Supplementary Information

Subjects. Study subjects included 52 infant male rhesus monkeys born between 2003 and 2015 in 24 half-acre (0.2 ha) field corrals, each comprising 80–150 animals of all ages and both sexes. Each subject was born to a different dam and sire. Animals were tested in the CNPRC BioBehavioral Assessment (BBA) program at a mean age of 112.98 days (range: 103–120). All animals were born into our "conventional" (i.e., not specific-pathogen-free [SPF]) colony at the CNPRC. Animals were selected to test specific hypotheses about a pattern of behavior referred to as behavioral inhibition; selection utilized a dataset that is not part of the present analysis (but which is described in Capitanio (2019)), and results of this analysis are not presented here. Because animals at the extremes were oversampled for that analysis, our principal component analyses were conducted by weighting the sample to match the distribution of these phenotypes in the population (based on data from n=1 345 non-SPF males). Animals classified as inhibited were given a weight of 0.52, animals in the middle of the distribution were given a weight of 2.11, and animals classified as least inhibited were given a weight of 0.29. Total sample size was n=52 for the RP data; for the HI data, where n=51, the weights were recalculated accordingly.

The BBA program, which comprises a series of highly standardized tests conducted over a 25 h period, has been described in detail elsewhere (Capitanio, 2017; Golub et al., 2009). Briefly, mothers and infants were net captured from their field corrals, separated from each other, and delivered to the testing room (infants) or to holding cages (mothers) outside the sensory range of the infants. Infants, which were

always tested in cohorts of 5–8 animals, arrived at 0900 h, and were housed individually in standard-sized holding cages (0.58 m×0.66 m×0.81 m, Lab Products, Maywood, NJ, USA) indoors. A variety of behavioral assessments were performed throughout the day, and early the next day. Infants were returned to their mothers at 1000 h the following day, where they were given 1 h to nurse prior to returning to their natal cages with their mothers. Each holding cage contained a stuffed cloth toy duck, a towel, and a novel object that the infants could manipulate. Infants were provided with water *ad libitum*, orange-flavored drink, fresh fruit, and commercial monkey chow.

Plasma cortisol. Blood was drawn on four occasions during the 25 h period. Sample 1 (1 mL) was drawn at 1100 h, approximately 2 h after subjects were separated and relocated, i.e., a stress sample. Sample 2 (0.5 mL) was obtained at 1600 h and reflected the animals' responses to sustained stress. Immediately after drawing sample 2, animals were injected intramuscularly (i.m.) with 500 mg/kg dexamethasone (American Regent Laboratories, Inc., Shirley, NY, USA). The third sample (0.5 ml) was taken at 0830 h the next morning, after which each subject was injected (i.m.) with 2.5 IU ACTH (Organon, Inc. West Orange, NJ, USA). The fourth sample (0.5 mL) was taken 30 min after ACTH administration.

In all cases, blood was drawn into non-heparinized syringes from a femoral vein following manual restraint and immediately transferred to tubes containing EDTA. Sample 1 (0.5 mL) was delivered to the CNPRC Clinical Laboratory for analysis (next section). The remaining 0.5 mL from sample 1, and samples 2, 3, and 4 were

spun in a refrigerated centrifuge at 4 °C for 10 min at 1 277 *g*. Plasma was removed and frozen at −80 °C until assay.

Up to 2013, cortisol concentrations were assessed using radioimmunoassay (RIA) kits from the Diagnostic Products Company (USA) (later Siemens) until they were discontinued. Beginning in 2014, samples were assayed using a chemiluminescent assay (Advia Centaur System, Siemens, Inc., USA) described in detail at www.healthcare.siemens.com/immunoassay/systems/advia-centaur-cp-immunoassay-sys (see also Vandeleest et al., 2019). Parallel runs using the two platforms on the same samples (n=32) resulted in a prediction equation (with R^2=0.88), which allowed us to convert the later cortisol values to the same scale observed in the RIA assays.

Hematology. One 0.5 mL aliquot from sample 1 was delivered to the CNPRC Clinical laboratory for a complete blood count (using an ABX Pentra 60C [Horiba Medical, Irvine, CA, USA] analyzer) with manual differential. Blood aliquots (50 μL) were directly labeled with phycoerythrin (anti-CD4-M-T477; BD Pharmingen, USA), peridinin chlorophyll-alpha protein (anti-CD8-SK1; BD Pharmingen, USA), fluorescein isothiocyanate (anti-CD3-SP34; BD Pharmingen, USA), and allophycocyanin (anti-CD20 Clone L27; BD Pharmingen, USA). A Coulter Qprep (Coulter Corp., Miami, FL, USA) was used to lyse the red blood cells and fix the samples in paraformaldehyde. Lymphocytes were gated by forward and side light scatter. A FACS Calibur flow cytometer (BD BioScience, San Jose, CA, USA) was used to phenotype the lymphocyte subsets.

Sample preparation for metabolomics assay. Banked plasma samples from sample

1 were centrifuged at 160 *g* for 5 min at 4 °C. Ice cold methanol (800 µL) was added to plasma (100 µL) and then vortexed. Samples were kept on ice for 1 h to allow proteins to precipitate. Afterwards, samples were centrifuged at 8 000 *g* for 10 min at 4 °C, with the supernatant then dried in a SpeedVac and stored at −20 °C until further processing.

Reversed-phase (RP) liquid chromatography online mass spectrometry analysis. Metabolite extracts were reconstituted in 50% methanol, centrifuged at 4 000 *g* for 4 min at room temperature, and then analyzed using an Agilent Technologies 1100 HPLC system (Agilent Technologies, Santa Clara, CA, USA) connected to a Bruker Impact II TOF MS system (Bruker Daltonics, Billerica, MA, USA) controlled by Hystar 3.2 Software (Bruker Daltonics). Reversed-phase liquid chromatography was carried out with a two eluent gradient (eluent A: 0.1% formic acid in water, eluent B: 0.1% formic acid in acetonitrile). The gradient was run at a flow rate of 500 µL/min at 40 °C using a C18 column, 100 mm length, 3 mm ID, 2.6 µm particle size, 100 Å poor size (Phenomenex Ltd., Aschaffenburg, Germany), and C18 guard column (Phenomenex Ltd.). Metabolites were eluted isocratically for 1 min with 99% eluent A followed by a 15 min gradient to 50% eluent B and maintenance at 50% eluent B. Eluents were infused splitless into the ESI source with an end plate offset of -500 V, a capillary voltage of –4 500 V in positive mode and 3 500 V in negative mode, nebulizer gas N_2 flow 10 L/min, 3.5 bar pressure, and 220 °C. Compass 1.9 software (Bruker Daltonics) was used for profile data acquisition with a spectra rate of 1 Hz in full scan and a mass range from 30–1 300 m/z. The MS2 spectral data of the five most

intense fragments were selected for fragmentation within a mass range of ±3 Da, 20 eV collision energy, and 5 Hz spectra rate. Mass accuracy was adjusted by internal calibration using sodium format clusters in ESI+ and ESI- mode with DataAnalysis 4.4 (Bruker Daltonics). After baseline correction and mass calibration, chromatograms were converted to mzXML files by MSConvert from ProteoWizard (http://proteowizard.sourceforge.net).

Hydrophobic interaction (HI) liquid chromatography online mass spectrometry analysis. Samples were analyzed with the same hardware as above. Hydrophobic interaction liquid chromatography was carried out with a two eluent gradient (eluent A: 5% acetonitrile, 10 mmol/L ammonium acetate pH 6.8, eluent B: 95% acetonitrile, 10 m mmol/L ammonium acetate pH 6.8). The gradient was run at a flow rate of 100 µL/min at 40 °C using a ZIC®-HILIC 2.1×100 mm column, 3.5 µm particle size, 100 Å pore size, and guard column (Merck KGaA, Darmstadt, Germany). Metabolites were eluted isocratically for 2 min with 95% eluent A followed by a 4 min gradient to 85% eluent B and another 6.5 min gradient to 21% eluent B. The sample flow was introduced splitless into the ESI source. Compounds were ionized with an end plate offset of –500 V and a capillary voltage of –4 500 V in positive mode and 4 000 V in negative mode. The nebulizer gas N_2 flow was 8 L/min with 1 bar pressure heated to 220 °C into the source. Profile data were acquired with a spectra rate of 1 Hz and a mass range of 50–1 300 m/z using Compass 1.9 software (Bruker Daltonics) in full scan MS1. Mass accuracy was adjusted by internal calibration using sodium format clusters in ESI+ mode with DataAnalysis 4.4 (Bruker Daltonics).

Mass spectrometry data processing. After calibration, chromatograms were converted to mzXML files by MSConvert (ProteoWizard, http://proteowizard.sourceforge.net, v3.0.9987). The converted mzXML files were imported to MZmine 2 (Pluska et al., 2010) and processed with the ADAP module (Chowdhury et al., 2009) to generate deconvoluted chromatographic peaks. The peak lists were further processed with the CAMERA module (Kuhl et al., 2012) to identify isotopes and feature groups. Aligned peak lists were annotated according to exact mass and retention time using an inhouse database. Final peak lists were exported to CSV files for statistical analysis. For data evaluation, CSV files were imported into Perseus (Tyanova et al., 2016) (http://www.biochem.mpg.de/5111810/perseus) and MetaboAnalyst (Chong et al., 2018) (http://www.metaboanalyst.ca). Both tools were used to perform normalization and scaling of the data as well as unsupervised and supervised statistics. For compound annotation, Human Metabolites Database (HMDB; Wishart et al., 2007) and Metlin Database searches were performed for exact parent MS2 spectral matches. In addition, MS2 spectra were matched to the HMDB database (Bruker Daltonics) and an inhouse database generated with standard compounds.

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