BRAIN RESEARCH 1385 (2011) 163–174

available at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/brainres

BRAIN RESEARCH

Research Report

Antenatal maternal stress alters functional brain responses in adult offspring during conditioned fear

Theodore R. Sadler[a], *Peter T. Nguyen*[b], *Jun Yang*[b], *Tina K. Givrad*[e], *Emeran A. Mayer*[f,g], *Jean-Michel I. Maarek*[e], *David R. Hinton*[a], *Daniel P. Holschneider*[b,c,d,e,*]

[a]*Department of Pathology, USC Keck School of Medicine, Los Angeles, CA 90033, USA*
[b]*Department of Psychiatry and the Behavioral Sciences, USC Keck School of Medicine, Los Angeles, CA 90033, USA*
[c]*Department of Cell and Neurobiology, USC Keck School of Medicine, Los Angeles, CA 90033, USA*
[d]*Department of Neurology, USC Keck School of Medicine, Los Angeles, CA 90033, USA*
[e]*Department of Biomedical Engineering, USC Viterbi School of Engineering, Los Angeles, CA, 90089, USA*
[f]*Center for Neurobiology of Stress, UCLA, Los Angeles, CA, 90095, USA*
[g]*Department of Medicine, UCLA, Los Angeles, CA, 90095, USA*

ARTICLE INFO

Article history:
Accepted 29 January 2011

Keywords:
Prenatal
Stress
Brain mapping
Cerebral blood flow
Amygdala
Fear

ABSTRACT

Antenatal maternal stress has been shown in rodent models and in humans to result in altered behavioral and neuroendocrine responses, yet little is known about its effects on functional brain activation. Pregnant female rats received a daily foot-shock stress or sham-stress two days after testing plug-positive and continuing for the duration of their pregnancy. Adult male offspring (age 14 weeks) with and without prior maternal stress (MS) were exposed to an auditory fear conditioning (CF) paradigm. Cerebral blood flow (CBF) was assessed during recall of the tone cue in the nonsedated, nontethered animal using the ^{14}C-iodoantipyrine method, in which the tracer was administered intravenously by remote activation of an implantable minipump. Regional CBF distribution was examined by autoradiography and analyzed by statistical parametric mapping in the three-dimensionally reconstructed brains. Presence of fear memory was confirmed by behavioral immobility ("freezing"). Corticosterone plasma levels during the CF paradigm were measured by ELISA in a separate group of rats. Antenatal MS exposure altered functional brain responses to the fear conditioned cue in adult offspring. Rats with prior MS exposure compared to those without demonstrated heightened fear responsivity, exaggerated and prolonged corticosterone release, increased functional cerebral activation of limbic/paralimbic regions (amygdala, ventral hippocampus, insula, ventral striatum, and nucleus accumbens), the locus coeruleus, and white matter, and deactivation of medial prefrontal cortical regions. Dysregulation of corticolimbic circuits may represent risk factors in the future development of anxiety disorders and associated alterations in emotional regulation.

* *Corresponding author at:* University of Southern California, 1333 San Pablo St., BMT 403, MC9112, Los Angeles, CA 90033, USA. Fax: +1 323 442 1587.
E-mail address: holschne@usc.edu (D.P. Holschneider).

0006-8993/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.brainres.2011.01.104

1. Introduction

Maternal psychological stress during pregnancy has been implicated as a risk factor for the development of affective disorders and schizophrenia in exposed offspring, as well as for decrements in their cognitive and language abilities (Koenig et al., 2002, Kofman, 2002, Weinstock, 2008, Laplante et al., 2008). Antenatal maternal stress (MS) has been shown to elicit changes in brain structure (Kawamura et al., 2006, McClure et al., 2004, Murmu et al., 2006, Salm et al., 2004, Wiggins and Gottesfeld, 1986, Zhu et al., 2004), brain neurochemistry (Adrover et al., 2007, Son et al., 2007, Van den Hove et al., 2006, Barros et al., 2006), stress hormone release (Kapoor et al., 2006) and behavior (Bowman et al., 2004, Lemaire et al., 2000, Louvart et al., 2005, Sternberg and Ridgway, 2003, Takahashi et al., 1992). The effects early life stress has on adult brain functional activation are just beginning to be examined. Prior work examining the effects of MS on functional brain activation during an acute stress challenge has been limited to a few studies documenting alterations in c-fos expression in isolated brain areas (hypothalamus, locus coeruleus) (Del Cerro et al., 2010, Fujita et al., 2010, Humm et al., 1995, Viltart et al., 2006). Using a rodent model, our study is the first to examine functional brain activation using whole brain perfusion mapping during a stress challenge in adult rats with or without a prior history of MS.

2. Results

2.1. *Antenatal maternal stress heightens adult psychophysiological responses*

Effects of MS were examined in a classic auditory fear conditioning paradigm. During the baseline, prior to receiving tone/footshock pairings, MS and no maternal stress (NMS) rats were actively engaged in exploratory behavior in the training chamber with no significant group difference in anxiety-like responses (percent "freezing," range 0.0–26.0%, Fig. 1A). During the CF training phase, animals with prior exposure to MS showed greater anxiety-like behavior compared to NMS animals (freezing response during minutes 4–15; "MS," $F_{1,36}=13.2$, $P<0.001$). Surprisingly, the effects of MS were most apparent in animals that had not received footshocks (CON: MS vs. NMS, freezing 63.4±13.0% vs. 32.2±12.2%, mean±SD, Fig. 1B). This increased response may be attributed to the tone, which itself may have been interpreted as an unfamiliar, fearful stimulus. A ceiling effect was noted for rats exposed to the footshock (CF: MS vs. NMS, freezing 66.5±14.9% vs. 56.1±16.0%, Fig. 1A), with no significant group differences between MS/CF and NMS/CF, except during the initial freezing responses to the footshocks ("time×CF×MS," $F_{14,23}=2.6$, $P<0.05$). Similarly, histograms plotting the number of 30-second intervals displaying freezing behavior during training showed a significant separation in the MS and NMS animals that had not received the footshocks (MS/CON vs. NMS/CON, $P<0.05$), with a nonsignificant trend in the CF animals (MS/CF vs. NMS/CF) (Fig. 1A–B, right panels).

Twenty-four hours later during reexposure to the tone cue (recall), CF-trained animals had significantly elevated freezing behavior compared to controls ("CF," $F_{1,36}=58.9$, $P<0.0005$). Comparison of MS/CF to NMS/CF animals showed statistically significant differences in freezing between these groups ("MS," $F_{1,36}=10.8$, $P<0.002$, CF: MS vs. NMS, 99.3±1.4% vs. 88.0±12.4%, CON: MS vs. NMS, 65.9±12.2% vs. 44.0±13.0%, Fig. 1D–E, left panels). NMS/CF animals gradually increased their freezing response to near maximal levels (~97%) over two minutes, whereas MS/CF animals were 100% motionless starting with the first 30 seconds of tone playback, remaining so throughout the entire recall. Histograms also showed a significant separation in the MS and NMS populations during fear conditioned recall (MS/CF vs. NMS/CF, $P<0.05$; Fig. 1D–E, right panels).

Blood corticosterone (BC) results paralleled those noted in the behavioral scoring. Prior to CF training, BC levels of NMS and MS rats were both within ranges of previously reported basal values (24–56 ng/mL and 32–65 ng/mL, respectively) (Cordero et al., 1998). After the first tone/foot-shock pairing, BC levels increased to 68±15 ng/mL in NMS/CF rats, while levels in MS/CF animals dramatically increased to 235±64 ng/mL ("CF," $F_{1,28}=7.1$, $P<0.01$) (Fig. 1C). Significant differences in BC levels between MS/CF and NMS/CF rats persisted during the duration of tone exposure (MS/CF 230–280 ng/mL, NMS/CF 68–107 ng/mL), with continued elevation even 120 minutes after the last tone-shock pairing for MS/CF rats (142±58 ng/mL) ("MS," $F_{1,28}=9.9$, $P<0.004$). As noted in the behavioral measures, exposure to "tone alone" elicited greater BC levels in MS/CON than in NMS/CON animals (MS/CON 172±54 ng/mL, NMS/CON 57±20 ng/mL), suggesting that the auditory stimulus was perceived as being more stressful by animals with prior MS exposure. Twenty-four hours later during recall, significant effects were noted for MS and CF ("MS," $F_{1,28}=10.8$, $P<0.003$, "CF," $F_{1,28}=19.6$, $P<0.0005$). MS/CF rats again demonstrated elevations in BC levels compared to NMS/CF rats (MS/CF 136±19 ng/mL and NMS/CF 79±13 ng/mL) (Fig. 1F).

Fig. 1 – Psychophysiological responses of adult male rats, with previous exposure to maternal stress (MS) or no maternal stress (NMS) during a conditioned fear (CF) paradigm. TRAINING (top): Behavioral responses of (A) NMS/CF ($n=9$) and MS/CF ($n=14$) rats during CF training. (B) NMS/CON ($n=8$) and MS/CON ($n=11$) controls exposed to tone without footshock. The left panel depicts percent behavioral immobility (freeze) over time, with each point representing the group average (± SEM) over 30 s. The right panel depicts a histogram plotting the number of 30 s intervals displaying a specified percent of freezing behavior. Also shown are blood corticosterone levels during (C) CF training for NMS/CF ($n=8$), NMS/CON ($n=10$), MS/CF ($n=7$), and MS/CON ($n=7$) rats. RECALL (bottom): (D) Behavioral results and (E) corticosterone results during recall, i.e. reexposure to the tone alone 24 hours after CF training. The horizontal arrow indicates the time and duration of the tone exposure. The vertical dashed bar represents the time of radiotracer infusion. *Comparison of the specified group average with each of the comparison groups, $P<0.05$.