Chronic psychosocial stress in mice leads to changes in brain functional connectivity and metabolite levels comparable to human depression

Grandjean, Joanes ; Azzinnari, Damiano ; Seuwen, Aline ; Sigrist, Hannes ; Seifritz, Erich ; Pryce, Christopher R ; Rudin, Markus

Abstract: Human depression, for which chronic psychosocial stress is a major risk factor, is characterized by consistent alterations in neurocircuitry. For example, there is increased functional connectivity (FC) within and between regions comprising the default mode network (DMN) including prefrontal cortex and cingulate cortex. Alterations in network FC are associated with specific aspects of psychopathology. In mice, chronic psychosocial stress (CPS) leads to depression-relevant behavior, including increased fear learning, learned helplessness, fatigue and decreased motivation for reward. Using multimodal in vivo magnetic resonance imaging (MRI) and spectroscopy (MRS), we investigated CPS effects on function and structure in the mouse brain under light anesthesia. Mice underwent a baseline MRI/MRS session, followed by 15-day CPS (n=26) or control handling (n=27), and a post-treatment MRI/MRS session. In BOLD fMRI, relative to controls, CPS mice exhibited robust, reproducible increases in FC within 8 of 9 identified cortical networks, including the prefrontal and cingulate cortices that contribute to the "mouse DMN". CPS mice exhibited increases in between-network FC, including amygdala - prefrontal cortex and amygdala - cingulate cortex. MRS identified metabolic alterations in CPS mice as increased inositol levels in amygdala and increased glycerophosphorylcholine levels in prefrontal cortex. Diffusion-weighted MRI detected increased fractional anisotropic values in the cingulum. This study demonstrates that chronic psychosocial stress induces FC states in the mouse brain analogous to those observed in depression, as well as cerebral metabolism and white matter pathway alterations that contribute to understanding of pathological processes. It also demonstrates the importance of brain imaging to the establishment of valid animal models in translational psychiatry.

DOI: https://doi.org/10.1016/j.neuroimage.2016.08.013

connectivity and metabolite levels comparable to human depression. NeuroImage, 142:544-552. DOI: https://doi.org/10.1016/j.neuroimage.2016.08.013

Title: Chronic psychosocial stress in mice leads to changes in brain functional connectivity and metabolite levels comparable to human depression

Running title: fMRI in mouse chronic psychosocial stress model of depression

Authors: Joanes Grandjean[1], Damiano Azzinnari[2], Aline Seuwen[1], Hannes Sigrist[2], Erich Seifritz[3,4], Christopher R. Pryce[2,4]*, Markus Rudin[1,4,5]*

[1] Institute for Biomedical Engineering, University and ETH Zurich, CH-8093, Zurich, Switzerland
[2] Preclinical Laboratory for Translational Research into Affective Disorders, DPPP, Psychiatric Hospital, University of Zurich, August-Forel-Str 7, CH-8008, Zurich, Switzerland
[3] Department of Psychiatry, Psychotherapy and Psychosomatics (DPPP), Psychiatric Hospital, University of Zurich, Lenggstrasse 31, CH-8032 Zurich, Switzerland
[4] Center for Neuroscience Research, University and ETH Zurich, CH-8093, Zurich, Switzerland
[5] Institute of Pharmacology and Toxicology, University and ETH Zurich, CH-8093, Zurich, Switzerland

* Authors contributed to the work equally

Corresponding author:

Prof. Dr. Markus Rudin
Institute for Biomedical Engineering
University and ETH Zürich

AIC-ETH HCI D426
Vladimir-Prelog-Weg 4
CH- 8093 Zürich

Tel: +41 (0)44 633 76 04
Fax: +41 (0)44 633 11 87
e-mail: rudin@biomed.ee.ethz.ch

Abstract: 247 words

Body: 5854 words

Figures: 4

Table: 0

Supplementary material: 6

Abstract

Human depression, for which chronic psychosocial stress is a major risk factor, is characterized by consistent alterations in neurocircuitry. For example, there is increased functional connectivity (FC) within and between regions comprising the default mode network (DMN) including prefrontal cortex and cingulate cortex. Alterations in network FC are associated with specific aspects of psychopathology. In mice, chronic psychosocial stress (CPS) leads to depression-relevant behavior, including increased fear learning, learned helplessness, fatigue and decreased motivation for reward. Using multimodal *in vivo* magnetic resonance imaging (MRI) and spectroscopy (MRS), we investigated CPS effects on function and structure in the mouse brain under light anesthesia. Mice underwent a baseline MRI/MRS session, followed by 15-day CPS (n=26) or control handling (n=27), and a post-treatment MRI/MRS session. In BOLD fMRI, relative to controls, CPS mice exhibited robust, reproducible increases in FC within 8 of 9 identified cortical networks, including the prefrontal and cingulate cortices that contribute to the "mouse DMN". CPS mice exhibited increases in between-network FC, including amygdala - prefrontal cortex and amygdala - cingulate cortex. MRS identified metabolic alterations in CPS mice as increased inositol levels in amygdala and increased glycerophosphorylcholine levels in prefrontal cortex. Diffusion-weighted MRI detected increased fractional anisotropic values in the cingulum. This study demonstrates that chronic psychosocial stress induces FC states in the mouse brain analogous to those observed in depression, as well as cerebral metabolism and white matter pathway alterations that contribute to understanding of pathological processes. These findings demonstrate the importance of brain imaging to the establishment of valid animal models in translational psychiatry.

Introduction

Chronic psychosocial stress is a major etiological risk factor for a number of psychiatric disorders, most notably depression (Kendler et al., 2003; Kessler, 1997). Depression presents with core symptoms of low mood, reduced interest, and high fatigue (DSM-5, 2013), and therefore involves disturbances of negative- and positive-valence domains (Cuthbert and Insel, 2013). Neuroimaging studies comparing depressed patients with healthy controls have provided major *in vivo* evidence for functional, structural, metabolic and molecular markers in depression (Dutta et al., 2014; Ende et al., 2006; Price and Drevets, 2010). Functional magnetic resonance imaging (fMRI) comparing depressed patients to healthy volunteers revealed hyper-activity in subgenual anterior cingulate cortex (sgACC) and amygdala, and hypo-activity in dorsal medial prefrontal cortex (dmPFC) in response to emotional tasks (Drevets et al., 1992; Price and Drevets, 2010; Sheline et al., 2001). These observations have been rationalized in terms of altered neurocircuitry proposed to underlie specific depression psychopathologies, e.g. the amygdala – sgACC - PFC circuit for hyper-processing of negative stimuli (Disner et al., 2011). Imaging studies related to specific tasks have been complemented by resting-state (rs) fMRI, which provides information on the synchronicity of spontaneous activity thereby enabling the study of functional connectivity (FC) within and between networks. The method is of considerable interest for analyzing large-scale network alterations associated with specific mental states. For example, the default mode network (DMN), involving cingulate, prefrontal, parietal and temporal cortices (Broyd et al., 2009), is proposed to relate to self-absorption and rumination. In fact, FC within the DMN is increased by stress (Soares et al., 2013) and in depression (Grimm et al., 2009; Whitfield-Gabrieli and Ford, 2012). In addition to these analyses of the changes in brain function underlying depression, region-specific metabolic changes as derived from MRS have been reported for depressed patients (Hasler et al., 2007). These metabolic changes could be related to altered neuronal and glial function (Harrison, 2002; Rajkowska and Stockmeier, 2013; Yuksel and Ongur, 2010).

Comparative *in vivo* neuroimaging is probably the method most conducive to translating between the human evidence for altered brain states in psychiatric disorders and animal models thereof. If analogous brain changes can be identified, the animal models provide a unique opportunity to study the underlying cellular and molecular processes. In rats, a recent rs-fMRI study demonstrated that the

physical stressor of repeated immobilisation results in increased FC within the “DMN” as well as somatosensory-cortex and visual-cortex networks (Henckens et al., 2015). In mice, rs-fMRI has been applied to demonstrate that structurally connected brain areas also exhibit FC, that brain topology and networks exhibit homology with primate species (Stafford et al., 2014), and that network alterations are sensitive indicators of pathology (Grandjean et al., 2014b). In mice, chronic social stressors combined with behavioural tests have identified changes in emotional, motivational and cognitive states that are relevant to human psychopathologies. For example, chronic social defeat (CSD) (Golden et al., 2011) leads to social avoidance and decreased interest in gustatory reward (Covington et al., 2009; Krishnan et al., 2007). *In vivo* electrophysiology has provided evidence for altered PFC - amygdala coherence in terms of neuronal firing in CSD mice (Kumar et al., 2014). *Ex vivo* structural MRI identified correlations between CSD-induced social avoidance and the volumes of several sub-cortical brain regions (Anacker et al., 2016). Region-specific transcriptomics demonstrated altered expression of metabolic pathway genes in the nucleus accumbens in CSD mice (Krishnan et al., 2007). We have developed a modified version of the CSD manipulation, referred to here as chronic psychosocial stress (CPS): it comprises continuous distal exposure to dominant mice for 15 days and short daily episodes of proximate exposure and physical attack without wounding (Azzinnari et al., 2014). CPS mice demonstrate consistent increases in Pavlovian fear learning, learned helplessness and physical fatigue (Azzinnari et al., 2014; Fuertig et al., 2016), and consistent decreases in effortful motivation for reward and in cognitive decision making to obtain reward in operant tests (Bergamini et al., In press). These CPS effects are obtained without dividing mice into susceptible versus resilient sub-groups based on their subsequent passive avoidance of the aggressor mouse strain, the method used in the common CSD protocol (Golden et al., 2011). That is, we use an “inclusive” experimental design, as used extensively with other stressors e.g. chronic unpredictable mild stress, e.g. (Tye et al., 2013; Willner, 1997). Each of these behavioural effects of CPS is relevant to a psychopathology in major depression, e.g. (Cuthbert and Insel, 2013; Demyttenaere et al., 2005; Eshel and Roiser, 2010; Nissen et al., 2010; Pryce et al., 2011; Treadway, 2016). Transcriptomics identified, among other ontological groups, down-regulation of oligodendrocyte-myelin genes in amygdala and PFC in CPS mice (Azzinnari et al., 2014), and biochemical analysis has identified activation of immune-inflammation pathways in the periphery and brain (Fuertig et al., 2016). The application of *in vivo* high-resolution neuroimaging to study the effects of mouse CPS on brain FC has the potential to provide translational

evidence that this stressor induces *in vivo* functional and structural changes analogous to those identified in the human brain in depression.

Therefore, in this study we investigated CPS effects on the *in vivo* status of the mouse brain, using rs-fMRI to assess FC within and between networks, as well as voxel-based morphometry (VBM) and diffusion weighted imaging (DWI) to investigate macroscopic structure. In addition, neurochemical metabolite data were obtained using proton MRS for regions-of-interest (ROIs) in amygdala and medial PFC. The specific hypotheses tested were that: (i) CPS leads to robust changes in FC within and between networks; (ii) these correspond to the changes described for the analogous functional networks in depression in humans, and; (iii) functional alterations co-occur with depression-relevant structural and metabolic changes. Indeed, relative to control mice, CPS mice were found to exhibit increased FC within several cortical networks including those comprising the "DMN", and between amygdala and cortical networks; furthermore, metabolic effects were obtained in the amygdala and mPFC that provide insights into pathophysiological processes.

Methods and Materials

Mice and chronic psychosocial stress

This study was performed in accordance with federal guidelines and under a license (170/2012) from the Zürich Cantonal Veterinary Office. Young-adult male C57BL/6 mice bred in-house were studied (Azzinnari et al., 2014; Fuertig et al., 2016), maintained in type 2L cages in a ventilated cabinet (Scantainer, Scanbur Technology) on an inverted 12h day-night cycle, with food and water *ad libitum*. Chronic psychosocial stress was conducted as described previously (Azzinnari et al., 2014; Bergamini et al., In press; Fuertig et al., 2016). Briefly, each BL/6 (CPS) mouse was placed singly in the home cage of an aggressive CD-1 mouse, separated by a transparent, perforated divider. The CPS mouse was placed in the same compartment as the CD-1 mouse for either a cumulative total of 60-sec physical attack or 10 min maximum. To prevent bite wounds, the lower incisors of CD-1 mice were trimmed regularly. The CPS mouse x CD-1 mouse pairings were rotated so that CPS mice were placed in the home cage and confronted with a novel CD-1 mouse each day. This CPS procedure was conducted for 15 days, between 14:00-16:00 h. From day 16, each CPS mouse remained in one cage with the same CD-1 mouse without further attacks. Control mice remained in littermate pairs, the

standard condition in our laboratory, and were handled and weighed daily. The CPS protocol is modified from the standard CSD protocol (Golden et al., 2011) in terms of: timing the attacks and limiting maximum attack-time per mouse per day to 60 s (versus 10 min) and trimming the incisor teeth of CD-1 mice to prevent bite wounding (versus no teeth trimming and regular bite wounding). These modifications were combined with an increase in the duration of the stressor to 15 days (versus 10 days), thereby increasing comparability to other chronic rodent stressors such as chronic unpredictable mild stress (Willner, 1997). The rationale for the refinements of the CSD protocol is detailed in Azzinnari et al. (2014) and Fuertig et al. (2016). Mice were allocated to CPS and control groups by counterbalancing for both motor activity and body weight (Azzinnari et al., 2014). A total of 56 mice were studied, comprising n=26 in the CPS and n=27 in the control group. Three CPS mice were excluded according to predefined exclusion criteria: two exhibited continuous fighting back during physical attack and one mouse sustained injury; one control mouse was excluded from the MRS analysis due to spectrum artefacts in the baseline session. The study was conducted in two runs, which were identical except for the region of interest (ROI) for MRS: in Run 1 (CPS n=14, control n=15) the ROI was a 1.5µl voxel in the right amygdala, and in Run 2 (CPS n=12, control n=12) it was a 2.25µl voxel in the mPFC.

MRI and MRS

All mice were measured at two time points: the baseline session was conducted at 4 days prior to the onset of 15-day CPS/Control, and the post-treatment session at 1 day after CPS/Control completion. Anesthesia was induced with 3.5% isoflurane, mice were endotracheally intubated, positioned on a MRI-compatible cradle, and artificially ventilated at 80 breaths per minute, 1:4 O_2-to-air ratio, and 1.8 ml/h flow (CWE, Ardmore, USA), and isoflurane was reduced to 2%. The tail vein was cannulated, and an i.v. bolus of medetomidine 0.05 mg/kg and pancuronium bromide 0.2 mg/kg was injected, and isoflurane reduced to 1.5%. After 5 minutes, an infusion of medetomidine 0.1 mg/kg/h and pancuronium bromide 0.4 mg/kg/h was administered, and isoflurane further reduced to 0.5%. Sedation was set at these low levels to minimize the potential occurrence of neuronal and physiological confounding effects that have been described for either isoflurane or medetomidine at higher concentrations (Grandjean et al., 2014a). The use of mechanical ventilation and an i.v. line for medetomidine ensured accurate and reproducible anesthesia exposure and control of physiological variables including O_2 and CO_2 levels (Mueggler et al., 2003). Rectal temperature was maintained at

36.5 ± 0.5°C throughout measurements. Data acquisition had a total duration of 120 min (see below), after which recovery was monitored. The strict timeframe used (see below) ensured that the duration of anesthesia exposure at each scanning stage was equivalent across subjects and across baseline and post-treatment sessions. In control mice (n=27), body weight at baseline was 29.4±2.3 g and post-session was 30.6±2.3 g, and in CPS mice (n=26) was 29.9±2.3 g at baseline and 30.4±1.4 g post-session.

Data were acquired with a Bruker 94/30 Biospec spectrometer (Bruker BioSpin MRI, Ettlingen, Germany) operating at 9.4T, equipped with a BGA-S gradient system, a linear volume resonator coil for transmission, and a 2x2 phased-array cryogenic surface receiver coil. Images were acquired using Paravision 6 software. Tripilot images were acquired to ensure correct positioning of mice with respect to the coil and the magnet isocentre. Shim gradients were adjusted using a mapshim protocol, with an ellipsoid reference volume covering the entire cerebrum. Resting state fMRI was conducted for 6 min, with blood oxygenation level-dependent (BOLD) gradient-echo echo planar images (EPI) acquired using repetition time TR=1000ms, echo time TE=9.2ms, flip angle FA=90°, matrix size MS=90x70, field of view FOV=20x17.5 mm^2, slice number NS=12, slice thickness ST=0.5mm, slice gap SG=0.2mm, and bandwidth BW=250000.Hz, 360 volumes. 3D volumetric scanning was conducted for 16 min, with scans acquired using spin echo RARE sequence (Hennig et al., 1986), TR=2000ms, TE=48.6ms, MS 150x200x80, FOV=15x20x8mm^3, FOV saturation slice positioned on the inferior portion of the head. Diffusion weighted imaging (DWI) was conducted for 8 min, using multi-shot diffusion tensor imaging EPI sequence, 4 segments, TR=2000ms, TE=22ms, FA=90°, MS=128x128, FOV=20x17.5 mm^2, NS=20, ST=0.3mm, SG=0.15m, 5 volumes acquired with b=0s/mm^2, followed by 36 direction-encoded volumes with b-value=1000 s/mm^2. Finally, single voxel ^{1}H MRS was performed for 45 min in volumes of interest placed either in the right amygdala (voxel dimensions: 1x1x1.5mm^3) or in the mPFC (voxel dimensions 1x1.5x1.5mm^3) using a STEAM sequence (Frahm et al., 1989) with following parameters: TR=2500ms, TE=2.8ms, 1000 accumulations, excitation pulse bandwidth= 10kHz. VAPOR was used for water suppression and field maps were acquired for shimming. The decision to study the amygdala in the first run was based on the transcriptomic effects obtained for this ROI in a previous CPS study (Azzinnari et al., 2014), and the decision to study mPFC in the second run was based on prior transcriptomic effects (Azzinnari et al., 2014) and the connectivity effects obtained in the first run of this study.

Data processing

Study data are available in NIFTI format at the XNAT Data Repository (Project_ID: CSD_MRI_MOUSE).

FMRI images presented only minimal distortions due to the magnetic field inhomogeneity (Figure S1). The images were first temporally realigned, non-brain areas were masked using Bet (FMRIB Software Library v5.0, fsl.fmrib.ox.ac.uk), and then directly co-registered to the Australian Mouse Brain Mapping Consortium (AMBMC, http://www.imaging.org.au/AMBMC) MRI template, using linear affine and nonlinear greedy SyN diffeomorphic transformation metric mapping conducted with ANTS (Advanced Normalization Tools 2.1.0, picsl.upenn.edu/software/ants). Removal of unwanted confounds in the fMRI signal was performed using FIX (FMRIB's ICA-based Xnoiseifier, v1.062 beta) as in Zerbi et al. (Zerbi et al., 2015). In a previous group independent component analysis (ICA) conducted with the baseline fMRI scan of a sub-set of the control mice (n=15) from the present study, 17 plausible networks were identified (Zerbi et al., 2015). This network set was used as the spatial reference for (i) dual regression analysis of within-network effects and (ii) FSLNets (v0.6) analysis of between-network effects. In brief, the fMRI signal from each of the group-level networks for each mouse was extracted and used as a regressor to estimate individual-level representation for each network. To verify and complement the voxel-wise analysis, the average beta value estimates from the individual-level network maps were extracted using group-level network reference maps as ROIs, and are referred to in the text as ‘network strength’. The time courses extracted from each network during the first stage of the dual regression were further used to reconstruct between-network interaction strength for each pair of networks, using normalized full correlations.

Anatomical 3D volumetric images were converted to MINC format, and were registered iteratively: (i) to a reference image using 6 degrees of freedom (df) transform, (ii) by 12 df affine transformation between each pair of individual images, and (iii) with 3 consecutive non-linear transformations between each pair of images, using MICe-build-model and ANTS (Lerch et al., 2008). Region of interest analysis was performed using a set of ROIs derived from the Allen mouse brain atlas (http://www.mouse.brain-map.org) adapted to MRI space. Voxel-wise analysis from voxel-based morphometry analysis was performed on the scaled-Jacobians, blurred at 0.2 mm full widths at half maximum.

Diffusion weighted images were corrected for eddy current and the diffusion parameters were reconstructed in FSL. Axial diffusivity (D_{ax}), representing water diffusion along the main fiber orientation, was derived from the first eigenvalue, and radial diffusivity (D_{rad}), representing water diffusion across the main fiber orientation, was generated by averaging the last two eigenvalues estimated from the direction-encoded volumes. Fractional anisotropy (FA) was calculated from the three eigenvalues. The volumes acquired with b=0s/mm² were co-registered to an in-house reference template using ANTS. The computed transformations were applied to diffusion parameter maps to register individual diffusion maps in a standard reference space. FA, D_{ax}, and D_{rad} values were extracted using a mask with ROI placed on selected white matter fibers based on anatomical landmarks. The FA values were corrected with Fisher's z transform for bounded values, as a prerequisite for normal distribution assumptions, prior to statistical analysis.

In MRS, metabolites were quantified using LCmodel (Provencher, 1993) and expressed as ratio to the total creatine (phosphocreatine + creatine; tCr) level, assumed to be constant and not affected by CPS.

Statistical analysis

Voxel-wise statistics for within-network fMRI, VBM and DWI were performed using non-parametric permutation-based testing and a design matrix model of Treatment group (control or CPS), Session (baseline or post-treatment), and individual subject intercepts. Contrasts were designed to compare the interaction between Treatment group and Session. In the case of significant interaction, post-hoc pair-wise analysis was conducted within control and CPS groups. Correction for multiple comparisons was conducted using threshold-free cluster enhancement (TFCE). The statistical maps from dual regression analysis were further corrected with Bonferroni correction (threshold: p-value≤0.003). Findings are presented as color-coded p-value maps using a threshold set at the level of significance and shown as an overlay over the AMBMC template. Between-network analysis statistics were tested using the same design matrix, contrasts, and permutation-based testing, and corrected for multiple comparison using false discovery rate.

ROI statistics for within-network fMRI, VBM, DWI and MRS data were performed using R (3.0.1, The R Foundation for Statistical Computing, Vienna, Austria): a linear mixed model from lme4 package (version 1.1-7) and contrast analysis using the Multcomp package (version 1.3-1). Treatment group

and Session were modeled as fixed effects and animal intercepts as random effects. The factorial design and post hoc analysis were the same as those used for the voxel-wise analysis. QQ-plots were applied to confirm normal distribution, Tukey-Anscombe plots for homogeneity of the variance and skewness, and scale location plots for homoscedasticity. For MRS, the Cramér–Rao lower bound was used as a weighting factor in the linear model to minimize influence from values associated with larger estimation errors.

The null hypothesis, i.e., absence of Treatment group x Session interaction, was rejected at p-value ≤ 0.05, or according to thresholds set by the methods for multiple comparison correction. Descriptive statistics are given as mean ± 1 standard deviation.

Results

CPS mice exhibit increased functional connectivity within and between networks

CPS mice (1.9±0.6%) exhibited increased day-to-day body weight variation (delta) across the 15-days relative to control mice (1.3±0.4%) (*t*-test, $p<0.0001$). This is a consistent biomarker of CPS (Azzinnari et al., 2014; Fuertig et al., 2016), and provides evidence that baseline anesthesia and scanning did not impact on subsequent responsiveness to the CPS or control procedures.

The reference atlas was obtained by independent component analysis of the baseline fMRI scans performed on a sub-set of control mice (Figure S2) (Zerbi et al., 2015) and was applied for network FC analysis. Analysis of rs-fMRI data of 26 CPS and 27 control mice with dual-regression revealed a Treatment group X Session interaction effect for within-network FC and, post hoc, a post-treatment versus baseline increase in CPS mice specifically, in four sensory cortical networks, namely the supplementary, barrel field 1 and 2, and visual cortices, as well as in the cingulate cortex (Figure 1A). An example of the increase in within-network connectivity in CPS mice extracted from a ROI is given in Figure 1B for the supplementary cortex. In addition to these interaction effects that were sufficiently robust to survive all correction procedures, the interaction effect did not survive Bonferroni correction but did survive TFCE correction for several further networks, namely the motor, limb, and prefrontal cortices, and both dorsal and lateral striatal networks, for each of which FC was increased in CPS mice specifically (Figure S3). The within-network strengths extracted from the ROIs are detailed in

Table S1. Statistical analysis on values extracted from the ROIs confirmed the presence of a CPS mice-specific post-treatment increase in FC in cortical networks. Data acquisition was performed in two temporally separate runs (see Methods and Materials). Similar, significant interaction effects and network strength distributions were obtained in the two runs, indicating the robustness and reproducibility of the data; as an example, the run-specific findings for the supplementary cortex network are provided in Figure S4.

For the between-network analysis, interactions were computed using full correlations, resulting in a group-level interaction map (Figure S5). Hierarchical ordering of the interactions indicates the organization of between-network FC into four groups: a lateral cortical network including supplementary, motor, and barrel field 1 and 2 cortical components; a sub-cortical network comprising the piriform cortex, amygdala, the 3 striatal components, and the thalamus; an associative cortical network consisting of limb, visual, and auditory cortices; and fourthly a "default mode network" including prefrontal and cingulate cortices and ventral hippocampus. Statistical analysis indicated that significant Treatment group X Session interaction effects did not survive FDR correction. However, several between-network interactions were borderline to the level of significance after correction. Post hoc analysis revealed significant post-treatment increases in FC in CPS mice, surviving FDR correction, between prefrontal cortex and piriform cortex, prefrontal cortex and amygdala, ventral hippocampus and amygdala, cingulate cortex and piriform cortex, and cingulate cortex and amygdala (Figure 2).

CPS mice exhibit increased levels of inositol and glycerol-/phosphorylcholine

MRS was conducted in a ROI located in the right amygdala in Run 1 and in the mPFC in Run 2 (Figure 3 A, B). Local voxel shimming resulted in line widths of the water resonance (full width at half maximum FWHM) of 15.1±0.7 Hz for the amygdala and 10.5±0.4 Hz for mPFC ROIs. The high quality of the spectra allowed the unambiguous identification of the signals of 16 metabolites in each ROI (Figure 3 C, D). There was no significant Treatment or Session effect on the intensity of the total creatine resonance (phosphocreatine + creatine, tCr), which was subsequently used as a reference for estimating relative metabolite concentrations. In right amygdala (Figure 3C), there was a Treatment group X Session interaction for [inositol]/[tCr] ($p=0.03$), with post hoc testing demonstrating increased

inositol levels in CPS mice specifically (p= 0.0064, Figure 2E). In mPFC (Figure 3D), there was a Treatment group X Session interaction for glycerophosphorylcholine + phosphorylcholine [GPC+PCh]/[tCr] (p=0.01), in the absence of a group-specific post hoc effect (Figure 3F).

CPS mice exhibit unchanged brain ROI volumes and altered white matter structure in the cingulum

Non-linear transformation within the voxel-based morphometry analysis led to accurate co-registration of the individual volumes into a standard reference space. Using voxel-wise and ROI analyses, there was no evidence for CPS-specific effects on volume. Voxel-wise analysis of fractional anisotropy (FA) values derived from DTI did not reveal a CPS-specific effect following TFCE correction. However, *a priori* ROI analysis conducted for ROIs located in the minor forceps, anterior commissure, corpus callosum, external capsule, internal capsule, and cingulum (Figure 4A), revealed a Treatment group x Session interaction for the cingulum (p=0.004, Figure 4B), with CPS mice exhibiting an increase in post-session versus baseline values (p=0.014). There was no evidence for a CPS effect regarding values for axial diffusivity or radial diffusivity for any of the white matter structures evaluated, including the cingulum.

Discussion

Chronic psychosocial stress caused significant functional, structural, and metabolic alterations in the mouse brain. In particular, CPS mice exhibited increased resting-state functional connectivity primarily within cortical networks, but also between the amygdala and cortical and hippocampal networks. These changes reflect some characteristic network signatures found in human depression. Furthermore, specific metabolic and structural brain changes were observed that provide insights into some cellular and molecular processes induced by CPS. Building on the evidence that CPS induces depression-relevant behavioural phenotypes of increased Pavlovian fear learning, learned helplessness and fatigue and decreased motivation for reward (Azzinnari et al., 2014; Bergamini et al., In press; Fuertig et al., 2016), the present data constitute robust *in vivo* neuroimaging evidence for the

relevance of mouse CPS to the translational study of the neurobiology of depression pathologies, and their reversal.

Increases in within- and between-network functional connectivity in CPS mice

Nine cortical networks were identified, of which eight demonstrated increased within-network FC in CPS relative to control mice; these included some of the somatosensory cortices, e.g. supplementary, barrel field, and visual, and areas proposed to contribute to the "rodent default-mode network" ("DMN"), namely prefrontal and cingulate cortices (Gozzi and Schwarz, 2016; Sforazzini et al., 2014; Stafford et al., 2014). The present findings were robust and reproducible, as evidenced by the similar and significant CPS effects on network strengths obtained in each run, comprising 12-15 mice per CPS and control group per run, and with similar variances observed in the CPS and control data sets. These run sample sizes were similar to those used in previous studies of CPS effects on behavioural, transcriptomic and neuroinflammatory measures (Azzinnari et al., 2014; Bergamini et al., in press; Fuertig et al., 2016). A recent MRI study of chronic immobilization stress in rat, also using group ICA, identified similar networks and, in agreement with the present study, stressed rats exhibited cortical network-specific increases in FC in visual and somatosensory cortices as well as in "DMN" (defined as orbitofrontal, medial prefrontal, cingulate, retrosplenial parietal and medial visual cortices and hippocampus); motor, PFC and ACC, and four subcortical networks, were unaffected (Henckens et al., 2015). Therefore, both chronic physical stress in rat and CPS in mouse increased FC within cortical networks in lightly anesthetized subjects. Given that these two studies used different species, stressors, and anesthesia regimens but obtained somewhat parallel findings, it is unlikely that the effects observed where obtained due to stress-induced physiological effects (e.g. resting blood pressure, heart rate) that impacted on the fMRI data obtained.

These CPS findings are of translational interest, given that FCs within and between regions comprising the DMN, namely cingulate, prefrontal, parietal and temporal cortices, are also increased in humans in states of chronic stress or depression (Broyd et al., 2009; Grimm et al., 2009; Soares et al., 2013; Whitfield-Gabrieli and Ford, 2012). Connectivity within the human DMN is proposed to relate to self-absorption and rumination. Whilst it is a moot point as to whether such psychological processes pertain in rodents, they are clearly associated with increased sensitivity to negative valence (Cuthbert and Insel, 2013), a state that can be measured in rodents and, indeed, is increased in CPS mice, as

evidenced by increases in Pavlovian fear learning, learned helplessness and fatigue (Azzinnari et al., 2014; Fuertig et al., 2016). The PFC, in human and rodent, is also essential for cognitive processing of positive valence (reward), which is impaired in depressed humans and in CPS mice, as demonstrated in reversal learning tasks for example (Bergamini et al., In press; Taylor Tavares et al., 2008). The rodent evidence for a predominance of increased FC within cortical networks following stress suggests disruption of top-down cortical modulation of sub-cortical function. However, there were some CPS effects within sub-cortical networks also, including a relative increase in FC in dorsal and lateral striatum. The increased FC within dorsal striatum, an important motor region, could be relevant to the psychomotor retardation observed in CPS mice (Azzinnari et al., 2014).

In neuroimaging studies of depression, amygdala FC has received relatively little attention (Dutta et al., 2014), although PET-blood flow studies report increased amygdala blood flow in depressed patients, indicative of elevated activity (Drevets et al., 1992). In our study, whilst there was no detectable CPS effect on FC within amygdala, between-network analysis identified it to be a hub of CPS effects on the connectivity of networks with each other. Thus, CPS increased FCs between amygdala and each of prefrontal cortex, cingulate cortex and ventral hippocampus. In mouse, the amygdala, mPFC and hippocampus constitute a major circuit in the regulation of aversive stimulus processing, whereby input from the mPFC onto GABA interneurons in the basolateral complex of the amygdala constitutes major top-down inhibition of fear responding, learning and memory (Herry et al., 2008). CPS mice exhibit increased Pavlovian fear learning and learned helplessness (Azzinnari et al., 2014; Fuertig et al., 2016), effects which are likely to be underlain by increased amygdala (re)activity, and in turn to be partly the result of reduced top-down inhibition (Lüthi and Lüscher, 2014; Moscarello and LeDoux, 2013). Therefore, it will be important to build on the present evidence for increased between-network FC involving the amygdala in CPS mice in terms of understanding the changes in the excitation-inhibition between these networks. Interestingly, in CSD mice, an electrophysiology study also demonstrated increased coherence between the neuronal firing signals obtained from PFC and amygdala (Kumar et al., 2014). In human FC studies, the amygdala has been studied mainly in the affective network, depression-related changes in which remain to be investigated (Dutta et al., 2014). Nonetheless, the amygdala - hippocampus and amygdala – sgACC - PFC circuits proposed to underlie hyper-processing of negative stimuli in depression are characterized by changes in FC between regions analogous to those observed here in CPS mice (Disner et al., 2011). Using the

common CSD protocol, it was demonstrated that increasing ventral hippocampus - nucleus accumbens (ventral striatum) synaptic transmission using optogenetic methods induced a further increase in social avoidance in CSD mice (Bagot et al., 2015). Whilst optogenetic stimulation of a pathway in stressed mice is clearly a different situation to endogenous pathway activity in stressed mice, one can nonetheless extrapolate from this finding to the present study, and hypothesize increased ventral hippocampus - ventral striatum connectivity in CPS versus control mice; this was not observed. Using a two-day social defeat (so-called sub-threshold CSD), which itself does not induce social avoidance, it was demonstrated that optogenetic induction of phasic firing in ventral tegmental area (VTA) - nucleus accumbens neurons in such mice induced increased social avoidance, whilst in VTA - mPFC neurons it was optogenetic inhibition of phasic firing that induced increased social avoidance (Chaudhury et al., 2013). Because fMRI does not have sufficient resolution to allow identification of VTA, it is not possible to integrate the current study with these findings.

Etio-pathophysiological processes that underlie the CPS-induced increases in within-network FC might include a general increase in excitatory or decrease in inhibitory input within or on to the network. One candidate pathway is stress-induced activation of pro-inflammatory cytokines leading to increased blood and brain levels of kynurenines: both kynurenine and 3-hydroxy-kynurenine are increased in cortical and sub-cortical brain regions of CPS mice (Fuertig et al., 2016), and these are the precursors for the glutamate NMDA-receptor agonist quinolinic acid and antagonist kynurenic acid (Schwarcz et al., 2012). Given that the increase in FC in cortical networks was a generalized effect, it could be that the kynurenines, or other factors of course, are exerting their effects by altering the modulatory activity of one or more of the monoamine neurotransmitters. For example, structure-function changes in the mesocorticolimbic dopamine pathway have been reported for chronically stressed mice (Tanaka et al., 2012; Tye et al., 2013). With regard to processes that underlie the CPS-induced increases in between-network FC involving amygdala, in addition to increased levels of kynurenines, we have identified several additional candidates. Transcriptomics (RNA-seq) of amygdala tissue demonstrated that a number of genes encoding proteins either important in the regulation of or regulated by dopamine neurotransmission were down-regulated in their expression in CPS mice, e.g. *Drd2*, *Darpp-32*. Genes encoding proteins important for GABA neurotransmission, e.g. *Pvalb*, *Gabarδ*, were also down-regulated in CPS mice, as was the serotonin receptor, *Htr2a*, expressed primarily by GABA interneurons (Azzinnari et al., 2014). Furthermore, a number of genes specific to oligodendrocytes

including several encoding myelination proteins (e.g. *Mag*, *Mal*, *Mbp*, *Plp1*), exhibit down-regulated expression in amygdala and mPFC tissue from CPS mice (Azzinnari et al., 2014)(unpublished data), indicating the importance of including DTI in the present study (see below).

Metabolic and structural changes in CPS mice

MRS on the right amygdala identified increased levels of inositol in CPS mice. Human MRS studies have identified increased inositol in medial temporal lobe including amygdala (Venkatraman et al., 2009) and in fronto-cortical (PFC, ACC) gray and white matter (Caetano et al., 2005; Kumar et al., 2002) in depression, and in amygdala and thalamus in association with high depression symptoms in fibromyalgia (Valdes et al., 2010); decreased PFC-ACC inositol has also been reported in depression (Coupland et al., 2005). Inositol is an intracellular lipid and precursor of phosphatidylinositol (PI) structural lipids and inositol phosphate secondary messengers e.g. IP3 (Berridge, 2009; Berridge et al., 1989). Inositol and its metabolites are present in glia and neurons, including in myelin lipid membranes (Duarte et al., 2012). Increased inositol could reflect: inflammation resulting in activation of astrocytes and microglia in which inositol levels are relatively high; dysregulation of osmotic balance; and/or reduced metabolism of inositol to PIs related to deficient myelination (Duarte et al., 2012; Schmitt et al., 2015). As noted above, CPS mice exhibit increased levels of peripheral and central inflammatory factors (Azzinnari et al., 2014; Fuertig et al., 2016) and decreased expression of oligodendrocyte-myelination genes (Azzinnari et al., 2014; Fuertig et al., 2016). Both changes could contribute to the increased inositol signal in amygdala, therefore. It is also relevant that inhibition of inositol monophosphatase, leading to reduced inositol and IP3, is proposed to be the major mechanism-of-action of the mood stabilizer lithium (Berridge et al., 1989). MRS on mPFC identified increased GPC+PCh levels in CPS mice. Both metabolites contribute to the MRS choline peak, whereas the major choline, phosphatidylcholine (PC), is MRS-invisible. PC is incorporated into cell and myelin membranes, whilst cytosolic GPC and PCh are linked to both its synthesis and degradation. The increase in GPC+PCh in mPFC of CPS mice reflects changes in phospholipid turnover in cell membranes, potentially including increased demyelination. In human depression, choline-containing compounds were actually decreased in mPFC and hippocampus and increased in putamen (Ende et al., 2007; Venkatraman et al., 2009).

Potentially related to the current MRS and previous transcriptome findings for altered myelination and membrane metabolism in CPS mice (Azzinnari et al., 2014; Schmitt et al., 2015), DTI revealed increased fractional anisotropy in the cingulum, specifically, in CPS mice. This FA increase is suggestive of changes in white matter structure, including fiber rearrangement. The majority of the evidence for white matter changes in depression is actually for decreased FA: this is the case for cingulum, as well as splenium of corpus callosum and external and internal capsules, in adults with (Xiao et al., 2015) and adolescents at risk of (Huang et al., 2011) depression.

There was no evidence for CPS effects on the volumes of murine brain structures. In depressed patients, there is evidence for decreased PFC volume, whereas the evidence for amygdala volume change is equivocal (Price and Drevets, 2010). There is a relatively large literature on stress and depression and hippocampal volume; whilst there are several reports of decreased hippocampal volume in depression (Price and Drevets, 2010), the evidence is again equivocal. Decreased hippocampal volume was reported in rat following chronic unpredictable mild stress for 8 but not 2 weeks (Luo et al., 2014), suggesting that a certain chronicity of stress might need to elapse to induce volume reduction. Chronic immobilization stress in rat was without effect on hippocampal volume (Henckens et al., 2015). In mice that were susceptible to CSD in terms of decreased social avoidance, there was no increase in hippocampal volume post- versus pre-CSD, whereas there was an increase in hippocampal volume in control and resilient CSD mice (Tse et al., 2014). An ex vivo MRI study reported that hippocampal volume correlated positively with social avoidance score in CSD mice (Anacker et al., 2016). In healthy humans, stress response in terms of cortisol increase correlated positively with hippocampal volume (Pruessner et al., 2007). In a combined sample of elderly depressed and non-depressed humans, there was a positive correlation between number of stressful life events and hippocampal volume (Zannas et al., 2013).

Conclusion

The present study demonstrates that chronic psychosocial stress results in cortical and sub-cortical changes in resting-state functional connectivity together with specific metabolic and structural changes, in the mouse brain. In several cases, the changes are analogous to those described in FC studies of the human brain in depression. Furthermore, the networks demonstrating CPS-induced FC changes are known to be intimately involved in the depression-relevant behavioural states described

for the CPS model. The combination of related behavioural and macroscopic neural circuitry phenotypes constitutes robust evidence for the face validity of this mouse model for translational studies of how chronic psychosocial stress leads to depression psychopathologies. Furthermore, despite some important differences to human depression, metabolic and structural brain findings for the CPS model can be aligned with previous gene expression findings, to yield insights into underlying mechanisms. Therefore, multimodal fMRI/MRI/MRS constitutes a fundamentally important approach for the translational study of human depression, and can be applied to increase understanding of its etio-pathophysiology and develop novel therapeutic approaches.

Funding and Disclosure

This research was supported by a fellowship grant for JG and DA from the Swiss Foundation for Excellence and Talent in Biomedical Research (to CRP and MR), and by project grants 31003A-141137 (to CRP and ES) and 310030-160310 (to MR) from the Swiss National Science Foundation.

References

Anacker, C., Scholz, J., O'Donnell, K.J., Allemang-Grand, R., Diorio, J., Bagot, R.C., Nestler, E.J., Hen, R., Lerch, J.P., Meaney, M.J., 2016. Neuroanatomic Differences Associated With Stress Susceptibility and Resilience. Biol Psychiatry 79, 840-849.

Azzinnari, D., Sigrist, H., Staehli, S., Palme, R., Hildebrandt, T., Leparc, G., Hengerer, B., Seifritz, E., Pryce, C.R., 2014. Mouse social stress induces increased fear conditioning, helplessness and fatigue to physical challenge together with markers of altered immune and dopamine function. Neuropharmacology 85, 328-341.

Bagot, R.C., Parise, E.M., Pena, C.J., Zhang, H.X., Maze, I., Chaudhury, D., Persaud, B., Cachope, R., Bolanos-Guzman, C.A., Cheer, J.F., Deisseroth, K., Han, M.H., Nestler, E.J., 2015. Ventral hippocampal afferents to the nucleus accumbens regulate susceptibility to depression. Nat Commun 6, 7062.

Bergamini, G., Cathomas, F., Auer, S., Sigrist, H., Seifritz, E., Patterson, M., Mocaer, E., Gabriel, C., Pryce, C.R., In press. Mouse chronic social stress reduces motivation and cognitive function in operant reward tests: a novel model for reward pathology and assessment of predictive validity with the antidepressant agomelatine. European Neuropsychopharmacology.

Berridge, M.J., 2009. Inositol triphosphate and calcium signalling mechanisms. Biochimica et Biophysica Acta 1793, 933-940.

Berridge, M.J., Downes, C.P., Hanley, M.R., 1989. Neural and developmental actions of lithium: a unifying hypothesis. Cell 59, 411-419.

Broyd, S.J., Demanuele, C., Debener, S., Helps, S.K., James, C.J., Sonuga-Barke, E.J., 2009. Default-mode brain dysfunction in mental disorders: a systematic review. Neurosci Biobehav Rev 33, 279-296.

Caetano, S.C., Fonseca, M., Olvera, R.L., Nicoletti, M., Hatch, J.P., Stanley, J.A., Hunter, K., Lafer, B., Pliszka, S.R., Soares, J.C., 2005. Proton spectroscopy study of the left dorsolateral prefrontal cortex in pediatric depressed patients. Neurosci Lett 384, 321-326.

Chaudhury, D., Walsh, J.J., Friedman, A.K., Juarez, B., Ku, S.M., Koo, J.W., Ferguson, D., Tsai, H.C., Pomeranz, L., Christoffel, D.J., Nectow, A.R., Ekstrand, M., Domingos, A., Mazei-Robison, M.S., Mouzon, E., Lobo, M.K., Neve, R.L., Friedman, J.M., Russo, S.J., Deisseroth, K., Nestler, E.J., Han, M.H., 2013. Rapid regulation of depression-related behaviours by control of midbrain dopamine neurons. Nature 493, 532-536.

Coupland, N.J., Ogilvie, C.J., Hegadoren, K.M., Seres, P., Hanstock, C.C., Allen, P.S., 2005. Decreased prefrontal Myo-inositol in major depressive disorder. Biol Psychiatry 57, 1526-1534.
Covington, H.E., Maze, I., LaPlant, Q.C., Vialou, V.F., Ohnishi, Y.N., Berton, O., Fass, D.M., Renthal, W., Rush, A.J., Wu, E.Y., Ghose, S., Krishnan, V., Russo, S.J., Tamminga, C.A., Haggarty, S.J., Nestler, E.J., 2009. Antidepressant effects of histone deacetylase inhibitors. J Neurosci 29, 11451-11460.
Cuthbert, B.N., Insel, T.R., 2013. Toward the future of psychiatric diagnosis: the seven pillars of RDoC. BMC Medicine 11, 126.
Demyttenaere, K., De Fruyt, J., Stahl, S.M., 2005. The many faces of fatigue in major depressive disorder. Int J Neuropsychopharmacol 8, 93-105.
Disner, S.G., Beevers, C.G., Haigh, E.A.P., Beck, A.T., 2011. Neural mechanisms of the cognitive model of depression. Nature Rev Neurosci 12, 467-477.
Drevets, W.C., Videen, T.O., Price, J.L., Preskorn, S.H., Carmichael, S.T., Raichle, M.E., 1992. A functional anatomical study of unipolar depression. J Neurosci 12, 3628-3641.
DSM-5, 2013. Diagnostic and Statistical Manual of Mental Disorders. 5th edn. Revision American Psychiatric Association, Washington, DC. American Psychiatric Association, Washington, DC.
Duarte, J.M., Lei, H., Mlynarik, V., Gruetter, R., 2012. The neurochemical profile quantified by in vivo 1H NMR spectroscopy. Neuroimage 61, 342-362.
Dutta, A., McKie, S., Deakin, J.F., 2014. Resting state networks in major depressive disorder. Psychiatry Res 224, 139-151.
Ende, G., Demirakca, T., Tost, H., 2006. The biochemistry of dysfunctional emotions: proton MR spectroscopic findings in major depressive disorder. Prog Brain Res 156, 481-501.
Ende, G., Demirakca, T., Walter, S., Wokrina, T., Sartorius, A., Wildgruber, D., Henn, F.A., 2007. Subcortical and medial temporal MR-detectable metabolite abnormalities in unipolar major depression. Eur Arch Psychiatry Clin Neurosci 257, 36-39.
Eshel, N., Roiser, J.P., 2010. Reward and punishment processing in depression. Biol Psychiatry 68, 118-124.
Frahm, J., Bruhn, H., Gyngell, M.L., Merboldt, K.D., Hanicke, W., Sauter, R., 1989. Localized high-resolution proton NMR spectroscopy using stimulated echoes: initial applications to human brain in vivo. Magn Reson Med 9, 79-93.
Fuertig, R., Azzinnari, D., Bergamini, G., Cathomas, F., Sigrist, H., Seifritz, E., Vavassori, S., Luippold, A., Hengerer, B., Ceci, A., Pryce, C.R., 2016. Mouse chronic social stress increases blood and brain kynurenine pathway activity and fear behaviour: both effects are reversed by inhibition of indoleamine 2 3-dioxygenase. Brain, Behavior, and Immunity 54, 59-72.
Golden, S.A., Covington, H.E., 3rd, Berton, O., Russo, S.J., 2011. A standardized protocol for repeated social defeat stress in mice. Nat Protoc 6, 1183-1191.
Gozzi, A., Schwarz, A.J., 2016. Large-scale functional connectivity networks in the rodent brain. Neuroimage 127, 496-509.
Grandjean, J., Schroeter, A., Batata, I., Rudin, M., 2014a. Optimization of anesthesia protocol for resting-state fMRI in mice based on differential effects of anesthetics on functional connectivity patterns. Neuroimage 102 Pt 2, 838-847.
Grandjean, J., Schroeter, A., He, P., Tanadini, M., Keist, R., Krstic, D., Konietzko, U., Klohs, J., Nitsch, R.M., Rudin, M., 2014b. Early alterations in functional connectivity and white matter structure in a transgenic mouse model of cerebral amyloidosis. J Neurosci 34, 13780-13789.
Grimm, S., Boesiger, P., Beck, J., Schuepbach, D., Bermpohl, F., Walter, M., Ernst, J., Hell, D., Boeker, H., Northoff, G., 2009. Altered negative BOLD responses in the default-mode network during emotion processing in depressed subjects. Neuropsychopharmacol 34, 932-943.
Harrison, P.J., 2002. The neuropathology of primary mood disorder. Brain 125.
Hasler, G., van der Veen, J.W., Tumonis, T., Meyers, N., Shen, J., Drevets, W.C., 2007. Reduced prefrontal glutamate/glutamine and y-aminobutyric acid levels in major depression determined using proton magnetic resonance spectroscopy. Archives of General Psychiatry 64, 193-200.
Henckens, M.J.A.G., van der Marel, K., van der Toorn, A., Pillai, A.G., Fernandez, G., Dijkhuizen, R.M., Joels, M., 2015. Stress-induced alterations in large-scale functional networks of the rodent brain. NeuroImage 105, 312-322.
Hennig, J., Nauerth, A., Friedburg, H., 1986. RARE imaging: a fast imaging method for clinical MR. Magn Reson Med 3, 823-833.
Herry, C., Ciocchi, S., Senn, V., Demmou, L., Muller, C., Luthi, A., 2008. Switching on and off fear by distinct neuronal circuits. Nature 454, 600-606.
Huang, H., Fan, X., Williamson, D.E., Rao, U., 2011. White matter changes in healthy adolescents at familial risk for unipolar depression: a diffusion tensor imaging study. Neuropsychopharmacology 36, 684-691.

Kendler, K.S., Hettema, J.M., Butera, F., Gardner, C.O., Prescott, C.A., 2003. Life event dimensions of loss, humiliation, entrapment, and danger in the prediction of onsets of major depression and generalized anxiety. Arch Gen Psychiatry 60, 789-796.
Kessler, R.C., 1997. The effects of stressful life events on depression. Annual Review of Psychology 48, 191-214.
Krishnan, V., Han, M.-H., Graham, D.L., Berton, O., Renthal, W., Russo, S.J., LaPlant, Q., Graham, A., Lutter, M., Lagace, D.C., Ghose, S., Reister, R., Tannous, P., Green, T.A., Neve, R.L., Chakravarty, S., Kumar, A., Eisch, A.J., Self, D.W., Lee, F.S., Tamminga, C.A., Cooper, D.C., Gershenfeld, H.K., Nestler, E.J., 2007. Molecular adaptations underlying susceptibility and resistance to social defeat in brain reward regions. Cell 131, 391-404.
Kumar, A., Thomas, A., Lavretsky, H., Yue, K., Huda, A., Curran, J., Venkatraman, T., Estanol, L., Mintz, J., Mega, M., Toga, A., 2002. Frontal white matter biochemical abnormalities in late-life major depression detected with proton magnetic resonance spectroscopy. Am J Psychiatry 159, 630-636.
Kumar, S., Hultman, R., Hughes, D., Michel, N., Katz, B.M., Dzirasa, K., 2014. Prefrontal cortex reactivity underlies trait vulnerability to chronic social defeat stress. Nat Commun 5, 4537.
Lerch, J.P., Carroll, J.B., Spring, S., Bertram, L.N., Schwab, C., Hayden, M.R., Henkelman, R.M., 2008. Automated deformation analysis in the YAC128 Huntington disease mouse model. Neuroimage 39, 32-39.
Luo, Y., Cao, Z., Wang, D., Wu, L., Li, Y., Sun, W., Zhu, Y., 2014. Dynamic study of the hippocampal volume by structural MRI in a rat model of depression. Neurol Sci 35, 1777-1783.
Lüthi, A., Lüscher, C., 2014. Pathological circuit function underlying addiction and anxiety disorders. Nature Neuroscience 17, 1635-1643.
Moscarello, J.M., LeDoux, J.E., 2013. Active avoidance learning requires prefrontal suppression of amygdala-mediated defensive reactions. The Journal of Neuroscience 33, 3815-3823.
Mueggler, T., Baumann, D., Rausch, M., Staufenbiel, M., Rudin, M., 2003. Age-dependent impairment of somatosensory response in the amyloid precursor protein 23 transgenic mouse model of Alzheimer's disease. J Neurosci 23, 8231-8236.
Nissen, C., Holz, J., Blechert, J., Feige, B., Riemann, D., Voderholzer, U., Normann, C., 2010. Learning as a model for neural plasticity in major depression. Biological Psychiatry 68, 544-552.
Price, J.L., Drevets, W.C., 2010. Neurocircuitry of mood disorders. Neuropsychopharmacol 35, 192-216.
Provencher, S.W., 1993. Estimation of metabolite concentrations from localized in vivo proton NMR spectra. Magn Reson Med 30, 672-679.
Pruessner, M., Pruessner, J.C., Hellhammer, D.H., Bruce Pike, G., Lupien, S.J., 2007. The associations among hippocampal volume, cortisol reactivity, and memory performance in healthy young men. Psychiatry Res 155, 1-10.
Pryce, C.R., Azzinnari, D., Spinelli, S., Seifritz, E., Tegethoff, M., Meinlschmidt, G., 2011. Helplessness: a systematic translational review of theory and evidence for its relevance to understanding and treating depression. Pharmacol Ther 132, 242-267.
Rajkowska, G., Stockmeier, C.A., 2013. Astrocyte pathology in major depressive disorder: insights from human postmortem brain tissue. Current Drug Targets 14, 1225-1236.
Schmitt, S., Castelvetri, L.C., Simons, M., 2015. Metabolism and functions of lipids in myelin. Biochimica et Biophysica Acta 1851, 999-1005.
Schwarcz, R., Bruno, J.P., Muchowski, P.J., Wu, H.-Q., 2012. Kynurenines in the mammalian brain: when physiology meets pathology. Nature Reviews Neuroscience 13, 465-477.
Sforazzini, F., Schwarz, A.J., Galbusera, A., Bifone, A., Gozzi, A., 2014. Distributed BOLD and CBV-weighted resting-state networks in the mouse brain. Neuroimage 87, 403-415.
Sheline, Y.I., Barch, D.M., Donnelly, J.M., Ollinger, J.M., Snyder, A.Z., Mintun, M.A., 2001. Increased amygdala response to masked emotional faces in depressed subjects resolves with antidepressant treatment: an fMRI study. Biol Psychiatry 50, 651-658.
Soares, J.M., Sampaio, A., Marques, P., Ferreira, L.M., Santos, N.C., Marques, F., Palha, J.A., Cerqueira, J.J., Sousa, N., 2013. Plasticity of resting state brain networks in recovery from stress. Frontiers in Human Neuroscience 7, 1-10.
Stafford, J.M., Jarrett, B.R., Miranda-Dominguez, O., Mills, B.D., Cain, N., Mihalas, S., Lahvis, G.P., Lattal, K.M., Mitchell, S.H., David, S.V., Fryer, J.D., Nigg, J.T., Fair, D.A., 2014. Large-scale topology and the default mode network in the mouse connectome. Proceedings of the National Academy of Sciences of the United States of America 111, 18745-18750.
Tanaka, K., Furuyashiki, T., Kitaoka, S., Senzai, Y., Imoto, Y., Segi-Nishida, E., Deguchi, Y., Breyer, R.M., Breyer, M.D., Narumiya, S., 2012. Prostaglandin E2-mediated attenuation of mesocortical dopaminergic pathway is critical for susceptibility to repeated social defeat stress in mice. J Neurosci 32, 4319-4329.

Taylor Tavares, J.V., Clark, L., Furey, M.L., Williams, G.B., Sahakian, B., Drevets, W.C., 2008. Neural basis of abnormal response to negative feedback in unmedicated mood disorders. Neuroimage 42, 1118-1126.
Treadway, M.T., 2016. The Neurobiology of Motivational Deficits in Depression--An Update on Candidate Pathomechanisms. Curr Top Behav Neurosci 27, 337-355.
Tse, Y.C., Montoya, I., Wong, A.S., Mathieu, A., Lissemore, J., Lagace, D.C., Wong, T.P., 2014. A longitudinal study of stress-induced hippocampal volume changes in mice that are susceptible or resilient to chronic social defeat. Hippocampus 24, 1120-1128.
Tye, K.M., Mirzabekov, J.J., Warden, M.R., Ferenczi, E.A., Tsai, H.-C., Finkelstein, J., Kim, S.-Y., Adhikari, A., Thompson, K.R., Andalman, A.S., Gunaydin, L.A., Witten, I.B., Deisseroth, K., 2013. Dopamine neurons modulate neural encoding and expression of depression-related behaviour. Nature 493, 537-543.
Valdes, M., Collado, A., Bargallo, N., Vazquez, M., Rami, L., Gomez, E., Salamero, M., 2010. Increased glutamate/glutamine compounds in the brains of patients with fibromyalgia: a magnetic resonance spectroscopy study. Arthritis Rheum 62, 1829-1836.
Venkatraman, T.N., Krishnan, R.R., Steffens, D.C., Song, A.W., Taylor, W.D., 2009. Biochemical abnormalities of the medial temporal lobe and medial prefrontal cortex in late-life depression. Psychiatry Research: Neuroimaging 172, 49-54.
Whitfield-Gabrieli, S., Ford, J.M., 2012. Default mode network activity and connectivity in psychopathology. Annual Review of Clinical Psychology 8, 49-76.
Willner, P., 1997. Validity, reliability and utility of the chronic mild stress model of depression: a 10-year review and evaluation. Psychopharmacology (Berl) 134, 319-329.
Xiao, J., He, Y., McWhinnie, C.M., Yao, S., 2015. Altered white matter integrity in individuals with cognitive vulnerability to depression: a tract-based spatial statistics study. Sci Rep 5, 9738.
Yuksel, C., Ongur, D., 2010. Magnetic resonance spectroscopy studies of glutamate-related abnormalities in mood disorders. Biol Psychiatry 68, 785-794.
Zannas, A.S., McQuoid, D.R., Payne, M.E., Steffens, D.C., MacFall, J.R., Ashley-Koch, A., Taylor, W.D., 2013. Negative life stress and longitudinal hippocampal volume changes in older adults with and without depression. J Psychiatr Res 47, 829-834.
Zerbi, V., Grandjean, J., Rudin, M., Wenderoth, N., 2015. Mapping the mouse brain with rs-fMRI: An optimized pipeline for functional network identification. Neuroimage 123, 11-21.

Figure legends

Figure 1. Functional networks exhibiting increased within-network resting-state functional connectivity in CPS mice. Dual regression analysis was conducted using a Treatment group x Session model in 27 control (CON) and 26 CPS mice. (A) There was a significant Treatment group x Session interaction in the supplementary, barrel field (1) and (2), visual, and cingulate cortical networks that survived threshold-free cluster enhancement and Bonferroni correction. Voxels where the Treatment group x Session interaction effect survived p-value correction are shown as color-coded p-values, orange-yellow denoting an increase in the CPS group relative to baseline and control. (B) Network strength extracted from the supplementary cortex ROI as an example of a network exhibiting a Treatment group x Session interaction. Post hoc testing demonstrated a significant increase in post-session compared to baseline connectivity in CPS mice. ** $p < 0.01$, *** $p < 0.001$.

Figure 2. Between-network analysis reveals a significant increase in interaction strength in the post-session relative to baseline in the CPS group (n=26), but not in control group (n=27), for: (A) prefrontal cortex – piriform cortex; (B) prefrontal cortex – amygdala; (C) ventral hippocampus – amygdala; (D) cingulate cortex – piriform cortex; (E) cingulate cortex – amygdala. Representations of the networks involved in each interaction are shown as color-coded overlays on coronal anatomical slices.

Figure 3. MR spectroscopy in the amygdala and prefrontal cortex and identification of metabolite levels impacted by chronic psychosocial stress. A single voxel (represented in yellow) was positioned in the (A) right amygdala ($1x1x1.5mm^3$) in Run 1 (control n=15, CPS n=14) and (B) medial prefrontal cortex ($1x1.5x1.5mm^3$) in Run 2 (control n=12, CPS n=12). The anatomical region of interest from the Mouse Brain Atlas is shaded in blue and shown as an overlay on an anatomical image. Chemical shift spectra included distinct peaks corresponding to 16 identified neurochemicals, and a representative example from a control mouse is given for amygdala (C) and mPFC (D). E) For amygdala, there was a Treatment group x Session interaction for the estimated inositol concentration relative to total creatine (tCr) concentration and post hoc testing identified a post-session increase relative to baseline in CPS mice specifically. F) For mPFC, there was a Treatment group x Session interaction for the estimated glycerophosphocholine + phosphocholine (GPC+PCh) concentration relative to total creatine reflecting an increase in CPS mice compared to baseline and controls. * $p < 0.05$.

Figure 4. Fractional anisotropy and identification of white matter alteration in the cingulum in CPS mice. (A) Axial anatomical slice with Mouse Brain Atlas delineation as an overlay and corresponding fractional anisotropy map showing the underlying white matter structure. The location of the cingulum ROI is shown in yellow. (B) In data from 27 control and 26 CPS mice, for cingulum specifically, there was a Treatment group x Session interaction, and post hoc testing identified a post-session relative to baseline increase in fractional anisotropy in CPS mice specifically. Distance (mm) is indicated relative to Bregma.