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EMOTION REGULATION EFFECTS OF INFANT-DIRECTED SINGING: EVIDENCE FROM INFANTS AND CAREGIVERS

by

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A thesis submitted in conformity with the requirements

for the degree of Master of Arts

Graduate Department of Psychology

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Emotion regulation effects of infant-directed singing: Evidence from infants and caregivers Master of Arts, 1999 Tali Shenfield Graduate Department of Psychology University of Toronto

Abstract

The present study explored the utility of salivary cortisol to index emotional correlates of maternal singing to infants. Saliva samples from mother and infant were collected immediately before the singing episode and 10 min after it ended. Mothers (N=31) sang songs of their choice to their 5- to 7-months-old infants for 10-min. A control group of mothers (N=30), who remained seated alone in a quiet room, provided saliva samples at similar times. The singing and non-singing mothers did not differ in pre- to post-test cortisol changes, with both groups showing a significant decrease in cortisol levels. The change in infants' cortisol values did not exceed chance levels. When the videotapes of singing episodes were coded for maternal sensitivity or responsiveness to infant signals, maternal sensitivity was found to be significantly correlated with infants' cortisol changes but not those of mothers. Specifically, more sensitive interactions tended to elevate infants' cortisol levels, and less sensitive interaction tended to depress them.

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Introduction

The establishment of consistent and positive interactions with a caregiver early in life is thought to provide the basis for healthy social and emotional development. Throughout the world, caregivers interact vocally with preverbal infants by means of baby talk, also known as motherese (for a review, see Fernald, 1991), and by means of singing (for a review, see Trehub & Trainor, 1998). In both modes, the goal is to modulate infants attention, arousal, and affect. In various laboratory studies, infants have shown attentional and affective responsiveness to maternal speech (Cooper & Aslin, 1990; Fernald & Kuhl, 1987) and singing (Trainor, 1996; Trehub & Henderson, 1994). One factor that may attenuate the responsiveness to such stimuli is the typical use of audio recordings involving unfamiliar female voices (mothers of other children) rather than "live" performances by the infant's mother (as in Fernald & Kuhl, 1987; Trainor, 1996). In principle, infants' emotional responses could be assessed by means of autonomic measures. Accordingly, the primary goal of the present study was to explore infants' affective responses to their caregivers' sung performances by means of changes in salivary cortisol levels. A secondary goal was to explore the affective consequences of infant-directed singing on the singer herself by means of the same salivary cortisol measure.

Music Perception in Infancy

Recent research indicates that infants are surprisingly adult-like in their perception of musical patterns. Moreover, the practice of assessing infants' responsiveness to infantdirected speech and song by means of differential visual attention to a loudspeaker or sound source (e.g., Fernald & Kuhl, 1987; Trainor, 1996) or by means of infants' facial expressions (Fernald, 1993; Werker & McLeod, 1989) may obscure the full extent of infants' response

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(Trehub, 1993; Trehub, 1999). For example, infants typically encode melodies on the basis of global features like melodic contour (i. e., successive directional changes in pitch), which involve relations between tones rather than local features such as absolute pitch (Trehub, Bull, & Thorpe, 1984). Although contour is their dominant focus, infants can encode intervals (i. e., the exact pitch relations between two simultaneous or successive tones) if the tones are related by small-integer frequency ratios, such as octaves (12 semitones; 2:1 ratio), perfect fifths (7 semitones; 3:2 ratio), and perfect fourths (5 semitones; 4:3 ratio) rather than large-integer frequency ratios such as the tritone (6 semitones; 45: 32 ratio) (Schellenberg & Trehub, 1996). Furthermore, infants are also sensitive to aspects of scale structure, performing better on scales with unequal steps (e.g., Western and non-Western scales) than on those with equal steps (Trehub, Schellenberg, & Kamenetsky, 1999). With respect to temporal features, infants are sensitive to the rhythmic organization of tone sequences (Thorpe & Trehub, 1989; Thorpe, Trehub, Morrongiello, & Bull, 1988; Trehub & Thorpe, 1989) and to the phrase structure of long musical passages (Jusczyk & Krumhansl, 1993; Krumhansl & Jusczyk, 1990). The presence of these abilities early in life suggests the possibility of biological predispositions for processing certain aspects of musical input 4 (Trehub, 2000; Trehub & Trainor, 1993).

Early musical preferences. Because infants are capable of perceiving many subtleties of musical patterns, it is conceivable that they might prefer some types of music over others. In fact, infants exhibit attentional preferences for recordings of women singing to an infant over the same women singing without an infant audience (Trainor, 1996). The presumption is that infants respond to differences in the emotional expressiveness of these performances. Moreover, infants show greater affective responses to lullabies than to adult songs or play songs, and to mothers' singing than to fathers' singing (Trehub & Henderson, 1994). Infants also prefer higher- over lower-pitched versions of the same song by the same singer (Trainor & Zacharias, 1998). Nevertheless, the preferences in all cases have been very small, perhaps because of the aforementioned limitations of stimuli (audio recordings) and response measures (typically visual attention to the sound source).

Parallels with speech. There are a number of parallels between infant-directed (ID) music and speech. ID speech is generally characterized by high pitch, smooth and simple pitch contours, rhythmicity, slow tempo, slurred words, and repetitiveness (Fernald, 1984; Fernald & Simon, 1984; Stern, Spieker, & MacKain, 1982). In fact, these features contribute to the song-like quality of ID speech. Moreover, soothing ID utterances, with their low pitch, limited dynamic range, gentle tone, and falling pitch contours (Fernald, 1989; Fernald & Simon, 1984) have parallels with lullabies (Trehub & Schellenberg, 1995; Trehub & Trainor, 1998). Just as structural features of ID speech are present across cultures (Fernald et al., 1989), so are they evident in lullabies and play songs (Trehub, Unyk, & Trainor, 1993; Unyk, Trehub, Trainor, & Schellenberg, 1992). On the basis of the universality of several ID speech features (Fernald, 1991; Papousek, Bornstein, Nuzzo, Papousek, & Symmes, 1990), the observed parallels with non-human vocal signals (Fernald, 1992), and infants' preference for ID over adult-directed (AD) speech (Cooper & Aslin, 1990; Fernald, 1985; Fernald & Kuhl, 1987), Fernald (1992) proposed motherese as a biologically determined form of speciesspecific caretaking behavior.

The characteristic modifications of maternal prosody may serve to communicate emotional meaning as well as modulating attention and arousal in the preverbal infant (Fernald, 1989). Perhaps the prosody of infant-directed speech conveys emotional information in a characteristic way that is recognizable across cultures (Frick, 1985). Similarly, universal qualities in infant-directed songs may be suggestive of comparable functions of emotion and attention regulation (Trehub, Unyk & Trainor, 1993a).

Singing to infants: The performer. As is the case for speech, there is evidence that caregivers fine-tune their performances to suit the needs of their young listeners. For example, mothers' versions of the same song are noticeably different in their infant's presence or absence (Trehub, Unyk, & Trainor, 1993b). Mothers' and fathers' performances to infants are distinguishable from attempts to reproduce those performances in the infant's absence (Trehub et al., 1997). Typically, singers use a slower tempo and higher pitch level when singing directly to their infant, which results in performances that adults judge to be more loving (Trainor, 1996), emotionally engaging (Trehub et al., 1997). Moreover, caregivers' ID versions of songs exhibit minor perturbations of frequency and intensity that are linked to heightened emotionality (Trainor, Clark, Huntley, & Adams, 1997; Trehub & Trainor, 1998).

To some extent, mothers make subtle adjustments to the ability and needs of their audience. For example, (Bergeson & Trehub, 1999) found that mothers sang the same song at a higher pitch level for their infant than for their preschooler, but they articulated the words more clearly for their preschooler.

Although singers may alter their ID performances to accomplish specific caregiving goals, the modifications may reflect, in part, the singer's own emotional state. For example, aspects of infant's appearance may act as innate releasing stimuli (Alley, 1981; Lorenz,

1943), generating increased emotional engagement on the part of the caregiver and particular vocal consequences (Trehub & Trainor, 1998).

Singing to infants: The listener. When infants are presented with recordings of women's infant-present and infant-absent versions of the same song, they exhibit more sustained attention to the infant-present version (Trainor, 1996). Moreover, infants show more vocalization, movement, and self-focused attention during soothing performance of a song compared to playful performances of the same song (Rock, Trainor, & Addison, 1999). They also exhibit greater enjoyment while listening to lullabies than to play songs or adult songs and to women' ID singing than to men's ID singing (Trehub & Kamenetsky, in preparation).

Fernald (1992) suggests that higher pitch is one of the salient features that promotes preferential responding to ID speech. In fact, Trainor and Zacharias (1998) found that infants showed more sustained attention to higher-pitched than to lower pitched versions of the same songs by the same singers.

Emotion Communication in Music

<u>Music and emotion.</u> Traditionally, music is considered a medium for emotional communication and is associated with the expression and the induction of emotions (Dowling & Harwood, 1986). "Music can serve as a way of capturing feelings, knowledge of feelings, or knowledge about the form of feelings, communicating them from the performer or the creator to the listener..." (Gardner, 1993, p. 124).

Adults reliably identify the intended affective meaning of musical passages (Behrens & Green, 1993; Dowling & Harwood, 1986; Lundin, 1967; Radocy & Boyle, 1988). A number of features of Western tonal music have been associated with specific emotional

interpretations (Rigg, 1964; Gabrielsson & Juslin, 1996). For example, "serious" or "solemn" music is typically slow, low-pitched, with regular rhythms and consonant harmonies. "Sad" music is also slow and low-to-moderate in pitch, but is more likely to be in a minor mode (as opposed to major), and to include dissonance. By contrast, "happy" music tends to be fast, high-pitched, in the major mode, with moderate variations in timing, bright timbre, and little dissonance. "Exciting" music is fast, loud and may contain dissonance. There are indications that musical performances expressing happiness, sadness, anger, and fear are more transparent than those expressing more complex emotions because of their phylogenetic history and intrinsic relation to expression (Gabrielsson & Juslin, 1996).

Adults are sufficiently sensitive to nuances in musical performance so that they reliably identify appropriate, exaggerated, and neutral expressiveness,, basing their judgments on variations in tempo, vibrato, and timing (Kendall & Carterette,1990). In principle, listeners' ability to grasp the emotional interpretation of a musical piece should be enhanced when the composer is the performer. In fact, adults are highly accurate at identifying musicians' improvisations that express sadness, anger, and fear (Behrens & Green, 1993). Furthermore, sadness was identified most accurately when the improvisations were performed with violin or voice; anger was most readily identified when performed on timpani, and fear when performed on violin. Thus, timbre supplements other features of the performance in emotional communication. To some extent, however, emotional meaning in music is determined by cultural conventions (Gregory & Varney, 1996).

<u>Vocal expression of emotion.</u> Although Darwin (1872/1965) proposed an equal role for vocal and facial expression in the communication of emotion, research has focused largely on facial expression. Nevertheless, a number of investigators have identified cues in prosodic and paralinguistic aspects of speech. Listeners are highly accurate at identifying emotion in speech that has been electronically filtered to remove linguistic cues (Scherer, 1979, 1981). Presumably, the emotional state of the talker has noticeable acoustic consequences. Several cues are thought to be critical for the recognition of emotion. For example, high pitch level, wide pitch range, pitch variability, loud sound, and rapid tempo tend to be indicative of high levels of arousal; low levels of those parameters are associated with low arousal (Scherer, 1986). Moreover, specific prosodic contours (i.e., patterns of pitch and loudness over time) are thought to express specific emotional states (Williams & Stevens, 1972). For example, falling contours are associated with pleasantness and rising contours with surprise and fear (Scherer, 1974). Tone of voice or timbre, which is defined by spectral structure (i.e., amount of energy at different frequencies), is also implicated in the transmission of emotional content (Lieberman & Michaels, 1962), with positive emotions reflected in relatively few component frequencies (Scherer & Oshinsky, 1977) or greater energy in higher component frequencies (Frick, 1985).

According to Frick (1985) most prosodic means of expressing emotion in speech are not conventional but stem from the bodily movements, postures, and patterns of muscular tension associated with different emotions. Facial expression in particular alters vocal cues to affect by changing the shape of the vocal tract (Laver, 1980; Schrerer, 1986). For example, smiling tends to shorten the vocal tract raising the formant or resonant frequencies (Tartter, 1980; Tartter & Braun, 1994). Scherer (1986) contends on that changes in the somatic and autonomic nervous systems (SNS and ANS respectively) have distinctive influences on vocal production, with the SNS mainly responsible for patterns of muscular tension and ANS being implicated in respiration, mucus and saliva secretion, and muscle tonus. Moreover, these subsystems have mutual influences.

Prosodic and paralinguistic aspects of speech have distinct response profiles. Moderately intense sounds induce cardiac deceleration, an orienting response, in contrast to higher intensity signals, which induce cardiac acceleration, a defensive reaction (Berg, 1975). Moreover, stimuli with gradual onsets (i.e., rise times) elicit eye opening and orienting, whereas more abrupt onsets elicit eye closing and withdrawal (Kearsley, 1973). It is conceivable then, that similar mechanisms underlie infants' response to vocal music.

Measuring the emotional impact of music. Attempts to describe emotional responses are likely to involve physiological manifestations, subjective feelings, cognitive appraisal, and facial expressions (Izard, Kagan, & Zajonc, 1984; Plutchik & Kellerman, 1980; Scherer & Ekman, 1984). To date, most studies of emotion in music have involved emotional judgments or reports of subjective emotional experiences. These methods depend on accurate judgments or self-perceptions coupled with the ability for translation of these judgments or perceptions. It is surprising, then, that there has been little effort to assess emotional responses to music by physiological means. Instead, some researchers have asked participants to report noticeable physical reactions while listening to music (Goldstein, 1980; Panksepp, 1995; Pignatiello, Camp, Elder, & Rasar, 1989; Sloboda, 1991). Many people have reported "thrills" (a physical sensation of pleasurable "shivers" or "tingles" running from the nape of the neck down the spine). According to Goldstein (1980), "the typical stimulus that elicits a thrill is a confrontation with an emotional stirring situation or event, such as a natural scene of transcendent beauty, a magnificent work of art or drama, a musical passage, a poignant personal encounter" (p.127). Sloboda (1991) found that "peak" emotional experiences to music often included laughter, tears, and a lump in a throat in addition to "thrills". Moreover, Sloboda's (1991) listeners could locate the precise musical events giving rise to specific physical responses. For example, tears were most often evoked by passages containing sequences and appogiaturas, while shivers were most reliably induced by new or unexpected harmonies.

Although the aforementioned phenomena are in accord with popular conceptions of emotional reactions to music, they are, at best, indirect measures of physiological responses to music. By contrast, Pignatiello et al. (1989) measured heart rate, systolic and diastolic blood pressure, finger pulse, and respiration rate in adults after an exposure to music expressing elation, neutrality, or sadness. Music expressing elation and sadness produced bidirectional responses for heart rate and systolic blood pressure, indicating the specificity of physiological reactions to the intended emotional mood of the music.

In recent years there has been a revival of interest in music as an adjunct to the therapeutic process (e.g., Brodsky, 1989; Cook, 1986; Rider, 1985), with music thought to be capable of altering the function of immune and stress systems. For example, Barlett, Kaufman, and Smeltekop (1993) found that relaxing music produced favorable changes in the biochemical agents mediating the immune/stress response, such as an increase in interleukin-1 and a decrease in cortisol levels. Similarly, Field, Martinez, Nawrocki, Pickens, Fox, and Schanberg (1998) reported that short-term music listening altered electrophysiological and biochemical measures of depression in chronically depressed adolescents. In particular, Field et al. (1998) observed an attenuation of right frontal EEG activation and a decrease in cortisol levels.

In short, these findings indicate the potential of psychophysiological phenomena to supplement the usual self-reports of responses to music.

HPA Axis Activity as an Indicator of Psychological Processes

Physiological correlates of emotion. As early as 1890, William James described the role of bodily responses in emotion experience and suggested that different emotions are associated with unique patterns of physiological activity. The emergence of the two-factor theory of emotion (Schacter & Singer, 1962), which emphasized the role of cognition in emotion experience, supplanted the earlier view. Nevertheless, in the past decades the work of Ekman, Levenson, and colleagues (for a review, see Levenson, 1992) has renewed interest in James' original account. According to current views, the differentiation of emotion is evident at the level of central nervous system (CNS), the autonomic nervous system (ANS) and the endocrine system.

The development of neuroimaging techniques in recent years has facilitated the description of subtle neural dynamics related to various emotional states. For example, different forms of positive affect can be distinguished based on the extent to which the emotion is associated with approach behavior (Davidson, 1993). In contrast, the patterns of the ANS activity seem to be associated with broader categories of emotions. For instance, similar sympathetic reactions may occur in response to anger and aggression. Changes in the activity of the autonomic nervous system are necessary to provide metabolic support for actions characteristically associated with particular emotional states (Levenson, Ekman, & Friesen, 1990). Thus, autonomic responses are determined by prototypical action tendencies associated with the corresponding emotion. For example, in response to induced fear, the autonomic nervous system reacts by increased cardiovascular activity, increased blood

supply to skeletal muscles, as well as elevation in circulating levels of epinephrine and cortisol. These metabolic changes prepare the organism for the demands of the subsequent behavioral response. There is evidence for distinctive patterns of autonomic activity for negative emotions such as anger, fear, and disgust and, possibly, sadness (Ekman, Levenson, & Friesen, 1983; Levenson, Carstensen, Friesen, & Ekman, 1991; Levenson, Ekman, & Friesen, 1990). Although, relatively little is known about autonomic correlates of positive affect, a reliable difference has been found between manifestations of negative emotions and the positive emotion of happiness (Levenson, 1994). It has been suggested that positive emotions do not show a characteristic physiological pattern, because they are not associated with actions that require metabolic support (Levenson, 1988). There is some suggestion, however, that they have a normalizing function, acting as "undoers" of the autonomic activation produced by negative emotions (Fredrickson & Levenson, 1992).

In this model, the function of certain positive emotions would be to restore the organism to its pre-arousal state in a more efficient and rapid manner than would be the case if the negative emotions were allowed to run their natural course (Levenson, 1994, p. 256).

The endocrine system is intricately involved in communication between the CNS and ANS. Some hormones are produced in the brain and act on tissues throughout the body, while others are produced by the body organs and travel to the brain in the bloodstream. As with the ANS, the endocrine system responds to environmental changes in a more generalized manner than the CNS. As a result the endocrine system is often discussed as part of the ANS. One system that is responsible for the continuous, two-way feedback between the CNS and peripheral organs is a hypothalamic-pituitary-adrenal (HPA) axis. This complex appears to be especially sensitive to psychological stimulation, and may, therefore, be implicated in emotional reactions to music.

HPA axis: structure, function, and characteristics. The HPA axis is a complex neuroendocrine pathway that includes multiple endocrine glands and several different hormones. First, corticotropin-releasing hormone (CRH) is produced in the hypothalamus by CRH-secreting neurons, and released into a specialized bloodstream circuit that conveys it to the pituitary gland. In response to the release of CRH, the pituitary releases adrenocorticotropin hormone (ACTH) into the bloodstream, which in turn acts on the adrenal glands and triggers the production of cortisol. Cortisol is the main glucocorticoid hormone secreted by the adrenal glands that exerts a wide range of effects on various organs. It increases the rate and strength of heart contractions, sensitizes blood vessels to the actions of norepinephrine, and affects many metabolic functions. Furthermore, cortisol is a potent immunoregulator and antiinflammatory agent, which prevents the immune system from overreacting to injuries and external agents.

As an end product of HPA activity, cortisol can be used as an indicator of its function. Since the introduction of an effective method for measuring corticosteroids concentrations in saliva in the late 1970s, this approach has gained considerable popularity in psychobiological research. The simple and unobtrusive procedures for collecting saliva made this method suitable for research with all age groups, and for healthy as well as clinical populations. Moreover, because trained medical personnel are not required for sampling of saliva, the method is suitable for laboratory and natural settings.

It has become clear that cortisol concentrations in saliva reliably reflect hormonal plasma values (Riad-Fahmy, Read, Walker, & Griffiths, 1982; Vining & McGinley, 1987).

Once secreted into the blood stream, approximately 90% of the endogenous hormone is bound to the blood-borne carrier proteins, while only 5-10% of it circulates unbound. Only this latter fraction can pass through the secretory endpiece of the salivary glands due to its small molecular size and highly lipid-soluble composition, which enables the molecule to rapidly diffuse through the lipid-rich cell membrane via passive intracellular diffusion (Kirschbaum & Hellhammer, 1989). However, only the unbound fraction of the total cortisol is biologically active and can act on the target tissues, leading to a broad spectrum of physiological effects (for reviews see Bondy, 1985, Ekins, 1990; Munck, Guyre, & Holbrook, 1984). Correlations between salivary and plasma cortisol levels are high, ranging from $\underline{r} = 0.54$ to $\underline{r} = 0.97$, with most investigators reporting $\underline{r} \ge 0.90$ (e. g., Ferguson, Price, & Wallace, 1980; Francis, et al., 1987; Hiramatsu, 1981; Kahn, Rubinow, & Davis, 1988).

Throughout the day, cortisol is tonically secreted by the adrenal cortex in a pulsatile fashion with about 15 distinct pulses detectable within a 24-hour cycle. In addition to the pulses, it exhibits a clear-cut circadian profile characterized by a substantial burst in early morning hours and subsequent gradual decreases through the day with the lowest hormonal levels found around midnight (Kirschbaum & Hellhammer, 1989). This pattern of normal cortisol secretion is regulated by two negative feedback systems, a fast rate-sensitive and a delayed proportional control system (Brandenberger, Follenius, & Muzet, 1984; Fehm, Voight, Kummer, & Pfeiffer, 1979). Even though there is considerable day-to-day stability of the rhythm (Walker, Riad-Fahmy, & Read, 1978), early morning measures seem to be the most reliable, while afternoon and evening values show greater sensitivity to environmental influences.

The adrenocortical response is characterized by considerable interindividual variations in both baseline levels and response magnitude. Several factors are presumed to account for these individual differences. For example, high correlations of baseline and response levels of adrenocortical activity have been observed in monozygotic but not dizygotic twins (Kirschbaum & Hellhammer, 1993), implying considerable genetic influence of HPA function. Similarly, sex differences in cortisol response have been observed repeatedly. When baseline levels are similar, adult males normally release 1.5-2 fold more cortisol than females following psychological stress (Kirschbaum, Wust, & Hellhammer, 1992) even if the stress is only anticipated. Even male newborns exhibit greater increases in cortisol levels than do female newborns during a brief physical examination (Gunnar et al., 1989). The presence of this difference in the neonatal period suggests a physiological origin, but further research is needed to confirm this hypothesis. Repeated exposure to nicotine leads to chronically elevated levels of ACTH and/or cortisol, which results in reduced responsiveness of the axis (Kirschbaum, Wust, & Strasburger, 1992; Weidenfeld, Bodoff, Saphier, & Brenner, 1989). Thus, factors that contribute to interindividual variability must be controlled in studies that use adrenocortical response as the dependent variable.

Mother-infant attachment and HPA axis activity. The importance of the development of a strong socio-emotional bond between mothers and their infants has been well documented in both human and animal research (Ainsworth, 1979; Goldberg, 1993; Kraemer, 1992a; Ruppenthal, Arling, Harlow, Sackett, & Suomi, 1976). In research with non-human primates, socially-compromised infants showed an array of long-lasting negative consequences, including deficits in social behavior, sexual behavior, problem solving, responses to novelty and stress, and measures of emotional regulation (for a review, see Suomi, 1982). The implication is that, during early development, mothers play a regulatory role in a number of psychobiological systems (Hofer, 1987; Kraemer, 1992a/1992b), including the HPA axis, and that some forms of deprivation may permanently alter their function (Hill, McCormack, & Mason, 1973; Mason, 1979). For example, peer-reared rhesus monkeys failed to show an appropriate HPA stress response as compared to mother-reared counterparts (Clarke, 1993).

Maternal behavior and feelings, in turn, are highly influenced by their hormonal profile. Among human mothers, greater feelings of attachment to infants are associated with greater elevation in the estradiol to progesterone ratio (Fleming, Ruble, Krieger, & Wong, 1997) and greater elevation in cortisol during the postpartum period (Fleming et al., 1993; Fleming, Steiner, & Corter, 1997). Specifically, levels of cortisol on days 2-3 postpartum were positively correlated with mothers' attraction to infant-related cues, sensitivity to differences in infants' cry quality, ability to recognize their own infant based on body odor, and positive interaction with the infant. However, mothers' interaction and physical contact with their infants induce declines in circulating cortisol levels (Fleming, Steiner, Corter, Ruble, Krieger, & Wong, in preparation). Furthermore, mothers who reported greater attraction to their infants' odors showed larger decreases in cortisol levels after holding their infants than did mothers who were less attracted to their infants' odors. Thus, it appears that the HPA axis plays a major role in the regulation of maternal responses and attitudes toward newborns in the postpartum period.

<u>Cortisol as a marker of psychological changes.</u> The normal pattern of cortisol activity is altered continuously by a wide spectrum of external and internal factors, such as health status, medications, affective state, cognitive processes, physical exercise, pregnancy, and smoking. Mason (1968), reviewing more than 200 papers on the psychoendocrinology of the HPA axis, concluded that "psychological influences are among the most potent natural stimuli known to affect pituitary-adrenal cortical activity" (p. 595). Among the psychological variables with a potential for mediating HPA functioning, the stress response has been studied most extensively for its ability to override the feedback systems and enhance the frequency and amplitude of cortisol pulses (e.g., Kirschbaum & Hellhammer, 1994; Mason, 1968, Sharpley & McLean, 1992). This interest is understandable in view of the negative consequences of chronic cortisol hypersecretion on cognition, mood, and behavior.

There have been two main approaches to studying the physiological correlates of the stress response: (1) the observation of changes in selected physiological measures in response to experimentally induced stress and (2) the comparison of measures in groups with or without chronic stress (e.g., clinical patients, animals living in overcrowded cages, circumcision). On the whole, situational factors experienced as novel, unpredictable, or uncontrollable are associated with increased cortisol output. For example, the anticipation of public speaking is one stress manipulation that enhances salivary cortisol levels (Bassett, Marshall, & Spillane, 1987). Similarly, prolonged anxiety associated with the anticipation of surgery or other painful procedures has been shown to elevate cortisol levels (Ben-Aryeh, Roll , & Kahana , 1985; Manyande et al., 1995; Villani et al., 1996). Furthermore, university students' anticipation of examinations is consistently associated with increased cortisol secretion (Jones, Copolev, & Outch, 1986; Hellhammer et al., 1985).

Although the aforementioned changes in HPA axis activity were in the same direction, there was considerable variability in the magnitude of individual responses. Kirschbaum and Hellhammer (1989) suggest that the stressfulness of an event is determined not by the strength of noxious stimulation but by the extent to which it is perceived as unpleasant. Thus, adrenocortical responses to stress are mediated by emotional processing of stressful stimuli. For example, a film with disturbing content elicited enhanced cortisol levels when projected to the right hemisphere but decreased cortisol levels when projected to the left hemisphere (Wittling & Pfluger, 1990).

The adrenocortical response to stress is essential for survival, but chronically elevated cortisol levels can lead to deleterious changes in a number of vital systems. A brief stress response mobilizes energy resources that are necessary for action and serves a homeostatic function by mediating other stress-sensitive mechanisms including the catecolamine system, the endogenous opiate system, and the immune system (Munk, Guyre, & Holbrook, 1984). Chronically high cortisol levels, however, may result in decreased energy, loss of concentration, and increased depressive affect (Wolkowitz & Weingartner, 1988). In addition, long-term elevation of cortisol may interfere with the consolidation and storage of memories and inhibit REM sleep (Gunnar, Malone, Vance, & Fisch, 1985).

In contrast to the extensive literature on the reaction to stress in the HPA axis, there is relatively little research on the consequences of pleasurable stimulation in this system. One reason is methodological: prior to the introduction of salivary cortisol measurements, reactions to pleasant stimuli were often overridden by negative reactions to blood sampling procedures. Nevertheless, there is suggestive evidence that pleasurable stimulation lowers cortisol levels, an effect opposite to the stress response. For example, shifting food-deprived rats from low- to high-rate reinforcement leads to reductions of cortisol concentrations (Levine & Coover, 1976). In human adults, the induction of deep hypnotic trance is associated with a substantial decrease in cortisol levels (Sachar, Fishman, & Mason, 1965).

The observed effect is thought to arise from the tension-relieving or euphoric aspects of deep hypnosis. Similar reductions have been found in response to Disney nature films (Handlon et al., 1962).

More recent studies with salivary cortisol measures have revealed similar findings. For example, massaging infants alleviated anxiety and lowered cortisol level in the elderly (Field, Hernandez, Quintini, Schanberg, & Kuhn, 1998). Drops of hormonal concentrations in hypertensive adults followed 8 weeks of relaxation training (McGrady, Yonker, Tan, Fine, & Woerner, 1981). Depressed adolescents who listened briefly to music sessions showed reduced adrenocortical activity compared to similarly depressed adolescents who were simply instructed to relax (Field, Martinez, Nawrocki, Pickens, Fox, & Schanberg, 1998). In view of the limited research in this domain, more research is needed to identify the stimulus characteristics that can influence HPA axis activity.

HPA axis activity and emotion regulation in infancy. Adrenocortical measures have been widely used in studies of early emotional development (for a review, see Stansbury & Gunnar, 1994). As is the case with adults, the HPA response in children is thought to arise from the perception of a stimulus as noxious, novel, uncertain, or incongruent (Hennessy & Levine, 1979). When novel experiences occur in a pleasant emotional context, they may lead to reductions rather than elevations in cortisol relative to basal levels. Thus, a first-time swimming experience produced reductions in cortisol levels in infants 6-13 months of age (Hertsgaard, Gunnar, Larson, Brodersen, & Lehman, 1992). The critical aspect of uncertainty is not the novelty of an event, but rather "uncertainty about how to control or influence the stressful event and one's behavioral and emotion reactions to it. Thus, uncertainty about the effectiveness of emotion regulation processes may be the critical mediating factor" (Stansbury & Gunnar, 1994, p.123). From this perspective, the stress response is more sensitive to the perception of control than to the actual circumstances of control.

In general, negative affect in children is associated with elevations in cortisol, as is the case with adults, but there are occasional dissociations of behavioral and physiological responses (Levine, Wiener, Coe, Bayart, & Hayashi, 1987). When newborns are repeatedly exposed to psychological stressors, for example, their HPA response habituates, even though their crying continues (Gunnar, Connors, & Isensee, 1989). Although the effect of control on children's HPA activity has not been addressed directly, there are indications that developing emotional control may mediate their stress response. For example, crying in response to blood-sampling procedures was found at 4 years of age, when cortisol levels were low, however, crying was substantially less for 6-year-olds whose adrenocortical response reached its peak (Gunnar, Marvinney, Isensee, & Fisch, 1989). One suggestion is that children who attempt to restrict overt signs of distress but cannot maintain complete self-control exhibit dissociations between their overt stress responses and adrenocortical responses (McCoy & Masters, 1985). In line with this explanation, older children who had low levels of crying and of cortisol were able to generate and verbalize strategies for controlling their behavior. Thus, children's belief that they could cope with the situation played a role in alleviating their physiological stress response.

There are suggestions that HPA activity is influenced by distraction or attention regulation (Thompson, 1994). For example, reduced cortisol levels from a first-time swimming experience in infancy may be attributable to the attentional consequences of this activity. (Hertsgaard et al., 1992). Similarly, massage therapy during a 6-week period had positive behavioral and physiological effects in infants of depressed mothers, including reductions in crying and cortisol levels (Field et al., 1996). Moreover, listening to a maternal heartbeat, but not a Japanese drum, following a heelstick procedure had soothing effect on newborns, as reflected in stabilized salivary cortisol levels (Kurihara et al., 1996). Presumably, social support increases infants' perceived control over their environment and their own internal state. Thus, a playful babysitter increases infants' ability to cope with a 30min separation from mother, as reflected in a reduced stress response. (Gunnar, Larson, Hertsgaard, Harris, & Brodersen, 1992).

The aforementioned studies imply that the internal processes and behaviors that play a role in emotion regulation are often accompanied by modifications of HPA activity. Although, the nature of this relation is unclear, what is clear is that the HPA system can be used as a marker of emotional and attentional processes in infancy. Measures of HPA activity are of particular interest when verbal communication is impossible (as with prelinguistic infants) and for identifying potential dissociations between behavioral and physiological responses.

Factors mediating HPA activity in infants. As is the case with adults, numerous factors contribute to interindividual variability in adrenocortical responsiveness, including temperament, experiential history, developmental level, current resources and strategies, and physiological differences in neuroendocrine and associated systems (for a review, see Gunnar, 1990). For example, a powerful stimulus can elicit an HPA response in some but not all participants, so that a significant between subjects variability is common. Nevertheless, some factors have predictable effects on HPA function. For example, infant boys have stronger adrenocortical stress responses than do infant girls, a phenomenon that parallels adult male/female differences (Davis & Emory, 1995). Adult-like circadian rhythms are

evident, as reflected in burst in adrenocortical activity in the early morning followed by a gradual decrease throughout the day by 3 - 6 months of age (Lewis & Thomas, 1990; Spangler, 1991). The development of this circadian rhythm seems to be correlated with sleeping and feeding patterns, the 24-hour sleep-wake cycle, and the mother's adrenocortical activity. Activities such as morning naps and car trips result in dramatic drops in cortisol concentrations, independent of whether infants sleep or not during the car trip (Larson, Gunnar, & Hertsgaard, 1991).

In a longitudinal study of HPA activity from 2 to 15 months of age, Gunnar et al. (1996) observed two pronounced shifts in the system. Between 2 and 4 months of age, the originally labile HPA system undergoes a dampening of adrenocortical responsivity to external stressors, stabilizing at approximately 6 months of age. Between 6 and 15 months of age, adrenocortical responsivity declines further so that infants show null adrenocortical reactions to physical examinations and inoculations. Relations between behavioral distress and HPA response also undergo developmental change. At 2 months of age, distress during inoculations is closely associated with cortisol response, but between 6 and 15 months of age, infants show increased behavioral distress together with a decline of adrenocortical reactivity. It is not clear, however, whether the apparent dissociation of behavioral and physiological reactions to stress reflects a general developmental trend, habituation, or increased ability to cope with pain. Comparable declines in HPA response have been reported for infants between 2 and 6 months of (Lewis & Ramsay, 1995; Lewis & Thomas, 1990).

In short, because of the sensitivity of HPA to psychological stimuli, it is potentially suitable for assessing reactions to aversive and pleasant stimuli on the part of preverbal

infants. In general, unpredictable, negative, and threatening situations as well as anxious and depressive mood states are associated with elevations in cortisol level. By contrast, pleasant and relaxing stimuli that engage attention are associated with reductions in cortisol concentration. Although causal relations between emotion and HPA activity remain unclear, it seems that adrenocortical activation can influence and be influenced by emotions and their correlates.

Emotion in the context of singing to infants. Emotions are thought to underlie the primary motivational system for human behavior, whose major function is the organization of traits and dimensions of personality (Izard, 1971; Izard, 1991; Malatesta, 1990; Tomkins, 1962). According to this view, relations between emotion and behavior develop early in life and remain relatively stable over time (Plutchik, 1980). Darwin (1872/1965) posited that emotional expression serves two primary adaptive functions from early infancy: social communication and the regulation of emotional experiences. In this context, attentional behaviors are thought to function as "emotional receptors" that create an interface between an antecedent event and an individual (Saarni, 1993). In this light, infants' attention to maternal singing is suggestive of emotional reaction to such stimulation. It could be argued, then, that singing to infants in the course of caregiving creates a framework for positive emotional interaction, which facilitates infant-caregiver communication and affective regulation. Because positive mood lowers the threshold for social interaction (Izard, 1993), routine singing to infants should foster social bonds between infant and caregiver, potentially shaping the infant's personality in the direction of extraversion. Thus, such early experiences may have long-lasting influences on children's personal and social development.

Affectively charged vocalizations are thought to be jointly determined by internal states ("push effects") and external situational demands ("pull effects") (Pittam & Scherer, 1993). Thus, it is possible that caregivers' inclination to sing to infants is determined, in part, by their personal emotional agenda, a process that elicits bidirectional affective responses. There is evidence, for example, that singing to infants can be an outlet for the singer's personal emotions (Trehub & Trainor, 1998). A non-comprehending listener can provide a context for the expression of thoughts and feelings, which, in turn, regulate caregiver's affective state. Thus, internal motivation on the part of the caregiver acts as a "push" force, whereas positive feedback from infants' attention acts as a "pull" force.

The purpose of the present study was to explore the emotional consequences of singing for preverbal infants and their caregivers. In principle, singing to preverbal infants might induce positive affective reactions in both infant and caregiver, regulating emotion, arousal, and attention. Salivary cortisol concentrations were measured before and after the singing interaction to index emotional arousal in each member of the infant-caregiver dyad. Because the target population was healthy and no stress-inducing procedures were involved, it was difficult to predict the direction of change in cortisol concentrations. If singing had an optimizing effect on arousal, it could lead to increases or decreases in cortisol concentrations, depending on the initial state. Infants were videotaped during the singing episode to facilitate the identification of possible behavioral correlates of adrenocortical responses.

Method

Participants

Participants in the experimental group included 31 middle-class mothers ($\underline{M} = 32$ years) and their 5- to 7- month-old infants. The age of infant participants was influenced by

several factors. First, HPA axis activity undergoes dramatic changes from 2 to 4 months of age, stabilizing toward 6 months of age with the emergence of a regular circadian rhythm in cortisol production (Lewis & Thomas, 1990; Spangler, 1991). Second, infants in this age range can maintain an awake-alert state for extended periods of time, as required for the experimental procedure. Third, these infants would have had a considerable experience with vocalization in general and maternal singing in particular (Trehub & Trainor, 1998). The control group included 30 middle-class mothers (M = 32 years) with young children (3 - 6 years of age); however, their children did not participate in the present experiment. All mothers and infants were in good health and medication free, according to maternal report. Moreover, all mothers were non-smokers.

Apparatus

Testing was conducted in a quiet room. Mothers in the experimental group were seated on a couch facing their infant, who was seated in a baby chair. A Sony CCD--TR500 video carnera was focused on the infant. Saliva samples from mothers were obtained by means of salivette kits (Sarstedt Canada Inc., St. Laurent, Quebec), which included a syringe, an insert tube, and a 1-in cotton roll. With infants a sterile 6-in cotton dental roll (Richmond Dental) replaced the 1-in roll for safety reasons. The salivettes were frozen at -20°C for subsequent assay. On the day of the assay, the salivettes were thawed and centrifuged for 15 min, 1000g at 4°C. As a result, the saliva passed through the hole in the bottom of the insert tube and into the syringe. Salivary cortisol levels were estimated with solid-phase ¹² I radioimmunoassays (Coat-a-Count, Diagnostic Products Corp., Los Angeles, CA). Samples were assayed in duplicate and discrepant duplicates were reassayed.

Procedure

Test sessions were scheduled between 9:30 and 11:30 a.m., because it is known that in the morning cortisol levels are less sensitive to environmental factors than in the afternoon (Walker, Riad-Fahmy, & Read, 1978). Mothers were instructed to refrain from any oral activity, including eating, drinking, chewing gum, or brushing teeth, for the hour preceding their scheduled appointment. They were asked to restrict their infants' feeding and drinking during the same period. Mothers were also encouraged to avoid singing and music listening on the morning of the test. Finally, they were asked to inform the researcher of any changes in their own or their infants' health status in the period between the scheduling of the appointment and the test date.

Mothers were told about our interest in internal (physiological) and external (behavioral) effects of singing to infants. For the experimental group, the mother and infant proceeded into the test room. Control group mothers proceeded into another room, where they were watching their children being tested in another study on a TV monitor. Mothers were shown how to collect saliva from their infant (where applicable) and from themselves, and pre-test (baseline) saliva samples were taken. Mothers removed a small cotton roll from the tube provided and chewed it for 1 min, replacing it in the insert tube, placing it in the syringe, and capping it. To collect saliva from infants, mothers placed a 6-in cotton roll in their infant's mouth, so that the infant could suck it or chew on it for 1 min. The saliva-saturated portion of the cotton roll was clipped off, placed in the insert tube of the salivette, and capped. Subsequently, experimental mothers were asked to sing or hum one or more songs of their choice to the infant, continuing their singing for 10 min. Mothers were assured that the quality of their singing was not of interest, and that they should sing casually, as they

do at home. In addition, mothers were asked to refrain from touching the infant during this 10-min period. After providing the instructions, the experimenter left the room and returned 10 min later. Singing episodes were videotaped for subsequent coding. Following the 10-min singing episode, mothers completed a background questionnaire unrelated to the present study, until 20 min from the onset of singing had elapsed, after which a second saliva sample (post-test) was obtained from mother and infant. Control mothers were cued to gather their own saliva samples at comparable periods, that is, two samples separated by 20 min.

Results

Mothers were excluded from the final data set because of insufficient saliva for salivary cortisol analysis (<u>n</u> = 7) or pre-test cortisol levels greater than two standard deviations above the mean (<u>n</u> =1). Pre-test, or baseline, cortisol levels were significantly greater for singing mothers (<u>M</u> = 5.90; <u>SD</u> = 2.53) than for non-singing mothers (<u>M</u> = 4.12; <u>SD</u> = 2.22), <u>t</u> (1, 52) = 2.71, <u>p</u> < 0.01. Changes from pre- to post-test levels of salivary cortisol did not differ between singing mothers (<u>M</u> = -1.03; <u>SD</u> = 1.53) and non-singing mothers (<u>M</u> = -0.90; <u>SD</u> = 2.11), <u>t</u> (1, 52) = 0.26, ns. In both cases, however, significant reductions in cortisol levels were evident, <u>t</u> (1, 26) = 3.74, <u>p</u> < 0.001 for singing mothers, and <u>t</u> (1, 24) = 2.14, <u>p</u> < 0.04 for non-singing mothers (see Figure 1).

Infants were excluded from the final data set because of insufficient saliva for laboratory analysis (n = 5). Changes from pre- to post-test levels (M = -0.03; SD = 0.29) did not significantly exceed the value expected by chance (0), \underline{t} (1, 25) = 0.11, ns.

To evaluate the possibility that cortisol responses of mothers or infants were associated with maternal singers' sensitivity to changes in infant state, videotapes of the singing sessions were coded by two independent observers who used somewhat different coding systems. Participants who ignored the experimenter's instructions to avoid physical contact with the infant (n = 10) were excluded from this analysis. One observer viewed the first, third, and fifth minutes

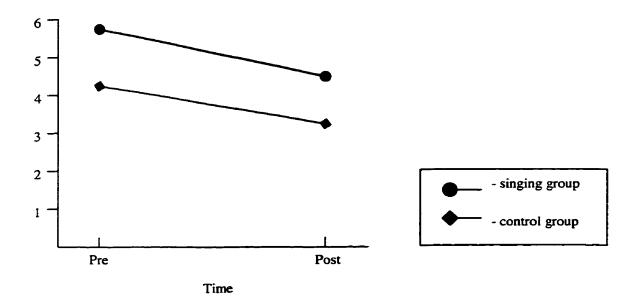


Figure 1. Pre- and Post-Test Values of Cortisol for Mothers

of each test session recording all instances of infant distress or loss of interest exceeding 5 s in duration. This observer also coded mothers' behavior for 5 s following these instances of distress or loss of interest. Specifically, he coded whether or not mother responded by changing her song or performing style to gain infant's attention.

This system, which involved fixed sampling period yielded different numbers of distress episodes for each dyad. A second observer also coded the first, third, and fifth minutes of the test session. In some instances, however, this observer continued coding infant and maternal behavior until 10 distress episodes were accumulated (or the test session ended). Otherwise the same coding procedure was employed. Thus, the second scheme equated dyads on the number of distress episodes rather than a specific sampling period (3 min). The proportion of distress episodes on which mothers responded yielded a score of maternal sensitivity. Thus, the two coding systems yielded two ratings for each mother (see Table 1). These slightly different ratings by different observers, one employing a fixed sampling period and the other a fixed number of distress episodes, were highly correlated, $\underline{r} = .86$, $\underline{p} < 0.0001$. The second rating system was used for all further analyses (although both systems yielded comparable results).

Maternal sensitivity scores were unrelated to changes in maternal cortisol levels, \underline{r} (22) = .26, ns. However, maternal sensitivity scores were positively correlated with changes in infant

Table 1

Two Ratings of Infants' Distress and Maternal Sensitivity

No	Incidents of distress		Interventions		Sensitivity	
	Rater 1	Rater 2	Rater 1	Rater 2	Rater 1	Rater 2
1	4	10	4	9	1.00	.90
2	6	10	3	7	.50	.70
3	15	10	7	5	.47	.50
4	6	10	1	1	.17	.10
5	5	10	1	2	.20	.20
6	10	10	7	8	.70	.80
7	7	10	2	5	.29	.50
8	8	10	0	1	0	.10
9	4	9	2	2	.50	.22
10	3	7	1	2	.33	.28
11	4	10	0	1	0	.10
12	3	6	3	5	1.00	.80
13	8	10	4	5	.50	.50
14	5	6	4	5	.80	.83
15	5	10	1	2	.20	.20
16	2	8	1	5	.50	.63
17	4	4	3	4	.75	1.00
18	4	10	1	2	.25	.20
19	• 8	10	4	8	.50	.80
20	6	10	4	4	.66	.40
21	12	10	10	8	.83	.83

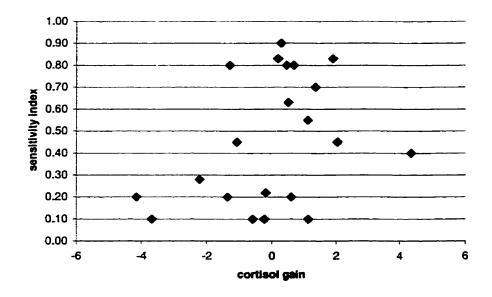


Figure 2. Relationship Between Maternal Sensitivity And Infant Pre- to Post-Test Change of Cortisol Values

Discussion

The purpose of the present study was to explore the utility of salivary cortisol as a marker of emotional response on the part of healthy unstressed maternal singers and infant listeners. Contrary to the prediction, salivary cortisol changes were not sensitive to the singing manipulation. It remains to be determined whether salivary cortisol changes would be evident when baseline hormonal levels of mothers or infants are elevated because of natural or experimental stressors. A number of factors have been shown to normalize elevated cortisol levels in infants such as maternal heartbeat sounds following a heelstick procedure (Kurihara et al., 1996). Perhaps singing, which is known to soothe distressed infants (Trehub & Trainor, 1998), would generate reductions in the elevated cortisol levels that result from separation distress (Gunnar et al., 1989; Hertsgaard et al., 1995).

As can be seen in Figure 1, singing and non-singing mothers differed in their initial and final levels of salivary cortisol. It is possible that the performing on videotape in an unfamiliar setting was mildly stressful, as is mere anticipation of mental arithmetic tasks (Bassett, Marshall, & Spillane, 1987; Sharpley & McLean, 1992). By contrast, mothers in the control group had no demands placed on them and were free to relax. Alternatively, mothers in the experimental group might have had generally higher cortisol levels, due to the increased vigilance associated with caring for young infants.

Singing and non-singing mothers showed reductions of cortisol levels over the 20min period. The reduction may be attributable to normal diurnal variations in adrenocortical activity (Kirschbaum & Hellhammer, 1989). Alternatively, both groups may have experienced a reduction in initial stress levels as a result of being away from their usual responsibilities. Regardless, the change in cortisol levels could not be attributed to singing itself.

Observations of the videotaped sessions revealed differences in mothers' degree of involvement with their infants and their sensitivity to changes in infant state. Although mothers who were more attuned to their infants may have experienced greater enjoyment of the interaction, this situation did not translate to differential cortisol changes. Instead, maternal sensitivity was associated with changes in infant cortisol levels. The more responsive the mother, the greater increase in infants' cortisol levels (see Figure 2). Thus, infants of the most sensitive mothers showed increases in cortisol levels, while infants of the least sensitive mothers showed decreases in cortisol levels. All changes were small, however, and did not exceed normal diurnal fluctuations of this hormone.

There is no definitive interpretation of this pattern of changes in infant cortisol levels. One can speculate, however, that the strategies used by sensitive mothers to keep their infants content -- clapping, swinging their body, adding high-pitched interjections (e.g., hooray!) -were arousing for infants, and were, therefore, reflected in activation of infants' adrenocortical system. General arousal associated with positive or negative emotions can lead to activation of various physiological parameters including the HPA axis (DeJong, van Mourik, & Schellekens, 1973; Mason, 1968; Schachter, 1964; Sternbach, 1966). At times, this may result in contradictory psychological and physiological responses. For example, listening to preferred, relaxing music leads to reductions in state anxiety but to increases in autonomic and muscular activity (Davis & Thaut, 1989). The lack of consistent relations between psychological and physiological measures are consistent with Berlyne's (1971) theory of arousal and hedonic value, which holds that moderate increases from low arousal levels and decreases from very high arousal levels are perceived as pleasant. In the present study, then, infants of sensitive mothers may have experienced a moderate increase in arousal, which enhanced their enjoyment of the interaction. By contrast, infants of less sensitive mothers showed a decline in cortisol levels, perhaps because of their disengagement from the interaction.

In conclusion, the major finding to emerge from the present study was that maternal sensitivity during a singing episode had measurable consequences on salivary cortisol levels for non-distressed infants. It is likely that the arousing consequences of maternal behavior affected the direction and magnitude of infants' adrenocortical response. Although mothers reported that the singing episode was pleasant, it did not affect their cortisol levels. However, arousing actions of high-sensitivity mothers seemed to influence their infants' hormonal levels. Coding of infant affect over the course of the test session will reveal whether infant's behavioral changes parallel their cortisol changes. Perhaps increases in infant cortisol levels reflect increases in attention and engagement. If this notion is corroborated by behavioral data, it would provide support for the idea that moderate increases from normal arousal levels are associated with positive affect and pleasure.

Limitations of salivary cortisol measures in general and the present measure in particular became apparent in the course of the study. Instead of a single post-test measure time (20 min from the onset of singing in this study) multiple post-test would reveal the unfolding of the hormonal response. Such multiple samples would be feasible for mothers but not for infants. Second, if effects of stress-reduction are sought, then it would seem reasonable to experimentally elevate stress levels in the case of mothers and perhaps to wait for naturally occurring distress episodes in the case of infants. Finally, behavioral observations in conjunction with physiological measures would provide a richer framework

for interpreting observed changes in hormonal levels.

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