Order Number 9130346

Effects of stress on cortisol in preterm infants

Lutkus, Catherine Ann, Ph.D.

City University of New York, 1991

U·M·I

300 N. Zeeb Rd.
Ann Arbor, MI 48106

EFFECTS OF STRESS ON CORTISOL IN PRETERM INFANTS

by

Catherine Ann Lutkus

A dissertation submitted to the Graduate Faculty in Psychology in partial fulfillment of the requirements for the degree of Doctor of Philosophy, The City University of New York

1991

This manuscript has been read and accepted for the Graduate Faculty in Psychology in satisfaction of the dissertation requirement for the degree of Doctor of Philosophy.

4-8-91
Date

Chair of Examining Committee

April 8, 1991
Date

Executive Officer

Judith M. Gardner, Ph.D.

Sam J. Korn, Ph.D.

Victoria Luine, Ph.D.

Peter M. Vietze, Ph.D.

Supervisory Committee

The City University of New York

Abstract

EFFECTS OF STRESS ON CORTISOL IN PRETERM INFANTS

by

Catherine Ann Lutkus

Adviser: Professor Gerald Turkewitz

Although exposure to stress has been shown to affect cortisol regulation in adults and animals, the ability of the premature infant to maintain adaptive cortisol regulation after exposure to such stress on the NICU has not been studied. To assess effects on cortisol, salivary cortisol levels were measured in relatively healthy NICU premature infants (n = 38). Cortisol levels were measured under three conditions: Basal - 120 minutes after no disrupting event or behavioral distress; Stressor - 30 minutes after a heel prick procedure; Neurobehavioral (NB) - 30 minutes after a NB examination. Basal and stressor samples were collected weekly during the hospital stay and NB samples were collected once at discharge. In addition, as cortisol regulation could also affect the infants' attention regulation, infants were tested in a visual preference paradigm at discharge under three conditions: Less Aroused, Internally Aroused and Externally Aroused.

During the infants' stay, mean basal and stressor cortisol levels were found to decline to those tentatively reported in healthy full-term infants. In addition, the mean NB cortisol levels were found to be between mean basal and stressor cortisol levels at discharge with all conditions significantly different from each other. In the looking preference paradigm, looking preferences were significantly different as a function of arousal condition. The looking preferences in the internally and externally aroused conditions were correlated with basal cortisol levels. They were also correlated with stressor cortisol levels in the externally aroused condition. To the generalizability of the findings, an additional group of cocaine-exposed premature infants (n = 11) were tested. They too were found to have significantly lower mean NB and stressor cortisol levels as well as a difference in visual preferences in the internally and externally aroused conditions.

Overall, the data suggest that healthy premature infants are able to maintain adaptive cortisol regulation despite exposure to stress on an NICU. In addition, a relationship between cortisol and attentional regulation was established which could help understand other groups of infants.

Acknowledgements

First, I would like especially to thank my adviser Dr. Gerald Turkewitz for his advice and support and the freedom he gave me to pursue the work presented here. Second, I am completely indebted to Drs. Judith Gardner and Bernard Karmel for without their unwavering guidance, encouragement, and support this thesis simply would not have been possible. They were true friends and excellent mentors, and their daily companionship will be sorely missed. Third, special thanks also go to Dr. Edward Diamond and his staff at Mt. Sinai Medical Center for their helpfulness, patience, and instruction. Finally, I would also like to thank Dr. Luine for her advice and guidance as well as Dr. Vietze and Dr. Korn for their thoughtful comments.

I also deeply appreciate the help in data collection and support given particularly by Bonny Volk as well as the NICU medical staff at the St. Vincent Medical Center. The encouragement and advice from Dr. David Lewkowicz was also greatly appreciated as was the friendship and support given by Marcia Dalbeye, Ronnie Geva, Nancy McKeever, Dorene Miya, and Elise Murochick.

Last, but not least, I am forever thankful and grateful for the great unconditional love, patience, understanding, and encouragement given to me throughout this project by my avowedly greatest fans, my husband, Joe, and son, Alex.

Table of Contents

List of Tables

List of Figures

Introduction

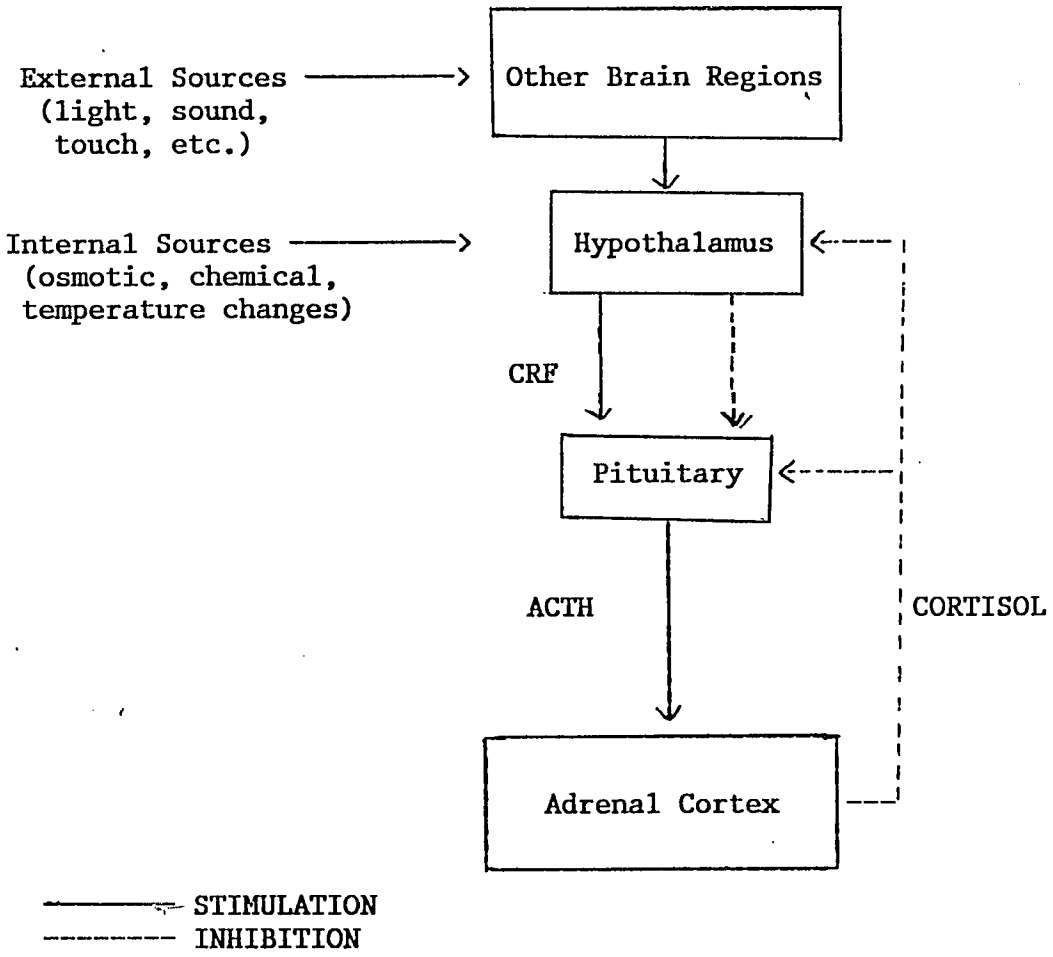
The ability to adapt successfully under different levels of stress is important not only for physical well-being but also for maintaining appropriate arousal levels needed to attend to relevant environmental events. The release of cortisol from the adrenal cortex contributes to successful adaptation under different levels of stress by sustaining the higher energy levels needed for awake behavior and preventing the over-reaction of other stress sensitive systems (Gunnar, 1986). Premature infants in a neonatal intensive care unit (NICU) are often exposed to seemingly high and prolonged periods of stress due to medical and environmental conditions. Moreover, because of the state of their development, premature infants may be even less prepared than full-term infants to deal with such an environment. As noted by Selye (1950), prolonged periods of stress have been shown to affect adaptive cortisol regulation by the adrenocortical system in both animal and human adults. The ability of the developing adrenocortical system to maintain adaptive cortisol regulation after exposure to prolonged stress during a period of such prematurity has not yet been reported in human infants.

In order to assess the effects of prolonged stress on the developing adrenocortical system, cortisol levels were measured under different levels of arousal and stress during the premature infants' NICU stay . Cortisol levels also were related to the infants' attentional responses under different arousal conditions to assess the relationship between cortisol regulation and the infants' ability to maintain arousal levels

needed to attend to relevant environmental events. These measures allowed for studying the immediate and longer term effects of prolonged exposure to stress on cortisol regulation in premature infants as well as on attentional responses.

Physiology of the Adrenocortical System

Production and release of cortisol occurs in the adrenal glands, which are located on top of the kidneys. The adrenal gland consists of an outer section, the adrenal cortex, and an inner section, the adrenal medulla (Gorbman, Dickhoff, Vigna, Clark, & Ralph, 1983). It is the adrenal cortex in the fasciculata and reticularis zones where glucocorticoids are produced of which the predominant one is cortisol. Regulation of cortisol release is controlled by a negative feedback system involving the hypothalamus and pituitary (see Figure 1) (Gorbman et al., 1983; Gunnar, 1986). For basal (resting) cortisol level, the neurosecreting cells in the hypothalamus secrete corticotrophin releasing factor (CRF) into the capillaries of a vascular portal system that carries CRF to the anterior pituitary. In the anterior pituitary, CRF stimulates the release of adrenocorticotrophic hormone (ACTH) into the general circulation where it activates the adrenal cortex cells to secrete cortisol. When the cortisol level reaches a certain concentration in the blood, an inhibition of CRF production occurs in the hypothalamus, which, in turn, decreases ACTH release resulting in a decrease of cortisol release.


External Sources
(light, sound,
touch, etc.)
Other Brain Regions
Internal Sources
(osmotic, chemical,
temperature changes)
Hypothalamus
CRF
Pituitary
ACTH
CORTISOL
Adrenal Cortex
STIMULATION
INHIBITION

In response to a stressful event, the hypothalamus is stimulated from other brain regions and increases CRF production which then decreases the inhibitory effect of cortisol on CRF production. This breaks the negative feedback loop resulting in prolonged, elevated cortisol levels. It is believed that a separate hypothalamic pathway from the one that regulates basal cortisol levels is activated to produce the elevated cortisol levels seen during stress. Since two separate pathways are involved in the regulation of cortisol level, it is possible to detect elevations in cortisol to a specific stressful event against the circadian changes in basal cortisol levels (Gunnar, 1986).

Prenatal and Neonatal Adrenocortical Responsiveness.

The morphology of the adrenals in the fetus and newborn is different from that in adults in both its size in relation to body size and its cell structure (Gorbman et al., 1983). The absolute size of the newborn's adrenal gland is equivalent to that of an 18 year old due to an additional region called the fetal zone. The fetal zone produces the steroids that are secreted into the placenta and converted by the mother into estriol, which is necessary for maintaining pregnancy. This region grows rapidly during the second and third trimester and secretes more steroids with growth. Since the steroids from the fetal zone and adrenal cortex are dependent on the same precursors for release from the adrenals, increases in the release of steroids from the fetal zone are likely to be accompanied by an increase in cortisol release from the adrenal cortex (Gorbman et al., 1983).

A cortisol surge during the end of pregnancy has been observed in almost all mammals. Although it provides a hormonal signal to end preg-

nancy in many mammals (Liggins, 1976), it apparently does not do so in humans (Sybulski, Goldsmith, Maughan, 1975). Cortisol is believed to induce many of the "finishing touches" in various organs in humans as it does in other mammals. The most studied cortisol effect is the stimulation of both the synthesis of surfactant and its release into the alveolar cells in the lungs as well as the inducement of cytodifferentiation of the alveolar cells (Liggins & Howie, 1972). In addition to the lungs, maturational processes in the liver, small intestine, and possibly the brain are also induced by cortisol (Liggins, 1976).

Unlike in adults, basal (resting) cortisol levels have not been found to vary in a circadian manner in newborns (Zurbrugg, 1976; Francis et al., 1987). This is consistent with the lack of newborn circadian rhythms observed in other systems such as the sleep/wakefulness cycle (Anders, 1978). Some observers have noted that the newborn appears to have two cortisol peaks per day. The time of the peaks, however, seems to be unrelated to diurnal, feeding or arousal patterns and varies randomly from infant to infant (Zurbrugg, 1976; Francis et al., 1987). It has been suggested that these peaks in cortisol release are possible precursors to the later marked circadian rhythm seen in older children and adults (Zurbrugg, 1976). The emergence of a circadian rhythm for cortisol production has been reported at different times but the most recent studies suggest that it appears around 3 months of age (Price, Close, & Fielding, 1983) and is firmly established by 6 months of age (Lewis & Thomas, 1990; Onishi et al., 1983).

A cortisol response to stress has been seen in infants as young as 26 weeks gestation by comparing the levels of cortisol of infants born

vaginally and via caesarean delivery (Murphy, 1982). Accounting for the anesthesia used, infants born by caesarean section (presumed to have been generally less stressed at birth) have lower cortisol levels than do vaginally delivered infants (Procianoy, Cecin, & Pinnheiro, 1983). Also, cortisol levels have been found to increase with degree of fetal distress shown by the infants during birth (Lagercrantz & Bistoletti, 1973; Sybulski et al., 1975). These cortisol responses have been shown to be the result of, and not the cause of, the delivery as spontaneous vaginally delivered infants have the same pattern of responses as do infants whose vaginal delivery was induced by administration of exogenous hormones (Lagercrantz & Bistoletti, 1973; Sybulski et al., 1975). Thus, the infant's release of cortisol that is related to degree of stress exposed to during birth, indicates that the infant is born with an adrenocortical system that is sensitive to stress intensity.

Studies with rat pups have supported the finding that the cortisol response to stress is present early in development. Two-day-old rat pups show cortisol responses to such stressors as electric shock and heat (Smotherman, 1983). In addition, both quantitative and qualitative developmental changes in the response have been found to occur in the rat pup (Schoenfeld, Leathem, & Rabii, 1980). Five- to 11-day-old rat pups show a decrease in both basal cortisol level and response to stress that corresponds to a refractory period in adrenal growth. The cortisol level and response has been shown to then increase until day 20. Qualitatively, the degree of cortisol response to different types of stressors may change with development. For example, from 1 to 5 days,

the cortisol response to a heat stressor is much greater than the response to electric shock. However, by 7 to 21 days, the cortisol response is the same for heat and electric shock. Thereby, the authors suggest that the maturation of the system may not be all or none but could involve several neural pathways which develop at different rates (Schoenfeld et al., 1980). Yet, it is just as likely that the effect could be due to differences in the rate of development of the heat and shock receptors as opposed to the adrenocortical system.

In full-term newborn infants, a cortisol response to stress has been found for different types of stressors by measuring the amount of cortisol found in the blood before and after the stressor. A measurable increase in the amount of cortisol in the general circulation occurs about 5 minutes after the event (Gunnar, Fisch, & Malone, 1985). The time course of this response has not yet been studied in individual infants but a group curve derived from healthy full-term infants has been established (Gunnar, Malone, Vance, & Fisch, 1985). As measured in blood, cortisol levels peak in the infant approximately 30 to 90 minutes following stress and then decline with the sharpest drop between 90 and 120 minutes after the stressor. Baseline levels seem to be reestablished by 150 minutes in most infants.

There may also be some behavioral mechanisms associated with stress that affect the regulation of cortisol release to stress. For example, quiet sleep (non-REM) has been found to be associated with the clearance of cortisol from the system following stress in infants. As noted by Emde, Harmon, Metcalf, Koenig, & Wagonfeld (1971), a stressful

event such as circumcision tends to be followed by increased quiet sleep. This is the behavioral state that is associated with the lowest level of cortisol (Anders, Sacher, Kream, Roffwarg, & Hellman, 1970). A study by Gunnar et al. (1985) has shown that in a group of healthy full-term males, the greatest decline in cortisol level corresponds with the period of the most quiet sleep, 90 to 120 minutes after circumcision. Since the body produces the lowest amount of cortisol during quiet sleep, the low production level during this state may be an aid in rapid clearance of the excess cortisol left from a stressful event, thus allowing a rapid and efficient return to basal cortisol levels following stress.

In animal studies, another factor that has been found to be involved in the regulation of cortisol in infants is tactile stimulation. In rat pups, tactile stimulation from the mother has been found to decrease the level of cortisol in the pups after exposure to a stressor such as electric shock (Smotherman, 1983; Stanton, Wallstrom, & Levine, 1987). A similar effect of tactile stimulation on cortisol level regulation has been noted in infant squirrel monkeys. During a separation-reunion paradigm, tactile contact with the mother during reunion seems to reduce or buffer the usual elevated cortisol response of both the infant and the mother to being separated (Mendoza, Smotherman, Miner, Kaplan, & Levine, 1978).

While regulation of cortisol by tactile stimulation has not been studied in human infants, growth hormone (which like cortisol has been shown to be regulated in rat pups by tactile stimulation (Schanberg &

Field, 1987)) has been studied in preterm infants. Supplemental stimulation provided to preterm infants in the form of gentle stroking has been shown to be associated with increases in growth and weight gain compared to infants who did not receive such stroking (Schanberg & Fields, 1987). The authors suggested that the growth and weight gain could be due to increases in growth hormone stimulated by the stroking. Based on possible regulation of growth hormone through tactile stimulation in human infants and rat pups, it is possible that other similar hormonal systems are regulated by tactile stimulation in human infants.

Besides physical stressors, novelty has been shown to affect the adrenocortical system in adult humans and rats (Hennessy & Levine, 1979; Mason, 1968). Some evidence for the effect of novelty on cortisol responses in newborn infants may be seen in a recent study on repeated discharge exams in healthy full-term infants (Gunnar, Connors, & Isensee, 1989). The infants were given a discharge exam on two consecutive days prior to hospital discharge and only showed a significant cortisol response to the exam on the first day suggesting rapid habituation of the cortisol response to the exam. The effect of novelty also has been seen in older 6- to 12- month old infants in their cortisol responses to swim classes (Gunnar, Hertsgaard, & Lehman, 1989).

While not reported in newborn infants, events that are only "psychologically" stressing also could stimulate cortisol release since they do in adults. In particular, cortisol seems to be released in adults when the emotions of fear, anxiety, and distress are expressed (Mason, 1968). However, studies of maternal separation, which elicit

these emotions, have not shown a clear cortisol response to this event in 9- to 12- month old human infants (Hertsgaard, Wanner, Jodl, & Mason, 1990; Gunnar, Mangelsdorf, Larson & Hertsgaard, 1989; Tennes, Downey, & Vernadakis, 1977). Large cortisol responses have been found to maternal separation in infant animals (Coe & Levine, 1981). Thus, in the neonatal period, it is not yet clear whether the adrenocortical system responds to events that are only "psychologically" but not physically stressful.

Association between Cortisol and Arousal Levels

In general, the level of cortisol has been found to increase as the infant changes from a less aroused to a more aroused state (Anders et al., 1970; Tennes & Carter, 1973; Gunnar, Fisch, & Malone, 1984). In an initial study on the relationship between cortisol and state, Anders et al. (1970) observed that for four healthy newborns cortisol levels in the blood were slightly but consistently higher during awake than during sleep states. In addition, a marked increase in cortisol was associated with the highest arousal state, crying, differentiating it from the other states. Similar findings were reported by Tennes & Carter (1973) and by Gunnar et al. (1985) with larger samples of newborns. It also has been found that 12-month-old infants continue to show the same relationship between cortisol and state (Tennes & Vernadakis, 1977). This association between cortisol and arousal levels has led to the suggestion that the metabolic actions of cortisol are involved in sustaining the higher level of energy needed for wakeful activity (Gunnar, 1986).

As discussed earlier, cortisol levels increase in infants in response to stressful events. In addition, infants show behavioral dis-

tress (an increased arousal level) by crying and increased movement in response to stressful events. It would be expected that the more distress an infant showed, the more cortisol he/she would release and vice versa, suggesting either a common underlying mechanism for both cortisol and arousal level, or a causal connection between the two. However, dissociations between the amount of cortisol and behavioral distress have been seen in several situations in both human and animal infant studies calling into question whether a simple relationship between cortisol and stress exists (see below).

In situations involving major stressors, such as circumcision, blood sampling, or birth, an increase in cortisol level is associated with an increase in behavioral distress (Gunnar, Fisch, Korvsvik, & Donhowe, 1981; Gunnar, Connors, Isensee, & Wall, 1988). However, while the amount of cortisol released seems to be related to the amount of stress the infant is exposed to (Gunnar et al., 1985), the magnitude of the cortisol response does not predict the amount of behavioral distress shown by the infant (Gunnar et al., 1984). That is, the infants who have the highest cortisol responses to a stressor are not necessarily the infants who demonstrate the most behavioral distress to the stressor. In addition, if an infant is calmed by being given a pacifier during a stressful procedure, a decrease in behavioral distress is not accompanied by a decrease in cortisol level (Gunnar et al., 1981). This suggests that the mechanism stimulated by nonnutritive sucking, which leads to behavioral calming, does not affect the release of cortisol under stress. This may be because nonnutritive sucking may primarily

stimulate the autonomic nervous system (Ignotoff & Field, 1979), which controls the release of catecholamines during stress, but may not necessarily stimulate the adrenocortical system.

Milder stressors such as a discharge exam, physical restraint, or a bath show even clearer dissociations between cortisol levels and arousal. First, the responses to these stimuli are highly variable in magnitude (Gunnar et al., 1988). For example, some infants show cortisol responses during a discharge exam that are as high as those measured during circumcision although they show much less behavioral distress, while other infants show little or no increase in cortisol during the exam (Gunnar et al., 1988). Also, providing infants with a pacifier, which behaviorally calms them, does not produce a corresponding decrease in cortisol level (Gunnar et al., 1984). Second, rather than being associated with behavioral distress, high cortisol levels are associated with more optimal performance in some infants during a discharge exam and Brazelton exam especially on self-quieting and state regulation items (Gunnar, Isensee, & Fust, 1987). Extremely healthy infants who had high cortisol levels demonstrated more organized behavior on the Brazelton exam while slightly less healthy infants who had high cortisol levels showed more behavioral distress. Therefore, although cortisol level can be associated with arousal level in the newborn, the two can not be used interchangeably for inferring how much stress the infant is experiencing. Thus, the association between cortisol and arousal level differs depending on the characteristics of the stimulus, the situation, and the infant.

It should be noted that studies with infant rhesus macaques and squirrel monkeys also have found similar relationships between cortisol and arousal levels. Usually when an infant monkey is separated from his mother in the absence of peers, both the infant and the mother show raised cortisol and behavioral distress levels (Coe & Levine, 1981). Over a period of time, the infant monkey will decrease his behavioral distress although cortisol levels will remain high if he is not in sensory contact with his mother (Coe, Wiener, Rosenburg, & Levine, 1985). However, if the infant can perceive the mother through either hearing or smell, the cortisol level decreases but the behavioral distress remains high (Coe et al., 1985). Therefore in monkeys, as with human infants, a one-to-one relationship between cortisol and arousal level does not always exist but rather, the association between cortisol and arousal level also appears to depend on the characteristics of the stimulus and situation.

Effects of Stress on Cortisol Responses

Animals exposed to long term chronic stress have been found to develop pathological changes in their adrenal cortex (Selye, 1950) indicating permanent changes in their cortisol release. Studies with rat pups also have shown that exposure to both mild (handling) and intense (electric shock) stressors can result in relatively permanent changes in their cortisol responses to later stressors compared to rat pups with no prior exposure to such stressors (Dienstbier, 1989; Levine, 1962; Levine & Mullins, 1968). As adults, the exposed rats showed lower cortisol responses to chronic stress situations and to mild acute stressors and

higher cortisol responses to more intense acute stressors compared to control rats (Levine, 1962; Levine & Mullins, 1968). While it is not clear for either full-term or premature infants what the effects of the stress from being on a NICU are on cortisol responses, a study on ill postterm infants has suggested an underresponsive adrenocortical system (Nwosu, Wallach, Boggs, & Bongivanni, 1975).

Investigation of the Relationship between Stress and Cortisol Levels in Present Study

In the present study, the general level of stress experienced by an infant in the NICU was estimated by the overall medical status of the infant. It was assumed that sicker infants would receive more frequent and painful medical procedures and handling compared to less ill infants. Such a measurement of general stress level is likely to provide only a very rough estimate of the actual stress experienced by any one infant. For instance, some infants who are only suspect or at risk for an illness may undergo painful diagnostic and screening tests but may not be judged as very sick and so, would receive a low score for stress level. Even more importantly, the degree of stress experienced for equivalent procedures could vary depending on the degree of illness of the infant and the skill of the caregiver. In addition, it is possible that other characteristics of the NICU environment may be stressful to the infant such as the noise and light levels (Lawson, Daum, & Turkewitz, 1977; Holmes et al., 1982).

In order to investigate the effects of stress on cortisol responses during the infant's stay on the NICU, both basal cortisol

levels and cortisol responses to a specific physically invasive stimulus were monitored throughout the infant's stay. In addition, cortisol responses to a less stressful noninvasive stressor were measured prior to discharge from the NICU. The basal cortisol level provided a measure of the overall effect on cortisol production levels while the cortisol responses to a specific stimulus examined how well the infant was able to respond and cope with a stressful event. It was expected that basal cortisol levels and responses to stress would change as the infants' medical status changed. A decrease in basal cortisol levels was expected as general stress level decreased. For the responses to specific stressors, it was expected that the higher and longer the level of stress the infant experienced, the greater an effect it would have on the infants' ability to cope adaptively to stressful events. However, the effect on cortisol responses could be expected in either direction with higher cortisol responses due to a greater responsiveness to stress or lower cortisol responses due to loss of adaptive cortisol responding, more graded cortisol responding, or habituation of the cortisol response to such stressful events (Dienstbier, 1989; Gunnar et al., 1989; Levine & Mullins, 1968; Selye, 1950).

Relationship between Arousal Levels and Attention.

In the neonatal period, infants appear to attend to stimuli based on the amount of overall stimulation rather than specific properties of the stimuli (Gardner & Karmel, 1984; Gardner, Lewkowicz, Rose, & Karmel, 1986; Gardner & Turkewitz, 1982; McGuire & Turkewitz, 1979; Turkewitz, Gardner, & Lewkowicz, 1984; Turkewitz, Lewkowicz, & Gardner, 1983). The

overall amount of stimulation seems to be determined by both internal, e.g. arousal level, and external stimulation. The combination of these sources of stimulation leads the infant to orient towards stimuli that provide an optimal or preferred level of stimulation for the infant (Gardner & Karmel, 1983; Gardner & Turkewitz, 1982). Thus, depending on the amount of stimulation from internal and external sources, the infant will shift his attention to different stimuli as opposed to preferring the same specific stimulus under all levels of arousal, thereby integrating his internal arousal level with external stimulation.

Either of these sources of stimulation can be systematically manipulated resulting in a corresponding change in the infant's attention. For example, in a visual preference paradigm, infants whose arousal level is manipulated by feeding and swaddling, will prefer slower temporal frequencies when tested prior to feeding and unswaddled, but will prefer faster temporal frequencies when tested just after feeding and swaddled (Gardner & Karmel, 1984). Since feeding and swaddling appear to lower an infant's arousal level, the infant is presumed to be more aroused internally before a feeding and unswaddled. A similar directional shift in visual preferences also is seen when arousal level is manipulated by external visual or auditory stimulation (Gardner et al., 1986; Lawson & Turkewitz, 1980; Lewkowicz & Turkewitz, 1981). That is, infants who are exposed to greater amounts of stimulation (internal or external) will prefer looking at less arousing events while infants who are exposed to lower amounts of stimulation will prefer looking at more arousing events. The lack of the ability to shift visual prefer-

ences under different arousal conditions has been suggested to indicate poor integration or organization of internal with external stimulation leading to later attentional problems, as this lack of visual shift is related to degree of brain insult in infants (Karmel, Gardner, & Magnano, in press).

Investigation of Cortisol levels and Attention in Present Study

Since cortisol levels have been found to increase as infants change from a less to a more aroused state (Anders et al., 1970; Tennes & Carter, 1973; Gunnar et al., 1984), an association between visual preferences and cortisol was expected in this study. The level of cortisol under different arousal levels may provide one of the internal physiological measures that determines the amount of overall stimulation of the infant. When cortisol levels are lower, the infant would attend to more stimulating stimuli and to less stimulating stimuli when cortisol levels are higher. Thus, the ability of the infants to regulate cortisol release to stressful events would be related to their ability to regulate their attention under different levels of arousal. Moreover, in infants who were not showing a visual shift under different arousal conditions, cortisol levels also may show fewer changes under different arousal conditions providing converging physiological and behavioral information on the infant's ability to regulate arousal and attention levels.

Methods

Subjects

Premature infants (n = 47) who were resident in the NICU nursery at St. Vincent's Medical Center were recruited. Relatively healthy in-

fants were chosen so that the infants would be medically stable to obtain cortisol measurements shortly after birth and so that cortisol levels would less likely be affected by medical problems. The criteria for selection was that the infants did not have evidence of any of the following conditions: 1. intraventricular hemorrhage with parenchymal involvement or venticular dilation, hydrocephlus, or seizures; 2. assisted ventilation for more than 48 hrs. or oxygen therapy for more than 1 wk.; 3. congenital or chromosomal abnormalities; 4. other major medical complications (e.g., necrotizing enterocolitis, cardiorespiratory arrest, sepsis, AIDs) 5. prenatal adrenocortical treatment; 6. maternal diabetes; 7. other major maternal or infant endocrine regulatory problems involving the adrenals, pituitary, thyroid, hypothalamus, or thalamus; 8. prenatal exposure to cocaine, opiates, or amphetamines. Of the 47 infants recruited, 9 were excluded due to an inability to obtain enough saliva in each condition for salivary cortisol analysis. The demographic data for the 38 infants, 21 females and 17 males, whose data are included are presented in Table 1. As seen in Table 2, the demographic characteristics of the 9 excluded infants are comparable to the other infants.

Cortisol Measurements

Collection Procedure. All cortisol levels were determined by measuring the amount of cortisol found in the saliva of the infants. The use of saliva samples has been found to be comparable to blood plasma samples (Raid-Fahmy, Read, Joyce, & Walker, 1981) with correlations between .76 and .83 reported in newborn infants (Francis, et al., 1987;

Table 1.

Demographic Data

Subject Number (N = 38)	BW (gm)	EGA (wk)	Apgar Scores 1-min	Apgar Scores 5-min	PNA[a] (day)	PCA[a] (wk)	Delivery[b] Type
1	1871	34	8	9	15	36	C\S
2	1980	35	9	9	12	36	C\S
3	2013	36	9	9	12	37	V
4	1700	35	9	9	24	38	C\S
5	2098	37	9	9	12	38	C\S
6	2608	35	9	9	6	35	V
7	2080	37	9	9	7	38	V
8	2268	35	7	9	10	36	C\S
9	1800	36	8	9	14	38	V
10	2126	34	9	9	12	35	C\S
11	1814	34	9	9	12	35	C\S
12	2013	36	8	9	5	36	V
13[c]	1701	34	9	9	25	36	C\S
14	1899	35	7	9	13	36	C\S
15	2760	36	6	9	12	37	V
16	1970	34	8	9	11	35	V
17	1956	36	8	9	14	38	V
18	2078	36	9	9	17	38	C\S

Table 1 (Continued)

19	2012	36	8	9	5	36	V
20[c]	1786	33	7	8	25	36	V
21	2395	36	9	9	9	37	V
22	2268	34	9	9	15	36	C\S
23	1899	33	9	9	21	36	C\S
24	1899	34	9	9	21	37	C\S
25	2673	35	8	9	6	35	C\S
26	1956	35	8	9	14	37	C\S
27	2155	35	5	8	12	36	C\S
28	1814	33	4	7	25	36	V
29	2013	34	7	8	19	36	V
30	1503	34	8	8	37	39	V
31	1332	30	9	9	53	37	C\S
32	1219	30	9	9	52	37	C\S
33	1446	31	7	7	40	36	V
34	2013	36	9	9	8	37	V
35[c]	1588	31	4	8	29	35	V
36[d]	2126	33	.	.	31	37	V
37	1928	36	9	9	16	38	V
38	2495	35	6	7	9	36	V
Mean	2005	34.6	8.1	8.7	17.4	36.6	20 V
SD	338	1.7	1.3	.6	12.0	1.1	18 C\S
Range	1219-2760	30-37	4-9	7-9	5-53	35-39	

[a] Postnatal age and postconceptional age at time of discharge from the NICU
[b] "V" stands for vaginally delivered and "C\S" stands for caesarean delivery
[c] Subjects not included in data analysis due to outlier cortisol values.
[d] No apgar scores were given as subject was born at home.

Table 2.

Demographic Data of Excluded Infants

Subject Number (N = 9)	BW (gm)	EGA (wk)	Apgar Scores 1-min	Apgar Scores 5-min	Delivery[a] Type
1	1850	32	9	9	V
2	2240	35	9	9	V
3	2381	35	8	9	V
4	1786	36	8	8	C\S
5	2211	34	8	9	C\S
6	1788	34	7	9	C\S
7	2126	35	8	9	V
8	2211	34	9	9	V
9	1871	33	7	8	V
Mean	2051	34.2	8.1	8.8	6 V
SD	227	1.2	.8	.4	3 C\S
Range	1786-2381	32-36	7-9	8-9	

[a] "V" stands for vaginally delivered and "C\S" stands for caesarean delivery

Gunnar et al., 1989). It has been suggested that saliva samples provide an even better estimate of plasma-free cortisol concentrations than blood plasma samples due to the low level of corticosteroid-binding globulin in saliva (Vining, McGinley, Maksvtis, & Ho, 1983; Laudat, Cerdas, Fournier, Guiban, Guilhaume, & Luton, 1988).

Saliva samples were collected by drawing at least 300 uL from the infant's mouth using a DeLee suction catheter modified to attach to a wall vacuum unit. Samples were collected while the infants were in their isolettes/cribs and then stored at -20 C degrees until analyzed (Raid-Fahmy, Read, Walker, & Griffiths, 1982). As relatively high levels of cortisol may be present in formula and breast milk (Kulski & Hartmann, 1973; Magnano, Diamond, & Gardner, 1989), samples were taken at least 2 hours after feeding (mean postfeed time = 177 ±33 min) to help reduce chances of formula or breast milk being present in the sample. In addition, the time of collection and the state of infant were recorded for each sample.

Basal Condition. Basal cortisol levels were measured throughout the infants' stay in the NICU nursery. Salivary samples were not taken for at least 120 minutes after the infants had experienced any event that was likely to cause a significant increase in cortisol levels (e.g. suctioning, I.V. insertion, blood drawn, bath, physical therapy). One hundred twenty minutes after a stressful event, cortisol levels have been shown to return to baseline levels in most healthy full-term infants (Gunnar et al., 1985). The first basal cortisol measurement was taken between 3 and 5 days after birth (mean = 4.1 ±.8 days) and then it was measured once weekly until the infant was discharged.

Stressor Condition. As with basal cortisol levels, responses to a stress-inducing event were measured throughout the infants' stay on the NICU. A heel-prick for drawing blood was used as the specific stress-inducing event as this procedure was performed routinely on all infants throughout their stay for a variety of medical tests. Salivary samples were taken 30 minutes following the heel-prick procedure. Only heel-prick procedures that were not preceded for 120 minutes by another event that could cause a significant increase in cortisol levels were used. As with basal measures, the first measurement was taken at 3 to 5 days after birth (mean = 3.9 ±.9 days) and then heel-prick responses were measured once weekly until the infant was discharged.

Neurobehavioral (NB) Condition. To measure cortisol responses to a noninvasive event, salivary samples were taken 30 minutes following the end of a standardized NB exam (Gardner, Karmel, Magnano, Norton, & Brown, 1990). The NB exam was performed on all infants just prior to discharge from the hospital. It was performed by trained experimenters in a separate room located on the NICU. The exam evaluated passive and active muscle tone as well as visual and auditory attention using items derived from the Einstein Neonatal Neurobehavioral Assessment Scale (Kurtzburg et al., 1979) and Katona's Activated Motor Patterns (Katona, 1983; Katona, 1988) (see Table 3 for items). The mean length of the exam was 18.6 ±5 minutes and the interobserver reliability was 94%.

Table 3.

Newborn Neurobehavioral Assessment[a]

I. ORIENTING	NORMAL	ABNORMAL
1. Visual: Hold semi-upright with head midline (waddled with pacifier)		
a. Fixation: Present checkerboard or bullseye vs blank, then reverse Right/Left positions	Prefers to gaze at pattern	No preference; prefers blank
b. Following: Move checkerboard or bullseye in arc from midline 45 deg Right then thru midline 45 deg Left, 2-3 times	Fixates in midline; Intermittent good or sustained following; R = L	No fixation; Transient, poorly organized following; R ≠ L
2. Auditory: Hold with head midline in palm of hand, each stimulus presented laterally 2-3 times on Right and Left		
a. Pillbox rattle (10 popcorns in plastic bottle)	Head turning > 90% of trials (may have 1-2 sec latency after eye brightening/searching; R = L	Only eye brightening R ≠ L
b. Bell		
c. Voice		

Table 3 (continued)

II. PASSIVE TONE/REFLEXES	NORMAL	ABNORMAL
1. Popliteal angle: Bend knee to chest, hold thigh while slowly extending lower leg	Angle at knee 45-150 deg; R = L	< 45 deg; > 150 deg; R ≠ L
2. Arm recoil: Flex arm with palm up for 5 sec, extend, then release	Brisk recoil; R = L	Slow recoil; R ≠ L
3. Grasp: Press palm without touching back of hand, pull up	Sustains/almost sustains weight; R = L	Weak; R ≠ L
4. Traction: Arm response during pull-to-sit (see head control items)	Moderate flexion; Some resistance to extension	Slight flexion then extension; Little or no resistance to extension
5. Tonic Neck Reflex (TNR): Turn head briskly to side and hold up to 30 sec	Releases "archer" position in < 30 sec; No TNR	Sustained TNR

Table 3 (continued)

6. Ventral suspension: Hold in air prone with hand under chest	Limb extension; Some head extension	Opisthotonia: Floppy
7. Rotation: Hold semi-upright and spin 360 deg	Ocular deviation to side of rotation, then to other side or nystagmus	No ocular responses
III. HEAD CONTROL	NORMAL	ABNORMAL
1. Activated pull-to-sit: Hold by hands/arms with head hanging off surface, wait for attempts to pull to sitting position,then assist	Some neck flexion; Pulls head up: feel tension in shoulders; Better with each attempt	Opisthotonia; Head lag; No attempt to pull head up
2. Sitting-in-air: Hold by thighs in sitting position, back against examiner's chest, then examiner extends arms so infant balanced in air	Starts with nose-in-navel, then comes to full sitting position	Opisthotonia; No attempt to lift head; Unable to straighten spine
3. Head extension:		
a. Observe while prone on horizontal surface, face down midline	Turns or lifts head off nose; Spontaneous or stimulated leg movements	No head extension; No or uncoordinated leg movements
b. Observe while prone facing up on 20-30 incline	Some head/neck extension	Opisthotonia; Floppy; No head/ neck extension

Table 3 (continued)

IV. EXTREMITY MOVEMENTS	NORMAL	ABNORMAL
1. Creeping: While prone, hold head up under chin parallel to surface	Tension in shoulders; Extension of fingers; Coordinated crawling movements of arms and legs; R = L	Asymmetry of movements; Differing quantity or quality of arms vs legs, R vs L; Fist always clenched; R ≠ L
2. Crawling: While holding under chin and belly, slowly drag fingers and toes along surface	Coordinated crawl with arms and legs; R = L	Frog-leg crawl; Hyperextension/flexion; Dragging of arms/legs; R ≠ L
3. Prone on 20-30 degree incline, facing downward: Observe	Alternating crawl pushing off with legs;R = L	Asymmetric crawl to side no matter which way head is turned
4. Placing and stepping: Hold under arms, stimulate top of feet against surface, then shift weight when steps (can do up incline)	Supports weight; walks with good flexion and extension of legs; R = L	Scissors; Weak leg movements; R ≠ L

Suggested sequence of testing: II(1,2); I(1a,1b,2a,2b,2c); II(3,4); III(1,2); II(5); III(3a); IV(3a); IV(1,2); II(6,7); III(3b); IV(3,4)

[a] From Gardner et al., 1990.

Cortisol Assay

A commercially available kit for the direct radioimmunoassay (RIA) of cortisol in plasma and urine (Double Antibody Cortisol RIA; Diagnostic Products Corporation, Los Angeles, CA) was used to analyze the amount of cortisol present in the saliva. The kit was modified for the low concentration of cortisol found in saliva by diluting the standards tenfold and by increasing the sample size four times to a 100 uL volume. This provided a similar ratio of cortisol standards to sample volume as used by Al-Hakiem and Abbas (1987), who adapted a plasma cortisol kit for use with saliva. To reduce within subject variance due to differences between assays, all cortisol samples collected for a subject were analyzed in the same assay. All samples were analyzed in duplicate with any samples differing more than 10% being retested if possible or eliminated. The intra-assay and inter-assay mean coefficients of variation were 3.3% and 10.1% respectively. The inter-assay variation was determined by running a control sample from pooled infant saliva with each assay.

Neonatal Special Care Score

The degree of general stress experienced by the infants during their stay on the NICU was assessed by the Neonatal Special Care Score (see Table 4) devised by Casaer et al. (1982). This scale measures the clinical condition of the infant on a day-to-day basis thereby providing an estimate of the degree of illness of the infant. The scale consists of 10 items that can be given a rating from 0 to 3 (because of their all or none properties, a few items are scored either 0 or 3) for a total of

Table 4.

Neonatal Special Care Score[a]

Item	Score			
	3	2	1	0
1. Respiration	Ventilator	CPAP	Extra Oxygen	Air
2. Circulation	Cardiac shock; Bradycardia		Cardiovascular liability	Normal
3. Feeding	Intravenous	Intravenous plus gavage or oral	Gavage	Oral
4. Nutritional Status	Poor			Good
5. Metabolic Homeostasis	Severe Deterioration		Bilirubin Phototherapy	Good
6. Neurological Excitability	Convulsions; Coma	Hyperexcitability; Apathy	Hyperexcitability on stimulation	Normal
7. Mobility	Hyperkinetic; Hypokinetic			Normal
8. Tonus	Hypertonic; Hypotonic			Normal
9. Laterality	Obviously present			Absent
10. Level of Care	Intensive	Medium Care	Crib	Discharged

[a] Adapted from Casaer & Eggermont (1985).

30 for the worst score and 0 as the best score. Five of the ten items involve the status of the major functions: respiration, circulation, feeding condition, nutritional status, and metabolic homeostasis. Another four items describe the spontaneous neurological behavior of the infant: neurological excitability, motility, tonus, and lateralization. The remaining item describes the overall care level of the infant. In addition, whether the infant was on the drug somophyline or under lights for bilirubin level elevation was recorded as these treatments were frequent on the NICU but are not on Casaer's scale. As somophyline is given to enhance cardiac functioning, a score of 1 was given for circulation if the infant was receiving the drug, and if the infant was receiving bilirubin treatment, a score of 1 was given for metabolic homeostasis.

Scores on each of the items were determined for the infants five times per week during their stay. From the scores on the items, a daily score was calculated. The interobserver reliability was 96% on 24 daily scores. The initial peak value of the neonatal scores taken on a daily basis during hospital stay on premature infants has been shown to be significantly correlated with the Obstetric Optimality Score (Casaer et al., 1982) and the slope of the line has been found to be related to intellectual and neurological evaluations at 7 and 8 months of age (Casaer, 1984).

Attention and Arousal Regulation

To assess the relationship between cortisol levels and attention under different levels of arousal, infants were tested in a visual preference paradigm as described by Gardner & Karmel (1984).

Apparatus. The visual preference apparatus consisted of a three-sided chamber lined with gray felt. The back panel of the chamber was covered with black posterboard and had two openings each covered with a light diffuser. Each opening measured 15.2 x 15.2 cm and the inner edges of the two openings were 30.4 cm apart. A centrally located peephole between the openings allowed for unobtrusive observations of the direction of the infant's gaze.

Stimuli. The stimuli were unpatterned light panels illuminated at different temporal frequencies. Stimuli were produced by two independent sets of white fluorescent lamps mounted inside boxes affixed to the openings behind the light diffusers. Most infants (n = 25) were presented with stimuli at temporal frequencies of 2, 4, and 8 Hz. As other studies have found no difference in the slopes of visual preferences as a function of stimuli used for this range of stimuli in healthy neonates (Karmel, Gardner, & Magnano, in press), 5 infants received stimuli of 1, 2, and 4 Hz and 5 infants received stimuli of 1, 2, 4, and 8 Hz in the course of testing. Square-wave modulated frequencies (a 50% duty cycle, when a duty cycle is defined as the proportion of the total cycle occupied by the stimuli) were used to equate for total stimulus luminance across frequencies as neonate's preferences are known to be affected by total luminance levels (Hershenson, 1964; Lewkowicz & Turkewitz, 1981). Critical flicker fusion (CFF) was assumed to be higher than the fastest frequency (8 Hz) as neonatal CFF has been estimated to be 35 Hz (see Regal, 1981). Frequency of temporal change was under the control of a microcomputer system designed to manipulate the stimuli for presenta-

tion, time the trials, and record the duration of looking to each stimulus as judged by the observer.

Procedure. Each infant was tested under three different arousal conditions: less aroused, externally aroused, and internally aroused. For the less aroused condition, infants were tested just after feeding while swaddled in a blanket. For the externally aroused condition, infants also were tested just after feeding while tightly swaddled but they were presented with an 8 Hz stimulus prior to each preference trial. For the internally aroused condition, infants were tested just prior to feeding while loosely swaddled.

In each condition for infants who received 3 stimuli (2, 4, and 8 Hz or 1, 2, and 4 Hz), there were six preference trials. Preference trials consisted of simultaneously presenting pairs of stimuli for 20 seconds. All possible pairs were presented twice. Thus, each infant was presented with a random order of six pairs of stimuli that were counterbalanced for side and sequence. For the 4 stimuli (1, 2, 4, and 8 Hz), twelve preference trials were given also consisting of all possible pairs twice for 20 seconds per pair in a random order balanced for side and sequence. In the externally aroused condition, there was an initial 8 Hz stimulation period which lasted for 20 seconds, with additional 10 second periods presented prior to preference trials.

During testing, the infant was seated in a semi-reclined position on the lap of an experimenter facing the stimulus panel of the looking box. The infant's head was held midline by the experimenter. In the prefeeding condition (internally aroused), the infants were given a

pacifier so that they would be calm enough to attend to the stimuli as well as to help prevent rooting responses. In other cortisol studies, providing infants with pacifiers to behaviorally calm them has not been found to affect their cortisol responses (Gunnar et al., 1984). Standing behind the looking box, another experimenter looked through a peep hole and recorded the direction and duration of the infant's looking by pressing buttons on the box connected to the computer. The experimenter began a trial by pressing the "start" button on the box when the infants had their eyes open and were not crying. Any trial in which total looking time was recorded to be less than 5 seconds was repeated. Inter-observer reliability using this apparatus has been found to be high ($r > .95$) (Gardner & Turkewitz, 1982).

Results

Salivary Cortisol

Preliminary Analysis. Cortisol values initially were examined for outliers that were potentially due to sampling close to a pulse of adrenocortical activity (Gunnar et al., 1989). This pulse release of cortisol into the circulation results in a sudden, temporary rise in free, unbound cortisol levels. As the ratio of unbound to bound cortisol is much higher in saliva compared to blood (Vining et al., 1983), the rise in salivary cortisol level can be temporarily quite high after a pulse of adrenocortical activity and could confound the infant's normal cortisol level in that condition. Outliers were defined as those cortisol values greater than 2 standard deviations above the mean, as determined separately for each condition (Basal, Stressor, and NB condi-

tions). Three infants had values in that range with two infants having 1 value and the other infant having 2 values. Two of the values were basal measurements and the others were a stressor measurement and a NB measurement. These three infants with outlier values were then eliminated from further data analysis. As the degree of variance was still high for the remaining subjects (n = 35), the cortisol values were transformed ($\log_{10}$) to help normalize the distributions. (The below analyses were also performed with the nontransformed cortisol values and the same pattern of significant relationships between variables was found .)

Basal and Stressor Cortisol Levels during NICU stay. To observe the degree of change in basal and stressor levels during the infants' stay on the NICU, the linear slopes of basal and stressor levels across days were calculated for each infant. Linear slopes of the NB cortisol levels could not be calculated as this measurement was taken only once during an infant's stay. The mean linear slopes were negative for both basal and stressor conditions (mean basal slope = -.07; mean stressor slope = -.08) with a significant Pearson correlation between the slopes ($r(35) = .53$, $p < .005$). To further analyze possible changes in basal and stressor cortisol levels during the NICU stay, the initial cortisol levels were compared to the levels just prior to discharge in a 2 (time of collection) x 2 (condition) analysis of variance (ANOVA) with repeated measures. The results yielded significant main effects of time of collection ($F(1,34) = 37.55$, $p < .001$) and condition ($F(1,34) = 34.43$, $p < .001$) but no interaction effect. Thus, both basal and stres-

sor levels decreased during the infants' stay on the NICU with basal levels remaining consistently lower than stressor levels (Figure 2). The means and standard deviations of initial and discharge cortisol levels are presented in Table 5 for each condition.

Basal, NB, and Stressor Cortisol Levels at Discharge. A one-way ANOVA with repeated measures showed a significant monotonic trend across the basal, NB, and stressor cortisol levels at discharge ($F(1,34) = 39.40$, $p < .001$). Planned comparisons, specifically linear contrasts on the dependent variables, found that the conditions, basal, NB, and stressor, were significantly different from each other (basal vs. stressor: $F(1,34) = 39.40$, $p < .001$; basal vs. NB: $F(1,34) = 10.21$, $p < .003$); NB vs. stressor: $F(1,34) = 19.44$, $p < .001$)). In addition, Pearson correlation coefficients revealed that the NB cortisol level was significantly related to the stressor cortisol level at discharge ($r(35) = .49$, $p < .005$) while none of the other comparisons of cortisol levels at discharge indicated associations.

Relationship to Neonatal Special Care Score. In order to compare the effect of general degree of stress on cortisol levels as assessed by the Neonatal Special Care Score (Casaer et al., 1982), the slopes of daily scores collected for each infant during the NICU stay were calculated (mean Casaer Daily Score slope = -.34) (see Figure 3). The slopes of the daily scores were then compared to the basal and stressor slopes by a one-way ANOVA with repeated measures. An overall significant difference was found among the three slopes ($F(1,34) = 26.43$, $p < .001$).

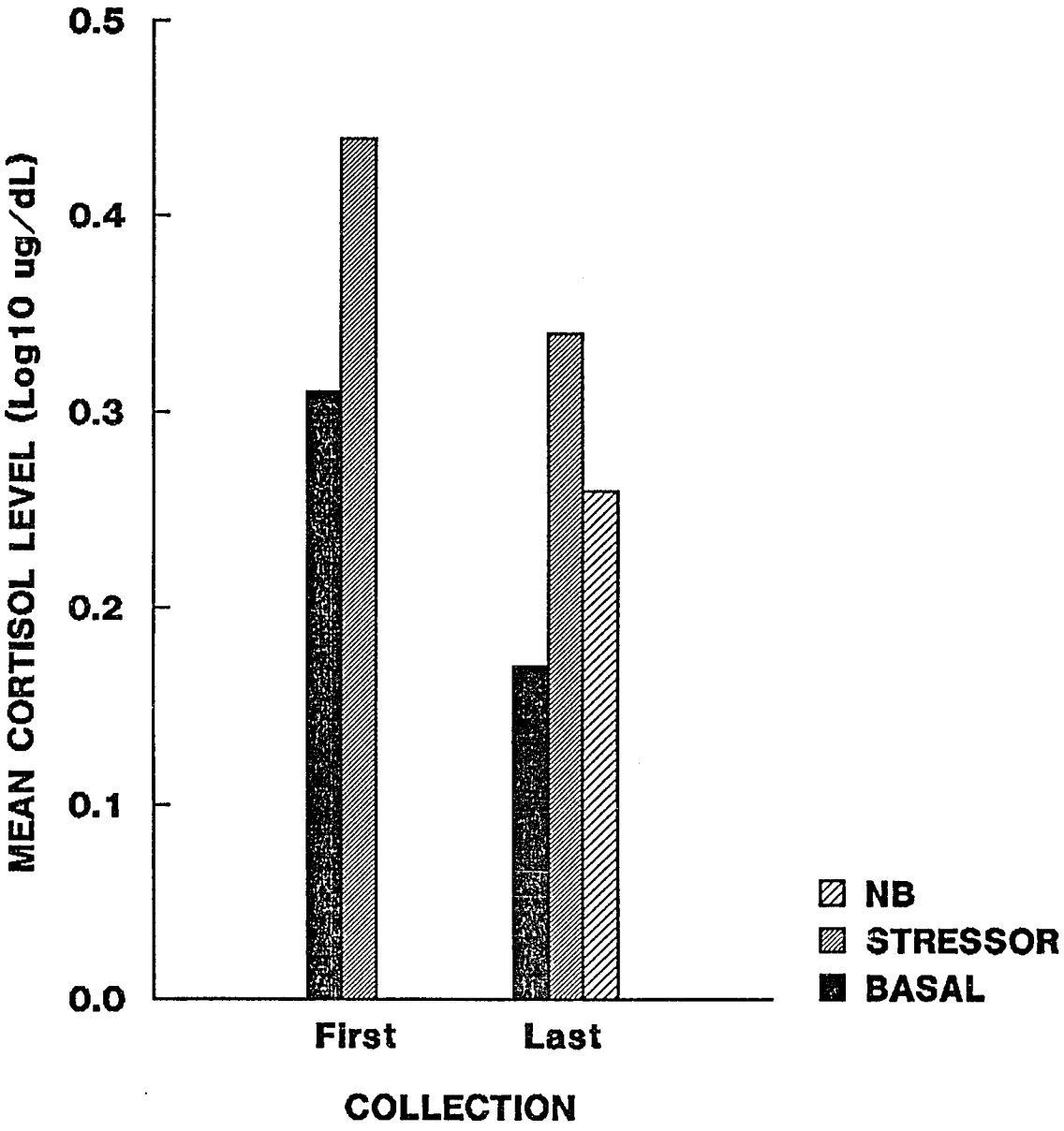
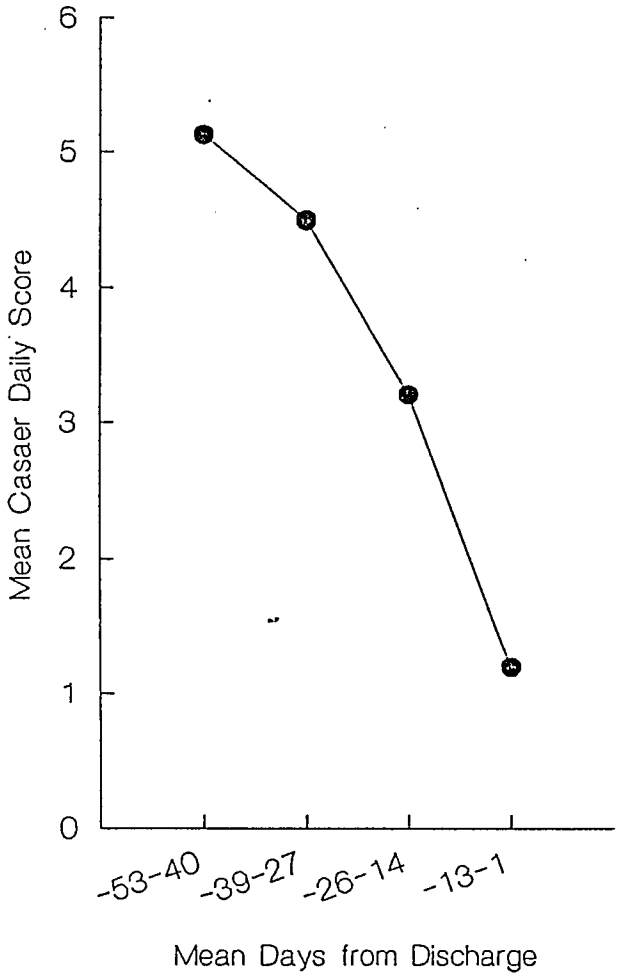

0.5
0.4
0.3
0.2
0.1
0.0
MEAN CORTISOL LEVEL (Log10 ug/dL)
First
Last
COLLECTION
NB
STRESSOR
BASAL

Table 5.

Mean Salivary Cortisol Levels (ug/dL)

Condition	Initial	At Discharge
	Mean SD	Mean SD
Basal	1.2 ± .9	.56 ±.6
NB	-	.86 ±.5
Stressor	2.0 ±1.3	1.3 ±.7


6
5
4
3
2
1
0
Mean Casaer Daily Score
-53-40
-39-27
-26-14
-13-1
Mean Days from Discharge

Planned comparisons, linear contrasts on the dependent variables, showed that significant differences existed between the daily score slope and the basal ($F(1,34) = 43.62$, $p < .001$) and stressor ($F(1,34) = 22.41$, $p < .001$) slopes but not between the basal slope and stressor slope. That is, the rate at which the infant's cortisol levels or responses to stress decreased during the NICU stay was not the same rate at which the daily scores decreased during the NICU stay. In addition, no significant correlations were found between the Neonatal Special Care daily score for the day the cortisol samples were taken and the basal and stressor cortisol levels for that day. Thus, while both the Casaer daily scores and the basal and stressor cortisol levels decreased during the infant's stay, the degree of change in the daily scores was not related to the degree of change in cortisol levels.

Correlations to Other Variables. In order to assess possible relationships between cortisol levels and potentially relevant variables in infant population, separate Pearson correlation coefficients were calculated between the initial and discharge cortisol levels in each of the conditions and the birthweight (BW), estimated gestational age at birth (EGA), Apgar scores, postnatal age (PNA) at discharge, and postconceptional age (PCA) at discharge. Point biserial coefficients also were calculated between cortisol levels and the sex of the infants. No significant correlations were found between the cortisol levels and any of the variables. However, it should be noted that there was not a wide variability in most measures in this healthy premature population (see Table 1). In addition, no significant correlations were found be-

tween the cortisol levels and the time of day that the sample was collected, the state of the infant at time of collection, or the time from the last feeding. A significant positive correlation was found between the length of NB exam and the NB cortisol level ($r(35) = .33$, $p < .05$).

Looking Preferences

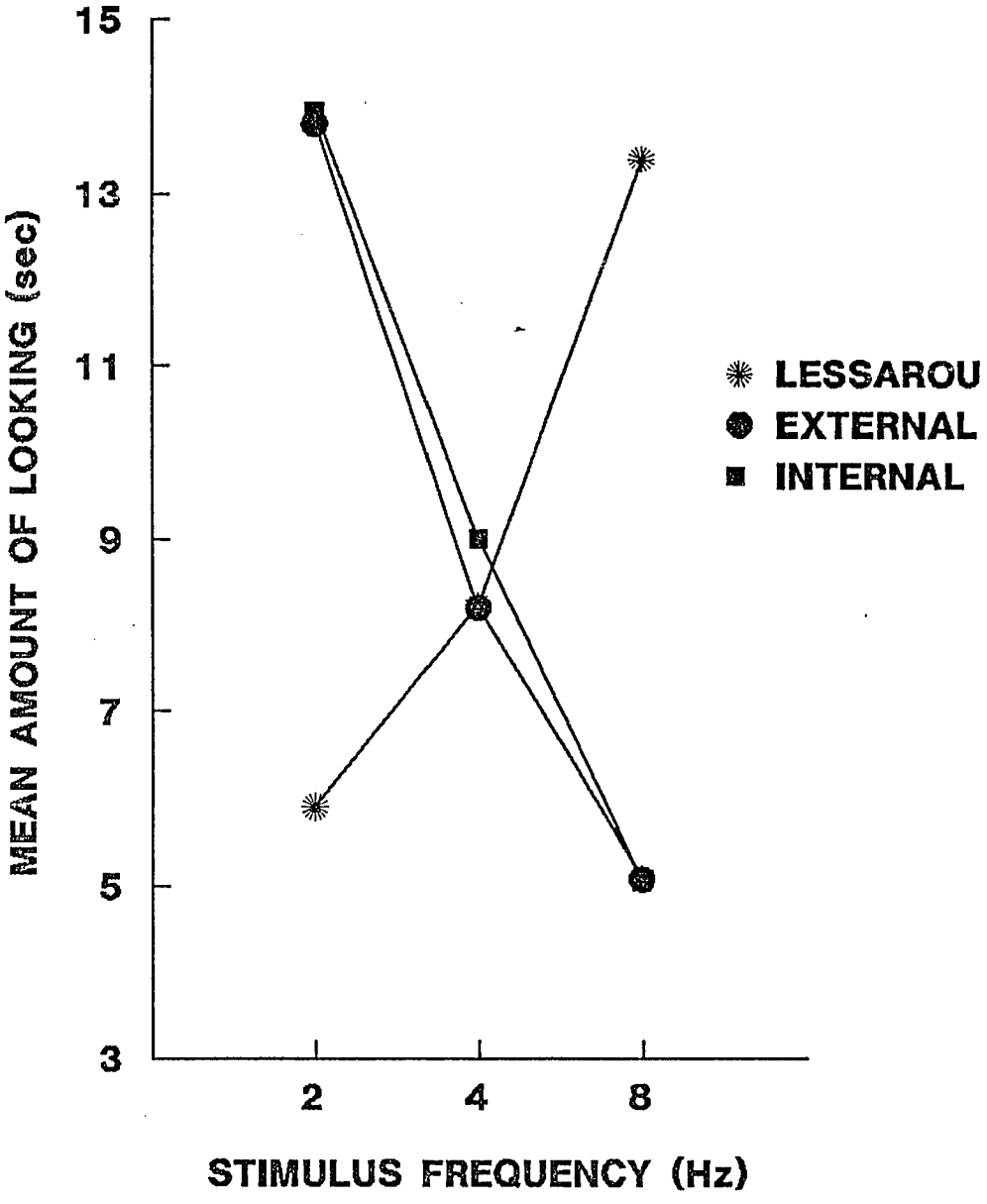
Preliminary Analysis. Since the infants had received different sets of visual stimuli in the course of testing (2, 4, and 8 Hz (n = 25); 1, 2, and 4 Hz, (n = 5); 1, 2, 4, and 8 Hz (n = 5)), visual preferences and cortisol levels between the three groups were compared to ensure that they were comparable. No significant differences were found for the looking preferences as a function of stimulus set ($F(2,32) = .18$, n.s.). Even though this may have been due to heterogeneity of variance due to disparate sample sizes and small n's in two sets, a similar lack of difference was found for healthy full-term infants (Karmel et al., in press). However, a significant difference in mean cortisol levels was found as a function of stimuli set ($F(2,32) = 6.74$, $p < .004$). Thus, the three groups could not be considered to be comparable and have their data combined. Therefore, since most infants (n = 25) received the 2, 4, 8 Hz stimuli, only looking preference data from these infants is presented. Further analysis of these data on the 25 infants revealed that one infant was greater than 2 standard deviations from the mean looking time to each of the stimuli in all looking preferences conditions. To normalize the data, this one outlier also was eliminated from the looking preference data analysis.

Effect of arousal level on looking preference. In order to look at the effect of arousal level on looking preference, linear slopes of

looking time across the visual stimuli were calculated for each of the arousal conditions (less aroused, internally aroused, and externally aroused). The mean linear slopes for each of the conditions were: less aroused = 1.26, internally aroused = -1.42, and externally aroused = -1.32 A major change in the direction of the slopes was found as a function of arousal with a strong positive slope in the less aroused condition and strong negative slopes in the more aroused conditions across the visual stimuli (see Figure 4). A one-way ANOVA with repeated measures yielded an overall significant difference among the three slopes ($F(2,46) = 116.17$, $p < .001$). Planned comparisons, linear contrasts on the dependent variables, revealed that the less aroused condition was significantly different from the more internally and externally aroused conditions ($F(1,23) = 136.71$, $p < .001$; $F(1,23) = 159.13$, $p < .001$) but that the two more aroused conditions were not significantly different from each other ($F(1,23) = .02$, n.s.). Thus, as seen in Figure 4, looking preferences shifted away from faster frequencies towards slower frequencies when the infants were more aroused compared to being less aroused. This replicates previous findings that looking preferences in the neonatal period are a joint function of the state of the infant and the characteristics of the stimulus (Gardner & Karmel, 1984; Gardner, Lewkowicz, Rose, & Karmel, 1986; Gardner & Turkewitz, 1982).

Relationship of Cortisol Level to Looking Preference.

In order to determine if a relationship existed between cortisol level and amount of preference shown for faster or slower frequencies in the different arousal conditions, Pearson correlation coefficients were


15
13
11
9
7
5
3
MEAN AMOUNT OF LOOKING (sec)
2
4
8
STIMULUS FREQUENCY (Hz)
LESSAROU
EXTERNAL
INTERNAL

calculated between the mean cortisol levels for each condition during the NICU stay and the calculated linear slopes for the three looking preference conditions. Significant correlations were found between the basal cortisol levels at discharge and the externally and internally aroused looking conditions ($\underline{r}(24) = -.43$, $p < .025$; $\underline{r}(24) = -.41$, $p < .025$). In addition, the stressor cortisol level at discharge was found to be correlated to the externally aroused condition ($\underline{r}(24) = -.44$, $p < .025$) but not to the internally aroused condition ($\underline{r}(24) = -.03$, n.s.). A similar pattern of correlations ($p < .010$) was found to initial cortisol levels with the initial basal cortisol level related to the two more aroused looking conditions and the initial stressor cortisol level related to the externally but not the internally aroused looking condition. No significant correlations were found for the less aroused looking condition. Thus, higher basal cortisol levels were related to a greater preference for slower frequencies in both more aroused looking conditions while higher cortisol levels following an invasive stressor were related to a greater preference for slower frequencies in only the externally aroused looking condition.

Summary of findings. Overall, the findings were that the mean basal and stressor cortisol levels declined during the NICU stay with the basal levels remaining significantly lower than stressor levels. In addition, the mean NB cortisol levels were found to be in between the mean basal and stressor cortisol levels at discharge with a significant monotonic trend across them. The Caesar Daily Scores and their change during the NICU stay were found to be unrelated to basal and stressor

cortisol levels or their changes during the stay. In the looking preference paradigm, the looking preferences in the more aroused (internal and external) conditions were found to be significantly different from the less aroused condition with the infants preferring the slower frequencies in the more aroused conditions and the faster frequencies in the less aroused condition. Moreover, significant correlations were found between the basal cortisol levels at discharge and the internally and externally looking conditions as well as between the stressor cortisol level at discharge and the externally aroused looking condition.

Generalization of findings. In order to see if the above findings could be generalized to other at-risk groups of infants, an additional 11 NICU infants were studied at time of discharge using the same procedures for cortisol measurements and looking preferences as previously described. The infants were similar demographically (see Table 6) except that they were exposed prenatally to cocaine as determined by a positive urine toxicology taken shortly after birth. None of the infants tested positive for other routinely tested drugs (opiates, heroin, and amphetamines) but most of the mothers also admitted to engaging in cigarette and alcohol use during pregnancy.

When the mean cortisol levels at discharge in the three conditions (basal, NB, and stressor) for these cocaine-exposed infants and the other infants were analyzed as a function of cocaine using an ANOVA with

Table 6.

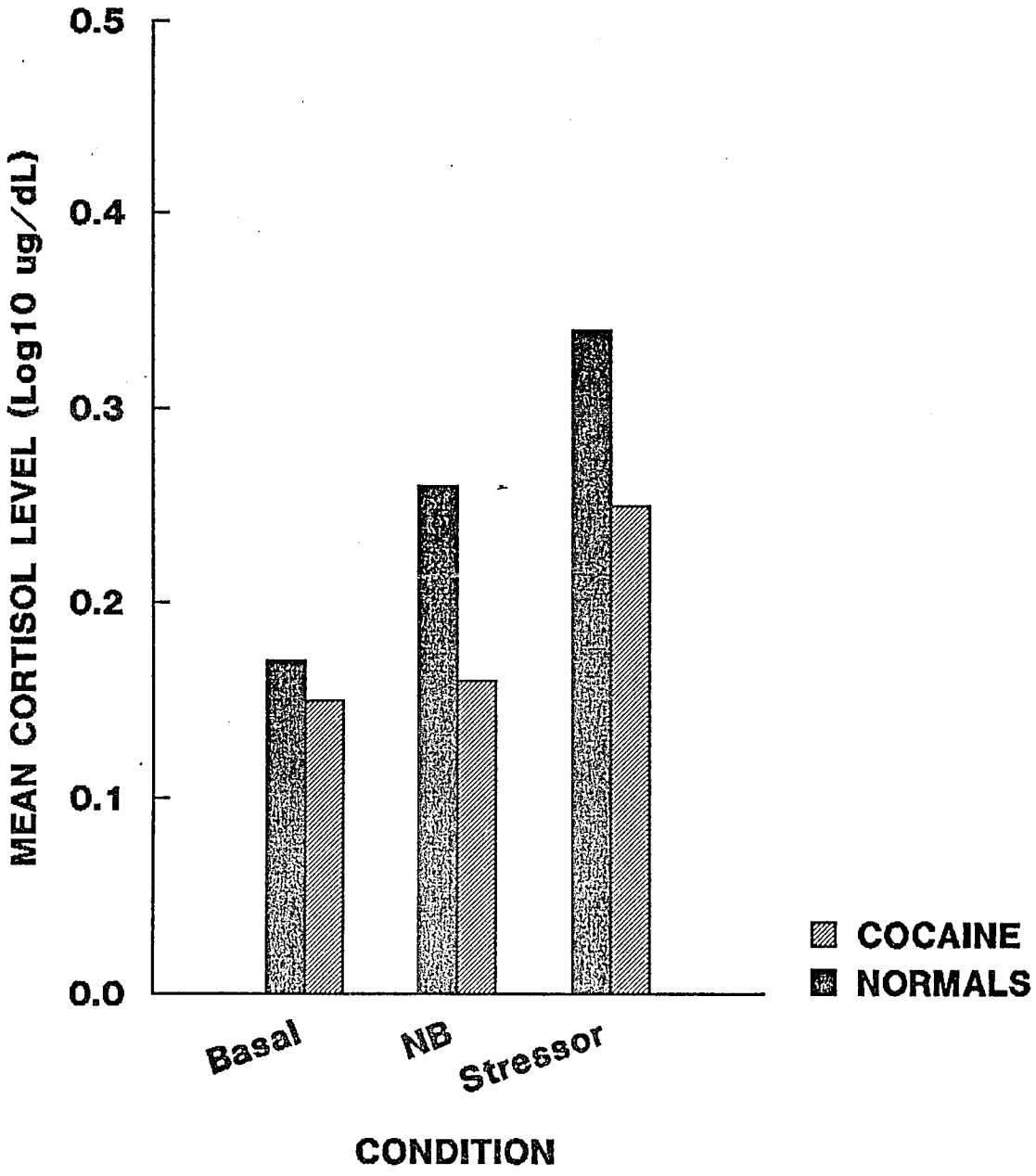
Demographic Data of Cocaine-Exposed Infants

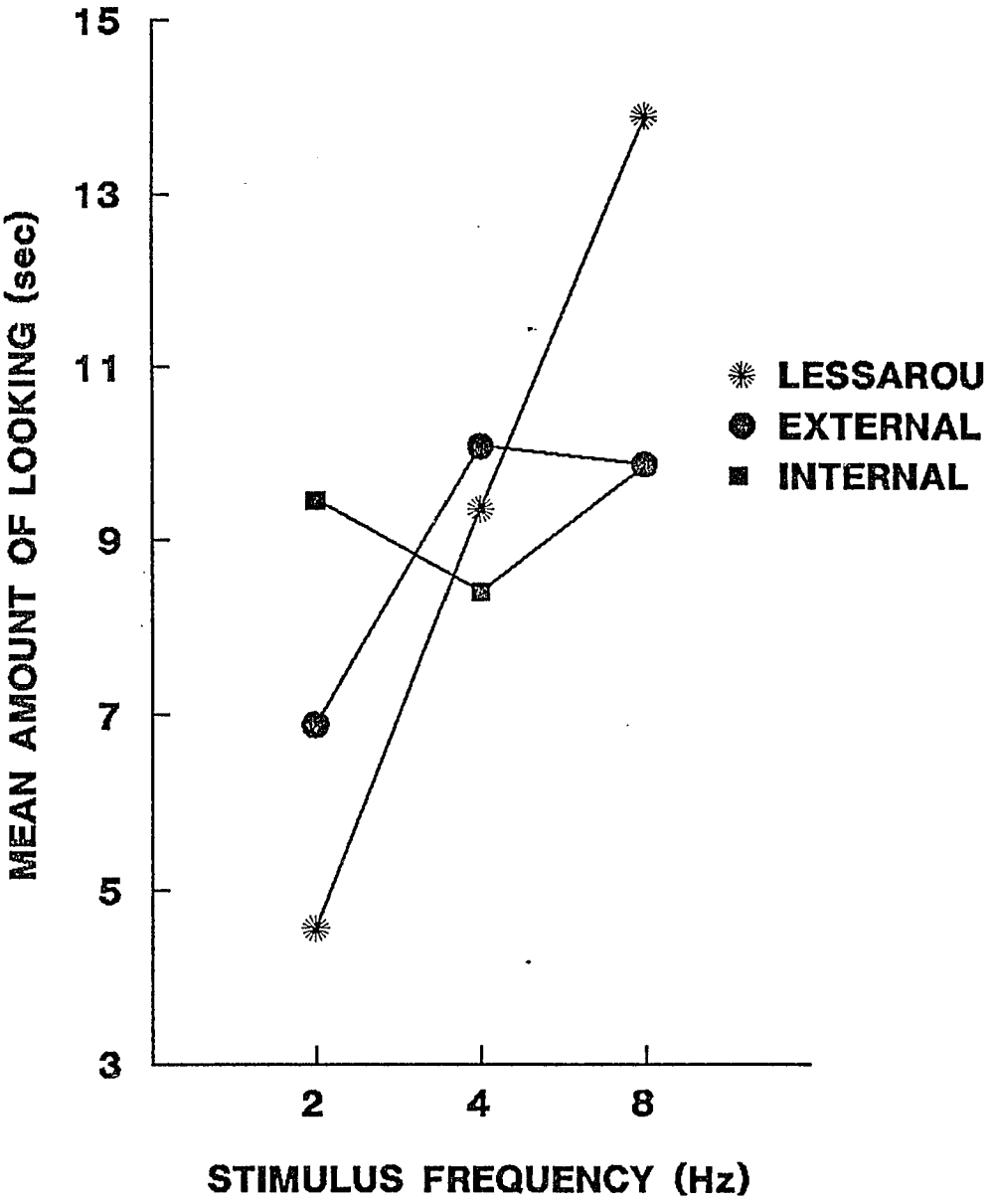
Subject Number (N = 11)	BW (gm)	EGA (wk)	Apgar Scores 1-min	Apgar Scores 5-min	PNA[a] (day)	PCA[a] (wk)
1	1984	34	9	9	11	35
2	1972	33	6	9	17	35
3	1534	31	5	8	28	35
4	2155	36	7	8	15	38
5	1729	37	8	8	27	40
6	1510	32	0	5	53	39
7	2098	33	8	8	22	36
8	2260	35	8	9	12	36
9	1360	32	9	9	36	37
10	1644	34	9	9	26	37
11	1783	34	1	5	26	37
Mean	1817	33.7	6.4	7.9	24.1	37.2
SD	295	1.8	3.2	1.5	11.9	1.4
Range	1360-2260	31-37	0-9	5-9	11-53	35-40

[a] Postnatal age and postconceptional age at time of discharge from the NICU

cocaine-exposure as a between-group variable and condition as a within-group variable, an overall effect of cocaine exposure was found ($F(1,44) = 6.76$, $p < .01$). Planned comparisons found that the cocaine-exposed infants had significantly lower cortisol levels in the NB ($F(1,44) = 7.75$, $p < .008$) and stressor ($F(1,44) = 5.09$, $p < 03$) conditions but did not have different basal cortisol levels compared to the noncocaine-exposed infants (see Figure 5). Thus, the cocaine-exposed infants appeared to have the same basal cortisol levels but to have significantly lower cortisol responses to the stressful conditions.

For the looking paradigm, the linear slopes of the visual preferences were calculated across the stimuli for each condition (internally, externally and less aroused) (see Figure 6). When the mean linear slopes between the cocaine-exposed and the other infants were analyzed as a function of cocaine-exposure using an ANOVA with cocaine-exposure as a between-group variable and the looking condition as a within-group variable, an overall significant effect of cocaine-exposure was found ($F(1,35) = 15.34$, $p < .001$). Planned comparisons found that the linear slopes of the externally and internally aroused conditions between the cocaine-exposed and noncocaine-exposed infants were significantly different ($F(1,35) = 48.34$, $p < .001$; $F(1,35) = 70.71$, $p < .001$) but that the linear slopes of less aroused condition were not different between the two groups of infants ($F(1,35) = 2.97$, n.s.) (see Figures 4 and 6). That is, the cocaine-exposed infants did not demonstrate a strong shift away from the faster frequencies towards the slower frequencies when they were more aroused (internally or externally) compared to being less


0.5
0.4
0.3
0.2
0.1
0.0
MEAN CORTISOL LEVEL (Log10 ug/dL)
COCAINE
NORMALS
Basal
NB
Stressor
CONDITION


15
13
11
9
7
5
3
MEAN AMOUNT OF LOOKING (sec)
2
4
8
STIMULUS FREQUENCY (Hz)
LESSAROU
EXTERNAL
INTERNAL

aroused. Thus, the cocaine-exposed infant's visual preferences appear to be less affected by arousal level compared to noncocaine-exposed infants.

Unlike the noncocaine-exposed infants, Pearson correlation coefficients between the mean basal, NB, and stressor cortisol levels and the linear slopes for each of the three looking preference conditions were not significant. This may be partly due to the small number of cocaine-exposed infants making significance hard to obtain. Nevertheless, cortisol responding was affected in these cocaine-exposed infants as seen in the decreased cortisol responses to stressful events and, as expected based on the relationship between cortisol levels and looking preferences found in the noncocaine-exposed infants, a parallel lower responsiveness to the more arousing conditions in the visual preference paradigm.

Discussion

Overall, the data suggest that adrenocortical responsiveness was not compromised in the preterm infants despite exposure to a potentially stressful atypical environment. During the infants' NICU stay, cortisol declined to levels approximating those reported in healthy full-term infants (see Table 7) with the difference between the basal and stressor levels remaining relatively constant. At time of discharge, the adrenocortical system appeared to be sensitive to the intensity of stress of the event. In addition, cortisol levels were found to be related to visual preferences under different levels of arousal.

Table 7.

Comparison of Mean Salivary Cortisol Levels (ug/dL) between Preterm Infants at Discharge and Healthy Full-term Infants

Source	Age of Infant	Condition			Comments
		Basal	NB	Stressor	
Present Study	35-40 wk PCA[a]	.56	.86	1.3	Preterm
Francis et al. (1987)	36-41 wk PCA	.46	-	-	Full-term
	36-41 wk PCA	.37[b]			
Gunnar et al. (1989)	38-41 wk PCA	.40	.82	-	Full-term; NB = discharge exam;
		.50[c]			
Lewis et al. (1990)	46-50 wk PCA	.74	-	1.35	Full-term; Stressor = DPT inoculation

[a]Postconceptional age of infant at time of salivary measurement
[b]Basal cortisol levels were measured in two separate groups of infants
[c]Basal cortisol levels were measured twice in the same group of infants

While the observed decrease in basal and stressor levels during the NICU stay could be suggestive of an age related change, this is unlikely since cortisol levels in the fetus significantly increase during the last weeks of pregnancy (Murphy, 1982). Also, no correlations were found between cortisol levels and the postconceptional age (PCA) or postnatal age (PNA) of the infant at the time the cortisol values were taken. The initial higher cortisol levels also were not likely the result of elevated responses to the birth process as the first values were collected after the time cortisol levels have been found to return to basal levels following birth in full-term infants (Gunnar, 1986). In addition, cortisol levels obtained in the middle of the NICU stay from infants who were there for several weeks tended to show a slow downward linear trend as opposed to a sudden drop in the levels found at discharge. Therefore, it seems likely that the changes were in response to decreases in amount of stress in the condition of the infant and the environment.

While the initial basal cortisol levels suggested that the infants were reacting to a general chronic type of stress in the NICU, the constant difference found in cortisol levels between the basal and stressor conditions indicated that adaptive cortisol responding was unaffected by this early exposure to stress. If the sensitivity of cortisol responding to an invasive physical stressor was affected by early exposure to stress, the amount of change in cortisol level from the basal level caused by a heel-prick would not be expected to remain the same (as was the case). In addition, cortisol levels were found not to be related

to the amount of stress the infant was exposed to as measured by either length of hospital stay or the Casaer scale score. If the cortisol responding was affected by early exposure to stress, correlations to those variables would have been expected.

The lack of effect of early exposure to stress on the adrenocortical system is more strongly supported by the responses to the NB exam observed at discharge. Since cortisol responses to milder, noninvasive stressors compared to more intense, invasive stressors have been found to be variable as well as affected by prior experiences in other studies (Gunnar et al., 1987; Gunnar et al., 1988; Gunnar et al., 1989), any effects of the early stress experienced on the NICU would likely be seen in the NB condition. However, cortisol levels following the NB exam and their relationship to the other conditions at discharge, which were significantly higher than basal levels but significantly lower than stressor levels, were similar to findings reported in full-term newborn infants (Gunnar et al., 1985). Moreover, the monotonic increase of cortisol level in response to different levels of stress indicates the adrenocortical system was likely to be responding in an adaptive, graded manner sensitive to the situation, rather than in only an all-or-none manner. It is possible, though, that the infants were individually responding in an all-or-none manner but when their responses were averaged together, it gave the appearance of graded responding to the different stress levels.

While the negative slopes of Caesar scale daily scores during the stay reflected the decrease in cortisol levels, these scores measuring

amount of stress the infant was exposed to as a function of illness were not found to be correlated with either mean basal or stressor cortisol levels or their degree of change. As the infants were relatively healthy, the small range in the scores values may have caused the lack of correlation. Out of a total possible score of 30 on the scale, the highest score obtained was 10 with a highest mean score of 6.1 (all infants had a score of 1 or 0 by time of hospital discharge). Thus, the scale was not likely to be sensitive enough to the smaller changes in condition and environment experienced by healthy premature infants to provide differential information within the population. While this scale perhaps could be effective for a sicker, less selective NICU population, a more sensitive scale would have been needed for this study to determine if any relationships existed between the infants' medical condition and cortisol level.

In the looking preference paradigm, an inverse relationship was found between the infant's arousal level and response to visual stimuli as has been reported by others (Gardner & Karmel, 1984; Gardner, Lewkowicz, Rose, & Karmel, 1986; Gardner & Turkewitz, 1982). In the less aroused condition (after feeding while swaddled), the infants preferred the faster frequencies while in the internally aroused (prior to feeding while unswaddled) and externally aroused (exposed to visual prestimulation) conditions the infants preferred the slower frequencies. In addition, the looking preference functions of the two more aroused conditions were the same suggesting that the infant's overall arousal level was determined equally by both internal and external sources of stimula-

tion. Thus, the visual preference was the outcome of the infant's integration of internal arousal with external stimulation to achieve an optimal or preferred level of overall stimulation for the infant (Gardner & Karmel, 1983).

As predicted, both cortisol levels and visual preferences changed as a function of arousal level and the two were related to each other. However, cortisol levels were found to be only associated with the more aroused conditions. Infants who had higher basal cortisol levels became more aroused by the internal and external stimulation as seen in greater shifts towards lower frequencies in their visual preferences. Thus, basal cortisol levels with visual attentional responses appear to provide information on the overall stimulation level of the infant in higher arousal situations.

Although the visual preferences were the same for the internally and externally aroused conditions, the stressor cortisol level at discharge was only correlated to the externally aroused condition. This suggests that while the overall arousal level was similar for the infants in the two conditions resulting in the same visual preferences, the two conditions may produce the same effect on arousal level through different, independent mechanisms. As both the heel-prick and visual prestimulation are external sources of stimulation, it is not unlikely that they would activate the same mechanisms. Due to the immediate effect of the visual prestimulation on the arousal level in the externally aroused condition, it was not likely that the effect on arousal was the result of a rise in cortisol levels as cortisol is not present until

several minutes following a stressor. Rather, it was more likely caused by the adrenomedullary system, which usually is activated by the same stimuli that activate the adrenocortical system and responds within seconds by releasing epinephrine following a stressor (Gunnar, 1986). Thus, as stressor cortisol level at discharge was found to be correlated with the externally aroused condition but not the internally aroused condition, it provided not only converging information with the visual preference functions on the infant's attentional processes but also differentiated between the two more aroused conditions, which has not been able to be accomplished prior to this with behavioral measures.

The findings with the cocaine-exposed infants suggest that cortisol measures in combination with behavioral measures can also be useful in studying other infants who may be at-risk for arousal regulation problems. While cortisol responsiveness in healthy premature infants did not appear to be affected by events experienced in the NICU, cortisol responsiveness was affected in the cocaine-exposed infants as seen by decreases in cortisol responses to normally stressful events. In addition, this low level of responsiveness to stress was also seen in the cocaine-exposed infants' attentional responses. It is not clear from this study whether these effects on cortisol and attentional responding in the cocaine-exposed infants are the direct result of cocaine exposure or are result of other factors generally associated with this infant population such as maternal use of alcohol, smoking, and poor nutrition during pregnancy. This relative underresponsiveness in stressful situations suggests that cocaine-exposed infants may need

more intense stimuli in order to maintain appropriate attentional responses to relevant environmental stimuli. These findings suggest a possible mechanism for some of the state regulatory and later attentional problems reported in cocaine-exposed infants and older children (Brown et al., 1989; Griffith, Chasnoff, & Freier, 1990; Neuspiel, Hamel, Hochberg, Green, & Campbell, 1989; O'Donnell, Gingras, Hume, & Stranger, 1989) but further investigation of older, cocaine-exposed children would be necessary and important.

In conclusion, by measuring the cortisol levels under different levels of stress, these studies were able to determine the ability of the developing adrenocortical system to adapt to stress, and the effects that earlier stress exposure may have on cortisol and arousal regulation. In healthy premature infants, the adrenocortical system did not appear to be affected by potentially stressful events normally experienced in the NICU. These infants were able to maintain adaptive cortisol responses during their NICU stay and to demonstrate appropriate cortisol and attentional responses at time of discharge from the NICU. In addition, a relationship between cortisol and attentional regulation to stressful events was established. Moreover, beyond the infants studied here, these findings suggest that cortisol measures can be used not only to understand but also to identify other groups of infants who may be at-risk for arousal regulation problems.

References

Al-Hakiem, M., & Abbas, S. (1987). Simple adaption of a radioimmunoassay kit for serum cortisol used to measure salivary cortisol. Clinical Chemistry, 33, 191-192

Anders, T., Sacher, E., Kream, J., Roffwarg, J., & Hellman, L. (1970). Behavioral state and plasma cortisol response in the human newborn. Pediatrics, 46, 532-537.

Anders, T. (1978). State and rhythmic processes. Journal of the Academy of Child Psychiatry, 17, 401-402.

Brown, E., Cole, J., Parker, S., Coulter, D., Corwin, M., & Aboagyne, K. (1989). Treatment of newborn narcotic abstinence (NA) fails to normalize infant behavior. Pediatric Research, 25, Abstract #56.

Casaer, P. (October, 1984). The neonatal evolution scale. Paper presented at the First International Conference on Neurohabilitation, Budapest, Hungary.

Casaer, P., Eggermont, E., Daniels, H., Devlieger, H., DeCock, P., Willekens, H., & Jaeken, J., (1982). The neurological assessment in the neonatal special care unit. Postgraduate Courses in Paediatrics, 55, 144-166.

Casaer, P. & Eggermont, E. (1985). Neonatal clinical neurological assessment. In Harel & Anastasiow (Eds.), The at-risk infant: Psycho/socio/medical aspects. Baltimore: Paul H. Brookes Publishing Co..

Coe, C. & Levine S. (1981). Normal responses to mother-infant separation in non-human primates. In D. Klein & J. Raplan (Eds.), Anxiety: New research and changing concepts (pp. 163-199). New York: Raven Press.

Coe, C., Wiener, S., Rosenburg, L., and Levine, S. (1985). Endocrine and immune responses to separation and maternal loss in nonhuman primates. In M. Reite & T. Filed (Eds.), The psychobiology of attachment (pp. 163-199). New York: Academic Press.

Dienstbier, R. (1989). Arousal and physiological toughness: Implications for mental and physical health. Psychological Review, 96, 84-100.

Emde, R., Harmon, R., Metcalf, D., Koenig, K., & Wagonfeld, S. (1971). Stress and neonatal sleep. Psychosomatic Medicine, 33, 491-497.

Francis, S., Walker, R., Raid-Fahmy, D., Hughes, D., Murphy, J., & Gray, O. (1987). Assessment of adrenocortical activity in term infants using salivary cortisol determinations. Journal of Pediatrics, 11, 129-133.

Gardner, J., & Karmel, B. (1983). Attention and arousal in preterm and full-term neonates. In T. Field & A. Sostek (Eds.), Infants born at risk: Behavior and development (pp. 69-98). New York: Grune & Stratton.

Gardner, J. & Karmel, B. (1984). Arousal effects on visual preferences in neonates. Developmental Psychology, 20, 374-377.

Gardner, J., Karmel, B., Magnano, C., Norton, K., & Brown, E. (1990). Neurobehavioral indicators of early brain insult in high-risk infants. Developmental Psychology, 26.

Gardner, J., Lewkowicz, D., Rose, S., & Karmel, B. (1986). Effects of visual and auditory stimulation on subsequent visual preferences in neonates. International Journal of Behavioral Development, 9, 251-263.

Gardner, J. & Turkewitz, G. (1982). The effects of arousal level on visual preferences in preterm infants. Infant Behavior and Development, 5, 369-385.

Gorbman, A., Dickoff, W. Vigna, S. Clark, N., & Ralph, C. (1983). Comparative Endocrinology. New York: John Wiley & Sons.

Griffith, D., Chasnoff, I., & Freier, C. (April, 1990). Developmental follow-up of cocaine exposed infants through age three years. Talk presented at the International Conference on Infant Studies, Montreal, Canada.

Gunnar, M. (1986). Human developmental psychoneuroendocrinology: a review of the research on endocrine responses to challenge and threat in infancy and childhood. In M. Lamb, A. Brown, & B. Rogogg (Eds.), Advances in developmental psychology, Vol. 4. Hillsdale, New Jersey: Lawrence Elbaum Associates.

Gunnar, M., Connors, J., & Isensee, J. (1989). Lack of stability in neonatal adrenocortical reactivity because of rapid habituation of the adrenocortical response. Developmental Psychobiology.

Gunnar, M., Connors, J., Isensee, J., & Wall, L. (1988). Adrenocortical activity and behavioral distress in human newborns. Developmental Psychology, 21, 297-310.

Gunnar, M., Fisch, R., Korsvik, S., & Donhowe, J. (1981). The effects of circumcision on serum cortisol and behavior. Psychoneuroendocrinology, 6, 269-275.

Gunnar, M., Fisch, R., & Malone, S. (1984). The effects of pacifying stimulus on behavioral and adrenocortical responses to circumcision. Journal of the American Academy of Child Psychiatry, 23, 34-38.

Gunnar, M., Fisch, R., & Malone, S. (1985). The psychobiology of stress and coping in the human neonate: Studies of adrenocortical responses to stress in the first week of life. In T. Field, P. McCabe, & N. Schneidermann, Stress and coping (pp. 179-196). Hillsdale, N.J.: Elbaum.

Gunnar, M., Hertsgaard, L., & Lehman, H. (April, 1989). Adrenocortical reactions to experiences: Correlates with emotional responding and temperament. Poster presented at the Society for Research in Child Development, Kansas City, MO.

Gunnar, M., Isensee, J. & Fust, L. (1987). Adrenocortical activity and the Brazelton Neonatal Assessment Scale: Moderating effects of the newborn's biomedical status. Child Development, 58, 1448-1458.

Gunnar, M., Malone, S., Vance, G., & Fisch, R. (1985). Coping with aversive stimulation in the neonatal period: Quiet sleep and plasma cortisol during recovery from circumcision. Child Development, 56, 824- 834.

Gunnar, M., Mangelsdorf, S., Larson, M., & Hertsgaard, L. (1989). Attachment, temperament, and adrenocortical activity in infancy: A study of psychoendocrine regulation. Developmental Psychology, 25, 355-363.

Hennessy, J., & Levine, S. (1979). Stress, arousal, and the pituitary-adrenal system: A psychoendocrine model. In J. Sprague, & A. Epstein (Eds.), Progress in psychobiological and physiological psychology. New York: Academic Press.

Hershenson, M. (1964). Visual discrimination in the newborn. Journal of Comparative and Physiological Psychology, 67, 326-336.

Hertsgaard, L., Wanner, M., Jodl, K., & Mason, L. (April, 1990). Group versus individual care: Effects on adrenocortical responses to brief maternal separation in human infants. Poster presented at the International Conference on Infant Studies, Montreal, Canada.

Holmes, D., Nagy, J., Slaymaker, F., Sosnowski, R., Prinz, S. & Pasternak, J. (1982). Early influences of prematurity, illness, and prolonged hospitalization on infant behavior. Developmental Psychology, 18, 744-750.

Ignatoff, E. & Field, T. (1979). Effects of nonnutritive sucking during tube feedings on the behavioral and clinical course of ICU preterm neonates. In L. Lipsitt & T. Field (Eds.), Infant behavior and development: Perinatal risk and newborn behavior. Norwood, N.J.: Ablex Publishing Corp.

Karmel, B., Gardner, J., & Magnano, C. (in press). Attention and arousal in early infancy. In M.J. Weiss and P.R. Zelazo (Eds.), Newborn attention: Biological constraints and the influence of experience. Norwood, N.J.: Ablex Publishing Corp.

Katona, F. (1983). An orienting diagnostic system in neonatal and infantile neurology. Acta Paediatrica Hungarica, 24, 299-314.

Katona, F. (1988). Developmental clinical neurology and neurorehabilitation in the secondary prevention of pre- and perinatal injuries of the brain. In P.M. Vietze and H.G. Vaughan (Eds.), Early identification of infants with developmental disabilities. Philadelphia: Saunders.

Kulski, J. & Hartmann, P. (1973). Changes in the concentration of cortisol in milk during different stages of human lactation. AJEBAK, 59, 769-778.

Kurtzberg, D., Vaughan, H., Daum, C., Grellong, B., Albin, S., & Rotkin, L. (1979). Neurobehavioral performance of low birthweight infants at 40 weeks conceptional age: Comparison with normal full term infants. Developmental Medicine and Child Neurology, 21, 590-607.

Lagercrantz, H. & Bistoletti, P. (1973). Catecholamine release in the newborn infant at birth. Pediatric Research, 11, 889-893.

Laudat, M., Cerdas, S., Fournier, D., Guiban, D., Guilhaume, & Luton, J. (1988). Salivary cortisol measurement: A practical approach to assess pituitary-adrenal function. Journal of Clinical Endocrinology and Metabolism, 66, 343-348.

Lawson, K., Daum, C. & Turkewitz, G. (1977). Environmental characteristics of the neonatal intensive-care unit. Child Development, 48, 1633-1639.

Lawson, K. & Turkewitz, G. (1980). Intersensory functions in newborns: Effect of sound on visual preferences. Child Development, 51, 1295-1298.

Levine, S. (1962). Plasma-free corticosteroid response to electric shock in rats stimulated in infancy, Science, 135, 795-796.

Levine, S., & Mullins, R. (1968). Hormones in infancy. In G. Newton & S. Levine, (1968). Early experience and behavior (pp. 168-197). Illinois: Charles C. Thomas.

Lewis, M. & Thomas, D. (1990). Cortisol release in infants in response to inoculation. Child Development, 61, 50-59.

Lewkowicz, D. & Turkewitz, G. (1981). Intersensory interaction in newborns: Modification of visual preferences following exposure to sound. Child Development, 52, 280-289.

Liggins, G. (1976). Adrenocortical-related maturational events in the fetus. American Journal of Obstetrics and Gynecology, 126, 931-941.

Liggins, G., & Howie, R. (1972). A controlled trial of antepartum glucocorticoid treatment for prevention of respiratory distress syndrome in premature infants. Pediatrics, 50, 515-519.

Magnano, C., Diamond, E., & Gardner, J. (1989). Use of salivary cortisol measurements in young infants: A note of caution. Child Development, 60, 1099-1101.

Mason, J. (1968). A review of psychoendocrine research on the pituitary-adrenal cortical system. Psychosomatic Medicine, 30, 576-608.

McGuire, I. & Turkewitz, G. (1979). Approach-withdrawal theory and the study of infant development. In M. Bortner (Ed.), Cognitive growth and development: Essays in memory of Herbert G. Birch. New York: Brunner/Mazel.

Mendoza, S., Smotherman, W., Miner, M., Kaplan, J., & Levine, S. (1978). Pituitary-adrenal response to separation in mother and infant squirrel monkeys. Developmental Psychobiology, 11, 169-175.

Murphy, B. (1982). Human fetal serum cortisol levels related to gestational age: Evidence of a midgestational fall and a steep late gestational rise, independent of sex or mode of delivery. American Journal of Obstetrics and Gynecology, 144, 276-282.

Neuspiel, D., Hamel, S., Hochberg, E., Green, J., & Campbell, D. (1989). Maternal Cocaine use and Infant Behavior Pediatric Research, 25, Abstract #84.

Nwosu, U., Wallach, E. Boggs, T., & Bongiovanni, A. (1975). Possible adrenocortical insufficiency in postmature neonates. American Journal of Obstetrics and Gynecology, 122, 969-974.

O'Donnell, K., Gingras, J., Hume, R., & Stranger, C. (1989). In utero cocaine exposure: Disruption of Fetal and Neonatal Behavior State organization. Pediatric Research, 25, Abstract #1132

Onishi, S., Miyazawa, G., Nishimura, Y., Sugiyama, S., Yamakawa, T., Inagaki, H., Katoh, T., Itoh, S., & Isobe, K. (1983). Postnatal development of circadian rhythm in serum cortisol levels in children. Pediatrics, 72, 399-404.

Price, D., Close, G., & Fielding, B. (1983). Age of appearance of circadian rhythm in salivary cortisol values in infancy. Archives of Disease in Childhood, 58, 454-456.

Procianoy, R., Cecin, S., & Pinheiro, C. (1983). Umbilical cord cortisol and prolactin levels in preterm infants: Relation to labor and delivery. Acta Paediatric Scand, 72, 713-716.

Riad-Fahmy, D., Read, G.F., Joyce, B.G., & Walker, R.F. (1981). Steroid immunoassays in endocrinology. In A. Vollar, A. Bartlett, & J.D. Bidwell (Eds.), Immunoassays for the 80's (p. 205-261). Baltimore: University Park Press.

Raid-Fahmy, D., Read, G., Walker, R., & Griffiths, K. (1982). Steroids in saliva for assessing endocrine function. Endocrine Reviews, 3, 367-395.

Regal, D. (1981). Development of critical flicker frequency in human infants. Vision Research, 21, 549-555.

Schanberg, S. & Field, T. (1987). Sensory deprivation stress and supplemental stimulation in the rat pup and preterm human infant. Child Development, 58, 1431-1447.

Schoenfeld, N., Leathem, J., & Rabii, J. (1980). Maturation of adrenal stress responsiveness in the rat. Neuroendocrinology, 31, 101-195.

Selye, H. (1950). Stress: The physiology and pathology of exposure to stress. Montreal: Acta Inc. Medical Publ..

Smotherman, W. (1983). Mother-infant interaction and modulation of pituitary-adrenal activity in rat pups after early stimulation. Developmental Psychobiology, 16, 169-176.

Stanton, M. Wallstrom, J., & Levine, S. (1987). Maternal contact inhibits pituitary-adrenal stress responses in preweanling rats. Developmental Psychobiology, 20, 131-145.

Sybulski, S., Goldsmith, W., & Maughan, G. (1975). Cortisol levels in the fetal scalp, maternal, and umbilical cord plasma. Obstetrics and Gynecology, 46, 268-271.

Sybulski, S. & Maughan, G. (1976). Relationship between cortisol levels in umbilical cord plasma and development of the respiratory distress syndrome in premature newborn infants. American Journal of Obstetrics and Gynecology, 125, 239-243.

Tennes, K., & Carter, D. (1973). Plasma cortisol levels and behavioral states in early infancy. Psychosomatic Medicine, 35, 121-128.

Tennes, K., Downey, K., & Vernadakis, A. (1977). Urinary cortisol excretion rates and anxiety in normal one-year-old infants. Psychosomatic Medicine, 39, 178-187.

Tennes, K. & Vernadakis, A. (1977). Cortisol excretion levels in one-year-old infants. Journal of Clinical Endocrinology and Metabolism, 44, 175-179.

Turkewitz, G., Gardner, J. & Lewkowicz, D. (1984). Sensory/perceptual functioning during early infancy: The implications of a quantitative basis for responding. In G. Greenberg & E. Tobach (Eds.), Behavioral evolution and integrative levels. Hillsdale, N.J.: Erlbaum.

Turkewitz, G., Lewkowicz, D., & Gardner, J. (1983). Determinants of infant perception. In J. Rosenblatt, R. Hinde, C. Beer, & M. Busnel (Eds.), Advances in the study of behavior (Vol. 13). New York: Academic.

Vining, R. McGinley, R., Maksvytis, J., & Ho, K. (1983). Salivary cortisol: A better measure of adrenal cortical function than serum cortisol. Annal of Clinical Biochemistry, 20, 329-335.

Zurbrugg, R. (1976). Hypothalamic-pituitary-adrenocortical regulation. Monographs in Pediatrics, 7, 12-21.