Maternal Endocrine Activation During Pregnancy Alters Neurobehavioral State in Primate Infants

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Key Words: infant, high risk • substance abuse

Objectives. The purpose of this study was to investigate whether maternal endocrine activation during pregnancy would affect the neurobehavioral state of primate offspring in a manner similar to that observed in human infants from pregnancies involving maternal substance abuse or maternal stress.

Method. Twenty-two rhesus monkey (Macaca mulatta) infants were derived from females administered either adrenocorticotrophic hormone (ACTH), which increased the mother's endocrine activity, or saline solutions for 14 consecutive days during mid-pregnancy. On days 15 and 30 postpartum, infants underwent brief separations from their mothers and were videotaped for later evaluation of neurobehavioral state.

Results. Infants from mothers administered ACTH spent significantly more time in a drowsy state than did controls (p < .04), and the increased drowsiness tended to be most pronounced during the postseparation period, when acute stress was highest. In contrast, controls remained in a more active alert state (p < .03), presumably searching for their mother, a species-typical adaptive response to maternal separation. Female infants spent more time in distressed state than did males on day 15, and the proportion of time in distressed state decreased in all infants after administration of 0.2 mL of 24% sucrose solution.

Conclusion. The results demonstrate that neurobehavioral state alterations are found in infants from mothers with increased endocrine activity during pregnancy. Neurobehavioral state disorganization can have an adverse impact on the human infant's concurrent and subsequent occupational performance. These findings establish the usefulness of the nonhuman primate model for advancing knowledge on early contributions to the development of human infant occupational behavior.

Occupational therapists provide essential services to infants and their family members when the child's occupational performance has been challenged by maternal substance abuse and other prenatal risk factors (Dunn, Campbell, Oetter, Hall, & Berger, 1989). However, successful therapeutic intervention can be implemented only to the extent that we understand the underlying causes of the dysfunction (Gorski, Lewkowicz, & Huntington, 1987). Because many questions remain unanswered about the etiology of childhood disabilities, further studies, such as the present study, that investigate causative factors for developmental disabilities are crucial for improving occupational therapy services for infants and their family members.

Children From Pregnancies Involving Maternal Stress or Substance Abuse

There is a growing appreciation among occupational
therapists that a large number of infants and children referred for occupational therapy services have impairments secondary to maternal substance abuse or maternal stress during the prenatal period. Research indicates that infants and children from pregnancies involving maternal substance abuse, stress, or both show a range of deficits, including growth deficiency and behavioral and intellectual impairments, that are secondary to the effects of the perturbation on brain development (Coles, 1996; Lobel, 1990; Coles, 1996; Day, Richardson, Geva, & Robles, 1994). These impairments often interfere with a child’s ability to perform in everyday contexts. For example, it is now known that fetal alcohol exposure contributes to fine and gross motor dysfunction, decreased attention span, hyperactivity, decreased intellectual capabilities, and disturbances in sleep patterns (Barr, Darby, Streissguth, & Sampson, 1990; Coles, 1996; Day, Richardson, Geva, & Robles, 1994; Larroque et al., 1995). These deficits in performance components can constrain the child in his or her ability to function in everyday activities.

Evidence suggests that as the child born to a mother who abused substances matures, his or her inability to master the tasks associated with the roles of his or her culture result in further delays or deficits. For example, longitudinal research indicates that fetal alcohol exposure has lifelong consequences for daily living skills and socialization skills (Streissguth, 1994). This could negatively affect the person’s ability to achieve a balance of occupational skills (work, play and leisure, activities of daily living) for achieving physical, mental, and socioemotional well-being.

Possible Mediating Factors

In evaluating the factors that may mediate the effect of prenatal stress and substance abuse on the fetus, maternal endocrine activation is a potential source. Substances of abuse and stressful events are known to have a marked effect on the body’s physiology and metabolic functions in activating the maternal endocrine system (Bandstra & Burkett, 1991; Coe et al., 1978; Weinberg & Bezio, 1987). This response includes increases in the levels of a number of hormones, including adrenocorticotrophic hormone (ACTH), from the pituitary. Although ACTH does not actually cross the placenta, it can have adverse effects on the developing fetus through its effect on adrenal hormone release (Wilke, Tseu, Rhee, & Fleming, 1982). In particular, higher cortisol levels may have many effects on the mother and can readily cross the placenta to affect the fetus. Moreover, primate studies have shown that synthetic corticoids, such as dexamethasone, when administered to female rhesus monkeys during mid-gestation, impair the development of brain areas in the offspring, inducing degeneration and depletion of the hippocampal pyramidal and dentate granular neurons (Uno et al., 1990; Uno et al., 1994).

Neurobehavioral State

To investigate whether and how maternal endocrine activation influences infant neurobehavioral state, we conducted repeated systematic observations of infant state behavior in rhesus monkeys from mothers administered ACTH or saline solution during pregnancy. Als’s findings on premature infants provide a useful model for understanding the infant’s capabilities to organize its behavior in response to environmental challenges (Als, 1986; Als, Duffy, & McAnulty, 1996; Als et al., 1986; Als et al., 1994). Briefly, Als and her colleagues proposed that infants interact with their environment through five interactive, hierarchically organized subsystems (physiologic, motor, state, attention, self-regulation). Optimal self-regulation (also referred to as neurobehavioral organization) occurs when stability is attained in all the subsystems. If an infant does not attain central nervous system (CNS) maturity, signs of disorganization can occur, causing difficulty in the infant’s ability to tolerate stimulation and maintain organization. When exposure to sensory stimulation exceeds the infant’s tolerance level, a state of physiologic and behavioral instability will develop. As stress further increases, self-regulation coping signals are typically seen, including leg bracing, hand-on-face, sucking, fis­tering, and shifting to lower behavioral states (drowsy or light sleep). If the stress continues to increase, the infant may manifest severe stress reactions, which can be dangerous in fragile neonates.

Some investigators have argued that the organization of neurobehavioral state influences neonates and infants’ behavior in a wide range of domains (Korner, 1969; Prechtl, 1974; Wolff, 1987). In fact, some researchers consider neonatal behavioral state to be the most important perspective from which to investigate the infant’s contribution to the mother-infant relationship and its own development (Spangler & Scheubeck, 1993).

In the neonate, Brazelton (1984) defined six behavioral states: (a) deep sleep, (b) light sleep, (c) drowsy, (d) quiet alert, (e) active alert, and (f) crying. Each state presumably reflects a particular mode of CNS activity. The emergence and stabilization of the quiet alert state—during the transition from sleep to crying and back to sleep—is a critical developmental milestone in the newborn. The newborn spends little time in the quiet alert state, but by the time he or she is 2 to 3 weeks of age, periods of quiet alertness have emerged and can be reliably maintained. Spending time in this state is critical for taking in cognitive and socioemotional information from the environment. In fact, individual differences in state organization in early infancy appear to predict later outcome (DiPietro & Porges, 1991). For example, Thoman (1990) found that infants with better state organization scored more positively on the Bayley Motor Scale. Hence, regulation
of state may serve as an index of the capacity to remain organized in a manner crucial to adaptive functioning (Fajardo, Browning, Fisher, & Paton, 1992).

Neurobehavioral State in At-Risk Infants

Infants who experienced prenatal perturbations, including exposure to substances of abuse, often appear to have difficulty maintaining a quiet alert, attentive state, usually fluctuating between crying and distress and a glassy-eyed, drowsy state (Chasnoff, Griffith, MacGregor, Dirkes, & Burns, 1989; Streissguth, Barr, & Martin, 1983). Because the quiet alert, attentive state is so critical for information processing, memory, and learning (Posner & Rothbart, 1980), such infants are at risk for altered developmental trajectories. Als (1991), for example, studied the behavior of preterm and full-term infants and reported that preterm infants showed poorer state and attention regulation, even in the absence of overt brain injury. Similarly, Streissguth et al. (1983) linked alcohol use during pregnancy to a low level of arousal in the newborn, with infants exposed to alcohol prenatally alternating between awake and drowsy states rather than between awake and crying. In addition, infants exposed to cocaine in utero exhibited impaired state and arousal regulation in response to novel stimulation (Chasnoff et al., 1989; see Stallings-Sables, 1993, for a review). However, others have found that the effects were not specific to prenatal alcohol exposure; after controlling for marijuana and tobacco use, there was no relationship between state organization and alcohol use (Richardson, Day, & Taylor, 1989).

Establishing a causal relationship between prenatal risk factors and poor infant outcomes can be difficult because a number of confounding variables can covary with prenatal events (see Zuckerman, 1991, for a review). For example, nutrition, maternal weight gain, obstetric medication, smoking, substance abuse, and socioeconomic status vary across women. In addition, all of these variables are known to covary with psychological stress, and each may contribute to poor fetal development (Brooke, Anderson, Bland, Peacock, & Stewart, 1989). To discern the relationship between prenatal risk factors and poor infant outcome, some researchers have turned to animal models, which afford the opportunity to systematically manipulate the variable of interest under more controlled conditions. Results can then be compared with those of control infants whose mothers were maintained under identical conditions in terms of nutrition, housing, and other factors (see Suomi & Higley, 1991, for a review).

Nonhuman Primate Model of Prenatal Stress

In our laboratory at the University of Wisconsin-Madison, we have developed a nonhuman primate model specifically aimed at addressing critical questions for occu-
signed to experimental and saline control groups and administered intramuscular injections of either ACTH (Acthar gel, 6 units) or saline (.25 ml) for 14 consecutive days during mid-pregnancy—days 120 to 134 postconception (the gestation period for rhesus monkeys is 165–170 days)—at either 0900 or 1600 hr. The injection involved briefly removing the animal to a nearby restraint cage. This mild handling disturbance was controlled for by the saline condition. The ACTH dose was selected on the basis of a pilot project indicating that 6 units of Acthar gel would increase cortisol for at least 4 hr but allow for a return to basal levels by the next morning (base = 23.2 μg%; 4 hr = 58.5 μg%; 24 hr = 27.1 μg%).

Postnatal infant evaluations. Infants were separated from their mothers for assessments on days 2, 15, and 30 (+1) postpartum by experimenters blind to the experimental conditions. On day 2, a blood sample was collected for evaluation of immune functions (Coe, Lubach, Karaszewski, & Ershler, 1996). On days 15 and 30, each infant was transported to a nearby testing room, and a 1-min videotape of the infant’s face and upper torso was made for later evaluation of its behavioral state. This test was followed by a 20-min assessment of neuromotor, interactive, and temperament characteristics and capabilities (IBAS: Schneider & Suomi, 1992). Then a 4-min videotape was made for evaluation of infant state. Day 15 was chosen for the evaluation because our previous work had indicated that this age is optimal for assessing a stable infant response (Schneider & Suomi, 1992).

Videotape session. All infants were filmed for approximately 5 min. The sequence consisted of five 1-min episodes that provided repeated observations of infants’ behavior after maternal separation, after a 20-min (IBAS) neurobehavioral assessment, during and after a postural challenge (inversion), and after administration of a sucrose solution. The inverted position was chosen to assess whether the treated infants would differ from controls in response to a postural challenge. In the inverted position, the static vestibular system can induce a physiologic response through the parasympathetic nervous system and reduce blood pressure, decrease muscle tone, and promote a generalized relaxation response (Farber, 1982). The sucrose administration was used to assess the quieting effects of intraoral sucrose on behavioral state (Bliss & Ciaramitaro, 1994).

For the film episodes, the infant was held close to the handler’s body at waist level. The first episode, referred to as postseparation, began within 2 min after the maternal separation. The infant was wrapped in a cloth diaper from the waist down, leaving the arms free to move, and held close to the handler’s body at waist level. The infant was filmed from a distance of 6 ft. Next, a 20-min neuropsychiatric test (IBAS) was administered to the infant. Immediately after the assessment, the infant was again wrapped in a diaper, and the remaining film episodes were completed without interruption. For the inversion episode, the infant was slowly lowered to an inverted position, facing head down toward the camera. After 1 min of filming, the infant was slowly returned to the upright position and filmed for 1 min (postinversion). Finally, .2 ml of a 24% sucrose solution was administered to the infant orally, referred to as postsucrose, and the infant was filmed for 1 min. The film episodes were referred to as follows:

1. Postseparation: the period immediately following maternal separation
2. Postneonatal: the period immediately following the 20-min administration of the IBAS
3. Inversion: the period during which the infant was turned upside down and maintained in that position for 1 min
4. Postinversion: the period following return to an upright position
5. Postsucrose: the period following oral administration of a few drops of 24% sucrose solution

Data Analysis

Frequency and duration of four observable behavior states (drowsy, quiet alert, active alert, distress) were scored from the videotapes with a laptop computer. Definitions of state were based on the work of Fragazy (1989) and Wolff (1987) (see Table 1). Percentage of time in state was used as the dependent measure. Interrater reliability was determined by two observers independently scoring the tapes of 10 infants and correlating the two sets of scores. Raters were trained to reliability, rs > .90. Mixed analyses of variance were used to investigate whether there were any effects due to prenatal treatment (ACTH treatment vs. saline treatment), gender (male vs. female), day of testing (day 15 vs. day 30), and episode (postseparation, postneonatal, inversion, postinversion, postsucrose) as repeated measures. A Fisher least significant difference test was used to conduct post-hoc pairwise comparisons, and the Greenhouse Geisser correction was used for repeated measures analyses (Keppel, 1991).

Results

Active Alert

Figure 1a depicts a significant interaction between episodes and prenatal treatment, indicating that ACTH-treated offspring remained in an active alert state for less time than did saline-treated infants after maternal separation and during the episode involving a postural challenge (inversion), F(4, 64) = 3.34, p < .03. No other differences were significant, including day of testing and gender.
The infants from both groups tended to spend less time in the drowsy state during inversion, \( F(4, 64) = 2.86, p = .056 \). There were no significant main or interactive effects for day of testing or gender (see Figure 1b).

**Quiet Alert**

Female infants remained in the quiet alert state less than did males on day 15, whereas males and females were comparable on day 30, \( F(1, 16) = 6.66, p = .02 \). No other differences were significant, including prenatal treatment or episodes (see Figure 1c).

**Distress**

Female infants remained in a distressed state more than males on day 15, whereas males and females were comparable on day 30, \( F(1, 16) = 10.61, p = .005 \). Overall, the proportion of time in a distressed state increased across episodes and decreased after the administration of the sucrose solution, \( F(4, 64) = 3.23, p = .04 \). There were no main or interactive effects of prenatal treatment (see Figure 1d).

**Discussion**

This study supports the hypothesis that infants have altered neurobehavioral state regulation if their mothers experienced endocrine system activation during pregnancy. Specifically, we found that ACTH, which increased the mother's endocrine activity, resulted in infants that remained in a drowsy state significantly more than did saline-treated controls. This finding of increased drowsiness was most pronounced during the postseparation period, when the acute stress resulting from the brief removal from the mother is greatest for the infant. In contrast, saline-treated control infants remained in a more active alert state, presumably searching for their mothers, a species-typical response to maternal separation.

The finding that infants from ACTH-treated pregnancies remained in a drowsy state more than did controls was consistent with human studies reporting poor state regulation in infants exposed to cocaine or alcohol in utero (Chasnoff, Burns, & Burns, 1987; Chasnoff et al., 1989; Corwin et al., 1992; Streissguth et al., 1983). These findings are also consistent with the fact that infant monkeys exposed to moderate levels of alcohol during pregnancy also show increased levels of drowsiness (Schneider, Rowghton, & Lubach, in press). Because alcohol, cocaine, and stress during pregnancy activate the maternal endocrine system, the data support the hypothesis that maternal endocrine activation is an important mechanism underlying the development of poor state regulation in fetal cocaine-exposed or alcohol-exposed offspring.

It is likely that the behavioral state alterations originated in the fetal period (see Arduini, Rizzo, & Romanini, 1995, for a review). In the human fetus, behavioral states are established by 36 weeks of gestation (Arduini et al., 1985). Behavioral states are reportedly disturbed in fetuses of diabetic mothers (Mulder, Visser, Bekedam, & Prechtl, 1987) as well as in fetuses with uteroplacental insufficiency (Van Vliet, Martin, Nijhuis, & Prechtl, 1985). Additionally, differences in fetal behavioral states distinguish hydrocephalic fetuses with a good prognosis from those with compromised outcomes (Arduini, Rizzo, Caforio, Romanini, & Mancuso, 1987). In the future, it may be possible to identify fetuses with altered behavioral states resulting from at-risk pregnancies in order to facilitate early implementation of occupational therapy services.

We also found that the proportion of time that the infant remained in a distressed state increased across episodes and decreased after the sucrose administration. The increase in distressed state across episodes was not surprising, given that the infants had been separated from their mothers for testing and were being handled by humans. However, the decrease in the proportion of time spent in a distressed state after the sucrose administration reflects a different process. It has been suggested that the reduction in distress after sucrose stimulation reflects an opioid hormone response, which induces a calming effect (see Blass & Ciaramitaro, 1994, for a review). In addition, distress reduction could be a consequence of the sucking response induced by the sucrose, which also stimulates calming gastrointestinal physiology (Uvnas-Moberg, 1989). Mammalian mothers routinely take advantage of this response to calm their agitated infants through nursing. Further, the sudden administration of the sweet-tasting solution could serve as a distraction from the distress of maternal

### Table 1

**Operational Definitions for State Measures**

<table>
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<tr>
<th>State Measure</th>
<th>Definition</th>
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<tr>
<td>Distress</td>
<td>Distress vocalizations; phasic motor activity; spasms; gagging; whining</td>
</tr>
<tr>
<td>Active alert</td>
<td>Continuous activity without signs of distress; movements may be phasic; eyes open, searching</td>
</tr>
<tr>
<td>Quiet alert</td>
<td>Absence of activity or distress; &quot;bright, shiny&quot; eyes scanning, focused; respirations constant; postural adjustments</td>
</tr>
<tr>
<td>Drowsy</td>
<td>Eyes half closed or opening and closing; state terminated when eyes focus or scan; slow postural adjustments</td>
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**Drowsy**

ACTH-treated infants remained in a drowsy state (\( M = 12.8\%, SD = 1.5\% \)) significantly more than did saline-treated infants (\( M = 6.2\%, SD = 1.1\% \)), \( F(1, 16) = 5.13, p = .056 \). There was also a marginally significant interaction in that ACTH-treated infants tended to spend more time in drowsy state than did saline-treated infants during the postseparation period, \( F(4, 64) = 2.62, p = .07 \).

The infants from both groups tended to spend less time in a drowsy state during inversion, \( F(4, 64) = 2.86, p = .056 \). There were no significant main or interactive effects for day of testing or gender (see Figure 1b).
Figure 1. Mean proportional time in neonatal state for infants from two pregnancy conditions (saline, n = 10; adrenocorticotropic hormone [ACTH], n = 12) across episodes (postseparation [post-sep], postneonatal [post-neo], inversion, postinversion [post-invers], postsucrose). Bars represent standard error.

separation. Because administration of the sucrose solution was not controlled by the administration of a nonsucrose solution, further studies are needed to resolve which of the three explanations is most plausible.

Although these results concur conclusions from research on human infants, it is also important to acknowledge that there are many differences, both developmentally and in the testing paradigm. Animal studies provide a useful model for studying phenomena in a simpler system under more controlled conditions; however, data generated from such studies need to be interpreted cautiously. In this particular study with monkeys, the findings highlight the potential importance of the drowsy-alert continuum as a predictor of infant well-being.

Increased drowsiness or low arousal may have important implications for subsequent social and cognitive development. A number of prenatal perturbations and toxicants may affect the fetus to alter the neural substrate underlying this neurobehavioral state. The present study suggests that the low arousal and increased drowsiness associated with in utero exposure to alcohol and other prenatal risk conditions may be due, in part, to maternal endocrine activation. Poor state regulation, or increased drowsiness, can have far-reaching effects because time spent in an optimal state is considered critical for sensory processing and fundamental for a positive developmental outcome (Prechtl & O’Brien, 1982; Thoman, 1990; Wolff, 1987).

Implications for Occupational Therapists
This study has important implications for occupational therapists. It provides direct evidence that infants from
mothers exposed to prenatal perturbations that activate the maternal endocrine system demonstrated increased drowsiness and that the increased drowsiness may be particularly salient under conditions of stress or challenge. Adequate state regulation allows the infant to engage with the parent in a system of reciprocity (Macyob, 1992) in which both are responsive to the other. This mutual reciprocal orientation serves as a foundation for a number of positive outcomes, launching the dyad into a positive developmental trajectory and paving the way for the occupational development of the young child.

In addition, this study supports the model proposed by Als and her colleagues, suggesting that if an infant is not functioning at the optimal level of CNS maturity, signs of neurobehavioral disorganization, such as shifting to a lower behavioral state (drowsiness or light sleep), may be demonstrated. Occupational therapists can assist infants with neurobehavioral instability, manifested by increased drowsiness, by modulating the environment to prevent sensory overload, positioning the infant, facilitating hand-to-mouth activities, encouraging clustering caregiving activities, and encouraging family members and caregivers to recognize infant cues and signals of disorganization and to respond in ways to help the infant become better organized (see Hunter, 1996; Vergara, 1993).

**Directions for Future Research**

Future studies are under way to investigate the effects of maternal endocrine activation on the monkeys' subsequent occupational performance. These studies involve repeated observations of monkeys from ACTH-exposed mothers and controls engaging in social interactions, performing cognitive tasks, and developing playful behavioral competencies. Finally, it is important to note that the nonhuman primate model, which provides the opportunity to systematically manipulate factors that potentially contribute to occupational behavior, provides an excellent methodology for advancing knowledge relevant to the study of early contributions to primate patterns of occupation.

**Acknowledgments**

Elizabeth Charlotte Rougham died on November 18, 1997. Beth was an extraordinary woman. Her dedication to occupational therapy and the rare spirit she brought to children and families will forever be a shining example to all of us who knew her and worked with her.

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