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Prenatal stress, moderate fetal alcohol, and dopamine system function in rhesus monkeys

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Abstract

This study examined the striatal dopamine system integrity and associated behavior in 5- to 7-year-old rhesus monkeys born from mothers that experienced stress and/or consumed moderate levels of alcohol during pregnancy. Thirty-one young adult rhesus monkeys were derived from females randomly assigned to one of four groups: (1) control group that consumed isocaloric sucrose solution throughout gestation; (2) stress group that experienced prenatal stress (10-min removal from home cage and exposure to three random loud noise bursts, gestational days 90 through 145); (3) alcohol group that consumed alcohol (0.6 g/kg/day) throughout gestation; or (4) combined alcohol plus stress group that received both treatments. The subjects were assessed for striatal dopamine system function using positron emission tomography (PET), in which the dopamine (DA)-rich striatum was evaluated in separate scans for the trapping of [^{18}F]-Fallypride (FAL) and 6-[^{18}F]fluoro-*m*-tyrosine (FMT) to assess dopamine D2 receptor binding potential (BP) and DA synthesis via dopa decarboxylase activity, respectively. Subjects were previously assessed for non-matching-to-sample (NMS) task acquisition, with ratings of behavioral inhibition, stereotypies, and activity made after each NMS testing session. Subjects from prenatal stress conditions (Groups 2 and 4) showed an increase in the ratio of striatal dopamine D2 receptor BP and DA synthesis compared to controls (Group 1). An increase in the radiotracer distribution volume ratios (DVRs), which is used to evaluate the balance between striatal DA synthesis and receptor availability, respectively, was significantly correlated with less behavioral inhibition. The latter supports a hypothesis linking striatal function to behavioral inhibitory control.

Keywords: Prenatal stress; Dopamine; Rhesus monkey; Striatum; Fetal alcohol exposure

1. Introduction

Increased violence and substance abuse in everyday life has led to a growing concern about the effects of psychosocial stress and alcohol exposure during pregnancy on child outcome. While it is well established that consumption of alcohol during pregnancy causes harm to the fetus [39], there are limited data on whether psychosocial stress interacts with these effects [36,37].

It is not a new idea that if a pregnant woman is exposed to stressful events in everyday life, her offspring might be at risk for learning, behavioral, and/or emotional disorders [31,42]. An important issue of concern relative to the present study is that maternal stress may contribute to child outcome by interacting with numerous other factors in a complex, mutually interacting process. Moreover, it is thought that exposure to prenatal stress and/or alcohol alters an individual's developmental trajectory through altered early brain development. We employ a primate model because in human studies, causal conclusions are difficult

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to reach due to the clustering of negative events in humans. Nonhuman primates have the advantage of gestation characteristics and early development similar to the human, and their shorter life span makes longitudinal studies somewhat easier to conduct.

Most animal studies of prenatal stress effects have been conducted in rodents. A variety of stressors have been employed, and they have been administered across a variety of times during pregnancy [35]. A number of rodent studies show that prenatal stress is associated with the abnormal regulation of the hypothalamic–pituitary–adrenal (HPA) axis. The underlying mechanism for the dysregulated HPA axis is not fully understood. Several lines of evidence, however, suggest that glucocorticoids or other substances that the mother produces as a result of the stressful experiences could cross the placenta during stress episodes and influence fetal brain development [15].

Studies have demonstrated that drinking alcohol during pregnancy can be harmful even in the absence of gross morphological defects associated with fetal alcohol syndrome (FAS) [24]. Recent findings from several animal models and from neuroimaging studies with children have indicated that several brain areas may be particularly vulnerable to the adverse effects of fetal alcohol exposure. Magnetic resonance imaging (MRI) studies of children with FAS indicated volume reductions in the basal ganglia after accounting for brain size [25,26]. Using positron emission tomography (PET), Loock et al. [22] reported reduced metabolic activity in the caudate nucleus of children with FAS. Prenatal alcohol exposure has also been found to result in alterations in a number of neurotransmitter systems, including dopamine [9,10,23,34,38,40,41].

Because prenatal stress can covary with fetal alcohol exposure, how fetal alcohol exposure and prenatal stress together affect fetal development is an issue of concern. Both prenatal stress and fetal alcohol exposure have been associated with altered dopaminergic function. For example, fetal alcohol exposure decreased D1 dopamine receptors in the hypothalamus and striatum in 18- to 35-day-old rodents [9]. Prenatal stress was associated with an increase in dopamine turnover in the right prefrontal cortex and a decrease in turnover in the left striatum in rats of both sexes [13]. In addition, an increase in the density of dopamine D2 receptors in the nucleus accumbens was reported in prenatally stressed male rats [16], while a bilateral decrease in dopamine turnover in the nucleus accumbens was reported in female rats [1].

To date, animal studies investigating how prenatal stress and fetal alcohol exposure together affect fetal development have produced mixed results. For example, restraint stress in mice increased the adverse effects of all-trans-retinoic acid, an essential chemical to which the early CNS is known to be highly sensitive and which is a teratogen in high concentrations [32]. Another study, however, reported that prenatal stress decreased the adverse effects of fetal alcohol exposure on sensorimotor development of rats [43]. We recently reported that prenatal stress in combination with moderate fetal alcohol exposure resulted in lowered birth weights in male rhesus monkey offspring and increased fetal loss [37]. The combination of prenatal stress and fetal alcohol exposure also increased the incidence of stereotypies and hyperactivity observed during cognitive testing in both male and female monkey offspring and increased the number of trials to criterion during the acquisition of non-matching-to-sample (NMS) task performance [36]. How prenatal stress with moderate prenatal alcohol exposure affects neurobiological function in monkeys is a question that remains unaddressed.

Our primary goal in the present study was to examine the consequences of prenatal stress, moderate fetal alcohol exposure, or the combination of both on striatal dopamine system function in adult rhesus monkeys. To assess striatal dopamine system function, we used PET. We chose to examine both D2 binding availability and DA synthesis in the striatum because it is rich in dopaminergic synapses and should therefore be sensitive to any treatment that alters DA function. The methodology for PET studies of these particular dopamine system components is well established, resulting from decades of research into neurodegenerative disease, such as Parkinson's. In addition, we investigated the balance of striatal DA synthesis and postsynaptic receptor density, given the documented compensatory relationship between these parameters. For example, it has been shown that a chronic decrease in dopamine production results in an increase in D2 receptor density [8]. The striatum, which consists of the caudate nucleus and putamen, receives inputs from all cortical areas and the thalamus and projects to frontal lobe areas (prefrontal, premotor, and supplementary motor areas). Those circuits regulate the cortex, play a role in predicting future events, and are involved in shifting attention sets, movement, and spatial working memory [17]. Therefore, alterations in striatal DA function may have widespread effects on behaviors associated with prenatal stress and/or fetal alcohol exposure, such as poor self-regulation, motor hyperactivity, and altered arousal.

A second purpose of our study was to examine the relationship between alterations in striatal dopamine system parameters and behavioral outcomes that were previously measured in these same animals. Recent theories of striatal function suggest that it is involved in inhibitory control, or the execution of a pattern of activity by suppressing another opposing pattern [28]. For example, studies by Ferry et al. [11,12] have supported the hypothesis that the striatum is involved in inhibitory control by demonstrating that lesions in the ventral striatum of macaque monkeys were associated with impairments in the ability to suppress responses to previously rewarded stimuli in a reversal task. In our prospective longitudinal study, we have conducted repeated observations of behavioral functions related to inhibitory control purported to be associated with striatal dopaminergic function. These include attention and motor function during early development [37] and aspects of

learning that require inhibitory control—an NMS task. The NMS task requires rule learning, shifting attention to the nonmatching stimulus, inhibitory control, and working memory—all functions dependent upon dopamine innervations in the basal ganglia, limbic, and prefrontal and frontal cortex [29,45]. Observers rated key aspects of behavior after each testing session [36].

2. Methods

2.1. Maternal stress and/or alcohol treatments

These studies were conducted in accordance with the University of Wisconsin-Madison Animal Care and Use Committee. Healthy adult female rhesus monkeys were identified within the breeding colony that consistently and voluntarily consumed 0.6 g/kg of a 6% v/v alcohol solution sweetened with 300 mg/100 ml NutraSweet (Equal Sweetener, Merisant US, Chicago, IL). Prior to breeding, blood samples that were obtained 60 min after consumption of 0.6 g/kg alcohol showed blood alcohol contents of 20–50 mg/dl. This dosage is comparable to an average-sized woman consuming two drinks daily. We define moderate alcohol consumption in accord with Dawson et al. [4], who classified moderate drinkers as those who consume 4 to 14 drinks per week. Females that voluntarily consumed alcohol before breeding were randomly assigned to the control group or one of three experimental groups in a 2×2 factorial design with prenatal stress and prenatal alcohol as the independent variables. One subgroup was also exposed to a mild 10-min psychological stressor during midlate gestation, Day 90 through 145 postconception (see below for details of prenatal stress treatment). We purposely avoided administering the stressor from conception to Day 90 or from Days 145 to 165 to avoid the risks of either early fetal loss or early parturition. The alcohol-consuming mothers voluntarily consumed the alcohol solution daily at 1600 h throughout gestation, beginning 5 days before breeding and ending at parturition. Water was available ad libitum, including during the period when the alcohol solution was available. The animals had no chow left by the time of day that the alcohol was introduced. The control and stress mothers consumed a sucrose solution that was designed to be approximately equivolemic and equicaloric (8 g/100 ml water) to the alcohol solution. During the remainder of the day, all females were housed under identical conditions, undisturbed except for necessary routine animal husbandry at all times except during the stress exposure.

The prenatal stress treatment for the experimental subjects was administered 5 times per week at approximately 1530 h. It involved removing the pregnant female from the home cage, placing her in a transport cage and taking her to a darkened room where three noise bursts were randomly administered over a 10-min period. The noise burst consisted of an alarm horn that produced a 1300 Hz sound of 115 dB intensity at 1 m. With this treatment plasma cortisol levels were previously found to increase from 25.2 ± 2.2 μg/dl at baseline to 34.8 ± 2.4 μg/dl poststress treatment (mean ± S.E.M.) [35].

2.2. Subjects

The offspring subjects in this study were 31 young adult (5–7 years old, mean = 6.13 years) rhesus monkeys (*Macaca mulatta*), 16 females and 15 males. Controls consisted of three males and seven females (Group 1; mean age = 6.40 years). The stress monkeys (Group 2; mean age = 6.50 years) consisted of five males and one female. Six monkeys, two males and four females, were born to the female rhesus monkeys given the alcohol treatment described above (Group 3; mean age = 6.60 years). Nine monkeys, five males and four females, were born to the female rhesus monkeys who experienced the alcohol plus stress treatment (Group 4; mean age = 5.0 years). These monkeys are members of an ongoing longitudinal study that investigates the effects of moderate-level fetal alcohol exposure, alone or in conjunction with prenatal stress, on development and neurobehavioral function. The rearing conditions and previous testing of these subjects were described in detail elsewhere [37]. Briefly, all infant monkeys were housed with their mothers in individual cages during the first 6 months of life. They were separated briefly from their mothers weekly and tested for neonatal neurobehavioral function during the first month of life. At 6 months, they were separated permanently from their mothers and reared in mixed-sex peer groups consisting of five to six monkeys from similar prenatal conditions. At the time of this study, they were pair-housed with same-sex peers from similar treatment groups. They were maintained on a diet of Purina Monkey Chow supplemented three times weekly with fresh fruit. All housing conditions were light- (16-h light and 8-h dark) and temperature- (21 ± 0.5 °C) controlled.

When the monkeys were 3 years old, they underwent cognitive and behavioral assessments in the Wisconsin General Test Apparatus (WGTA; described below). When they were 5–7 years old, they were assessed using the PET methodology described in the following section.

2.3. PET procedure

All procedures followed an overnight fast. Monkeys are typically fed chow at 0600 h each day. On the day before PET scans, all monkeys were supplemented with half rations at 1200 h and food- and water-deprived at 1630 h. On the morning of the PET scan, monkeys were anesthetized with ketamine (15 mg/kg), and an intravenous catheter was inserted to allow for the placement of PE tubing (ID: 0.76 mm; OD: 1.22 mm) into the saphenous vein. Monkeys were then intubated and transported to the PET facility. Upon arrival, isoflurane anesthesia was initiated at 3–5% and

maintained at 1.25–1.5% throughout the duration of the procedure (approximately 1.5 h). A fixed laser line reference was used to align each anesthetized animal in the PET scanner for horizontal slice imaging parallel to the orbital–meatus line at the center of the seven slice, 5.5-cm field of view in the ECAT 933 scanner.

Striatal DA synthesis was assessed using 6-[^{18}F]-fluoro-*m*-tyrosine (FMT) as a PET tracer. FMT imaging provides a quantitative measure of dopa decarboxylase activity, reflecting the enzymatic action required to produce dopamine. FMT gives a superior image contrast compared to the PET tracer currently used in most PET centers to assess DA synthesis, 6-fluoroDOPA [5]. The striatum–occipital cortex uptake ratio for FMT is about twice that found for 6-fluoroDOPA and is not confounded by nonspecific tracer metabolite uptake in the brain. The tracer used to assess dopamine D2 receptors was [^{18}F]-Fallypride (FAL), an F-18 labeled Raclopride analog developed by Mukherjee et al. [30]. FAL has a high affinity for dopamine D2 receptors and high brain uptake—almost 3 times higher compared to [^{11}C]Raclopride. The high affinity of FAL for dopamine D2 receptors suggests that changes in basal levels of endogenous dopamine do not affect the binding of FAL. Thus, measures of binding to D2 receptors using FAL are more directly related to total receptor density than those obtained using [^{11}C]Raclopride.

Radiotracers were administered as an intravenous bolus. Identical image acquisition protocols were used for the FAL and FMT tracers. Tracer injection of 5 mCi in 1–5 ml normal saline was followed by a dynamic sequence of images over 90 min, including a total of 13 frames with duration increasing from 2 to 10 min. At the end of the scanning, the PE tubing was removed. The animals were extubated and allowed to awaken, were returned to their transport cages, and were immediately transported to the animal care facility.

PET images were reconstructed from the raw data using the ordered subset estimation method (OSEM) [18]. Standard regions of interest (ROI) were placed on the occipital cortex (an area known to contain little significant D2 dopaminergic innervations) to produce reference region, time–activity curves for use as input functions in graphical analysis. Other ROIs were placed to cover both left and right caudate and putamen in the basal ganglia. Time–activity data for these ROIs were analyzed with the graphical method of Logan et al. [21]. Ratios of tracer total distribution volumes (DVRs) to those in the reference region were evaluated as described below. Our method assumes that the unbound components of the tracers are the same in the target regions (e.g., striatum) as in the reference region (occipital cortex). The DVR values can then be interpreted directly in terms of Fallypride binding potential (BP): Bmax/Kd = DVR-1, where Bmax is the mass-specific concentration of available receptors, and Kd is the receptor-ligand dissociation constant in that voxel.

The DVR can be extracted from dynamic imaging data by Eq. (1).

$$\frac{\int_0^T \mathrm{STR}(t)\mathrm{d}t}{\mathrm{STR}(t)} \approx \mathrm{DVR}\left[\frac{\int_0^T \mathrm{OC}(t)\mathrm{d}t}{\mathrm{STR}(t)} + \frac{\mathrm{OC}(t)}{\mathrm{STR}(t)k_2}\right] + \text{intercept} \quad (1)$$

In this case, the striatum activity concentration at time t is given by STR(t), with the occipital cortex concentration OC(t). The parameter k_2 is the efflux rate constant for wash out of the compound from the nonreceptor containing regions. For this analysis we assumed $k_2 = 0.163\ \mathrm{min}^{-1}$, the value for [^{11}C]Raclopride [21]. With the relatively large STR–OC ratios achieved with FAL and FMT, the k_2 term is a relatively small correction, and variations of a factor of 2 in k_2 from our assumed value result in less than a 7% change in the resulting DVR.

2.4. Cognitive task performance (NMS)

The protocol for cognitive testing and rating of behavior during testing has been described in detail elsewhere [36]. In brief, cognitive testing was conducted using the WGTA—an apparatus employed for assessing learning and memory capabilities in nonhuman primates [14]. The monkeys were placed individually in a cage opposite a human tester, who sat behind a one-way mirror and manipulated the stimuli and rewards. Testers were blind to the experimental conditions. Stimulus objects were plastic, three-dimensional, abstract-shaped objects varying in color, size, and texture.

In the acquisition phase of the NMS task, first, a sample object was presented over the central, baited food well of a tray containing three wells. The monkey was taught to displace the object for a food reinforcer. After a 10-s delay, during which time a screen between the monkey and the experimenter was closed, the sample object (unbaited) and a new object (baited) were presented simultaneously covering the lateral wells of the test tray. The correct strategy was to displace the new object for the food reinforcer. After a 20-s intertrial interval, a different sample object was presented in the center well, followed 10 s later by another recognition trial with another novel object. Placement of the sample and novel objects over the left and right wells was determined randomly.

Twenty trials, with 20-s intervals between trials, were presented 5 days a week until the monkeys reached a learning criterion of 90 correct responses in 100 consecutive trials (90% correct). The outcome measures employed for the acquisition phase of the NMS task were the number of trials taken by the monkey in reaching this criterion.

2.5. Behavioral ratings

Behaviors exhibited during testing that are associated with basal ganglia thalamocortical circuits and might affect

Table 1
Definition of items on behavioral rating scale

Items	Definition
Stereotypies	Repetitive (3 or more) rhythmic motor movements. (0 = none; 1 = approximately 25% of the time; 2 = approximately 50% of the time; 3 = continuous). Intermediate values were scored on half-point intervals.
Inhibition	Fearful, restrained behavior (0 = bold, not inhibited; 1 = fearful, inhibited approximately 25%; 2 = fearful, inhibited approximately 50%; 3 = extremely fearful, inhibited approximately 90%). Intermediate values were scored on half-point intervals.
Activity	Amount of gross body movement (0 = stays in one place; 1 = active approximately 25%; 2 = active approximately 75%; 3 = continual movement). Intermediate values were scored on half-point intervals.

performance of the cognitive task were also scored according to a standardized rating scale [36]. This scale was completed by the tester/observer for each monkey immediately following each testing session (Table 1). Behavioral inhibition is defined as restrained behavior. Lack of behavioral inhibition can be evidenced by increased stereotypies or repetitive motor patterns, and hyperactivity or increased gross motor body movement. All examiners were blind to the experimental conditions. Ratings were averaged across testing sessions for each task.

2.6. Statistical analysis

Treatment effects were analyzed by analysis of variance (ANOVA) for prenatal condition on PET dopamine data followed by *t* tests to determine which groups differed significantly from each other. To determine whether acquisition of task performance on the NMS task was related to D2 receptor–DA synthesis ratio, correlations were performed. To investigate potential relationships between behavior and D2/DA synthesis, DVRs and behavioral ratings were correlated with PET data using Spearman's rank order correlations. Finally, to explore whether the balance between DA synthesis and D2 receptor density was altered by the prenatal treatments, correlations between FAL DVR and FMT DVR were conducted within treatment groups.

3. Results

3.1. Positron emission tomography

The first four monkeys (two control and two alcohol plus stress) were scanned twice to ascertain that the measurements were reliable [33]. The percentage variation from the first test ranged from 6% to 10% with an average of 7.5%.

Fig. 1 shows the distributions, means, and S.E.M.'s of the DVR for the radiotracers measured by PET imaging that reflect the D2 receptor binding availability (indexed by FAL, Fig. 1a), DA synthesis (indexed by FMT, Fig. 1b), and the FAL–FMT ratio (Fig. 1c) for the four groups. In the current study, one female control monkey was rescanned because the results of the first scan were aberrant from the other control monkeys. Only the results from the second scan are included in the data analyses. The percentage

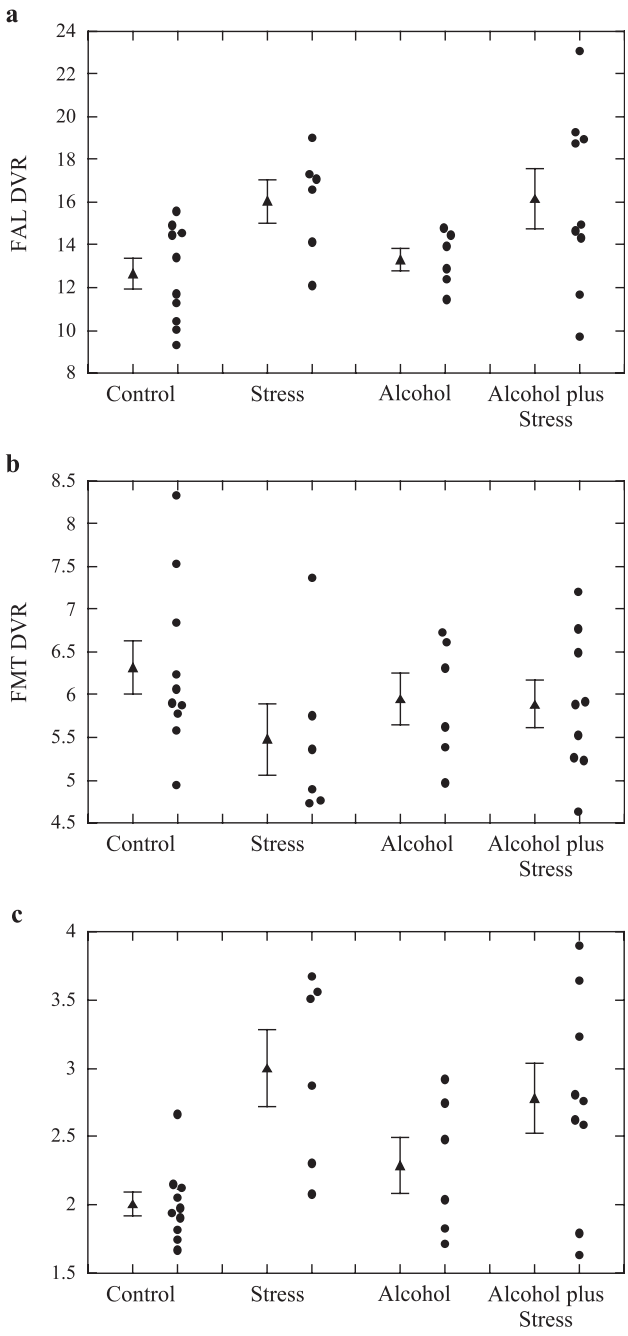

Fig. 1. Comparisons of dopaminergic parameters measured with PET in control versus alcohol plus stress groups. Group means and S.E.M. are shown. (a) DVR for FAL binding on postsynaptic D2 receptors; (b) DVR for FMT uptake indicating presynaptic dopamine synthesis; (c) DVR for these measures.

change across the two scans was 230%, out of the range of our previous reliability. We concluded that the first scan was erroneous. ANOVAs showed a significant effect of prenatal stress treatment on the FAL−FMT ratio, $F(1,27) = 12.33$, $P = 0.002$, and on FAL, $F(1,27) = 8.53$, $P = 0.007$, but not on FMT, $P > 0.15$. There were no significant main effects of prenatal alcohol treatment on any of the PET imaging variables (Ps>0.15), nor were the interactions of Prenatal Stress × Alcohol Treatment significant (Ps>0.15). The results were nearly identical when the analyses were conducted using the Brown−Forsythe ANOVA for unequal variances as well as with 20% trimmed means (all significant results reported above remained significant, although the P levels changed).

For FAL, pairwise tests of the differences between the group means showed that the control mean differed significantly from the stress ($t = 2.71$, $P = 0.022$) and alcohol plus stress ($t = 2.25$, $P = 0.044$) conditions but not from the alcohol condition ($P > 0.15$). The alcohol condition differed significantly from the stress condition ($t = 2.35$, $P = 0.049$), and the difference between the alcohol and alcohol plus stress condition approached significance ($t = 1.90$, $P = 0.086$). For FMT, none of the pairwise differences between groups approached significance (all Ps>0.15). Pairwise tests of group differences in the FAL−FMT ratio showed that the control group differed significantly from stress ($t = 3.40$, $P = 0.015$) and alcohol plus stress conditions ($t = 2.89$, $P = 0.016$) but not from the alcohol condition ($P > 0.15$). The difference between the alcohol and stress conditions approached significance ($t = 2.06$, $P = 0.069$).

Because of the possibility of sex differences in the neural substrates of prenatal stress [27,44], we examined the means for sex differences within a condition. We did not conduct ANOVAs with sex of animal as a factor because the female prenatal stress condition has one animal, and so the cell variance cannot be determined. In the alcohol plus stress condition, the female animals showed lowered FMT compared to the female control animals (5.17 vs. 6.32, $t = 2.59$, $P = 0.031$); whereas for the males, this was not found (6.46 vs. 6.31, $P > 0.15$). Furthermore, in the alcohol plus stress condition, the female animals showed an elevated FAL−FMT ratio relative to the female controls (3.075 vs. 2.088, $t = 3.76$, $P = 0.018$); whereas this was not the case for the males (2.547 vs. 1.822, $t = 1.75$, $P = 0.151$), although the difference was in the same direction as for the females. The female and male alcohol condition animals did not differ from their respective control groups in the ratio of FAL/FMT, FAL, or FMT (Ps>0.25). These sex differences should be considered tentative because of the small number of animals in each sex treatment combination.

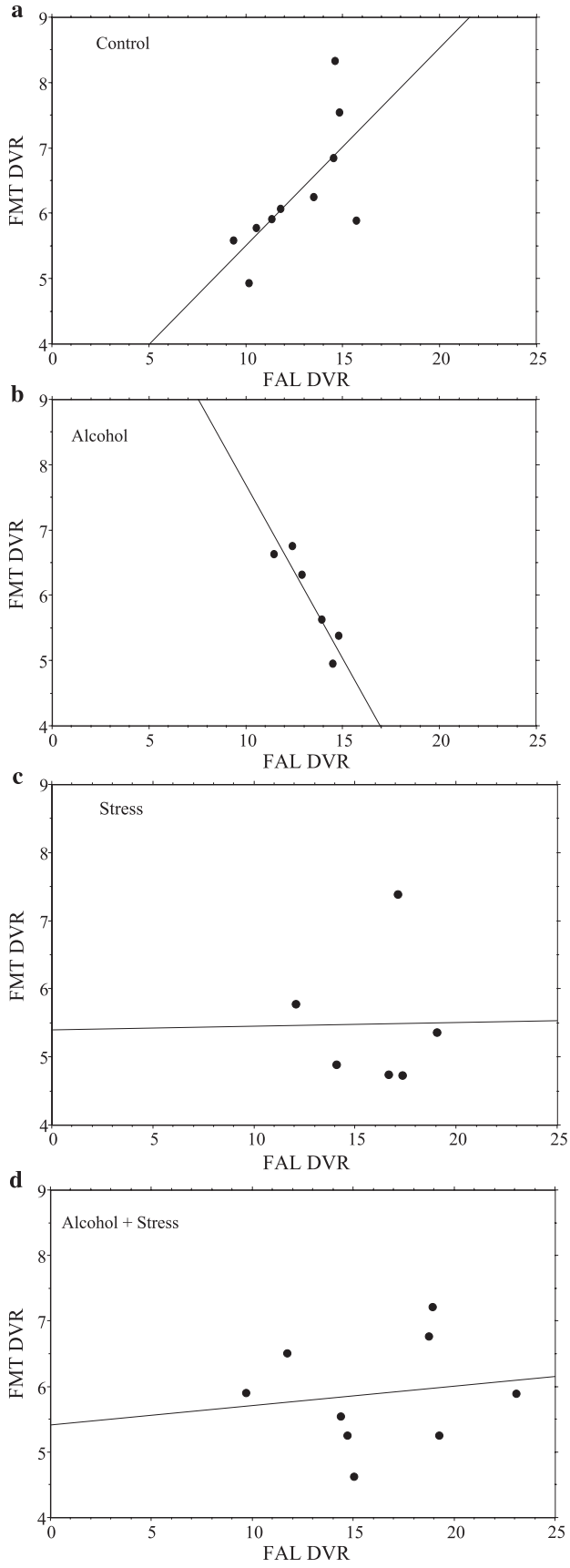

Fig. 2. (a) Relationship of DVR for FAL and FMT in control group; (b) DVR for FAL versus FMT in Alcohol group; (c) DVR for FAL versus FMT in stress group; (d) DVR for FAL versus FMT in alcohol plus stress group.

An important question is whether the treatment conditions alter the relationship between D2 receptor binding availability and DA synthesis. If there is a consistent balance between dopamine receptor density and DA synthesis, then a positive correlation between FAL and FMT PET measures is expected for control subjects. This is part of the rationale for taking the ratio of FAL and FMT DVRs. However, if the D2 neurotransmitter system compensates for alterations in DA synthesis by increasing the receptor binding sites, then the FAL and FMT variables should be negatively correlated. Fig. 2 shows the scatter plot of FAL and FMT with the treatment groups in separate panels. The Control Group showed the expected positive relationship between FAL and FMT (r=.687, P=0.03), whereas the three other treatment groups combined showed no relationship between FAL and FMT (r=−.046, P>0.15). A test of the difference in regression lines between control animals and the treatment animals was significant [$F(2,27)$=4.021, P=0.03]. Interestingly, the alcohol group showed a strong negative relationship between FAL and FMT (r=−.923). The test for difference in regression slopes between the alcohol and control groups was significant [$F(1,12)$=9.838, P=0.009, b=−1.635 and 1.556, for alcohol and control, respectively]. Although prenatal alcohol treatment did not alter the mean level of FAL, FMT, or their ratio, it may have perturbed the relationship between FAL and FMT.

3.2. Relationship between PET dopamine measures and learning task performance and behavior

Fig. 3 shows the correlations between the PET measures and variables from trials to criterion and task behavior on the NMS task that the animals learned when they were approximately 32–34 months old [36]. The figure also provides scatter plots for the FAL–FMT ratio with trials to criterion and with behavioral inhibition during task performance, and of FAL with stereotypies during task performance. The Spearman correlations are shown in the figure. The results showed that the relationship between the FAL–FMT ratio and trials to criterion was marginally significant (P<0.09), and the negative relationship between FAL–FMT ratio and behavioral inhibition, or suppression of irrelevant actions, was significant (P<0.03). Although there was no significant relationship with the ratio, there was a nonsignificant trend towards a positive relationship between FAL and stereotypies (r=.302, P=0.11) and between FAL and activity (r=.324, P<0.09). Activity and stereotypies were themselves highly correlated (r=.84).

To test whether the relationships between the PET measures and the behavioral ratings varied across treatment groups, we tested for differences between the regression for control versus treatment lines [7]. There was a marginally significant difference in the regression lines for control versus treatment animals for the regression of the FAL–FMT ratio on trials to criterion [$F(2,24)$=2.769, P=0.083; control intercept=1.55, treatment intercept=2.77, control

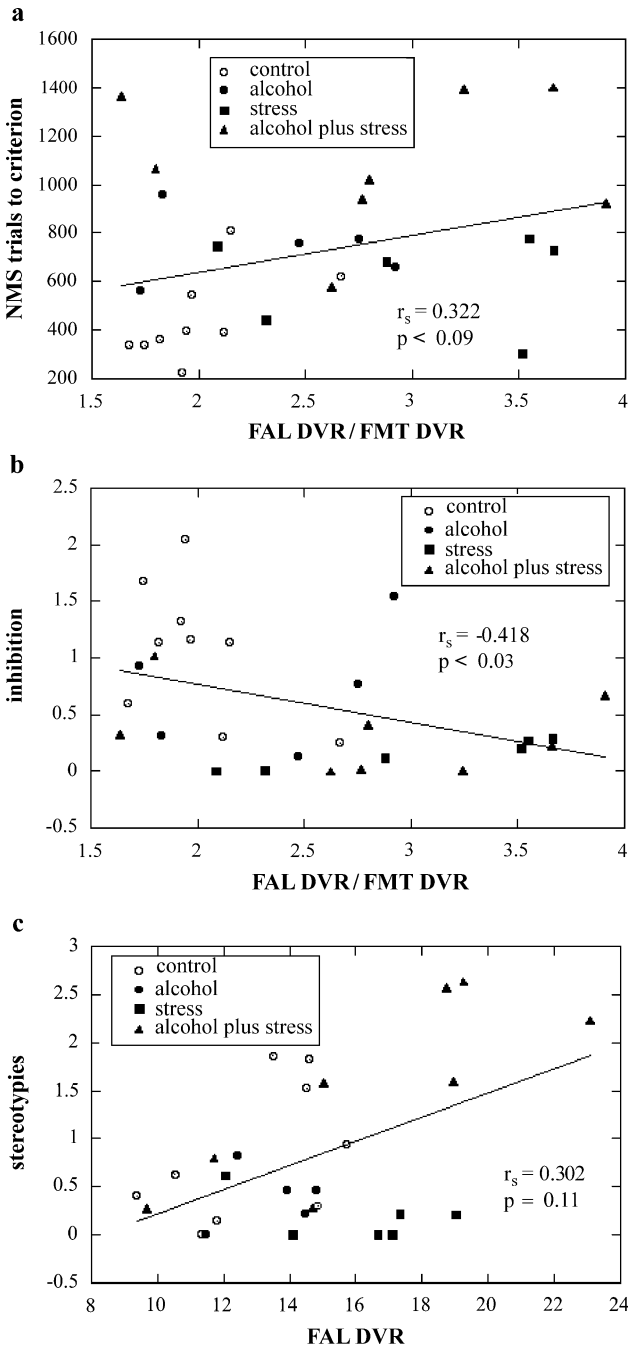

Fig. 3. PET measures of dopamine function versus behavior. (a) Dopamine balance reflected by FAL–FMT DVR versus trials to criterion for the NMS task. The criterion condition was 90% correct in consecutive 100 trials. (b) FAL–FMT DVR versus mean inhibition scores over all testing sessions, rated from 0 to 3 as defined in Table 1. (c) FAL–FMT DVR versus mean stereotypies score across testing sessions, rated from 0 to 3 as defined in Table 1.

b=0.0010, treatment slope=0.00003]. There was also a marginally significant difference in the regression lines for control and treatment animals and the relationship between stereotypies and FAL [$F(2,24)$=3.20, P=0.059; control intercept=11.56, treatment intercept=13.81, control

$b = 1.60$, treatment $b = 2.14$]. The regression lines for the control and treatment groups did not differ for the FAL–FMT ratio and inhibition ($P > 0.15$). These marginal differences in regression lines between control and treatment animals reflect primarily the differences in intercepts, as the slopes did not differ significantly (Ps>0.15). Hence, these trends suggest that the treatment effects on the FAL–FMT ratio or on FAL are related to the treatment effects previously obtained in trials to criterion, inhibition, and stereotypies [7].

4. Discussion

The aim of this study was to determine, under controlled laboratory conditions, whether prenatal stress and/or moderate-level maternal alcohol exposure would influence striatal dopamine function in young adult monkeys, assessed by PET. First, it was found that prenatal stress induced CNS alterations that were detected in measures of striatal dopamine system function. PET scans of the striatum showed an altered balance of D2 receptor binding (FAL) to DA synthesis (FMT) in the prenatal stress-exposed animals compared to controls. Second, monkeys that had higher striatal D2 receptor binding to DA synthesis ratios were less behaviorally inhibited during cognitive testing.

The finding that monkeys from the prenatal stress-exposed pregnancies showed altered striatal dopamine function was consistent with findings of altered dopamine system function in laboratory rats from prenatally stressed pregnancies [1,13,16]. We detected altered ratios between D2 receptor sites and presynaptic DA synthesis and increased D2 receptor density in prenatal stress conditions compared to controls. Up-regulation of D2 receptor density may be a feedback response to conditions of lower synaptic dopamine levels in the prenatal stress-exposed monkeys. This effect could also be related to other mechanisms involved in dopamine regulation not studied here, such as re-uptake site density.

In contrast to our results on other assessments of these monkeys (e.g., infant neurobehavioral functioning [37], responses to the stress of social separation, learning performance, and behavior [36]), the measures of mean levels of D2 striatal brain function in the prenatal alcohol condition did not differ significantly from the control condition. One possible interpretation is that D2 function in the striatum is one locus of prenatal stress effects but does not appear to be highly susceptible to continuous alcohol exposure throughout gestation. Based on work with other species, such as rodents, the timing of gestational exposure has a strong influence by which brain regions and neurotransmitter systems are affected [44]. In this study, the prenatal stress treatment occurred during the latter half of gestation, between Days 90 and 145 of the 165-day gestation period. Unpublished data from our separate study of the gestational timing of alcohol exposure, in a separate cohort of monkeys, indicate that striatal D2 function is affected by late gestational alcohol exposure but not by either early gestation or continuous exposure.

Furthermore, although we found no significant effect of prenatal alcohol exposure alone on the mean FAL, FMT, and ratio measures of striatal D2 functioning, it may be inappropriate to draw conclusions based on statistical nonsignificance. This is especially true in a relatively new area of research, such as imaging of specific neurotransmitter functioning in nonhuman primates. Nonhuman primate research can be quite powerful in that it allows experimental control that eliminates many confounding variables that are inevitably present in human research, but because of the necessity for relatively small sample sizes, caution is always in order in interpreting nonsignificance. On the other hand, our study assessed 31 animals, which is the largest sample size of neuroimaging studies of nonhuman primates.

Further studies are needed to explore the possible effects of fetal alcohol exposure and prenatal stress on different dopaminergic receptor subtypes. Because neurotransmitter systems are interactive, up-regulation of one type of dopamine receptor might be associated with down-regulation of another dopamine receptor subtype. For example, studies in rhesus monkeys have shown that chronic treatment with the dopamine D2 antagonist, Haloperidol, which displays a high affinity for the D2 dopaminergic receptor site in the neostriatum and nucleus accumbens, increases D2 receptor density but decreases D1 receptors in prefrontal and temporal association areas [20].

The trend indicating that striatal D2 receptor–DA synthesis ratio was marginally associated with trials to criterion for the NMS task and significantly associated with reduced behavioral inhibition supports findings linking striatal dopamine system function with inhibitory control. Close examination of the NMS task indicates that its performance requires the subjects to remember whether an object was seen on a preceding trial, learn the rule that the nonmatching object conceals the reward, and shift attention from the matching to nonmatching object—functions that are dependent on limbic, basal ganglia, and prefrontal and frontal cortex [29]. Some researchers have emphasized the role of ventral and orbital prefrontal cortex in inhibitory control [6,19], while others have focused on the basal ganglia in the model of inhibitory mechanisms [3]. The latter model of inhibitory control involves the basal ganglia in suppression of irrelevant action, while the frontal cortex is thought to be involved in representing and maintaining relevant information. According to Casey's model, the ability to attend to certain information as opposed to competing information increases as a result of development of the basal ganglia thalamocortical loops. More specifically, "the frontal cortex projects to the basal ganglia, then thalamus and the loop is closed with a projection back to the frontal cortex" (Casey, p. 240) [2]. This implies that structures of the basal ganglia, such as the striatum are presumably involved in tasks that are regarded as assessing prefrontal functioning. Further-

more, because the basal ganglia thalamocortical circuits are proposed to underlie inhibitory control, disruptions of one or more of these circuits could contribute to a range of developmental disorders characterized by poor inhibitory control [3]. Some of these pathways may involve striatal D1 receptor systems, which were not assessed in this study.

Future studies will employ pharmacological challenges to test the hypothesis that impaired striatal dopaminergic function subserves the behavioral effects we have found. The similarity between the human and nonhuman primate brain suggests that the results obtained in our monkey study will be relevant to understanding the effects of prenatal stress in pregnant women on the brain integrity and behavioral functioning of their children. Finally, the longitudinal nature of our study and the condensed lifespan of monkeys provide an opportunity to consider how maturation and environmental challenges could result in further shifts in neurochemical balance, an issue that is of utmost importance in understanding the course of prenatal stress effects in humans.

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References

[1] S.J. Alonso, E. Navarro, M. Rodriguez, Permanent dopaminergic alterations in the n. accumbens after prenatal stress, Pharmacol. Biochem. Behav. 49 (1994) 353–358.

[2] B.J. Casey, Disruption of inhibitory control in developmental disorders: a mechanistic model of implicated fronto-striatal circuitry, in: J.L. McClelland, R.S. Siegler (Eds.), Mechanisms of Cognitive Development: Behavioral and Neural Perspectives, Erlbaum, Mahwah, NJ, 2001, pp. 327–349.

[3] B.J. Casey, N. Tottenham, J. Fossella, Clinical, imaging, lesion, and genetic approaches toward a model of cognitive control, Dev. Psychobiol. 40 (2002) 237–254.

[4] D.A. Dawson, B.F. Grant, S.P. Chou, Gender differences in alcohol intake, in: W.A. Hunt, S. Zakhari (Eds.), Stress, Gender, and Alcohol Seeking Behavior, USDHHA, Bethesda, 1995, pp. 1–21 (NIAAA Research Monograph No. 29 NIH Pub. No. 95-3893).

[5] O.T. De Jesus, C.J. Endres, S.E. Shelton, R.J. Nickles, J.E. Holden, Evaluation of fluorinated *m*-tyrosine analogs as PET imaging agents of dopamine nerve terminals: comparison with 6-fluoroDOPA, J. Nucl. Med. 38 (1997) 630–636.

[6] A. Diamond, Developmental time course in human infants and infant monkeys, and the neural bases of inhibitory control in reaching, Ann. N.Y. Acad. Sci. 608 (1990) 637–669.

[7] W.J. Dixon, BMDP Statistical Software Manual, 1&2, University of California, Berkeley, CA, 1990.

[8] G.A. Donnan, D.G. Woodhouse, S.J. Kaczmarczyk, J.E. Holder, G. Paxinos, P.J. Chilco, A.J. Churchyard, R.M. Kalnins, G.C. Fabinyi, F.A. Menelsohn, Evidence for plasticity of the dopaminergic system in parkinsonism, Mol. Neurobiol. 5 (1991) 421–433.

[9] M.J. Druse, N. Tajuddin, A.P. Kuo, M. Connerty, Effects of in utero ethanol exposure on the developing dopaminergic system in rats, J. Neurosci. Res. 27 (1990) 233–240.

[10] K.L. Farr, C.Y. Montano, L.L. Paxton, D.D. Savage, Prenatal ethanol exposure decreases hippocampal 3H-glutamate binding in 45-day-old rats, Alcohol 5 (1988) 125–133.

[11] A.T. Ferry, C.Y. Montano, L.L. Paxton, D.D. Savage, Effects of excitotoxic lesions in the ventral striatopallidal thalamocortical pathway on odor reversal learning: inability to extinguish and incorrect response, Exp. Brain Res. 131 (2000) 320–335.

[12] T. Ferry, D. Ongur, X. An, D.L. Price, Prefrontal cortical projections to the striatum in macaque monkeys: evidence for an organization related to prefrontal networks, J. Comp. Neurol. 425 (2000) 447–470.

[13] E. Fride, M. Weinstock, Alterations in behavioral and striatal dopamine asymmetries induced by prenatal stress, Pharmacol. Biochem. Behav. 32 (1989) 425–430.

[14] H.F. Harlow, J. Bromer, A test-apparatus for monkeys, Psychol. Rev. 2 (1938) 434–436.

[15] C. Henry, G. Guegant, M. Cador, E. Arnauld, J. Arsaut, M. Le Moal, J. Demotes-Mainard, Prenatal stress in rats facilitates amphetamine-induced sensitization and induces long-lasting changes in dopamine receptors in the nucleus accumbens, Brain Res. 685 (1995) 179–186.

[16] C. Henry, M. Kabbaj, H. Simon, M. Le Moal, S. Maccari, Prenatal stress increases the hypothalamic–pituitary–adrenal axis response in young and adult rats, J. Neuroendocrinol. 6 (1994) 341–345.

[17] M.T. Herrero, C. Barcia, M. Navarro, Functional anatomy of thalamus and basal ganglia, Child's Nerv. Syst. 18 (2002) 386–404.

[18] H.M. Hudson, R.S. Larkin, Accelerated image reconstruction using ordered subsets of projection data, IEEE Trans. Med. Imag. 13 (1994) 601–609.

[19] M.D. Iversen, M. Mishkin, Perseverative interference in monkeys following selective lesions of the inferior prefrontal convexity, Exp. Brain Res. 11 (1970) 376–386.

[20] M.S. Lidow, P.S. Goldman-Rakic, A common action of clozapine, haloperidol, and remoxipride on D1 and D2-dopaminergic receptors in the primate cerebral cortex, Proc. Natl. Acad. Sci. 91 (1994) 4353–4356.

[21] J. Logan, J.S. Fowler, N.D. Volkow, G.J. Wang, Y.S. Ding, D.L. Alexoff, Distribution volume ratios without blood sampling from graphical analysis of PET data, J. Cereb. Blood Flow Metab. 16 (1996) 834–840.

[22] A. Loock, J.L. Conry, D.B.K. Li, C.M. Clark, Disregulation of caudate/cortical metabolism in FAS: a case study, Alcohol. Clin. Exp. Res. 17 (1993) 485.

[23] S.E. Maier, W.A. Chen, J.R. West, Prenatal binge-like alcohol exposure alters neurochemical profiles in fetal rat brain, Pharmacol. Biochem. Behav. 55 (1996) 521–529.

[24] S.N. Mattson, A.M. Goodman, C. Caine, D.C. Delis, E.P. Riley, Executive functioning in children with heavy prenatal alcohol exposure, Alcohol. Clin. Exp. Res. 23 (1999) 1808–1815.

[25] S.N. Mattson, E.P. Riley, T.L. Jerrigan, A. Garcia, W.M. Kaneko, C.L. Ehlers, K.L. Jones, A decrease in the size of the basal ganglia following prenatal alcohol exposure: A preliminary report, Neurotoxicol. Teratol. 16 (1994) 283–289.

[26] S.N. Mattson, E.P. Riley, E.R. Sowell, T.L. Jerrigan, D.F. Sobel, K.L. Jones, A decrease in the size of the basal ganglia in children with fetal alcohol syndrome, Alcohol. Clin. Exp. Res. 20 (1996) 1088–1093.

[27] C.M. McCormick, J.W. Smythe, S. Sharma, M.J. Meaney, Sex-specific effects of prenatal stress on hypothalamic–pituitary–adrenal responses to stress and brain glucocorticoid receptor density in adult rats, Brain Res. Dev. Brain Res. 84 (1995) 55–61.

[28] J.W. Mink, The basal ganglia: focused selection and inhibition of competing motor programs, Prog. Neurobiol. 50 (1996) 381–425.

[29] M. Mishkin, B. Malamut, J. Bachevalier, Memories and habits: two neural systems, in: G. Lynch, J.L. McGaugh, N.M. Weinberger (Eds.),

Neurobiology Learning and Memory, Guilford Press, New York, 1984, pp. 65–77.
[30] J. Mukherjee, Z.Y. Yang, R. Lew, T. Brown, S. Kronmal, M.D. Seiden, L.S. Seiden, Evaluation of D-amphetamine effects on the binding of dopamine D-2 receptor radioligand, 18F-fallypride in nonhuman primates using positron emission tomography, Synapse 27 (1997) 1–13.
[31] K.M. Paarlberg, A.J. Vingerhoets, G.A. Dekker, H.P. Van Geijn, Psychosocial factors and pregnancy outcome: a review with emphasis on methodological issues, J. Psychosom. Res. 39 (1995) 563–595.
[32] J.F. Rasco, R.D. Hood, Enhancement of the teratogenicity of all-trans-retinoic acid by maternal restraint stress in mice as a function of treatment timing, Teratology 51 (1995) 63–70.
[33] A.D. Roberts, O.T. De Jesus, M.L. Schneider, M.J. Schueller, S.E. Shelton, R.J. Nickles, Dopamine system characterization of rhesus monkeys exposed to moderate dose alcohol in utero, J. Nucl. Med. 40 (1999) 108 (Society of nuclear medicine 46th annual meeting, Los Angeles, CA).
[34] P.K. Rudeen, J. Weinberg, Prenatal ethanol exposure: changes in regional brain catecholamine content following stress, J. Neurochem. 61 (1993) 1907–1915.
[35] M.L. Schneider, C.F. Moore, Effect of prenatal stress on development: a nonhuman primate model, in: C.A. Nelson (Ed.), Minnesota Symposium on Child Psychology, Erlbaum, Mahwah, NJ, 2000, pp. 201–243.
[36] M.L. Schneider, C. Moore, G.W. Kraemer, Moderate alcohol during pregnancy: learning and behavior in adolescent rhesus monkeys, Alcohol. Clin. Exp. Res. 25 (2001) 1–10.
[37] M.L. Schneider, E.C. Roughton, G.R. Lubach, Moderate alcohol consumption and psychological stress during pregnancy induces attention and neuromotor impairments in primate infants, Child Dev. 68 (1997) 747–759.
[38] T.A. Slotkin, G.A. Barnes, E.C. McCook, F.J. Seidler, Programming of brainstem serotonin transporter development by prenatal glucocorticoids, Brain Res. Dev. Brain Res. 93 (1996) 155–161.
[39] A.P. Streissguth, F.L. Bookstein, P.D. Sampson, H.M. Barr, Attention: prenatal alcohol and continuities of vigilance and attention problems from 4 through 14, Dev. Psychopathol. 7 (1995) 419–446.
[40] N. Tajuddin, M.J. Druse, In utero ethanol exposure decreased the density of serotonin neurons: maternal ipsapirone treatment exerted a protective effect, Brain Res. Dev. Brain Res. 117 (1999) 91–97.
[41] T.D. Tran, S.J. Kelley, Alterations in hippocampal and hypothalamic monaminergic neurotransmitter systems after alcohol exposure during all three trimester equivalents in adult rats, J. Neural Transm. 106 (1999) 773–786.
[42] P.D. Wadhwa, Prenatal stress and life-span development, in: H.S. Friedman (Ed.), Encyclopedia of Mental Health, Academic Press, San Diego, CA, 1998, pp. 265–280.
[43] G.R. Ward, P.E. Wainwright, Prenatal ethanol and stress in mice: 1. Pup behavioral development and maternal physiology, Physiol. Behav. 45 (1989) 533–540.
[44] M. Weinstock, E. Matlina, G.I. Maor, H. Rosen, B.S. McEwen, Prenatal stress selectively alters the reactivity of the hypothalamic–pituitary adrenal system in the female rat, Brain Res. 595 (1992) 195–200.
[45] S.P. Wise, E.A. Murray, C.R. Gerfen, The frontal-cortex–basal ganglia system in primates, Crit. Rev. Neurobiol. 10 (1996) 317–356.