Behavioural Brain Research 316 (2017) 225–233

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

Altered neural connectivity in adult female rats exposed to early life social stress

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HIGHLIGHTS

- Early life social stress induces changes in resting state functional connectivity.
- The prefrontal cortex and hippocampus exhibited particularly robust changes.
- Stress affects connectivity in social and depression relevant brain regions.

ARTICLE INFO

Article history:
Received 22 April 2016
Received in revised form 24 August 2016
Accepted 25 August 2016
Available online 1 September 2016

Keywords:
Social stress
fMRI
Resting state functional connectivity
Prefrontal cortex
Hippocampus
Nucleus accumbens

ABSTRACT

The use of a variety of neuroanatomical techniques has led to a greater understanding of the adverse effects of stress on psychiatric health. One recent advance that has been particularly valuable is the development of resting state functional connectivity (RSFC) in clinical studies. The current study investigates changes in RSFC in F1 adult female rats exposed to the early life chronic social stress (ECSS) of the daily introduction of a novel male intruder to the cage of their F0 mothers while the F1 pups are in the cage. This ECSS for the F1 animals consists of depressed maternal care from their F0 mothers and exposure to conflict between their F0 mothers and intruder males. Analyses of the functional connectivity data in ECSS exposed adult females versus control females reveal broad changes in the limbic and reward systems, the salience and introspective socioaffective networks, and several additional stress and social behavior associated nuclei. Substantial changes in connectivity were found in the prefrontal cortex, nucleus accumbens, hippocampus, and somatosensory cortex. The current rodent RSFC data support the hypothesis that the exposure to early life social stress has long term effects on neural connectivity in numerous social behavior, stress, and depression relevant brain nuclei. Future conscious rodent RSFC studies can build on the wealth of data generated from previous neuroanatomical studies of early life stress and enhance translational connectivity between animal and human fMRI studies in the development of novel preventative measures and treatments.

1. Introduction

The use of a variety of neuroanatomical techniques has led to a greater understanding of the adverse effects of stress on psychiatric health. One recent advance that has been particularly valuable is the development of resting state functional connectivity (RSFC) in clinical studies. This technique measures intrinsic neural connectivity through the measurement of spontaneous fluctuations in BOLD activity in different brain regions [1,2]. RSFC analysis allows for the simultaneous assessment of long term changes in multiple neural circuits involved in psychiatric etiology. This method has recently been adapted to imaging in conscious rodents [3,4], and is a valuable tool to enhance the translational value of behavioral neuroscience studies of rodent models of psychiatric illness. Comparisons of clinical and animal model RSFC data will enhance our understanding of susceptibility and resilience, pathological etiology, and treatment response. When compared to other methods of assessing neural activity and connectivity (immunohistochemistry, various tract tracing techniques, pcr for neural activity), a single RFSC study can add a temporal dimension, even allowing the longitudinal collection of several months or years' worth of data, a scale typically not possible with other time course approaches such

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http://dx.doi.org/10.1016/j.bbr.2016.08.051
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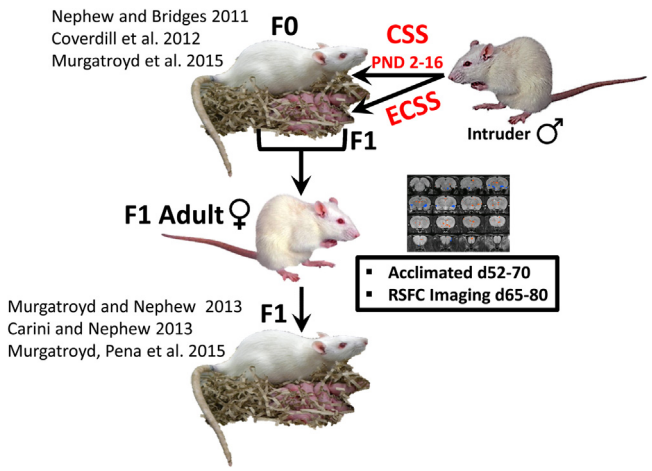

Fig. 1. The chronic social stress (CSS) model of postpartum depression and anxiety. F0 dams and their F1 pups are exposed to novel male intruder stress for one hour/day during postnatal days 2–15. This social stress is an early life chronic social stressor (ECSS) for the F1 generation. Both the F0 and F1 dams exhibit depressed maternal care and increased maternal anxiety. The current study focused on female F1 adults, who were imaged for resting state functional connectivity (RSFC).

as electrophysiological methods. Given similar financial resources, collecting similar amounts of data using other techniques may be impossible. In addition, RSFC in conscious rodents presents tremendous potential for enhanced etiological relevance through the longitudinal assessment of the effects of stress on multiple neural networks at several life history stages.

We have developed an ethologically relevant transgenerational model of the effects of chronic social stress (CSS) in postpartum depression and anxiety [5–9] (Fig. 1). Exposure of F0 dams to the chronic social stress of a daily exposure to a novel male intruder depresses maternal care, impairs lactation in both the F0 dams as well as their female F1 offspring, where CSS is an early life CSS (ECSS) Neuroendocrine studies of the F1 offspring of stressed F0 dams have revealed several behaviorally relevant changes in gene expression in a select set of nuclei involved in both the control of social behavior and the stress response that parallel similar findings in human studies of depression, anxiety and autism [5]. However, given that tissues were sampled at the end of lactation, it is unclear when the neural changes in gene expression occurred; for example, if they were present prior to gestation.

Recent study of the effects of a single traumatic stress exposure in rats indicated that it can cause long-term changes in the RSFC between the amygdala and mPFC, providing additional, clinically relevant support for the face and construct validity of this animal model of PTSD [10]. The development of RSFC in related animal models of psychiatric illness is postulated to enhance the identification of susceptibility indicators and the identification of effective preventative measures and treatments. The current study sought to build on this finding and apply RSFC to the study of the long term effects of early life stress, a mechanism relevant to a vast array of psychiatric disorders. It was hypothesized that animals exposed to ECSS would exhibit substantial changes in connectivity between several nuclei implicated in the early life stress associated disorders in both humans and animal models, including components of the Default Mode Network (DMN) and limbic network.

2. Methods

2.1. Animals

Sprague Dawley rats (Charles River, Wilmington, MA) in this study were maintained in accordance with the guidelines of the Committee of the Care and Use of Laboratory Animals Resources, National Research Council, and the research protocol was approved by the Tufts University and University of Massachusetts Institutional Animal Care and Use Committees. For an overview of the CSS paradigm, see Fig. 1. "CSS dams" refers to the adult females exposed to CSS during lactation (F0), and "ECSS females" refers to the adult female offspring of the CSS dams (F1); the focus of the present study. The F0 CSS stage of the study was conducted at Tufts University and the F1 pups were transported 10 miles at 30–40 days of age to the University of Massachusetts Center for Comparative Neuroimaging. The sample size for F1 control and ECSS female groups was 14.

2.2. ECSS model: creation of F0 dams and F1 females

F0 Dams (Charles River, Wilmington, MA) mated at Tufts University were subjected to the CSS protocol at the Cummings School (previously described) [7,9] consisting of placing a similarly sized (220–300 g) novel male intruder into a lactating female's home cage for 1 h from days 2–16 of lactation. Control dams were not exposed to the CSS protocol, and were only tested for maternal care and maternal aggression between 0800 and 1200 on days 2, 9, and 16 of lactation (both control and CSS dams were tested for maternal care and maternal aggression on these days). The F1 pups were left in the cage during the intruder presentation and the CSS F1 pups were exposed to depressed maternal care from their F0 mothers and the daily conflict between the mother and the male intruder (Early life CSS, ECSS) [7,9]. The F1 control and ECSS females of the current study were the offspring of the F0 control and CSS dams; the differences between the treatments of the control and ECSS F1 females were limited to the exposure of the ECSS F1 females to depressed maternal care and daily conflict between their F0 mothers and the male intruders during age 2–16 days. The F1 control and ECSS animals were treated identically after the age of 16 days. All F1 (CSS and control) females were transported to the UMass CCNI at 30–40 days of age (10 miles from the Cummings School), quarantined for 21 days, and then acclimated to the imaging procedures for 8 days.

2.3. Acclimation to imaging procedures

A total of 27 F1 adult females (65–90 days old) were exposed to the imaging protocol (13 CSS and 14 control). Before the imaging experiment, these rats were acclimated to the environment and imaging acoustic noise produced by the MR scanner using the procedure previously described [4]. Briefly, rats were anesthetized with isoflurane (2%) and secured in a head holder using plastic bite bar and ear bars. EMLA cream (Lidocaine 2.5% and Prilocaine 2.5% cream, Hi-Tech Pharmacal Co., Inc.) was topically applied to relieve any pain associated with the head holder. Animals were then placed into a black opaque tube (mock scanner) with tape-recorded scanner noise played. Animals were acclimated for eight days, one session per day. The time of acclimation was 15 min on day 1 with an increment of 15 min per day up until day 4. A maximum of 60 min was used on days 4–8, and all animals completed the acclimation procedures successfully.

2.4. Imaging

Animals were imaged at 65–90 days of age to assess the long term effects of early life stress on adult RSFC. All MR images were acquired on a 4.7T/40 cm horizontal magnet (Oxford, UK) interfaced with a Biospec Bruker console (Bruker, Germany) and equipped with a 20G/cm magnetic field gradient. A custom built 1H radiofrequency (RF) volume coil was used. Anatomical images were acquired using a multi-slice fast spin-echo sequence (RARE) with the parameters: repeti-