating the maximum threshold that left one of the networks connected (that is, it was possible to reach any node in the network from any other node).

Immunohistochemistry. Mice were killed under Euthasol then transcardially perfused with PBS with heparin. Brains were removed and drop fixed in 4% PFA for 48 h. After fixation, brains were washed with PBS, cryoprotected with 30% sucrose, then frozen in O.C.T. compound (Sakura Finetek) and sliced (40 μm) with a cryostat (Leica). Free-floating sections were maintained in PBS + Azide (0.02%) until further processing. Immunohistochemistry for c-fos (1:1000 dilution; Millipore) on free-floating sections was performed as previously described38.

Depletion of meningeal T cells. A rat monoclonal antibody to murine VLA4 (clone PS/2) was affinity purified from hybridoma supernatants and was used with the permission of K. Ley (La Jolla Institute of Allergy and Immunology, San Diego, CA). Mice were given two separate injections (intraperitoneal; 0.2 mg in saline per mouse), 4 days apart, of either anti-VLA4 or rat anti-HRP for control (clone HRPN; BioXcell), then tested 24 h after final injection.

Dissection of meninges and flow cytometry. Meninges were dissected as previously described4. Briefly, after killing and perfusing, skulls caps were removed by making an incision along the parietal and squamosal bones. The meninges were removed from the internal side of the skull cap and gently pressed through a 70 μm nylon mesh cell strainer with sterile plastic plunger (BD Biosciences) to isolate a single cell suspension. Cells were then centrifuged at 300g at 4°C for 10 min, resuspended in cold FACS buffer (pH 7.4; 0.1 M PBS; 1 mM EDTA; 1% BSA), and stained for extracellular markers using the following antibodies at a 1:200 dilution: CD45 PerCP-Cy5.5 (eBioscience), TCR Vβ5 (BD Bioscience), CD4 FITC (eBioscience), L/D Zombie NIR (BioLegend). To measure intracellular IFN-γ, single-cell isolates from meninges were maintained in T-cell isolation buffer (RPMI + 2% FBS, 2 mM l-glutamine, 1 mM sodium pyruvate, 10 mM HEPES, 1× non-essential amino acids, and 1× Antibiotic-Antimycotic (Thermo Fisher) and stimulated with PMA/ionomycin (Cell Stimulation Cocktail – eBioscience) + 10 μg/ml brefeldin A at 37°C for 48 h. After fixation, brains were washed with PBS, cryoprotected with 30% sucrose, then frozen in O.C.T. compound (Sakura Finetek) and sliced (40 μm) with a cryostat (Leica). Free-floating sections were maintained in PBS + Azide (0.02%) until further processing. Immunohistochemistry for c-fos (1:1000 dilution; Millipore) on free-floating sections was performed as previously described38.

To measure expression of IFN-γ receptors, brains were removed and placed in Neurobasal media containing 10% fetal bovine serum. The meninges were removed from the brain and the frontal cortex was micro-dissected under a dissection microscope. Using a 2 ml dounce homogenizer, brains were homogenized in Neurobasal media with 50 U/ml Dnase I. The homogenate was passed through a 70 μm nylon mesh filter and washed with cold FACS buffer. INFγR1 were labelled with anti-INFγR1 Biotin (BD Pharmingen; GR20) or rabbit anti-INFγR2 (Santa Cruz; M-20). Cells were washed with FACS buffer and incubated with FITC conjugated streptavidin or 488 conjugated chicken anti-rabbit. Next, cells were washed again then incubated with CD11B PE-Cy5 (eBioscience), L/D Zombie NIR (BioLegend), and Hoechst for 45 min. Cells were washed, then permeabilized and fixed with Cytofix/Cytoperm (BD Biosciences). After another wash with permeabilization wash buffer (PBS with 10% fetal bovine serum, 1% sodium azide, and 1% saponin; pH 7.4), cells were incubated overnight with NeuN PE (Millipore). Cells underwent a final wash and again passed through a 70 μm nylon filter. Samples were run on a flow cytometer Gallios (Beckman Coulter) then analysed using FlowJo software (Treestar).

SCID repopulation. SCID mice were repopulated with cells from spleen and lymph nodes (axillary, brachial, cervical, inguinal, and lumbar). Spleen and lymph nodes were collected from a 3-to 4-week-old donor and passed through a 70 μm nylon mesh cell strainer with a sterile plastic plunger. ACK buffer was used to lyse red blood cells before washing with saline. Cells were counted on an automated cell counter (Nexcelom) and injected (intravenously) at 5 × 106 cells in 250 μl of saline. Control mice were injected with 250 μl of saline only. For injections, the animal technician was blinded to the genotype of the mice and the content of the injection. Thus, all groups were handled identically. Mice aged 3–4 weeks were carefully placed into a tail vein injection platform. Their tails were briefly warmed using a heating pad and saline with cells or saline alone was slowly injected into the tail vein using a 28-gauge needle. After the injection, mice were returned to their home cage.
IFN-γ injections. Mice were anaesthetized with a ketamine/xylazine (ketamine (100 mg/kg) and xylazine (10 mg/kg)) injection (intrapерitoneally) or isoflurane (2%), then placed into a stereotaxic frame with the head at an approximately 45° angle. The skin above the cisterna magna was cleaned and sanitized before a 1 cm incision was made. The underlying muscles were separated with forceps, retracted, and a small Hamilton syringe (33-gauge) was used to slowly inject 1 μl of saline or IFN-γ (20 ng/μl) into the cisterna magna. After injection the syringe was held in place for 5 min to avoid back-flow of CSF. After the syringe was removed, mice were put back in place and skin was sutured. Mice were placed on a heating pad and given ketoprofen and buprenorphine for recovery.

Induced seizures. IFN-γ was injected into the CSF through the cisterna magna as described above, 24h before inducing seizures. Control mice were injected with the same volume of saline. To induce seizures, mice were injected with PTZ (40 mg/kg; intraperitoneally). After injection, mice were placed into an empty housing cage and recorded for video analysis. Seizures were analysed by a blinded observer using a behaviour scoring system previously published.

Diazepam treatment. Diazepam (1.25 mg/kg) was delivered intraperitoneally for 30 min before testing for social behaviour.

Fluorescence in situ hybridization. Mice were euthanized then transcardially perfused with PBS with heparin followed by 4% PFA. Brains were then removed and drop fixed in 4% PFA for 24h, frozen in OCT, and 12μM sections were cut on a cryostat. Fluorescence in situ hybridization was performed using RNA ISH tissue assay kits (Affymetrix) following the manufacturer’s protocol. Tissues were treated with protease for 20 min at 40°C. Images at 63× magnification were acquired on a Leica TCS SP8 confocal system (Leica Microsystems) using LAS AF Software.

AAV delivery. AAV1.hSyn.hGFP-Cre.WPRE.SV40 and AAV1.hSyn.eGFP.WPRE.bGH were purchased from Penn Vector Core. Ifne2-LoxP mice were purchased from the Jackson Laboratory. After 1 week of habitation, mice were anaesthetized with 2% isoflurane and injected bilaterally with 2 × 10^11 genome copies of AAV virus in 1 μl at stereotactic coordinates +2.5 μm bregma A/P, 0.25 μm lateral, 1.25 μm deep.

Measuring inhibitory currents. Visualized whole-cell patch-clamp recordings were performed on layer II/III prefrontal cortical neurons prepared from acute brain slices (adult) using the protective recovery method. Recordings were performed in 34°C artificial cerebrospinal fluid (ACSF) containing (in mM) 131/5 NaCl, 25 NaHCO3, 12 n-glucose, 2.5 KCl, 1.25 NaH2PO4, 2 CaCl2, and 1 MgCl2. ACSF also contained 3 mM kynurenic acid to block synaptic excitation and 2.5 Mm NO-711 to enhance tonic inhibition. Slices were incubated in this ACSF for 5–10 min before placement in the recording chamber. The patch pipette solution with elevated chloride content (in mM) 140/5 NaCl, 1 MgCl2, 10 HEPES, 0.05 EGTA, 2 Mg-ATP, and 0.4 Mg-GTP once. On-line recordings, baseline holding current in ACSF was measured for 3.5 min, after which ACSF containing IFN-γ (20 mg/ml) was applied for 8.5 min and then washed. Data presented show the mean holding current during the last minute of control (ACSF) and drug (ACSF + IFN-γ) conditions.

RNA isolation and sequencing. Eight-week-old male mice were purchased from the Jackson Laboratory and housed in standard housing boxes with either four mice per cage or isolated for 6 days. Mice were anaesthetized as described above and the PFC was microdissected under a dissection microscope. RNA was isolated using an RNAeasy mini kit (Qiagen) and a cDNA library was generated with a TrueSeq Stranded mRNA Library Prep Kit (Illumina) with Agencourt AMPure PX beads for PCR cleanup. Samples were loaded onto a NextSeq 500 High-output 75 cycle cartridge and sequenced on a NextSeq 500 (Illumina).

Transcriptome analysis. Raw FASTQ sequencing reads were chastity filtered to remove clusters having outlying intensity corresponding to bases other than the called base. Filtered reads were assessed for quality using FastQC tools. Reads were mapped to the UCSC genome build using STAR tools. Reads overlapping UCSC mm9 gene regions were counted using featureCounts tools. The DESeq2 Bioconductor package was in the R statistical computing environment was used for normalizing count data, performing exploratory data analysis, estimating dispersion, and fitting a negative binomial model for each gene comparing the expression from the PFC of mice in a social environment versus isolation. After obtaining a list of differentially expressed genes, log(fold changes), and P values, Benjamini–Hochberg false discovery rate procedure was used to correct P values for multiple testing. A gene set enrichment analysis (GSEA) algorithm was applied to identify the enrichment of transcriptional signatures and molecular pathways in PFC transcriptomes of mice exposed to group and isolation housing conditions. Four thousand seven hundred and twenty-six publicly available transcriptional signatures were obtained from the molecular Signature Database C2 version 4.0, and GSEA was used to examine the distribution of these curated gene sets in lists of genes ordered according to differential expression between group and isolation housing conditions. We analysed statistics by evaluating nominal P value and normalized enrichment score (NES) on the basis of 1,000 random sample permutations.

Meta-data analysis. The custom-made IFN-γ and pathogen-induced transcriptional signatures (Supplementary Table 2) were generated by retrieving genes upregulated at least twofold following IFN-γ stimulation or pathogen infection. All custom signatures were derived from publicly available transcriptomes downloaded from Gene Expression Omnibus (GEO). Specifically, mammalian IFN-γ transcriptional signatures were derived from transcriptomes GSE33057, GSE19182, GSE35659, GSE4710, GSE5633, and GSE35635. Zebrafish IFN-γ transcriptional signature was used as described. Drosophila pathogen-induced JAK/STAT-dependent transcriptional signatures were derived from transcriptomes GSE54833 and GSE8282.

A GSEA algorithm was applied to identify the enrichment of custom-made mammalian IFN-γ transcriptional signatures in the publicly available brain cortex transcriptomes of mice and rats exposed to social aggregation, sleep deprivation, stress, psychostimulants (DOI, cocaine, amphetamine, methamphetamine, methylphenidate, caffeine, nicotine, and modafinil), antipsychotics (olanzapine, haloperidol, and risperidone), anticonvulsants (levetiracetam, phenytoin, ethosuximide, and oxcarbazepine), and antidepressants (iproniazid, moclobemide, paroxetine, and phenelzine). In total, 41 transcriptomes were analysed as indicated in Supplementary Table 2. We analysed statistics by evaluating nominal P value and NES on the basis of 1,000 random gene set permutations.

Social interactions in Drosophila melanogaster flies play an important role in courtship, mating, egg-laying, circadian timing, food search, and even lifespan determination. Notably, domesticated zebrafish strains demonstrate higher social interaction and social novelty preference compared with wild zebrafish strains. Therefore, a GSEA algorithm was used to identify the enrichment of zebrafish IFN-γ transcriptional signature in the publicly available whole-brain transcriptomic profiles of behaviourally distinct strains of domesticated and wild zebrafish (Supplementary Table 7). The brain transcriptomic profiles of domesticated (Scientific Hatcheries (SH) and Transgenic Mosaic 1 (TM1)) and wild (Nadia, Gaighata) zebrafish strains were derived from publicly available transcriptome data set GSE38729. We analysed statistics by evaluating nominal P value and NES on the basis of 1,000 random gene set permutations.

Promoter motif analysis. A GSEA algorithm was used to identify genes that are differentially expressed in brain transcriptomes of mice and rats exposed to social aggregation, domesticated zebrasfish strains, and low-aggressive Drosophila melanogaster populations, compared with their control counterparts. High-scoring differentially expressed ‘leading-edge’ social genes were selected on the basis of their presence in the IFN-γ and pathogen-induced transcriptional signatures. Specifically, as shown in Fig. 3, we have identified 31, 48, 14, and 53 leading-edge genes in brain transcriptomes of mice and rats exposed to social aggregation, domesticated Zebrafish strain, and low-aggressive Drosophila melanogaster population, respectively. We next extracted promoter sequences of 200 bp upstream of transcription start sites of these ‘leading-edge’ genes using UCSC Genome Browser (https://genome.ucsc.edu/). The MEME suite was then used to discover overrepresented transcription factor binding motifs, as described. MEME parameters used were any number of motif repetitions per sequence, with a minimum motif width of 5 bases and maximum motif width of 15 bases. The discovered MEME motifs were compared using Tomtom analysis. In this case, the Tomtom motif similarity analysis ranks the MEME motif most similar to the vertebrates in vivo and in silico. The statistics were determined using Euclidean distance.

Circos plot. A GSEA algorithm was applied to identify the enrichment of IFN-γ, IL-4/IL-13, IL-17, and IL-10/TGF-β signalling pathways in the brain transcriptomes of rodent animals exposed to social aggregation, stress, psychostimulants, and antidepressants. All custom signatures were derived from publically available rodent transcriptomes downloaded from Gene Expression Omnibus. Statistical significance of GSEA results was assessed using 1,000 sample permutations. A NES greater than 1.5 and a nominal P value less than 0.05 was used to determine pairwise transcriptome connectivity. A Circos graph was generated using Circos package 0.68.12 (ref. 66).
Statistics. Data were analysed using the statistical methods stated in each figure legend. For the three-chamber assay, a two-way ANOVA was performed using genotype/treatment and sociability as main effects, followed by applying a Sidak’s post hoc comparison to assess if the group had a significant social preference. Before running an ANOVA, an equality of variance was determined by using a Brown–Forsythe test. Bars display the means, and error bars represent ranges of the standard error of the mean. For rsfMRI, data were analysed using a one-way ANOVA followed by a post hoc Tukey’s test. The box and whisker plots extend to the 25th and 75th percentiles and the centre line indicates the mean. The whiskers represent the min and max data points. Data for seizure latency were analysed using a two-way ANOVA with repeated measures followed by Sidak’s post hoc test. Additional details of statistical analysis are supplied in Supplementary Table 8.
Extended Data Figure 1 | SCID mice have no observable anxiety, motor, or olfactory deficits. a, The three-chamber sociability assay was used to test social behaviour. b, Neither wild-type nor SCID mice had a side bias in the habituation phase (empty cups) of the three-chamber assay (n = 6; repeated at least three times). c, There was no effect of genotype on distance travelled in the three-chamber assay during the habituation phase (n = 6; repeated at least three times). d, Both wild-type and SCID mice had an olfactory preference to urine, suggesting normal olfactory behaviour (n = 8 mice per group; ANOVA for urine preference F1,28 = 31.01; P < 0.0001; ***P < 0.001, **P < 0.01, Sidak’s post hoc test; single experiment). e, Percentage time spent in the open arms of plus-maze (n = 22 mice per group; pooled two independent experiments). f, Number of entries into the open arms of the plus-maze (n = 22 mice per group; pooled two independent experiments). g, Total arm entries of plus-maze (n = 22 mice per group; pooled two independent experiments). h, Percentage time spent in the centre of the open field (n = 22 mice per group; pooled two independent experiments). i, Total ambulatory distance in the open field (n = 22 mice per group; pooled two independent experiments). j, Latency to fall off the accelerating rotarod (n = 8 mice per group; single experiment). k, SCID mice spent less time investigating each other than wild-type mice spent investigating each other when placed into a novel social environment (n = 5 mice per group; repeated-measures ANOVA for genotype F1,21 = 5.708 *P < 0.05; single experiment). l, Repopulated SCID mice have similar numbers (m) and percentages (n) of meningeal T cells as wild-type mice (n = 4–5 mice per group; repeated at least three times). Cells were gated on singlets, live, CD45⁺, and TCR.
Extended Data Figure 2 | Neuroanatomical structures analysed by rsfMRI. 

a, Regions of interests (ROIs) were generated using The Mouse Brain by Paxinos and Franklin as a reference. Representative slices were extracted from ref. 32. Abbreviations are as follows: FrA, frontal association cortex; PrL, prelimbic cortex; OrbC, orbital cortex; OB, olfactory bulb; MC, motor cortex; SocC, somatosensory cortex; Ins, insula; PirF, piriform cortex; CpU, caudate putamen; Acb, accumbens; ACC, anterior cingulate cortex; dHip, dorsal hippocampus; T, thalamus; Amyg, amygdala; EntC, entorhinal cortex; Hyp, hypothalamus; VisC, visual cortex; SupC, superior colliculus; PAG, periductal grey; DpMe, deep mesencephalic nucleus; vHip, ventral hippocampus; SNR, substantia nigra; VTA, ventral tegmental area; CB, cerebellum; BS, brain stem.

b, Connectivity of local PFC/insula nodes. Correlation thresholds were applied to visualize the strength of the connection. Connections that pass a high threshold are shown in red; connections that pass a lower threshold are shown in dashed grey. SCID mice have aberrant hyper-connectivity in the PFC ($n = 8–9$ mice per group; $P < 0.05$, Jennrich test; two pooled independent experiments).

c, $c$-fos$^+$ cells in the hippocampus ($n = 9–10$ mice per group; single experiment).
Extended Data Figure 3 | Acute reduction of meningeal T cells with anti-VLA4. a, Anti-VLA4 depletes meningeal T cells. Meninges were dissected and single-cell suspensions were immunostained. T cells were gated on live, single, CD45⁺, TCR⁺ events and counted by flow cytometry. b, Acute injection of anti-VLA4 reduced the amount of TCR⁺ T cells in the meninges (n = 4 mice per group; *P < 0.01; repeated at least twice).
Extended Data Figure 4 | Circos plot showing the connectivity of Th1 response and social aggregation. Labels are shown for the data sets analysed and presented in Fig. 1h.
Extended Data Figure 5 | T cells in the meninges produce IFN-γ and IFN-γ-deficient mice have normal levels of anxiety and motor behaviour. a, A substantial percentage of meningeal T cells produce IFN-γ. Cells were gated for live, singlets, CD45⁺, and TCR⁺. Ifng⁻/⁻ mice were used to gate for IFN-γ staining. b, Percentage time spent in open arms of the plus-maze (n = 20 mice per group; pooled two independent experiments). c, Entries into the open arms of plus-maze (n = 20 mice per group; pooled two independent experiments). d, Total entries into all arms of the plus-maze (n = 20 mice per group; pooled two independent experiments). e, Percentage time spent in the centre of the open field (n = 20 mice per group; pooled two independent experiments). f, Total ambulatory distance in the open field (n = 20 mice per group; pooled two independent experiments). g, Latency to fall off the accelerating rotarod (n = 8 mice per group; single experiment).
Extended Data Figure 6 | IFN-γ signalling is necessary for normal social behaviour. a, Repopulating SCID mice with wild-type lymphocytes rescued a social preference; repopulating with Ifng−/− lymphocytes did not rescue a social preference; ANOVA for social behaviour F₁,₁₄ = 11.99; P = 0.0038 (**P < 0.01; n = 8 mice per group; single experiment). b, Connectivity of local PFC/insular nodes. Correlation thresholds were applied to visualize the strength of the connection. Connections that pass a high threshold are shown in red; connections that pass a lower threshold are shown in dashed grey. Ifng−/− mice have more connections than wild-type mice (Jennerich test; P = 0.0006). These connections were reduced by IFN-γ (Jennerich test; P = 0.02; pooled two independent experiments). c, Ifngr1−/− mice have social deficits (n = 6 mice per group; ANOVA for interaction P = 0.01; ***P < 0.01 Sidak’s post hoc test) that were not rescued by injecting IFN-γ into the CSF (d; n = 5–6 mice per group; ANOVA for interaction P = 0.01; ***P < 0.01 Sidak’s post hoc test; single experiment). e, Il4−/− mice spend more time than wild-type mice investigating a novel mouse; ANOVA for genotype F₁,₃₂ = 5.397; P = 0.0267 (*P < 0.05 Sidak’s post hoc test; n = 16–18 mice per group; pooled three independent experiments).
Extended Data Figure 7 | Gating strategy for neurons and microglia. Brain homogenates were stained and analysed by flow cytometry. Cells were gated on nucleated, singlets, and live. Neurons were then gated on NeuN-positive and microglia on CD11B-positive cells.
Extended Data Figure 8 | IFN-γ signalling in microglia is not necessary for normal social function. Mice deficient for STAT1 in microglia have normal social preference (n = 9 mice per group; ANOVA for Cre $F_{1,16} = 1.809$ and sociability $F_{1,16} = 30.10; P < 0.0001; **P < 0.01; ***P < 0.001$ Sidak's post hoc test; pooled two independent experiments).
Extended Data Figure 9 | Deleting IFNGRI by AAV transduction.
Mice were injected with AVVs expressing Cre and GFP under a synapsin promoter. a, GFP fluorescence in the PFC. Atlas image adapted from 2015 Allen Institute for Brain Science, Allen Brain Atlas. Available from: http://www.brain-map.org. b, GFP fluorescence is only observed in NeuN⁺ neurons, not Iba⁺ microglia (top, 20 ×; bottom 63 × objective).
Extended Data Figure 10 | IFN-γ increased the number of c-fos+ cells in layer I of the PFC. 
a, IFN-γ was injected into the CSF (into the cisterna magna) 2 h before killing and processing brains for immunohistochemistry. Slices were stained for c-fos. Atlas image adapted from 2015 Allen Institute for Brain Science, Allen Brain Atlas. Available from: http://www.brain-map.org. 
b, Total c-fos+ cells in layer I of the PFC (n = 3 mice per group; *P < 0.05; single experiment). Holding current pre and post IFN-γ application on acute slices from the PFC (c) and somatosensory cortex (d; n = 6 neurons from three mice). 
c, VgatCre::Ifngr1fl/fl mice. IFN-γ increased tonic inhibition in Cre+ mice (n = 6–7 cells from four mice per group; **P < 0.01 Sidak’s post hoc test).