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Authors

Avishai-Eliner, Sarit
Hatalski, Carolyn G
Tabachnik, Elvan
et al.

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Differential regulation of glucocorticoid receptor messenger RNA (GR-mRNA) by maternal deprivation in immature rat hypothalamus and limbic regions

Sarit Avishai-Eliner[a,b], **Carolyn G. Hatalski**[a], **Elvan Tabachnik**[b], **Mariam Eghbal-Ahmadi**[a], and **Tallie Z. Baram**[a,*]

[a] Departments of Pediatrics and Anatomy and Neurobiology, University of California at Irvine Med. Sci. I, 4475; UCI Irvine, CA 92697-4475, USA

[b] Hebrew University, Jerusalem and Kaplan Medical Center, Rehovoth, Israel

Abstract

Maternal deprivation (MDep) of neonatal rats significantly influences the hypothalamic–pituitary–adrenal (HPA) axis. This study hypothesized that GR-mRNA modulation constituted an early, critical mechanism for the acute effects of MDep on neuroendocrine stress-responses. GR-mRNA hybridization signal in hippocampal CA1, hypothalamic paraventricular nucleus (PVN) and frontal cortex was significantly reduced immediately following 24 h MDep. In amygdala, cingulate cortex, PVN and CA1, apparent gender-dependent MDep effects on GR-mRNA expression were observed, without significant differences in absolute levels. Thus, rapid, region-specific MDep effects on GR-mRNA expression in HPA-regulating areas are shown, consistent with involvement of GR-expression in mechanisms of MDep influence on HPA tone.

Keywords

Glucocorticoid receptor messenger RNA; Rat; Stress; Maternal deprivation; Hippocampus; Hypothalamic paraventricular nucleus; Amygdala central nucleus

Stress-responses during postnatal days (PND) 3–14 in the rat are characterized by altered stress-related gene regulation and attenuated hormone secretion [1,10,16]; the latter is augmented by maternal deprivation (MDep) [1,9,10,15]. In contrast to established enhancement of hormone secretion, the acute effects of MDep on *central* HPA components are not completely understood. For example, CRH-mRNA levels in 24 h MDep 9-day old rats were unchanged [1,14] or reduced [9]. Thus, MDep-induced CRH-mRNA changes did not correlate with enhanced hormonal responses, suggesting that mechanisms by which MDep influences the latter may not involve CRH gene expression.

Glucocorticoids (GC) and GR provide attractive candidate mechanisms for mediating MDep effects on hormonal stress responses. GR regulates stress-associated secretion of CRH and ACTH [6,18], acting at hippocampal [5,8] and hypothalamic PVN levels [6,12,18]. In addition, GR-mRNA expression in neonatal rat amygdaloid central nucleus (ACE) [13,19] and frontal and cingulate cortices [13] may participate in MDep influence on HPA tone. Therefore, this study aimed to determine whether 24 h MDep on PND8, influenced GR-

*Corresponding author. Fax: +1-949-824-1106; tallie@uci.edu.

mRNA levels in HPA-regulating regions. In addition, based on reports of gender-related effects of MDep [11], males and females were analyzed separately.

Pups were offspring of timed-pregnancy Sprague–Dawley dams [1–3]. Birth-date of pups was considered PND0; litters were culled to 12 and mixed among experimental groups. Cages were maintained as described [1–3,17–19], and procedures were approved by Institutional ACC. On the morning of PND8, rats were either placed individually in beakers, kept together separated from the dam, or left undisturbed in home cages with dams [1]. Deprived rats were kept euthermic and on a 12 h light schedule. On the morning of PND9, undeprived pups were sacrificed within 3 min of disturbance [17], followed by MDep pups. Trunk blood was collected for ACTH and corticosterone analysis using radioimmunoassay (INCSTAR, Stillwater, MN and ICN Irvine, CA, respectively) [1–3,17]. Because plasma corticosterone levels of individually- and group-MDep rats did not differ, these groups were combined.

Brains were rapidly dissected and processed for in situ hybridization (ISH) following previously described procedures [1–3,17–19], and using a riboprobe complementary to 490 bases of rat GR gene [19]. Semiquantitative analysis of GR-mRNA was performed by two investigators (one unaware of treatment), as described previously [3,17]. Optical density (OD) of GR-mRNA signal in digitized images (corrected for background and calibrated using ^{14}C standards) was determined over hippocampal CA1 (schematized in Fig. 1), dorsomedial parvocellular PVN, frontal and cingulate cortices and ACE. For each region, 4–6 brains/group and 12–22 matched sections (3–5 sections/brain) were analyzed. Statistical significance ($p < 0.05$) was determined by unpaired Student's *t*-test with Welch's correction when indicated [2].

GR-mRNA levels were significantly reduced (65%) in PVN (Fig. 1A,C,E), and in CA1 (Fig. 1B,D,E) of MDep rats. MDep also reduced GR-mRNA levels in frontal (33%), but not cingulate cortex (Fig. 1E). In ACE, a trend towards GR-mRNA upregulation was not statistically significant ($p = 0.14$). MDep did not elevate morning basal plasma ACTH levels, but significantly increased corticosterone (7.40 ± 0.42 and 1.40 ± 0.20 μg/dl in deprived and controls, respectively, $p < 0.0001$), consistent with previous reports [1,2,10,14,15].

Absolute GR-mRNA levels did not differ between males and females in either MDep or control groups. (Fig. 2A,B). However, when the MDep effect on males and females was analyzed separately, apparent gender-related influences were noted in ACE, PVN, CA1 and cingulate cortex (Fig. 2C): the PVN–GR-mRNA ratio of controls/MDep was 3.8 for males and 1.65 for females, suggesting a larger effect of MDep on GR-mRNA in males. In ACE, the ratio: deprived/control was 1.25 for males and 2.4 for females, indicating a more robust enhancing effect of MDep on ACE–GR-mRNA in females (Fig. 2C). We emphasize that no statistical significance is attached to this analysis, because it is based on means of a small number of samples. However, the variances (*F*-test) for PVN and ACE did not differ significantly between male and female groups in either control (PVN $p = 0.14$; ACE $p = 0.44$) or MDep groups (PVN $p = 0.4$; ACE $p = 0.25$). Trends for gender-specific MDep effects were apparent also in CA1 and cingulate-but not frontal-cortex (Fig. 2C). Notably, no gender-related differences in basal plasma corticosterone were observed (not shown).

This study demonstrates differential modulation of GR-mRNA expression in HPA-regulating brain regions by 24 h MDep on PND8. GR-mRNA levels in hippocampus, PVN and frontal cortex of male and female MDep rats were decreased compared with controls. At least two alternative mechanisms may underlie this rapid reduction of hippocampal GR-mRNA: (1) MDep enhancement of plasma corticosterone levels which downregulate

hippocampal GR gene expression [5,8]. This negative feedback is developmentally regulated, commencing between PND 7–14 [5,18], and *reduces negative input* to peptidergic PVN neurons, thus *increasing* stress-responses [18]. Alternatively, MDep may enhance stress-responses via *direct* increase of PVN–CRH-mRNA expression and stress-induced CRH release. However, reports of unchanged [1,14] or reduced CRH-mRNA [9] after MDep are inconsistent with this alternative.

Reduced hippocampal GR-mRNA levels in rats undergoing MDep on PND7 or 11 were previously shown on PND20 [14] but were not measured earlier in that study [14]. Thus, *immediate* down-regulation of hippocampal GR-mRNA, shown here, together with earlier findings of unchanged PVN–CRH-mRNA levels [1,14], strongly suggest that GR modulation may be an early, key step mediating MDep effects on HPA tone. Also, finding decreased PVN–GR-mRNA levels after MDep suggests important 'local' GR feedback regulation of PVN–CRH-mRNA, as shown in adult [6,8,12] and immature rat [18].

MDep-induced reduction of cortical GR-mRNA, shown here, is consistent with studies implicating cortical-GR expression in long-term influences of neonatal manipulations on HPA tone [7]. Thus, enhancement of GR-mRNA levels has led to decreased HPA tone [7], whereas reduced expression (reported here) should enhance HPA sensitivity.

Observed gender-related effects of MDep on GR-mRNA levels in PVN, ACE, CA1 and cingulate cortex amplify reports on differential effects of maternal adrenalectomy (GR-mRNA levels increased in female offspring only, [4]), and of MDep during PND3 (increased hippocampal GR levels in adult females, decreased in males [11]). These findings are consistent with influences of sex-steroid milieu on perinatal regulation of GR-mRNA [4].

In summary, acute, differential regulation of GR-mRNA levels in HPA-regulating regions by MDep, combined with lack of early changes of PVN–CRH-mRNA, strongly implicate rapid, early alterations in GR expression in the mechanisms by which MDep alters HPA tone in the immature rat.

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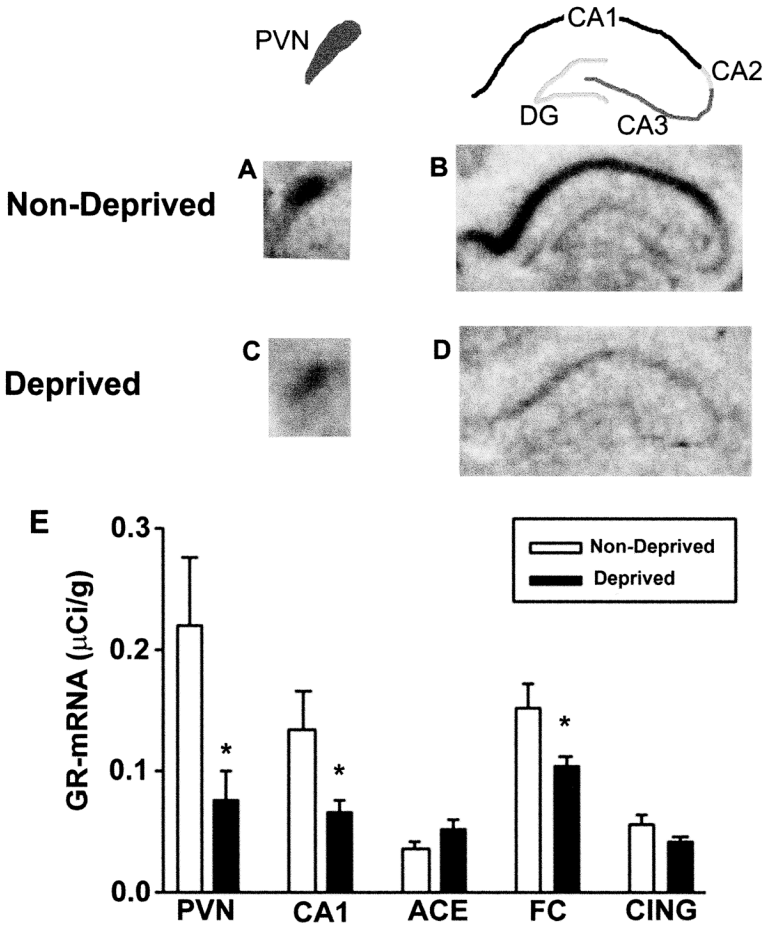

Fig. 1.

GR-mRNA levels in maternally-deprived and non-deprived 9-day old rats. Following ISH for GR-mRNA, optical density was measured over paraventricular nucleus (PVN), hippocampal CA1, amygdaloid central nucleus (ACE), frontal-(FC) and cingulate cortex (CING). Autoradiographs show that GR-mRNA signal in PVN of deprived pups, (C) was lower than in controls (A). GR-mRNA signal over CA1 of controls (B) was stronger than in deprived pups (D). (E) Quantitative analysis of GR-mRNA signal. Mean ± S.E.M. of data from 12–22 sections from 4–6 brains per group; *$p < 0.05$.

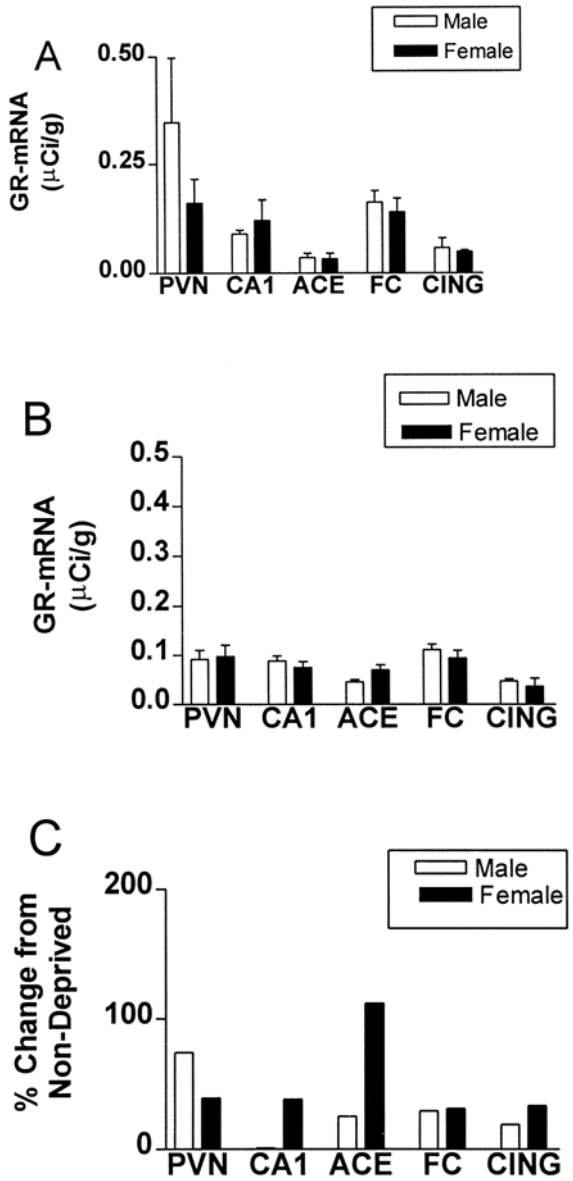

Fig. 2.

Gender-related effects of maternal deprivation on GR-mRNA levels. GR-mRNA levels did not differ between males and females in either controls (A) or deprived rats (B). (C) Reflects separate analyses of deprivation-effect on GR-mRNA levels in males and females. Gender-related differences in the magnitude of GR-mRNA level changes from the non-deprived baseline are apparent in ACE and PVN (see text for statistical considerations).