Journal of Neuroscience Research 76:488–496 (2004)

Early Adoption Modifies the Effects of Prenatal Stress on Dopamine and Glutamate Receptors in Adult Rat Brain

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Stressful stimuli during pregnancy induce complex effects that influence the development of offspring. These effects can be prevented by environmental manipulations during the early postnatal period. Repeated restraint during the last week of pregnancy was used as a model of prenatal stress, and adoption at birth was used to change the postnatal environment. No differences were found in various physical landmarks, except for testis descent, for which all prenatally stressed pups showed a 1-day delay in comparison with control rats, regardless of the postnatal adoption procedure. Levels of dopamine (DA) D_2 and glutamate (Glu) N-methyl-D-aspartate (NMDA) receptors were differentially regulated in different forebrain regions of cross-fostered adult offspring. Increased concentrations of cortical D_2 receptors detected in stressed pups, raised by a gestationally stressed biological mother, were not detected when the pups were raised by a control mother. Control pups raised by a foster mother whether gestationally stressed or not had higher levels of NMDA receptors in cortical areas. These findings suggest that the normal expression of DA and Glu receptors is influenced by in utero experience and by lactation. The complex pattern of receptor changes reflects the high vulnerability of DA and Glu systems to variations both in prenatal and in postnatal environment, particularly for cortical D_2 receptors and NMDA receptors in cerebral cortex and nucleus accumbens. In contrast, testis descent appears to be more susceptible to prenatal than to postnatal environmental events.

Key words: dopamine D_2 receptors; glutamate NMDA receptors; testis descent; prenatal stress; cross-fostering; quantitative autoradiography

Offspring of pregnant female animals exposed to stress prenatally have higher vulnerability to anxiety, abnormal circadian rhythm, impaired sexual function, and enhanced propensity to self-administer drugs of abuse (Henry et al., 1995; Vallée et al., 1999). Such vulnerability may result from abnormalities in the development and integration of forebrain dopamine (DA) and glutamate (Glu) projections during the prenatal period. In this regard, prenatal stress altered DA neurotransmission, evidenced by a higher DA turnover in prefrontal cortex and a lower turnover in striatum and nucleus accumbens (Fride and Weinstock, 1988; Alonso et al., 1997). In addition, DA D_2 and D_3 receptors were increased and decreased, respectively, in nucleus accumbens of mature adults subjected to restraint prenatal stress (Henry et al., 1995). We have recently assessed the effects of prenatal stress on DA and Glu receptor expression in adult rats subjected to repeated restraint stress during the last week of pregnancy and found that these animals have higher levels of DA D_2 and Glu metabotropic group III receptors in cortical regions and higher concentrations of N-methyl-D-aspartate (NMDA) receptors in cerebral cortex and basal ganglia compared with their control littermates (Berger et al., 2002).

Several behavioral strategies have been employed to modify the postnatal environment, namely, postnatal handling, early adoption (or cross-fostering), and maternal

Contract grant sponsor: Agencia Nacional de Promoción Científica y Tecnológica; Contract grant number: PICT 02287; Contract grant sponsor: CONICET; Contract grant number: PIP 6266.

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Received 2 December 2003; Revised 5 February 2004; Accepted 17 February 2004

Published online 9 April 2004 in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jnr.20119

separation; all of them have been reported to exert profound behavioral and neuroendcrinological effects in adulthood. For example, postnatal handling improved the performance of cognitive tasks in mature adults subjected to prenatal stress (Meaney et al., 1988; Escorihuela et al., 1995). Handled rats exhibited attenuated fearfulness in novel environments, decreased hypothalamic-pituitary-adrenal (HPA) axis responses to a wide variety of stressors (Meaney et al., 1996), and increased glucocorticoid receptor gene expression in all cell fields of the hippocampus (O'Donnell et al., 1994). Early adoption was reported to decrease novelty-induced locomotion, improve recognition performance in the Y-maze (Barbazanges et al., 1996), and reduce the secretion of corticosterone produced by novelty-induced stress (Maccari et al., 1995). In addition, Ellenbroek and colleagues (2000) showed that cross-fostering decreased adult susceptibility to apomorphine in an apomorphine-susceptible rat strain. Maternal separation of infant rats for a 24-hr period altered normal brain development by accelerating death of neurons and glia (Zhang et al., 2002) and decreasing glucocorticoid receptors, mineralocorticoid receptors, corticotropin-releasing hormone, and proopiomelanocortin transcripts in the brains of maternally deprived mice (Schmidt et al., 2002).

Therefore, early environment can contribute to the development of individual differences in neuroendocrine responses to stressful stimuli. Postnatal handling had been reported to attenuate the effects of prenatal stress on HPA response, block the increased pain thresholds induced by prenatal stress (Smythe et al., 1994), reverse age-related HPA dysfunctions, and reduce cognitive impairments (Vallée et al., 1999). Moreover, Maccari et al. (1995) found that adoption prevented prolonged stress-induced corticosterone secretion and blocked the increase in type I corticosteroid receptors in the hippocampus of adult rats subjected to prenatal stress. However, no other parameters were studied in models of early adoption in prenatally stressed rats.

In this study, we extended our recent findings on the enhanced expression of DA and Glu receptors in adult rats subjected to prenatal stress (Berger et al., 2002) by describing region-specific changes in D_2 and NMDA receptors in brains of adult rats exposed to several prenatal/postnatal manipulations. These manipulations included exposure to prenatal stress (or no stress prenatally), followed by postnatal care by a biological mother, a gestationally nonstressed foster mother, or a gestationally stressed foster mother. We hypothesized that adoption of a prenatally stressed offspring by a foster control mother would inhibit the effects of prenatal stress. Consequently, adoption of a control offspring by a gestationally stressed mother might mimic the effects of prenatal stress. The experimental manipulation of adoption per se was included for comparison.

MATERIALS AND METHODS

Materials

R,S-(+/–)-[N-methyl-^{3}H]nemonapride (YM-09151-2; specific activity 81.4 Ci/mmol) and [3-^{3}H](+)-5-methyl-10,11-dihydro-[5H]-dibenzo[a,d]cyclohepten-5,10-imine (MK-801; 23.9 Ci/mmol) were purchased from New England Nuclear (Boston, MA). Tritium-sensitive Hyperfilm and tritium autoradiography standards were purchased from Amersham (Arlington Heights, IL). D-19 photographic developer and fixative were from Eastman-Kodak (Rochester, NY).

Chemicals and drugs included ketamine hydrochloride, spermine tetrahydrochloride, 1,3-ditolylguanidine (DTG), pindolol, S(–)-sulpiride from Sigma-RBI (Natick, MA); ethylenediaminetetraacetic acid (EDTA) from Fischer Scientific (Fair Lawn, NJ); and L-glutamic acid (Glu), L-glycine hydrochloride, cation hydrochlorides, and Tris-(hydroxymethyl)aminomethane-HCl (Tris) from Sigma (St. Louis, MO).

Animals

Virgin female Wistar rats weighing 250 g were obtained from the local vivarium. Vaginal smears were collected daily for 8 days before mating to determine the stage of the estrus cycle and the day of conception. On the day of proestrus, sexually experienced male Wistar rats weighing 400 g were introduced for mating. Vaginal smears were taken on the following morning. The day on which spermatozoa were found in the smear was designated day 1 of pregnancy.

Prenatal Stress and Adoption Procedures

Pregnant female dams (n = 16) were randomly assigned to control or prenatal stress group. The control group was left undisturbed in the home cage; the other group was subjected to restraint stress. Rats were transferred to an experimental room where the selected stress paradigm was applied. Pregnant females were placed individually in a plastic, transparent restrainer fitted closely to body size for three periods (45 min each) per day (9 and 12 AM and 5 PM) between the 14th and the 21st days of pregnancy. This type of stress was selected because it influences the fetus indirectly via direct stress on the mother (Ward and Weisz, 1984). The sessions were performed in a lighted environment. No other subjects were present in the experimental room during stress exposure. At the end of the stress session, animals were returned to the animal housing room and were then individually housed with ad libitum access to food and water. Constant light/dark cycles (on at 06:00, off at 20:00) and controlled temperature (21–25°C) were maintained. All procedures were in agreement with standards for the care of laboratory animals as outlined in the NIH *Guide for the Care and Use of Laboratory Animals*. Care was taken to minimize the number of animals used.

On the day of parturition, litter characteristics were recorded and litters were culled to 10 pups, maintaining similar numbers of males and females, whenever possible. No differences in litter sizes were found between stressed and nonstressed animals. Control or prenatally stressed pups were maintained with their biological mother or adopted by a foster control or gestationally stressed mother, resulting in six possible combinations: 1) control pups left undisturbed with the biological control mother (C); 2) control pups reared by a foster control mother (C/C); 3) control pups reared by a foster gestationally stressed mother (C/S); 4) prenatally stressed pups reared by the biological stressed mother (S); 5) prenatally stressed pups reared

TABLE I. Experimental Groups

Prenatal treatment	Postnatal care	Group name
Control	Biological mother	C
Control	Control foster mother	C/C
Control	Gestationally stressed foster mother	C/S
Stress	Biological mother	S
Stress	Control foster mother	S/C
Stress	Gestationally stressed foster mother	S/S

by a foster control mother (S/C); and 6) prenatally stressed pups reared by a foster gestationally stressed mother (S/S; Table I).

Four litters were maintained for each experimental group. To prevent litter effects, at most two male pups from same litter were tested in adult life. Pups were placed in the cage of the adoptive mother within the first day after birth. During this procedure, the mothers were briefly (less than 1 min) removed from their cages. The offspring were weaned 21 days after birth, and only male offsprings were selected for receptor autoradiographic studies. At most five male pups were placed per cage and left undisturbed until postnatal day (PND) 90.

Neonatal Physiological Markers and Sexual Development

Body weight of each litter was measured at PND 10, 30, and 90. In addition, each litter was evaluated for development of physical landmarks, including pinnae detachment (PND 1–5), eye opening (PND 13–15), and testis descent (PND 22–29).

Pinna detachment. The appearance of pinna detachment was recorded as the age when the pinnae of both ears unfolded to a fully erect position. Ears were inspected from PND 1 until the entire litter exhibited complete unfolding.

Eye opening. Eye opening was defined as any visible break in the membrane covering the eye. Eyes of each animal were examined from PND 13 until both eyes of every animal in every litter were opened.

Testis descent. Testis descent was defined as the days when both testes fully descended into the scrotal sac and could be palpated while males were held vertically under their forelimbs. The test was initiated on PND 22.

Quantitative Autoradiographic Assays

At PND 90, rats were killed by rapid decapitation; their brains were then quickly removed, frozen by immersion in Freon (−40°C), and stored at −85°C. Frozen coronal sections (10 μm) were cut in a cryostat at –20°C, mounted on gelatin-coated microscope slides, and stored at −85°C until use. On the day of the experiment, slides were thawed at room temperature (RT). Receptor binding levels were measured in medial prefrontal cortex (MPC), dorsal frontal cortex (DFC), nucleus accumbens core (NAcC), and CA_1 region of the hippocampus for DA D_2 receptors and MPC, DFC CA_1, caudate-putamen (CPu), and NAcC and shell (NAcS) for Glu receptors. These areas were selected based on our recent study showing an increase in D_2 and NMDA receptors in prenatally stressed group (S) compared with control (C) group (Berger et al., 2002). In addition, these selected cortical, limbic, and extrapyramidal brain regions mediate cognitive, emotional, and motor behaviors that are typically disturbed in patients with psychotic disorders (Benes, 2000; Baldessarini and Tarazi, 2001).

D_2 Receptor Binding

Sections were first preincubated for 1 hr at RT in 50 mM Tris-HCl buffer (pH 7.4) containing 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, and 1 mM $MgCl_2$. Sections were incubated for 1 hr at RT in the same buffer containing 1.0 nM [^{3}H]nemonapride with 0.5 μM DTG and 0.1 μM pindolol to mask sigma ($\sigma_{1,2}$) and $5HT_{1A}$ sites, respectively. Nonspecific binding was determined with 10 μM S(–)-sulpiride. After incubation, slides were washed (2 × 5 min) in ice-cold buffer, dipped in ice-cold water, and air dried (Defagot and Antonelli, 1997; Tarazi et al., 1998a). Although the resulting radioligand binding may include traces of binding to D_3 or D_4 sites, most of the signal is believed to represent D_2 receptors (Tarazi et al., 1998a).

NMDA Receptor Binding

Sections were preincubated for 60 min at RT in 50 mM Tris-HCl buffer (pH 7.4), then incubated for 150 min at RT in fresh buffer containing 10 nM [^{3}H]MK-801 and 100 μM L-Glu, 100 μM glycine, 1 mM EDTA, and 75 μM spermine to enhance the binding of [^{3}H]MK-801 to its site within the open cation channels associated with NMDA receptors. Nonspecific binding was determined by including 20 μM ketamine. After incubation, slides were washed in ice-cold 50 mM Tris-HCl buffer, twice for 20 min, and dried (Tarazi et al., 1998b, 2003).

Film Exposure and Image Analysis

Radiolabelled slides and calibrated [^{3}H] standards (Amersham) were exposed to Hyperfilm (Eastman-Kodak) at 4°C for 4−6 weeks. Films were developed in Kodak D-19 developer and fixative. Optical density (OD) in brain regions of interest was measured with a computerized densitometric image analyzer (MCID-M4; Imaging Research, St. Catharines, Ontario, Canada). Brain regions of interest were outlined and their ODs measured (see Fig. 1; Berger et al., 2002; Tarazi et al., 2003). Left and right sides of two contiguous sections (four measurements per subject-brain) represented total binding, and two other sections represented nonspecific binding; the four determinations were averaged for each subject. OD was converted to nCi/mg of tissue with calibrated [^{3}H] standards, and, after subtracting nonspecific from total binding, specific binding was expressed as fmol/mg tissue.

Statistical Analysis

Statistics were analyzed by using Statistica 5.5 (StatSoft, Inc). A three-way analysis of variance (ANOVA) was used to analyze the effects on weight and physical landmarks of prenatal treatment (A) and postnatal adoption procedures (B), with litter nested in A × B (Table II). A two-way ANOVA was used to analyze the effects of prenatal treatment and postnatal adoption procedures on the expression of dopamine and glutamate receptor in different forebrain regions (see Figs. 2, 3). Those effects that were determined to have a significant interaction were further tested by using simple effects and Tukey's post hoc test for significance. Comparisons were considered significant at $P < .05$.

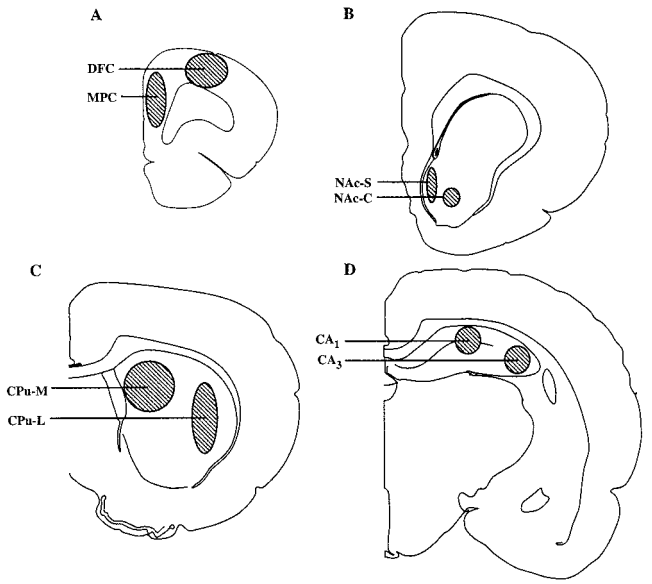

Fig. 1. Sites for autoradiographic analysis of rat brain regions. Samples included 10-μm coronal sections from A-P 3.2–4.2 mm (**A**), A-P 1.7–2.2 mm (**B**), A-P 0.2–0.7 mm (**C**) anterior and A-P –3.3 to –3.8 mm posterior (**D**) to bregma, according to Paxinos and Watson (1982). MPC, medial prefrontal cortex; DFC, dorsal frontal cortex; CA_1, CA_3, regions of the hippocampus; CPuL, caudate putamen lateral; CPuM, caudate putamen medial; NAcC, nucleus accumbens core; NAcS, nucleus accumbens shell.

RESULTS

Physical Landmarks

Animal weight and physical landmarks are presented in Table II. No significant differences were found in body weights of offspring in any experimental situation at PND 10, 30, and 90 (Table II). Moreover, the days of eye and ear opening were similar among groups. No significant interaction was found between prenatal and postnatal treatment in the average day of testis descent. However, all prenatally stressed groups showed a significant delay in testis descent (1 day later) compared with prenatally nonstressed groups, independently of the cross-fostering procedure (25.38 ± 0.24 vs. 24.49 ± 0.24; $P < .05$).

Receptor Autoradiography

D_2 dopamine receptor. [^{3}H]nemonapride binding in MPC, DFC, and the hippocampal CA_1 region displayed similar changes (Fig. 2). In the three areas, [^{3}H]nemonapride binding did not significantly change in a control offspring reared by its biological control mother (C) or by a gestationally stressed mother (C/S). However, if the control offspring was reared by a foster control mother (C/C), [^{3}H]Nemonapride binding increased significantly. In contrast, [^{3}H]nemonapride binding was similar in prenatally stressed offspring reared by a biological mother (S) and prenatally stressed offspring reared by a foster gestationally stressed mother (S/S). Both, S and S/S, differed significantly from a prenatally stressed offspring reared by a foster control mother (S/C). Therefore, enhanced D_2 receptor expression in a prenatally stressed rat returned to control values when reared by a foster control mother.

In our previous study (Berger et al., 2002), NAcC showed a significant increase of S over C. However, in the present study, a statistically nonsignificant increase (19%; $P = .07$) in D_2 receptor binding was observed. No other differences were observed among any of the different situations tested in this area (Fig. 2).

NMDA glutamate receptors. Cortical areas (MPC and DFC) showed the same pattern of changes for NMDA receptors (Fig. 3). Control offspring reared by a foster control mother (CC) and control offspring reared by a gestationally stressed mother (C/S) showed similar levels of MK-801 binding compared with stressed offspring reared by their biological stressed mothers (S). Levels of cortical NMDA receptors in three groups were significantly higher than control offspring reared by their biological mothers (C) and stressed offspring reared by foster mothers gestationally stressed (S/S) or not (S/C).

In contrast, NMDA receptors in hippocampal area CA_1 were significantly reduced (by 33%) in control offspring reared by a foster control mother compared with C. No changes in NMDA receptor binding were observed when offspring were reared by a prenatally stressed mother (C/S). Prenatally stressed offspring reared by a stressed foster mother (S/S) showed similar levels of NMDA receptor compared with S, in contrast to a significant decrease (18%) in NMDA receptors when the stressed pup was reared by a control mother (S/C; Fig. 3).

Concentrations of NMDA receptors remained unchanged in NAcC and NAcS of C, C/C, and S/C. Significantly higher levels were obtained for C/S and S/S. However, the increase of NMDA in NAcC and NAcS of S was significantly higher than that in any other group (Fig. 3). Caudate-putamen showed no significant interaction between control and stress groups and was not included in Figure 3.

DISCUSSION

Physical Landmarks

Prenatal stress and early adoption did not produce any difference in whole-body weight or day of ear and eye opening among all the situations tested. However, testis descent showed a delay of 1 day in all prenatally stressed offspring independent of the status of the mother that reared the offspring. These observations suggest that the proposed prenatal stress-induced blockade of testosterone surge, which normally occurs between days 18 and 19 of gestation (Ward and Weisz, 1984), is probably the key factor that controls testis descent and is not altered by postnatal environment. Biagini and Pich (2002) found that another postnatal procedure, such as maternal separation, did not modify time to testis descent. In addition, daily stress episodes of maternal deprivation were not sufficient to affect the development of reproductive functions in the rat (Lau et al., 1996). Consequently, stress applied during

TABLE II. Animal Weights and Physical Landmarks*

Test	C	C/C	C/S	S	S/C	S/S
Weight (g)						
PND 10	15.1 ± 0.7 (4; 26)	15.1 ± 0.9 (2; 10)	14.9 ± 0.6 (2; 8)	13.9 ± 0.3 (4; 22)	14.4 ± 0.3 (2; 13)	15.9 ± 0.3 (2; 15)
PND 30	63.3 ± 2.5 (4; 26)	63.7 ± 1.8 (2; 10)	67.2 ± 2.6 (2; 7)	63.9 ± 0.8 (4; 21)	58.8 ± 2.2 (2; 13)	65.6 ± 1.7 (2; 15)
PND 90	315.0 ± 8.3 (4; 24)	293.6 ± 8.1 (2; 9)	296.7 ± 8.5 (2; 7)	309.6 ± 3.1 (4; 21)	271.2 ± 4.3 (2; 13)	294 ± 7.3 (2; 15)
Eye opening (day)	16.7 ± 0.6 (3)	17.0 ± 1.4 (2)	16.5 ± 0.7 (2)	16.7 ± 0.5 (4)	17.5 ± 2.1 (2)	16.0 ± 0.0 (2)
Ear opening (day)	4.0 ± 0.4 (3)	4.0 ± 0.5 (2)	4.0 ± 0.5 (2)	4.25 ± 0.3 (4)	4.5 ± 0.5 (2)	3.0 ± 0.5 (2)
Testis descent (day)	24.77^{a} ± 0.36 (4; 26)	24.20^{a} ± 0.33 (2; 10)	23.86^{a} ± 0.14 (2; 7)	25.62^{b} ± 0.37 (4; 21)	25.31^{b} ± 0.55 (2; 13)	25.07^{b} ± 0.35 (2; 14)

*Values are reported as mean ± SEM from (n_1; n_2), where n_1 represents number of litters and n_2 represents number of rats, except for eye and ear opening, for which data were registered by litter. Means with no superscript letter in common were significantly different ($P < .05$). PND, postnatal day.

postnatal ages did not alter the maturation of reproductive functions or disrupt sexual behaviors in the offspring (Lau et al., 1996). In agreement with these studies, our findings further indicate that testis descent impairment elicited by prenatal stress is not altered by postnatal procedures.

Dopamine D_2 Receptor

DA D_2 receptors in cortical and hippocampal areas (MPC, DFC, and CA_1) showed a similar trend of changes in prenatally stressed rats that were reared either by the biological or by a foster mother (S and S/S), suggesting that adoption per se (S/S) did not induce any changes in cortical and hippocampal D_2 receptor levels compared with S. However, both groups showed an increase in D_2 receptors compared with C. Interestingly, D_2 receptors returned to control (C) levels when prenatally stressed rats were reared by a control mother (S/C) in all three areas studied. These findings suggest that both prenatal and postnatal experiences of a prenatally stressed offspring influence the expression of D_2 receptor level in adulthood. Previous studies drew a correlation between postnatal experiences and maternal behavior and care during the nursing period. Studies on maternal care were conducted by using two groups of high- or low-licking/grooming arched-back nursing (LG-ABN) mothers (Caldji et al., 2000). Quality of nesting/nurturing maternal behaviors appeared to be programming stress reactivity as well as modulating the expression of certain receptors in the adult offspring (Caldji et al., 2000). Another study examined the maternal behavior in mice, subjected to stress during pregnancy, that raised either biological or adopted pups in a model similar to ours (Meek et al., 2001). The authors reported deficits in nurturing/nesting behaviors in nonstressed dams raising stressed pups and stressed dams raising nonstressed pups, although some differences were found in terms of pup retrieval and aggressive behaviors. Interestingly, stressed dams raising stressed pups behaved like nonstressed animals raising nonstressed young (Meek et al., 2001). However, the amount of care provided by parents is determined through a complex interaction of offspring signals and responses by parents to those signals (Agrawal et al., 2001). It seems, therefore, that the outcome on D_2 receptor expression might be influenced both by prenatal experience and by postnatal maternal care, which in turn are influenced by the pup's experience. This may contribute to the observed differences in cortical D_2 receptors in control or stressed offspring in spite of being raised by a foster control mother (C/C vs. S/C). A similar situation was observed with C/S vs. S/S.

However, the final expression of any receptor in the adult brain is the result of prenatal experience as well as consecutive influences received during other critical periods in life, such as puberty. It is well established that plasticity of the brain continues during puberty by a coordinated process of overproduction and elimination of excessive synapses and receptors. These developmental events appear to shape brain architecture by fostering coordinated establishment of neuronal circuits involving selected neuronal components and receptor types (Miller, 1988; Tarazi and Baldessarini, 2000). In addition, sex hormones levels change substantially during this period as a result of the onset of puberty. Several behavioral and biochemical alterations exerted by prenatal insults were seen only after puberty (Henry et al., 1995; Diaz et al., 1997). Preliminary studies from our laboratory (Barros et al., 2002) showed that DA receptor overexpression seen in prenatally adult rat brains was not observed before puberty, suggesting that an early modification of neuronal reactivity to hormones and neurotransmitters may modify the normal modulation by sex steroids during puberty. Therefore, the long-term effects of prenatal stress may result from disrupting the hormone-neurotransmitter imprinting of the neuroendocrine system (Reznikov et al., 2001).

Alterations in the functional state of the HPA axis have been reported to be a consequence of pre- and postnatal events (Weinstock, 2001). Several groups have investigated the possible relationship between an impaired control of corticosterone secretion in prenatally stressed animals and long-term changes in DA systems (for review see Diaz et al., 1997). Increases in circulating corticosterone level were reported to increase DA release in a state-dependent manner (Wolkowitz et al., 1986; Tanganelli et al., 1990; Piazza et al., 1996) and to modulate DA receptor density and affinity (Faunt and Crocker, 1988; von Euler et al., 1990; Biron et al., 1992; Lammers et al., 1999). Although two studies (Kurosawa et al., 1980; Leret

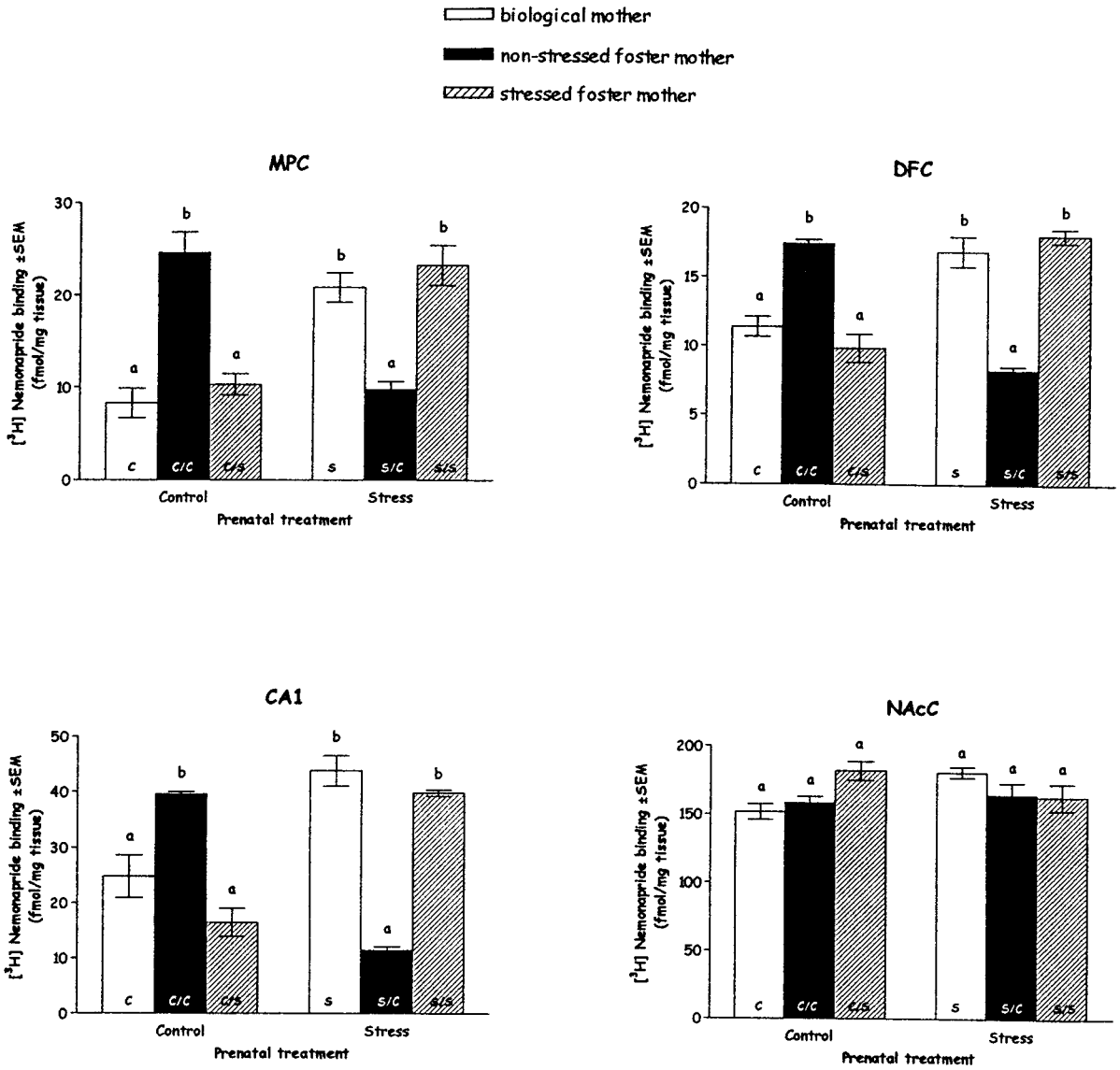

Fig. 2. [^{3}H]nemonapride binding in adult prenatally nonstressed rats raised by their biological mother (C), adult prenatally nonstressed rats adopted by a control nonstressed mother (C/C), adult prenatally nonstressed rats adopted by a mother stressed during pregnancy (C/S), adult prenatally stressed rats raised by their biological mother (S), adult prenatally stressed rat adopted by a control foster mother (S/C), and adult prenatally stressed rat adopted by a mother stressed during pregnancy (S/S). Data are mean ± SEM from n = 3–7 (each n represents one offspring randomly chosen from four different litters in each experimental group). Groups with no letters in common are significantly different ($P < .05$).

et al., 1993) suggested that the developing DA system is sensitive to maternal glucocorticoids, the exact mechanisms by which glucocorticoids modify DA receptors in prenatally stressed rats remain to be investigated. Insofar as both the mesolimbic and the nigrostriatal DA pathways express glucocorticoid receptors, alterations in corticosterone levels may influence DA receptors through an indirect action at glucocorticoid (GR) receptors. A previous study by Maccari et al. (1995) showed that type I corticosterone receptors were decreased in the hippocampus of prenatally stressed rats, but adoption blocked the effects of prenatal stress in rats adopted by either control nonstressed or stressed mothers. In our hands, adoption of a prenatally stressed offspring by a control mother but not by a foster prenatally stressed mother prevented the observed increase in DA receptors. However, the effects of prenatal stress and early adoption on corticosterone and DA receptors observed in both studies are difficult to correlate. Another hypothesis suggested that prenatal stress-induced corticosterone secretion can act directly alter dopamine receptors (Lammers et al., 1999). However, our recent results showed that corticosterone inhibited D_4 receptor (mem-

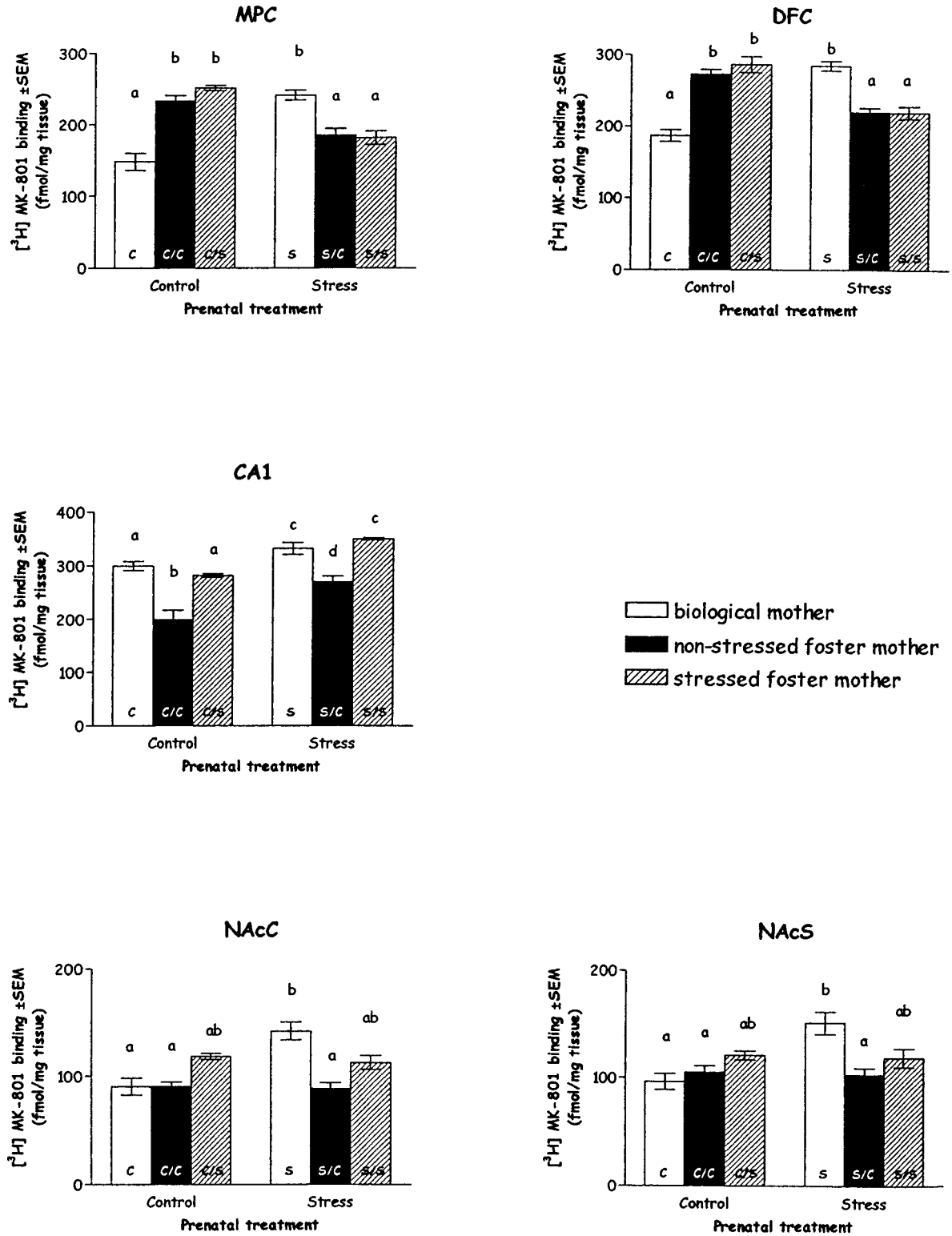

Fig. 3. [^{3}H]MK-801 binding in adult prenatally nonstressed rats raised by their biological mother (C), adult prenatally nonstressed rats adopted by a control nonstressed mother (C/C), adult prenatally nonstressed rats adopted by a mother stressed during pregnancy (C/S), adult prenatally stressed rats raised by their biological mother (S), adult prenatally stressed rat adopted by a control foster mother (S/C), and adult prenatally stressed rat adopted by a mother stressed during pregnancy (S/S). Data are mean ± SEM from n = 3–5 (each n represents one offspring randomly chosen from four different litters in each experimental group). Groups with no letters in common are significantly different ($P < .05$).

ber of the DA D_2-like receptors) expression in immortalized cell lines from cerebral cortex of normal mouse fetuses (Barros et al., 2003), suggesting that overexpression of DA D_2 receptors in prenatally stressed offspring is not directly promoted by corticosterone.

Blockade of the stress-induced increase in D_2 receptor expression appears to be specific to cortical areas; D_2 receptors in NAcC did not show any significant change. These specific changes in cortical DA receptors after prenatal stress might be relevant to studies that link alterations in cortical dopaminergic neurotransmission with neuropsychiatric disorders.

Glutamate NMDA Receptors

NMDA receptors showed the same response to cross-fostering procedures in both cortical areas measured (MPC and DFC). In these two areas, NMDA receptors were overexpressed in adopted control offspring independent of the foster mother experience. In contrast, the adopted stressed offsprings (S/C and S/S) showed a decrease in NMDA receptors compared with stressed offspring raised by their biological stressed mother (S). These results suggest that cortical glutamate receptors are particularly sensitive to cross-fostering manipulations independent of the prenatal experience of the foster mother.

The CA_1 area of the hippocampus showed a pattern of changes different from that in the cortical areas: adoption per se decreased NMDA receptors in control offspring but adoption by a control mother blocked the increase in NMDA receptor levels in stressed offspring. NAc was also sensitive to adoption procedures both in control and in stressed offspring. Stressed offspring were more susceptible than control offspring to adoption by either a normal or a stressed foster mother (S/C and S/S).

Disturbances in glutamate neurotransmission have been hypothesized to contribute to the pathophysiology of psychotic or major affective disorders, including schizophrenia (Tamminga, 1998; Carlsson et al., 1999). In consideration of the high expression of glutamate receptors in forebrain areas and their pivotal role in modulating glutamatergic neurotransmission (Monaghan et al., 1989), this perinatal susceptibility to the interactive effects of prenatal stress and early adoption that are expressed in adult life in limbic and basal ganglia structures of the offspring brain further support the hypothesis that stress exacerbates neuropsychiatric disorders such as schizophrenia (Gispen-de Wied, 2000; Del Arco and Mora, 2001).

CONCLUSIONS

In the present study, we investigated whether early cross-fostering procedures may alter the expression pattern of DA and Glu receptors observed in adult individuals subjected to prenatal stress. We found that changes elicited by prenatal stress on one of the sexual maturation indicators, testis descent, are not modified by postnatal manipulations. In contrast, stress-induced increase in the expression of D_2 and NMDA receptors in specific brain areas of adult brain can be modulated by postnatal manipulations. The fact that the prenatal stress-induced increase in the expression of these relevant synaptic receptors can be modified through changes in the postnatal environment indicates the need for future studies to determine: 1) the importance of environmental manipulations on normal development and sprouting of catecholaminergic and glutamatergic neuronal elements; 2) the relevance of early experience on neuropsychiatric disorders that involve dopamine and glutamate, such as schizophrenia and other psychotic disorders; and 3) the possibility of normalizing the negative outcome of prenatal insults with the appropiate postnatal modifications.

ACKNOWLEDGMENTS

Additional grant supprt for this work was provided by "Beca Ramón Carrillo-Arturo Oñativia, Ministerio de Salud de la Nación" and Committee for Aid and Education in Neurochemistry (CAEN-ISN; to M.C.A.), Fundación Antorchas MH-068359 (to V.G.B.), and Stanley Medical Research Institute and NARSAD Young Investigator Award (to F.I.T.).

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