1. **Supplementary Methods**

**1.1 Validation of Poly I:C**

*Poly I:C increases pro-inflammatory cytokines.*

We validated the cytokine response to POL (n=4) and SAL (n=3) in a separate cohort of pregnant dams. On GD 9, POL was injected and three hours later trunk blood was collected through live cervical dislocation (sFig 1).

**Supplementary Table 1**

Average concentrations of cytokines for POL and SAL dams. Concentrations of <0.64 indicate the cytokine was undetectable in the sample. IL = Interleukin; TNF-ɑ = Tumor necrosis factor-alpha

|  | **Average IL-1B pg/mL (range) [n undetectable]** | **Average IL-6 pg/mL (range) [n undetectable]** | **Average IL-10 pg/mL (range) [n undetectable]** | **Average TNF-ɑ pg/mL (range) [n undetectable]** |
| --- | --- | --- | --- | --- |
| **POL (n=4)** | 72.03 (1.24 - 142.81) [n = 2] | 5819.96 (4552.18 - 6513.61) [n = 0] | 135.85 (56.73 - 187.5) [n = 0] | 75.1 (45.72 - 90.51) [n = 0] |
| **SAL (n=3)** | 96.62 (96.62) [n = 2] | 53.94 (49.83 - 56.59) [n = 0] | 20.51 (16.4 - 24.62) [n = 1] | 12.04 (2.18 - 21.89) [n = 1] |

**1.2 Pups per Dam**

**Supplementary Table 2**

Number of dams per condition and number of male/female pups per dam. Where possible, 2 males and 2 females were used per dam.

| **Dam** | **2** | **4** | **6** | **15** | **17** | **20** | **1** | **3** | **8** | **12** | **16** | **18** |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Condition** | **POL** | **POL** | **POL** | **POL** | **POL** | **POL** | **SAL** | **SAL** | **SAL** | **SAL** | **SAL** | **SAL** |
| **N females** | 1 | 2 | 2 | 2 | 0 | 3 | 2 | 2 | 2 | 2 | 2 | 0 |
| **N males** | 2 | 1 | 2 | 2 | 2 | 1 | 2 | 2 | 2 | 2 | 0 | 2 |

**1.3 Functional MRI processing**

The functional image had the following parameters: EPI; in-plane resolution 0.25 x 025 mm, 0.5 mm slice thickness, TR/TE = 1000 ms/15 ms, matrix size = 96 x 40, voxel dimensions = 0.25 mm x 0.25 mm, ~8 min total. Functional images (n=80) were exported as DICOMs and converted to the Neuroimaging Informatics Technology Initiative (NIFTI) format for processing. Preprocessing was performed with the in-house software, Rodent Automated Bold Improvement of EPI Sequences, version 0.2.0 (RABIES; <https://github.com/CoBrALab/RABIES>).

In brief, anatomical and functional images for each mouse and time point were submitted in the Brain Imaging Data Structure (BIDS) format. Preprocessing comprised bias-field correction, motion-realignment and susceptibility distortion correction based on symmetric image normalization (SyN). Images were submitted to a melodic group independent component analysis to derive principal components. If BOLD activity was represented in the fMRI signal, then we would expect visualizations of components to reflect known networks present in the mouse resting state data [(Grandjean et al. 2020)](https://paperpile.com/c/1prxJP/gpbK). As no known network could be visually identified, the fMRI data were not examined further.

**1.4 Model Comparison**

Two linear mixed effects models were compared with the Akaike Information Criteria (AIC). The first included an interaction between time (age in days) and treatment with sex as a covariate and random effects of litter and subject ID (Model 1). The second included a three-way interaction between time (age in days), treatment, and sex with the same random effects (Model 2). In the R code, defaults were set to SAL and female so effects show POL and sex in comparison.

Model 1: Ysubject,j = β0 + β1sexMsubject,j + β2groupPOLsubject,j + β3agesubject,j +

β4age:group\_POLsubject,j + vsubject + vlitter + εsubject,j

Model 2: Ysubject,j = β0 + β1sexMsubject,j + β2groupPOLsubject,j + β3agesubject,j + β4age:group\_POLsubject,j + β5sexM:group\_POLsubject,j + β6age:sexMsubject,j + β7age:group\_POL:sexMsubject,j + vsubject + vlitter + εsubject,j

Where β is coefficient for fixed effects, v is the coefficient for random effects, *j* is a repeated measure (timepoint) and ε is the random error.

At each voxel, models were compared with AIC. Model 1 was ultimately selected, as it fit the majority of voxels as visualized in sFig 7. Additionally, regions of particular interest, such as the cortex and hippocampus were better fit by Model 1. Furthermore, Model 2 better fit voxels at the dorsal edge of the brain, more subject to artifacts and sensitive to global rain size differences (not the focus of the present work).

**1.5 Background for MRS**

**1.5.1. Generation of metabolite basis sets**

Using the FID-A Simulation toolbox in Matlab (version R2019a) we simulated the metabolite functions for quantification. The simulations implemented the exact waveforms of the PRESS refocusing pulse used in the experiment [(Mao et al. 1988; Fowler et al. 2020)](https://paperpile.com/c/1prxJP/zipd+nqGW0), as well as the TE and TR used experimentally. Previously published chemical shifts and J-coupling constants were used in the simulations [(Govindaraju et al. 2000)](https://paperpile.com/c/1prxJP/7EOE). Metabolites were simulated with Lorentzian lineshapes and linewidths of 2 Hz, including 18 metabolites: alanine (Ala), aspartate (Asp), creatine (Cr), γ-aminobutyrate (GABA), glucose (Glc), glutamine (Gln), glutamate (Glu), glycerophosphocholine (GPC), glutathione (GSH), lactate (Lac), Ins, NAA, N-acetylaspartylglutamate (NAAG), phosphocholine (PCh), phosphocreatine (PCr), phosphoethanolamine (PE), serine (Ser), taurine (Tau).

**1.5.2 Test-retest**

Six adult C57/BL6/J mice (3 males, 3 females) were scanned with the same acquisition parameters and protocol for MRS as the experiment (voxel size = 1.2 x 2.6 x 2.6 mm3 in the ACA; Point Resolved Spectroscopy sequence [PRESS; TR/TE=3000/8.5 ms, 256 averages]). After the acquisition the mouse was taken completely out of the scanner, then placed back in and a second acquisition was run. Any variation between the metabolite concentrations is attributable to changes in scanner artifacts, mouse positioning, or voxel placement, affording an estimate of the reliability of metabolite concentration estimates over time.

**1.6 Behavioral Testing**

**1.6.1 Open Field Test:**

Results from the OFT were assessed by comparing the time spent and frequency of entry into the anxiogenic center zone (~40% of a 45x45 cm2 grey plastic arena) compared to the corners and edges. Mice were allowed to explore the arena for 15 minutes under bright light.

**1.6.2 Three chambered social preference and social novelty task**

For the three chambered social approach task, mice were habituated (ten minutes) under red light to a three-chamber plastic box (26 (l) x 21.6 (w) x 21.6 (h) cm) with divider panels that have open doors, with a black wire container (9.5 (h) 7.6 (d) cm) in the two extreme chambers. Trial one measured social preference (ten minutes). Interactions with an age-matched (∓ 2 weeks) same-sex stranger mouse were compared to interactions with a non social object (rubber frog of similar size to an adult mouse, each under wire containers). Trial two measured preference for social novelty, and interactions were compared between the same stranger mouse and a novel stranger mouse (which replaced the non social object).

**1.6.3 Prepulse Inhibition**

Sensorimotor gating to acoustic startle was measured with the PPI task using commercially available startle chambers (San Diego Instruments, San Diego, CA). Startle response to an acoustic stimulus (120 dB) was compared to trials in which the startle stimulus was preceded by a 30 ms prepulse stimulus ranging from 3-15 dB above background noise (73-85 dB; 5 trials/stimulus, 3 dB increments) [(Guma et al. 2021)](https://paperpile.com/c/1prxJP/miLV).

**1.7 Power analysis for sample size**

*Power analysis.* In order to design a study with an appropriate sample size, data from a previous study from our group [(Guma et al. 2021)](https://paperpile.com/c/1prxJP/miLV) was used to simulate the current experiment, allowing us to estimate the power of detecting group differences across various effect sizes. Using volume estimates from brain regions at two timepoints that matched the scanning days in the current experiment (PND 35 and 60), a longitudinal power analysis was performed using SIMR/1.0.4 in R/3.5.1.

To begin, we conducted a pseudo test-retest by including volume estimates from found data--SAL mice (21 males, 20 females)--at two adult timepoints (PND 60 and 90) when we would not expect gross volumetric changes and can attribute alterations in volume primarily to the effects of the scanner. After modelling volume changes with anatLm in the RMINC package, version 1.5.2.2, including age and sex as effects of interest, we estimated the effect size of the age term at each region with Hedge’s g\* [(Mouse-Imaging-Centre 2022)](https://paperpile.com/c/1prxJP/dKgk), where a g\* of one indicates the two groups differ by a single standard deviation. SFig 8a depicts a histogram of all g\*s produced by the analysis. The interquartile range is 0.75, spanning from -0.12 to 0.63, and demonstrating that for most of the data, the g\* is less than a single standard deviation, suggesting there is relatively little variability between the timepoints.

Following the pseudo test-retest, we implemented a power analysis, using the simulated data to query what power could be expected given sample sizes of ten males and ten females for POL and SAL groups for three standardized effect sizes: 0.45, 0.5, and 0.65 across all structures. SFig 8b shows violin plots with quartiles marked, demonstrating all structures had predicted power greater than 80%. Based on results from the power analysis, we selected a sample size of 10 pups per sex per condition.