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# Prenatal Stress Diminishes Neurogenesis in the Dentate Gyrus of Juvenile Rhesus Monkeys

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**Background:** *Early life stress, including during fetal development, has been hypothesized to predispose individuals to several illnesses and psychiatric disorders later in adulthood.*

**Methods:** *To determine whether prenatal stress alters neural, hormonal, and behavioral processes in nonhuman primates, pregnant rhesus monkeys were acutely stressed on a daily basis for 25% of their 24-week gestation with an acoustical startle protocol. At 2 to 3 years of age, hippocampal volume, neurogenesis in the dentate gyrus, and cortisol levels were evaluated in the offspring generated from stressed and control pregnancies.*

**Results:** *Prenatal stress, both early and late in pregnancy, resulted in a reduced hippocampal volume and an inhibition of neurogenesis in the dentate gyrus. These changes were associated with increased pituitary–adrenal activity, as reflected by higher cortisol levels after a dexamethasone suppression test, and also with behavioral profiles indicative of greater emotionality.*

**Conclusions:** *These findings indicate that the prenatal environment can alter behavior, dysregulate neuroendocrine systems, and affect the hippocampal structure of primates in a persistent manner.* Biol Psychiatry 2003; 54:1025–1034 © 2003 Society of Biological Psychiatry

**Key Words:** Neurogenesis, hippocampus, stress, pregnancy, cortisol, monkey

## Introduction

Despite decades of intensive research on psychiatric disorders, the etiology and precise biological mechanisms behind these conditions are still poorly understood. Mounting evidence indicates that psychiatric disorders not only have a neurochemical basis, but might also be

associated with morphologic alterations of the brain. Enlarged ventricles accompanied by a decreased size of certain cortical and limbic structures, including the hippocampus, have been described in patients suffering from major depressive disorder, schizophrenia, and posttraumatic stress disorder (Altshuler et al 2000; Bremner et al 1995, 1997, 2000; Gur et al 2000; Sheline et al 1996; reviewed by Manji and Duman 2001). Postmortem studies of patients with mood disorders and schizophrenia indicate both altered morphology and a decreased number and density of neurons (reviewed by Arnold 1999; Rajkowska 2000). Understanding the origin of these structural alterations might help us to prevent and treat these severe psychiatric disorders (McEwen 2000).

It has become increasingly evident that the antecedents of many illnesses begin in fetal life, and that prenatal events can bias our development toward either health or disease in the postpartum period. The potential vulnerability of the fetus to extrinsic factors is most apparent when teratogenic drugs or fetal alcohol exposure cause congenital malformations in infants, but even more moderate disturbances of the pregnant female have been found to affect fetal physiology (Lou et al 1994; Wadhwa et al 2001). For example, several retrospective studies have confirmed that maternal stress during pregnancy can both significantly increase the likelihood of premature delivery and small-for-date babies (Hedegaard et al 1993, 1996), as well as result in disturbed physical and/or psychological development during infancy (Clements 1992; Jones and Tauscher 1978; Lou et al 1994; Meijer 1985). It has also long been suspected that maternal stress and viral infections during pregnancy might be associated with increased risk for schizophrenia and affective disorders (Brown et al 2000; Huttunen and Niskanen 1978; Machon et al 1997; Myhrman et al 1996; van Os and Selten 1998; Watson et al 1999).

Many studies in animals have demonstrated that even in the absence of overt effects on length of gestation and fetal growth, maternal disturbance can influence the offspring's brain chemistry, endocrine function, emotionality, and learning ability (reviewed by Weinstock 1997). These findings suggest that the fetus is not completely buffered

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against stressful events experienced by the gravid female and indicate a need to clarify the range of in utero conditions that the fetus can tolerate and still maintain its normal development.

Our study was designed to specifically assess one sensitive and critical area of the developing brain, the hippocampus (Koehl et al 1999; Takahashi 1998). Previous research had demonstrated that exposure of the fetal monkey 1 month before term to just 2 days of dexamethasone (DEX), a synthetic corticosteroid drug, was sufficient to cause massive atrophy of hippocampal cells at birth (Uno et al 1990). Magnetic resonance imaging showed that the hippocampal volume had still not recovered by 2 years postpartum (Uno et al 1994). Although DEX is uniquely potent, especially because it is not readily metabolized by the fetus, these pharmacologic studies support the view that the placental transfer of maternal cortisol is a primary mediator of maternal stress effects on fetal development (Dodic et al 2002; Kay et al 2000; Pepe and Albrecht 1987). Additional stress-induced influences might occur because of acute hypoxic episodes, reduced placental transfer of nutrients, and altered fetal metabolism (Rees et al 1999; Walsh et al 1979). The sensitivity of the developing hippocampus is of particular concern, because it becomes an important brain center after birth, involved in the regulation of the hypothalamic–pituitary–adrenal (HPA) axis. Thus, we hypothesized that monkeys from disturbed pregnancies might evince signs of cortisol hyperactivity comparable to that seen in rats that are stressed early in the postpartum period (Meaney et al 1992; Plotsky and Meaney 1993; Weinstock 1997). Uno et al (1994) had reported that monkeys exposed to DEX as fetuses exhibited significantly larger cortisol responses to acute stressors when they were tested as juveniles. Similarly, we found previously that infant monkeys from stressed pregnancies remained more emotionally reactive and showed immune abnormalities that seemed to be associated with higher HPA activity (Clarke and Schneider 1993; Coe et al 1996, 2002b; Schneider 1992; Schneider et al 1999).

For the current study, we took advantage of techniques that allow one to assess new cell growth in the adult brain of vertebrates, including primates (Eriksson et al 1998; Gould et al 1999; Kornack and Rakic 1999). We hypothesized that the ability of certain brain regions to generate new cells during the postpartum period would be affected by the occurrence of fetal disturbance. Lemaire et al (2000) had already documented in rats that maternal stress during the last week of pregnancy affected neurogenesis in the pups, but this finding has not been replicated in primate infants, nor has anyone previously considered the issue of the timing of the disturbance during pregnancy. Our study compared the impact of equivalent periods of maternal disturbance experienced either early or late in

gestation. If prenatal stress does, in fact, alter the set point for cell growth in the hippocampus, it could give credence to many retrospective studies in humans suggesting that learning disabilities and even mental illness might have their origin in fetal life (Brown et al 2000; Clements 1992; Huttunen and Niskanen 1978; Myhrman et al 1996; van Os and Selten 1998; Watson et al 1999).

## Methods and Materials

### *Subjects and Housing*

Rhesus monkey infants (*Macaca mulatta*) were generated from three types of pregnancies, including a control and two prenatal disturbance conditions. Initially, 20 healthy male and female offspring were chosen for the hormone and behavior assessments when they were 2–2.5 years of age, before puberty. Then, a smaller subset of 12 was selected for the neuroanatomic evaluation because it required euthanasia. The sacrificed animals were age-, weight-, and sex-matched, including two males and two females from each rearing condition. Exclusion criteria included any history of serious clinical illness.

The mothers were laboratory-reared, multiparous females in a long-established breeding colony. They were time-mated with one adult male during the 4 days around ovulation to verify the date of conception for the pregnancy manipulations. The gravid females were maintained in undisturbed and standardized housing conditions, except on the acute disturbance days (see below). After birth, the infants were reared normally by the mother through 7 months of age and then transferred into small social groups comprising four to eight animals from both types of pregnancies. To facilitate the current blood and behavioral evaluations, they were re-housed as pairs. The husbandry conditions were in accordance with National Institutes of Health guidelines for the proper care and treatment of laboratory animals. Commercial monkey chow was provided daily, fruit was given three times per week, and water was available ad libitum. The light:dark schedule was 16:8 hours, with lights on at 6:00 AM; ambient room temperature was maintained at 21°C. All experimental procedures were approved by the Institutional Animal Care and Use Committee.

### *Prenatal Manipulations*

Juvenile monkeys from undisturbed, normal pregnancies (control) were compared with ones that had been disturbed for 6 weeks during the 24-week pregnancy, either early or late in gestation. The early stress period began on day 50 postconception and lasted until day 92, whereas late stress spanned days 105–147. These periods correspond to two distinct stages of cell growth and synaptogenesis in the fetal monkey cortex (Bourgeois et al 2000). Of specific importance, much of the cytoarchitecture and neurochemistry of the hippocampus is established in fetal monkeys by day 90, and in humans the cell proliferation in the dentate slows and mostly stops in the ventricular zone after midgestation (Berger et al 1993; Seress et al 2001). The pregnant female was acutely disturbed 5 days per week, Monday through Friday, by being moved to a darkened test room between 2:30

and 4:00 PM. While located there for 10 min in a small transport cage, she was intermittently aroused with an acoustical startle protocol (three 1-sec broadcasts of a 110-dB horn, randomly at 1–4-min intervals). Prior research using the same paradigm demonstrated that it significantly elevates maternal cortisol above the normal level for pregnant monkeys and can affect the infant's behavioral reactivity and immunity postpartum (Clarke and Schneider 1993; Coe et al 1996, 2002b). Both before and after the 6-week manipulations, the females lived undisturbed in their home cages until the natural birth of their infants.

### *Behavioral Characterization*

When the offspring were 2.0–2.5 years of age, each monkey's behavior was observed and recorded on a laptop computer across 12 5-min sessions. This took place over a 1-month period before the hormone assessments and 5-bromo-2'-deoxyuridine (BrdUrd) treatments. The observer scored a set of 10 predefined behaviors, which generated an activity profile and focused on behavior indicative of emotionality. The former included five mutually exclusive categories, ranging from asleep, inactive, and alert, to goal-directed movement. The mean duration of nondirected motoric activity has been presented as an index of emotionality, because it includes repetitive and stereotypic movement. Other normative behaviors were recorded, including eating, vocalization, grooming, and manipulative exploration of the physical environment. The duration of the latter behavior was selected for presentation because low scores usually occur in emotionally reactive animals that do not focus their attention as readily or investigate and attend as much to the cage milieu. The cages and animals were located in a large housing room and were not identified in any way that would allow the observer to readily know the experimental history of their prenatal conditions.

### *HPA Axis*

To characterize adrenal hormone secretion, plasma cortisol levels were next determined under two conditions. Blood samples were collected at 9:00–10:00 AM to assess basal levels, which also incorporated a measure of acute reactivity because it took approximately 5 min to collect blood from the awake monkey. Following a 1–2-week interval, samples were then obtained at 9:00 AM after a 12-hour overnight DEX treatment (dexamethasone sodium phosphate, .25 mg/kg, intramuscular [IM]). The latter condition should normally inhibit HPA activity and thus is a measure of the negative feedback sensitivity of the central nervous system to corticosteroid hormones. Dexamethasone in this dose range normally keeps cortisol suppressed below 5 µg/dL in monkeys (Hou et al 1996; Kay et al 2000). Cortisol was determined by enzyme-linked immunoabsorbent assay.

### *Histologic Procedures*

**BROMODEOXYURIDINE INJECTION AND IMMUNOCYTOCHEMISTRY.** Several months later, when they were 2.5–3.0 years of age, 12 of the monkeys were administered BrdUrd (75 mg/kg, intraperitoneal [IP]) on 3 consecutive days. To facilitate the injections and to provide analgesia, the monkeys were briefly

anesthetized with ketamine on each of these mornings (15 mg/kg, IM). Ketamine is rapidly catabolized by the liver and has a short half-life of 10–15 min, and thus the monkeys were conscious within 1 hour. Three weeks after these BrdUrd injections, the animals were perfused to obtain the brains for analysis. This was done with 4.0% paraformaldehyde in .1 mol/L phosphate buffer under ketamine (20 mg/kg) and deep sodium pentobarbital anesthesia (60 mg/kg). The 21-day survival time was chosen to allow for the determination of the phenotype of any newly generated cells. An oscillating tissue slicer was used to collect 40-µm coronal sections through the entire hippocampal formation. For quantification of BrdUrd-positive cells, the tissue was processed immunohistochemically with the peroxidase methods, according to our standard protocol (Gould et al 1999). In brief, the tissue was first treated by heating in .1 mol/L citric acid buffer (pH 6.0), followed by incubation in hydrogen peroxide, trypsin, and 2 mol/L hydrochloric acid (HCl), and then blocked in normal horse serum and incubated in mouse monoclonal antibody to BrdUrd (1:250; Novocastra, Newcastle, United Kingdom). These sections were processed with a Vectastain Elite ABC kit (Vector Laboratories, Burlingame, California), followed by a brown diaminobenzidine reaction, and finally counterstained for Nissl with cresyl violet.

For fluorescence immunocytochemistry, the sections were denatured in 2 N HCl for 30 min, rinsed, and incubated overnight at 4°C in rat monoclonal antibody against BrdUrd (1:200 + 0.5% Tween-20; Accurate, Westbury, New York). The sections were rinsed and incubated in biotinylated rat secondary antisera for 60 min (1:200; Chemicon, Temecula, California), rinsed, and incubated in streptavidin Alexa-568 (1:500; Molecular Probes, Eugene, Oregon). Then, rinsed and incubated in mouse anti-neuron-specific nuclear protein (NeuN) (1:200; Chemicon) for 2 days at 4°C. After several more rinses, sections were incubated in the appropriate secondary antisera conjugated to Alexa-488 for 30 min (1:500; Molecular Probes). As the final step, rinsed sections were mounted and dried, then preserved in 25% glycerol in Tris-buffered saline under a cover slip. Tissue stained for fluorescence was viewed with a BX-60 fluorescent microscope (Olympus, New Hyde Park, New York) and also with a confocal scanning laser microscope (Zeiss Axiovert 510 LSM; Carl Zeiss Microimaging Inc., Thornwood, New York; lasers: Argon 458/488, HeNe 543, UV 351/364) for verification of double labeling. Optical stacks of 1-µm sections revealing double-labeled cells were obtained and the images rotated in orthogonal planes to verify double labeling. Fifteen BrdUrd-labeled cells were identified per animal, and colocalization incidence with NeuN was expressed as a percentage. Glial markers were not investigated in this study.

**BrdUrd QUANTIFICATION.** Serial sections were collected throughout the entire anterior–posterior extent of the left hippocampal formation. From each animal, every 15th section was selected for quantification and processed for BrdUrd immunohistochemistry (a mean total of 20 sections per animal). To ensure objectivity, slides were coded before quantitative analysis, and the code was not broken until the analysis was completed. All BrdUrd-labeled cells were counted under 400× magnification, regardless of size or shape. The number of

BrdUrd-positive cells was assessed in the granule cell layer, together with the subgranular zone (defined as a two-cell-body-wide zone along the border of the granule cell layer) and separately in the hilus. Values are expressed as densities (number of BrdUrd-labeled cells/1 mm<sup>3</sup> of the granule cell layer).

**HIPPOCAMPAL VOLUME.** The volume of the hippocampal formation was estimated on the basis of the Cavalieri principle (Gundersen et al 1988). After randomly selecting a starting point, sections were taken at 1000- $\mu$ m intervals, and then cross-sectional areas were measured by outlining the boundaries of the hippocampal formation with the aid of the NeuroLucida system (Microbrightfield, Colchester, Vermont). Volumes were calculated by multiplying the cross-sectional area measurements with the intersection distance. Volumes are reported as mm<sup>3</sup>.

### Data Analysis

The results are presented as the mean  $\pm$  SEM. Prenatal treatment effects were assessed with one-way analysis of variance (ANOVA), followed by Tukey or Newman-Keuls post hoc analysis for examination of group differences.

## Results

### Behavior

Observation of the monkey's behavior indicated that the offspring of prenatally disturbed pregnancies engaged in significantly lower levels of focused exploration [ANOVA:  $F(2,17) = 4.53$ ;  $p < .05$ , followed by Newman-Keuls post hoc analysis: early stress  $q = 3.96$ ,  $p < .05$ ; late stress  $q = 3.34$ ,  $p < .05$  versus control] and tended to exhibit higher levels of nondirected locomotor behavior, such as pacing (Figure 1). This finding is in keeping with prior reports of altered emotionality after these types of gestational manipulations in monkeys (Clarke and Schneider 1993, Clarke et al 1994; Schneider 1992; Schneider et al 1999).

### HPA Axis

Monkeys generated from the early stress and late stress pregnancies had significantly higher cortisol levels than did the control monkeys [ANOVA:  $F(2,17) = 8.85$ ;  $p < .05$ , followed by Tukey post hoc analysis: early stress  $q = 5.12$ ,  $p < .05$ ; late stress  $q = 5.18$ ,  $p < .05$  versus control], which was a reflection of their higher basal levels at 9:00 AM and perhaps some influence of the 5-min handling required to collect blood from a conscious animal (Figure 2). Twelve hours after DEX administration, both the early stress and late stress monkeys had significantly higher cortisol levels than did the control monkeys [ANOVA:  $F(2,17) = 5.21$ ;  $p < .05$ , followed by Newman-Keuls post hoc analysis: early stress  $q = 4.47$ ,  $p < .05$ ; late stress  $q = 3.98$ ,  $p < .05$  versus control], indicating that their HPA

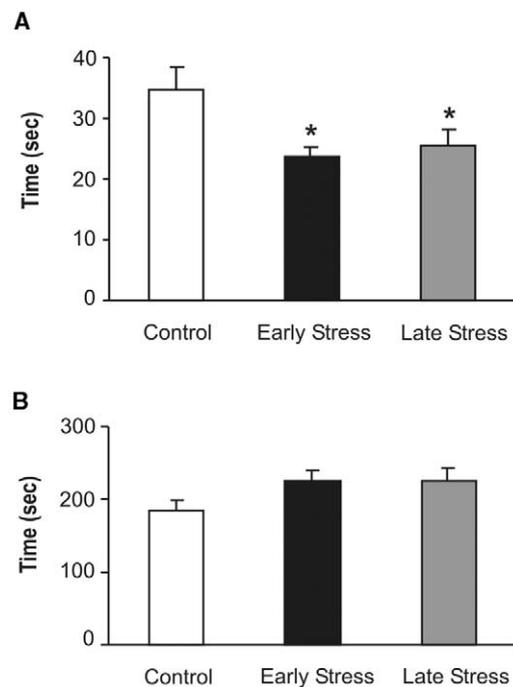


Figure 1. Exploratory behavior (A) and locomotor activity (B) based on 12 5-min observation sessions. Time spent in focused environmental exploration was significantly decreased in both the early and late stress monkeys as compared with control monkeys (one-way analysis of variance followed by Newman-Keuls multiple comparison test). Nondirected locomotor activity was exhibited 22% more by prenatally disturbed monkeys, a tendency that approached but did not reach significance with this small sample size. \*  $p < .05$ , significantly different from control monkeys.

axis was not as suppressed by the negative feedback of DEX on the central nervous system (Figure 2).

### Hippocampal Neurogenesis and Volume

We observed BrdUrd-labeled cells predominantly in the subgranular zone and only occasionally in the hilus of the hippocampus. Quantitative analysis of the BrdUrd-labeled cells in the dentate gyrus revealed that disturbance during either early or late pregnancy resulted in a dramatic 32% decrease in density relative to the undisturbed control monkeys (Figure 3). The ANOVA indicated that there was significant difference between the gestation conditions [ $F(2,9) = 6.85$ ;  $p < .05$ ], and Tukey post hoc comparisons verified that monkeys from both the early stress ( $q = 5.01$ ,  $p < .05$ ) and late stress pregnancies ( $q = 4.89$ ,  $p < .05$ ) showed lower neurogenesis than did control monkeys. No statistically significant difference was found with regard to the timing of the maternal stress during pregnancy. A similar pattern was observed in the hilus: both early and late stress monkeys had a lower density of BrdUrd-

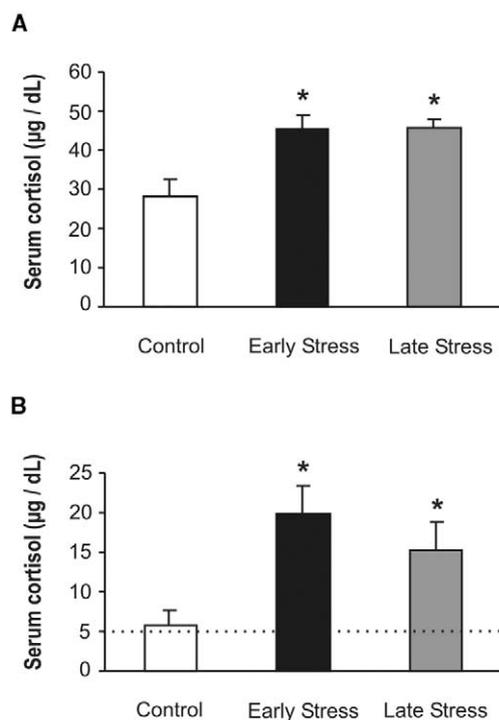


Figure 2. Basal cortisol levels (A) and values after an overnight dexamethasone suppression test (DST) (B). Animals generated from early and late stress pregnancies had significantly elevated cortisol levels, both at baseline and after the DST. Horizontal dashed line indicates the maximum level of plasma cortisol (5 µg/dL) considered normal (e.g., failure to stay suppressed). Results are presented as mean  $\pm$  SEM (µg cortisol/dL). For statistical comparisons, one-way analysis of variance was used, followed by Tukey's multiple comparison test. \*  $p < .05$ , significantly different from the control monkeys.

positive cells, by 23% and 21% respectively, but those decrements did not reach significance given the low number of labeled cells in this hippocampal region (data not shown). Furthermore, there was a significant correlation between plasma cortisol levels after the DEX suppression test and the number of BrdUrd-positive cells in the dentate gyrus ( $p < .05$ ).

Combined immunofluorescent labeling of random samples revealed that  $80\% \pm 7\%$  of the BrdUrd-labeled cells were also positive for NeuN in the control group, and similar ratios were observed in the early stress ( $82\% \pm 8\%$ ) and late stress ( $87\% \pm 7\%$ ) groups (Figure 4). Thus, these data indicate that similar proportions of labeled cells matured into neurons in all three groups of monkeys.

To determine whether prenatal stress influenced hippocampal volume, postmortem volumetry of the hippocampal formation was performed (Figure 3). Prenatal stress resulted in a mild but statistically significant de-

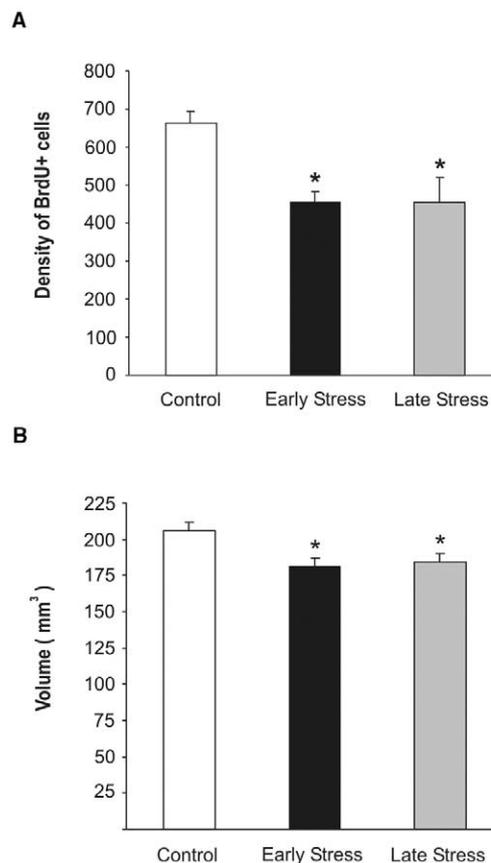


Figure 3. (A) Postnatal hippocampal neurogenesis was evaluated by quantifying 5-bromo-2'-deoxyuridine (BrdUrd)-positive cells in samples collected from the entire anterior–posterior extent of the hippocampal formation. Cell proliferation in the dentate gyrus was significantly less in monkeys from both early and late stress pregnancies. Results are the mean density  $\pm$  SEM (number of BrdUrd-positive cells/1 mm<sup>3</sup> of the granule cell layer). (B) Hippocampal volume measurements. Postmortem hippocampal volumetry revealed that prenatal stress resulted in a significantly reduced hippocampal volume in both early (–12%) and late stress monkeys (–10%). Results are the mean volume (mm<sup>3</sup>)  $\pm$  SEM. For group comparisons, one-way analysis of variance was used, followed by Tukey's post hoc analysis. \*  $p < .05$ , significantly different from control monkeys.

crease in the hippocampal volume [ANOVA:  $F(2,9) = 6.07$ ,  $p < .05$ ]. The hippocampal volume was 12% smaller in animals from the early stress group and 10% smaller in the late stress monkeys as compared with control monkeys. Tukey post hoc comparisons indicated that both of these volume reductions were statistically significant: early stress  $q = 4.46$ ,  $p < .05$  and late stress  $q = 4.04$ ,  $p < .05$ , when compared with control monkeys. The timing of the disturbance during pregnancy did not have a differential effect.



Figure 4. Confocal images of new neurons in dentate gyrus of a prenatally stressed subadult rhesus monkey. Granule cells labeled NeuN (green) and a newly generated neuron labeled with 5-bromo-2'-deoxyuridine (BrdUrd; red). The majority of BrdUrd-labeled cells in the dentate gyrus of all animals expressed the neuronal marker NeuN indicating a neuronal phenotype. No group differences were observed in the percentage of double-labeled cells. NeuN, neuron-specific nuclear protein.

## Discussion

Our study expands on previous prenatal stress findings in rodents and primates, which indicate that manipulations of pregnancy conditions can have lasting effects on behavior, hormone activity, and brain anatomy and function (Takahashi 1998; Weinstock 1997). Specifically, in the rhesus monkey, it had been shown that disturbance of the gravid female results in offspring with: 1) immature neuromotor reflexes at birth; 2) greater emotionality as infants; and 3) lymphocyte responses that were still abnormal at 2 years of age (Clarke and Schneider 1993; Coe et al 2002b; Schneider et al 1999). By using the DEX suppression test in the current study, we have now demonstrated that the experience of maternal stress during pregnancy also causes the offspring to have a different neural set point for the HPA axis. Specifically, they emerged from the pharmacologic inhibition after less than 12 hours, which is unusual for monkeys (Hou et al 1996). This result concurs with evaluations of adrenal function in rats after prenatal stress, and specifically with the effect of prenatal exposure to DEX on later stress reactivity in monkey infants (Bakker et al 1995; Koehl et al 1999; Uno et al 1994). It also seems to support the hypothesis that HPA hormones are involved in mediating some of the maternal stress effects on the fetus (Dodic et al 2002; Nyirenda and Seckl 1998; Wadhwa et al 2001).

Many studies have shown that administration of DEX or adrenocorticotrophic hormone to a gravid female can mimic the effect of nonspecific psychological stressors on the fetus (Rhees and Fleming 1981). Indeed, the deleterious consequences of transferring too much maternal cortisol to the fetal compartment seems to be so great that the placenta has developed enzymes to metabolize cortisol into less active forms, such as cortisone, and thereby might partially be able to protect the fetus (Seckl 1997). Nevertheless, subsequent to maternal stress, hormone alterations as well other physiologic changes, including reduced placental blood flow and oxygen availability, seem to be able to override this placental buffer and alter fetal development (Fujii et al 2003; Rees et al 1999). Further-

more, maternal stress might induce an imbalance in numerous other factors, such as excitatory amino acid neurotransmitters, neurotrophins, and corticotrophin-releasing hormone in the fetal central nervous system (Avishai-Eliner et al 2002).

There is now a substantial literature indicating that fetal exposure to DEX can have adverse effects on brain development, which must be weighed against its benefits when used in the neonatal intensive care unit to take advantage of its ability to promote rapid maturation of the baby's lungs (Matthews 2000). Fetal monkeys administered DEX, at doses 5–10 times higher than used with premature human babies, exhibited marked atrophy of hippocampal cells at birth, and their hippocampi failed to recover to normal size even at 2 years postpartum (Uno et al 1994). Here we report a 10%–12% decrease in hippocampal volume after prenatal stress. Although this reduction might seem modest, it should be noted that magnetic resonance imaging analyses revealing a similar amount of hippocampal atrophy in psychiatric patients has raised considerable concern (e.g., Bremner et al 1995, 1997; Gur et al 2000). It is of further interest that a recent clinical study suggested that the occurrence of smaller hippocampi might constitute a risk factor for the development of stress-related psychopathology (Gilbertson et al 2002). The brain alterations found in the current study are perhaps even more remarkable because this hippocampal vulnerability was found after psychological disturbance of a relatively moderate nature. It should be emphasized that the disturbance manipulations lasted only 10 min per day and spanned only 25% of pregnancy. Yet these prenatal stress conditions were sufficient to alter a fundamental and important aspect of brain function, the regenerative capacity to grow new cells. In a previous study of other infant monkeys, we reported that the same type of pregnancy manipulation also had effects on the size and shape of the corpus callosum (Coe et al 2002a). To this effect on interhemispheric connections, we now add a reduced set point for neurogenesis and a decreased hippocampal volume, which was manifest in both male and female off-

spring and seen equally after stress early and late in pregnancy.

The lack of an effect of gestational timing was somewhat surprising, given that there are very important differences in the neuronal growth within the fetal brain corresponding to the two periods we selected for the gestational manipulations (Berger et al 1993; Bourgeois et al 2000; Rakic and Nowakowski 1981). Thus, it would suggest that the lingering effect postpartum was due to a sustained shift in the developmental trajectory after birth, rather than just mediated by a single, punctate insult before term. Given that we have evidence of different behavioral responses, alterations in stress reactivity, and heightened HPA activity in the prenatally disturbed infants, these brain changes probably reflect multiple influences. It has already been shown that adrenal hormone feedback on the hippocampus can affect the ongoing rate of neurogenesis in the adult organism (Gould et al 1992). Moreover, in rodents there have been several studies demonstrating that mother–infant interactions play an important role in extending the effects of early rearing manipulations (Plotsky and Meaney 1993). Observations of the maternal behavior of our monkeys do not suggest that the prenatally stressed infants were treated differentially, but we do know that other aspects of the mother–infant relationship do serve to extend the gestational influence. For example, we have recently documented that infant's gut flora during the nursing period is different if they were generated from stressed pregnancies (Bailey et al, in press). Thus, many behavioral and physiologic processes contribute to sustaining the altered developmental trajectory of these infants.

Currently, adult hippocampal neurogenesis is appreciated to be an important, novel contributor to the plasticity of adult neural circuitry. This continuous production of granule cells is also accompanied by cell death, which results in a continuous turnover of cells in the dentate gyrus (Biebl et al 2000). A substantial portion of the newly generated cells die within the first few days after division, but a significant fraction of these newborn neurons do survive and are maintained (Kempermann et al 2003). The net effect is the stable integration of a low number of new neurons into existing neuronal networks. This dynamic balance between proliferation and cell death can be influenced by elevated levels of glucocorticoids resulting from stress exposure (Gould et al 1998, Sapolsky 2000). In the present experiment, the incidence of cell death was not evaluated, nor do we know the degree to which the contemporaneous cortisol levels in circulation drove the differences in neurogenesis. It should be emphasized, however, that the 3-week survival time after the BrdUrd injection is sufficiently long after cell division to indicate that the remaining fraction of new cells was already quite

stable (Cameron et al 1993; Kempermann et al 2003). This interval was also sufficiently long to minimize the potential influence of the ketamine anesthesia, which was administered to facilitate the humane injection of the BrdUrd. This could be viewed as methodical confound because ketamine is a potent *N*-methyl-D-aspartate (NMDA) receptor antagonist, and NMDA antagonist drugs are known to stimulate adult neurogenesis in the dentate gyrus (Cameron et al 1995; Nacher et al 2001); however, this factor was controlled because all monkeys received the same dose, and the ketamine would have been rapidly catabolized by the liver and excreted.

The functional role of the newly generated granule cells is still unknown, but recent studies suggest that they can play an active role in the formation of certain types of hippocampal-dependent memory (Shors et al 2001, 2002). In addition, several recent studies have suggested that altered rates of adult neurogenesis might contribute to the etiology or pathophysiology of some stress-related psychiatric illnesses, especially to mood disorders (Czéh et al 2001; Eisch 2002; Malberg et al 2000; Manji et al 2000). Thus, it is conceivable that individuals with a disturbed structural plasticity due to prenatal or early-life stress might be at greater risk for developing psychiatric illnesses. Now that the finding of prenatal influences on neurogenesis and hippocampal anatomy has been extended from the rodent (Lemaire et al 2000; Schmitz et al 2002) to the nonhuman primate, there is a compelling need to consider the implications for children.

The overall development of the hippocampal dentate gyrus in monkeys and humans is similar (Berger et al 1993), although it is difficult to specifically compare the rate of fetal growth and stage of maturity at birth. The formation of dentate granule cells starts as early as the E38 in the rhesus monkey, and the majority of the granule cells are generated between E60 and E120 (Rakic and Nowakowski 1981). In humans, the granule cell layer first appears during gestational weeks 13–14 (Humphrey 1967), and similar to rhesus monkeys, the majority of the granule cells are formed throughout the second trimester (Seress et al 2001). At term, approximately 50%–60% of the granule cells are present in the dentate gyrus of the rhesus monkey (Keuker et al 2003), and this number is approximately 70%–85% in humans (Seress, personal communication, February 27, 2003). In both monkeys and humans, the production and maturation of the granule cells thus continues well into the postnatal period (Eriksson et al 1998; Gould et al 1999; Kornack and Rakic 1999; Seress 1992; Seress et al 2001); however, we hopefully benefit even more from the fact that the human brain is extremely dependent on growth during the postpartum period. At birth, the brain of the neonatal monkey is 60% of adult size, whereas in humans it is only 24% of adult

size. Thus, there might be more capacity for recovery in children and a greater reliance on the rearing environment for creating the right milieu needed for healthy development. Even so, it is clear that we should strive for an optimal pregnancy environment followed by a good rearing environment, to ensure that there are not irreversible effects on brain development that could lead to learning or psychiatric disorders in adulthood.

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Marian Kramer died unexpectedly as he was finishing these experiments. The manuscript was written posthumously by his colleagues, who mourn the loss of this talented, young scientist.

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