Effects of Auditory Stimulation in Low and High Light Conditions on Behavioral and State Organization in Preterm Infants

By

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Abstract

The purpose of this study was to examine the effects of multi-modal stimulation (differing amounts of light and vocal stimulation) on preterm infants' behavioral and state organization. Specifically, we looked at the effects that supplemental vocal stimulation (taped female voice) had when varied in amount of exposure (three times a day versus once a day) and when provided in different lighting conditions ("typical illumination" versus " decreased illumination). Forty infants were placed in one of four groups: Standard Illumination/High Voice (SIHV), Standard Illumination /Low Voice (SILV), Decreased Illumination/High Voice (DIHV) and Decreased Illumination/Low Voice (DILV). Infants receiving standard illumination were exposed to the vocal stimulus in standard NICU lighting conditions (approximately 20 lux), whereas infants in the "low" lighting conditions were exposed to the stimulus in darkened conditions (approximately 3 lux). Infants receiving high vocal stimulation listened to a taped female voice three times a day, whereas infants receiving low vocal stimulation were exposed to the voice only once a day. Each infant received 10 minutes of exposure per session over five consecutive days. Infants were videotaped in their incubator for 10 minutes before, during, and after the stimulus exposure (total of 30 minutes) for each day. The videotapes were then scored on the infant's frequency of stress related behaviors and self-regulatory behaviors before, during, and after the stimulus for each day. Results indicated that both lighting levels and vocal stimulation altered preterm infants' stress and self-regulatory behaviors, and that these effects were dependent on both the day and the stimulus condition the infant was in. In addition, the vocal stimulation and lighting levels had an effect on the states that infants exhibited during and after the presentation of stimulation. These results suggest that the occurrence of different types and amounts of stimulation have an effect on behavioral organization of the preterm infant, and these effects are highly dependent on both history and context in which this stimulation is presented

Dedication

To my parents, Pirjo and John Strunk,

Thanks for the great 9 months, 3 day start,

And for being there every day after.

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Chapter 1

Introduction

The effects of environmental stimulation on neurobehavioral, sensory, perceptual and attentional regulation in both human and animal infants has received increasing attention in recent years. This issue becomes particularly important when addressing the needs of the early preterm human infant who, due to preterm status, ends up in the Neonatal Intensive Care Unit (NICU). Preterm infancy is typically defined as birth prior to 38 weeks gestation. However, concerns about the impact of sensory stimulation on the preterm infant tend to be primarily focused on infants born earlier than that cut off point. Unless there are specific medical complications, infants born later in gestation (for example, 36 – 37 weeks) often do not spend any significant time in the hospital or Neonatal Intensive Care Unit. These infants are typically assessed by medical staff to be sufficiently developed to cope with the challenges of adaptation to life outside the womb, much like a newborn full-term infant. While this may not always be accurate, most infants born later in the ‘preterm’ period can be discharged home and cared for by their parents with few complications. Therefore, concerns about sensory stimulation and the preterm infants tend to focus on the infants who are challenged by greater physiological problems (either due to the relative amount of development of the infant or specific medical complications) and spend time in the NICU. With regard to this population of preterm infants, investigators have questioned how the sensory environment, including the types and amount of stimulation, potentially impact the development of neural and behavioral organization of the preterm infant. Since the early preterm infant is removed from a rich yet limited sensory environment and required to develop with an altered sensory experience, significant debate has focused on what type of stimuli should be made available to the preterm infant (supplemental stimulation) and what stimuli should be limited or eliminated.

One area of focus has been on auditory stimulation. Since evidence suggests infants can hear during the last trimester of pregnancy and that the maternal voice is prevalent during that time, there is considerable debate about the use of supplemental vocal stimulation to foster appropriate development in preterm infants. However, research on this topic has been relatively scarce and poorly controlled. In addition, few studies have explored the effect of multimodal stimulation on the presentation of supplemental auditory stimulation. Thus, the focus of this study is to examine the effects of vocal stimulation on the regulatory behaviors of preterm infants, and to explore the moderating effects of the presence of light during vocal stimulation. The subsequent literature review discusses the effects of sensory stimulation during human fetal development, evidence from comparative work that suggests that altered sensory stimulation effects perceptual development, the sensory environment of the Neonatal Intensive Care Unit, and a discussion of some of the known effects of vocal and visual stimulation on the preterm infant.

Literature Review

I. Historical and Theoretical Perspectives

Historically, the early preterm infants were viewed as inadequate, underdeveloped full-term infants, with nervous systems that were too immature to be greatly affected by the environment or their experiences. However, it is now felt that the preterm infant is an organism well-equipped and adapted for the uterine environment (Als, 1982). Indeed, the uterine environment not only provides the fetus with a basic life support structure but is by its very nature a developmental context with limited sensory experiences. That is, the event of early birth thrusts the preterm infant into a vastly different environment than the typical prenatal environment, one in which the infant is ill-prepared to cope with even basic survival tasks. Instead of the 'normal' uterine environment, the preterm infant who ends up in the NICU depends on medical technology and support staff to provide not only life support but also to assist the infant in regulating his/her physiological/behavioral state and environment. With medical advancements that provide the ability to save infants as young as 22 weeks gestation, concerns about the impact of environmental stimulation take on greater importance.

As a consequence of these medical advancements, an ongoing controversy has emerged regarding what kind of care is appropriate for preterm infants. Korner (1990) notes that, historically, this debate has swung from both ends of a continuum. On one end, proponents argue that preterm infants need to be protected from the high and varied levels of sensory stimulation that are available in the Neonatal Intensive Care Unit (NICU). Conversely, other investigators suggest that providing stimulation might help promote appropriate physiological and behavioral development. Indeed, a wealth of comparative literature suggests that the perinatal sensory environment impacts the behavioral and perceptual development of neonates (Lickliter, 2000, for review). Given the apparent influence that the environment has on the developing fetus and infant, the debate concerns itself with the following questions:

A. What stimuli are most helpful/harmful for the preterm infant?

B. When do specific stimuli have the most negative/positive impact on the neonate?

C. How do we organize the sensory environment to minimize the negative impact of sensory stimulation while providing the infant with sensory stimulation that supports the process of sensory and perceptual development, as well as physiological and behavioral organization?

While various viewpoints exist regarding what type of sensory intervention should be provided to preterm infants, these positions are rarely guided by developmental theory or solid empirical research (Lickliter, 2000). In recent years, a shift has occurred in the study of development, with views on development moving away from simple cause-effect, or linear relationships, to viewing development as a dynamic, non-linear, multidetermined, experience-dependent process (Fisher & Biddell, 1998; Ford & Lerner, 1992; Oyama, 1985). From this developmental systems view, increasingly complex structures and functions are the result of coactions in the environment-organism system

(Gottlieb, 1991; Lickliter, 2000). This perspective is useful because it attempts to take into account the multiple variables and contexts that combine to support the emergence of phenotypic traits and characters.

The development of sensory systems in birds and mammals begins prenatally, although each sensory system does not develop simultaneously. Rather, the sequence of the development of sensory systems follows an invariant pattern, beginning with tactile and proceeding to vestibular, chemical, auditory, and visual system function (Alberts, 1984; Gottlieb, 1971). The neurodevelopment of the sensory systems was once viewed as a strictly maturational process in which developing neurological structures ultimately led to the orderly onset of function of the sensory systems. However, Gottlieb (1971) suggested that the development of the sensory systems is the result of a bi-directional relationship between structure and function. According to this view, the development of specific phenotypic features is not structurally determined (via genetic action), nor is it the result of specific environmental features. Rather, phenotypic features are the result of complex interactions of the genetic and biological structure of the organism, and the various environmental factors experienced during development (Lickliter & Banker, 1994). Thus, a developmental systems perspective (see Gottlieb, 1991) suggests that the outcome for specific sensory systems is the result of the influence of various factors or variables within the system (both internal and external), and the relationship of the factors or variables with other components of the larger developmental milieu.

II. Sensory Stimulation and the Uterine Environment

The *in utero* environment of mammalian infants is both a sheltered and rich complex sensory environment. The uterine environment provides a host of differential functions, providing protection from environmental stimulation, temperature control, nutritional resources, and state and hormone control (Als, 1995; Wittenberg, 1990). Fetuses receive tactile stimulation from amniotic fluid, vestibular and tactile stimulation from both their own movements and those of their mother, information regarding light-dark cycles and rhythmicity from the physiological changes in the mother as a result of her sleep-wake cycles, and auditory and tactile stimulation from the mother's vocalizations, heart beat, and borborygmi from digestion (Fifer & Moon, 1988; Reppert & Weaver, 1988; Smotherman & Robinson, 1988). This rich yet protected sensory environment has the potential to progressively influence the course of the sensory and perceptual development of the fetus and neonate.

The auditory system in the human fetus appears to be developed enough by 23 to 25 weeks gestation to result in fetal physiological reactivity to sound (Graven, 2000). Background noise levels in the human uterus have been measured over just 50 dB at lower frequencies, with occasional bursts over 70 dB (Abrams & Gerhardt, 2000). The auditory environment of the fetus contains sounds generated by the audible maternal pulse, the borborygmi from digestions, and the attenuated low-frequency sounds (<250 Hz) of the maternal voice and that of outside noise, filtered via the maternal tissue and amniotic fluid (Fifer & Moon, 1988; Hepper, Scott, & Shaidullah, 1993). In addition, changes in the relative amount of amniotic fluid and in the head position of the fetus may influence auditory development, tied to specialization of auditory neural development (Fifer & Moon, 1988; Previc, 1991).

Although the sound environment in the uterus is attenuated, sounds such as maternal voice are rich in the frequencies displayed, and the lower frequencies appear to be discriminated above the rest of the in utero background noise (Gerhardt & Abrams, 2000). The amount of external sound attenuation appears to be related to the origin of the sound source, the pressure variations within the uterus, and the type of sound stimulus (Abrams, Gerhardt, & Peters, 1995). Regardless of this sound attenuation, however, evidence suggests that even sound stimulation at lower frequencies can result in changes in fetal activity. For example, Lecanuet, Granier-Deferre, and Busnel (1995) conducted a historical survey of fetal responsiveness to startling and nonstartling auditory stimulation. These researchers found that presentation of either vibratory or air-coupled auditory stimulation (pure tones at or above 100 dB) to fetuses during the last trimester resulted in greater fetal movement (startle responses) and heart rate accelerations. Additional research indicates that sudden bursts of sound can cause changes in blood pressure, heart rate, respiration, and glucose consumption (Graven, 2000). Additional animal studies have indicated that exposure to intense low-frequency sounds can damage hair cells in the auditory system as well as result in nerve damage (Hall, 2000).

What remains unclear at this time is how the presence of the maternal voice affects the developing fetus in the prenatal environment. As noted previously, maternal voice is a stimulus that is present and readily discriminated in utero. Fifer and Moon (1988) note that maternal vocal input co-occurs with the diaphragm muscle movement and may result in greater amounts of kinesthetic and tactile stimulation associated with those sounds. This co-occurring, multimodal, and potentially arousing form of stimulation may promote greater responsiveness to maternal vocal stimulation than other prenatal sounds experienced by the infant.

Prenatal exposure to maternal speech is believed to play some role in newborn orientation to vocal stimulation and to ultimate language development (Hepper, Scott, & Shahidullah, 1993; Mehler, Jusczyk, Lambertz, Halsted, Bertoncini, & Amiel-Tison, 1988; Moon & Fifer, 2000) Several studies demonstrate that human newborns exhibit changes in attentional behavior (looking times, orientation, non-nutritive sucking) to auditory stimulation encountered prenatally. For example, DeCasper and Fifer (1980) found that when neonates are presented with recordings of the mother's voice versus that of another woman, they appeared to prefer the maternal vocal stimulation. In addition, human fetuses appear to be able to learn distinct features of speech. When human newborns were presented with either acoustic passages read to them *in utero* by their mother or passages that their mother had not recited, the newborns preferred to hear the familiar passages (DeCasper & Spence, 1986). The development of auditory perceptual abilities of the fetus likely underlies the prenatal auditory learning demonstrated in newborns. For example, Fifer and Moon (1995) showed that human fetuses also exhibit physiological changes to their mother's voice. Mothers in this study were directed to either speak using adult-directed speech, to whisper, or to remain silent alternating over a series of trials. During this time, fetal heart rate responses were recorded. Fifer and Moon found that fetuses exhibited significant heart rate decreases during times of maternal speech. Alternately, there was no difference in heart rate when the mothers were silent (baseline) or whispered, which has similar vestibular/tactile conditions as speech. Additional studies have indicated that the human fetus (at 35 weeks) is able to discriminate among different phonemes (Hepper et al., 1993) and that newborn infants can even discriminate between a change in the order of consonant presentation (Moon, 1985).

Fifer and Moon (1995) suggest that the perinatal response to mother's voice is indicative of a model for the effects of early auditory experience on the developing brain of the fetus. In essence, the maternal voice is a consistent, naturally occurring stimulus which is salient during a highly sensitive time period for the neurodevelopment of the fetus, and that there are immediate and enduring effects of this stimulation on the developing auditory system and the newborn's later perceptual and attentional preferences. For newborn infants, the maternal voice acts as a modulator of arousal, working to alert, soothe, calm, or maintain infant state. Newborn infants, both humans and other animals, exhibit differential responses to and proximity-seeking behaviors to maternal stimuli (such as odor, touch, visual stimulation, and maternal voice). According to Hofer (1988), these behaviors suggest that a network of maternal 'regulators' is already well established at birth for mammals. Prenatal exposure to maternal auditory stimulation (specifically maternal voice) may therefore contribute to the initial processes involved in developing reciprocal social interaction between the mother and infant, and (in the case of maternal voice) may facilitate the use of auditory stimulation in emotional and social regulation in the months following birth.

III. Sensory Stimulation and Early Birth

Taken together, these studies suggest that the mother's voice has the potential to shape the fetuses' early sensory and physiological functioning (DeCasper & Fifer, 1980; DeCasper & Spence, 1986; Fifer & Moon, 1988, 1995; Moon, 1985). It seems plausible that the interruption of normal fetal development via early birth might result in overall changes or deficits in preterm infants' emerging sensory and perceptual abilities. As the type of sensory stimulation changes, the relationship between that stimulus presentation and physiological and behavioral reactivity to those stimuli may also change. Thus, when an infant is born prematurely, it is no longer possible to experience the 'normal' uterine world that full-term infants experience during the course of late gestation. Preterm infants are exposed to a rich, varied, stimulation-loaded world at a time when critical neurological and physiological systems are at fragile states of development (Als, 1995). While both the uterine and terrestrial environments are rich in sensory stimulation, the concern surrounds the interaction of the type, variability, and amount of stimulation that is unique to the terrestrial world. In addition, depending on when the infant is born, neurological systems, hormone systems, cardiovascular systems, and respiratory systems can all be in different, and potentially vulnerable, points of development. This early exposure to *atypical* types and amounts of sensory stimulation, coupled with the loss of other reliable species-typical types of stimulation, compounded by the loss of state regulating stimuli available *in utero*, could leave the preterm infant with multiple perceptual and state regulatory tasks that it is challenged to perform effectively. As a result, Als (1995) has suggested that these early multisensory experiences in preterm infants may result in alternate paths of neural development.

Als (1982) formulated a theoretical model for infant development that focuses on the continuous interaction both internal to the organism and between the organism and the external environment. These include the autonomic system, the motor system, the state-organizational system, the attention/interaction system, and the self-regulatory (balancing) system. According to Als, a dynamic interplay exists between the environment and the human fetal brain, in which these different subsystems function and develop independently yet are continuously interacting with each subsystem and the environment (Als, 1982, 1995). Thus, various subsystems exist side by side, interacting with each other, but still differ in both their development and the effect that each subsystem has on the development of subsequent subsystems (Als, 1982). This perspective complements a developmental systems perspective, in that physiological and behavioral organization and development depend on multiple, dynamic factors (both internal and external to the organism) that co-act to result in organization, structure, and functional relationships.

The needs of the preterm infant cannot be readily determined by the standards for either typical fetal development or by the abilities a full-term infant displays. As noted previously, the preterm infant is an organism who is removed from the intrauterine environment for which he/she was well adapted, and emerges into an environment in which he/she is required to manage physiological and environmental demands. The impact of this disruption is complicated by when during the developmental process the infant is born. While medical technology can help assist the infant in basic physiological functions such as thermoregulation, respiration, and feeding, these standard care routines are not designed to assist the infant in its overall behavioral organization. Indeed, while medical routines are effective for the infants' immediate survival, they often can leave in their wake other chronic problems. For example, many preterm infants experience significant problems in bottle-feeding, especially after a long period of ventilator support. This problem may be due to level of illness, level of development, or to the development of oral aversion coupled with disassociation between hunger/feeding and the suck/swallow response. Thus, Als suggests that atypical experiences could result in altered developmental of neurological and behavioral organization.

Various forms of sensory stimulation appear to have different effects on the organization, structure and functional integration of neural systems and the development of behavioral coordination in preterm infants. The process of early neurological organization might be influenced by both the timing of certain types of stimulation (when in the developmental process they take place) and the nature of the stimulation (how much or what type). For example, it appears that preterm human infants experience earlier than normal visual stimulation, altered types and amounts of auditory stimulation, and reduced levels of vestibular stimulation. While minimal visual stimulation may not result in large changes in neurological structure and function, altering the intensity, timing, and amount of visual stimulation during the perinatal period *may* have profound effects (Als, 1995; Korner, 1990; Lickliter, 2000). Carefully analyzing the types, intensity, and timing of sensory stimulation has been addressed via comparative work and may be important to consider when examining the effects of unusually early stimulation on preterm infants' development.

IV. Differential Effects of Early Sensory Stimulation: Comparative Research

Some clarification of how early sensory experience affects the developing fetus and infant can been revealed via careful examination of comparative developmental research. The prenatal environment of both avian and mammalian species provides a wide variety of tactile, vestibular, chemical, and auditory sensory information (DeCasper & Fifer, 1980; Gottlieb, 1971; Hepper, Scott, & Shahidullah, 1993; Lickliter, 1993, 1995; Smotherman & Robinson, 1986). When considering the development of specific sensory modalities, it is important to examine the effects of the type, amount, and timing of these various forms of stimulation on the functioning of each sensory system. In addition, given the sequential onset of development of the specific sensory modalities prenatally, it is also important to examine the differential effects of the development of earlier developing sensory systems on later developing sensory modalities and vice-versa. This is more readily accomplished in animal studies, where researchers can introduce ‘prematurity’ in a controlled environment and systematically vary the types and amounts of stimulation provided.

Turkewitz and Kenny (1982) suggest that the limitations induced by the invariant temporal sequence of sensory development may actually be adaptive for the young organism, as these limitations in sensory functioning reduce the type and amount of information to which the organism is exposed at any given time in the process of early development. From this view, the immaturity of later-developing sensory systems limits the amount of sensory information that the organism receives, reducing and regulating competition between the differing sensory modalities (Lickliter, 1993; Lickliter & Banker, 1994). Therefore, limitations on sensory experience (resulting from the relative immaturity of the later-developing sensory modalities) provide structure and organization to the process of sensory development. In turn, these limitations help determine early intersensory relationships and buffer the embryo and fetus during sensitive periods of sensory development (Turkewitz & Kenny, 1982, 1985).

Within the context of this hypothesis, the early presentation of sensory stimulation associated with a later developing sensory system might not only impact the development of that system, but also interfere with the ongoing development of the earlier developing sensory systems. This might be especially true if that sensory modality is at a sensitive stage of development, when key structural and neural pathways are being laid down. According to this view, preterm stimulation of a later developing sensory system potentially alters the development of earlier sensory systems because the stimulation of the later-developing system results in a 'taking over' of neural space typically appropriated by the earlier developing sensory system (Radell & Gottlieb, 1992; Turkewitz & Kenny, 1982). A weaker form of this hypothesis suggests that the effects of premature stimulation are not necessarily permanent, but rather are the result of attentional difficulties caused by concurrent stimulation to two or more modalities (Radell & Gottlieb, 1992). The implication of this view is that a functional recovery can be made, and that the stimulation does not necessarily impact overall neurological structure, or if it does that structural change does not have a lasting impact on functional capacity.

A number of studies have examined the relationship between both early sensory stimulation and sensory deprivation on subsequent sensory and perceptual development in both altrical and precocial animal infants. This body of work has found a relationship between the type, timing, and amount of stimulation provided and subsequent patterns of early perceptual and behavioral development. For example, Kenny and Turkewitz (1986) examined the effects of early visual simulation on the development of homing behavior in rat pups. Homing behavior, or orientation to and seeking of the nest site, in normal rat pups proceeds along a fairly consistent developmental path. Rat pups exhibit a gradual increase in the amount of homing behavior they exhibit, peaking around 14-15 days after birth, which is followed by a gradual decline in this behavior. Since home orientation is initially related to thermal and olfactory cues, Kenny and Turkewitz hypothesized that exposing rat pups to early visual stimulation via early opening of the pups' eyelids should result in interference with the normal development and behavior mediated by the olfactory and thermal systems and a decrease in overall homing behavior. They found that visually exposed pups did not exhibit the typical decrease in homing behavior found in normal pups, and that this maintenance of homing behavior appeared to be the result of a visual dominance overriding typical reliance on olfactory cues. Thus, the unusually early exposure to visual stimulation appeared to influence the normal reliance on olfactory cues and competed with those cues in mediating home orientation in the period following birth.

Lickliter (1990a) found similar results when examining the effects of atypical early visual stimulation on the species-typical auditory and visual preferences of bobwhite quail chicks. In this study, Lickliter presented embryos with patterned visual stimulation during the 24-36 hours prior to hatching, and then tested these chicks at 24 or 48 hours post-hatching. Unmanipulated bobwhite quail chicks exhibit species-specific preferences for the auditory maternal call alone (Lickliter & Virkar, 1989). However, chicks exposed to early visual stimulation did not respond to the auditory call alone. The early-exposed chicks required both the auditory stimulus (maternal call) and a visual stimulus (stuffed maternal hen) to exhibit a maternal preference. This suggests that the early exposure to visual stimulation, prior to normal onset of visual functioning, can result in an acceleration in intersensory functioning. Lickliter (1990b) subsequently found that the mechanism for the apparent acceleration of intersensory functioning was related to an acceleration in visual functioning rather than a reduction in auditory responsiveness. As a result of early visual experience, the chicks' overall behavior pattern became organized to use the available visual information (Lickliter, 1990b). Chicks appeared more visually oriented in early postnatal development when compared to control chicks. In addition, while the prenatally exposed chicks did respond to the auditory information alone, there was a significantly faster decline in responsiveness to the maternal auditory call than that seen in normally reared chicks. These results indicate that the effects of early visual stimulation can exert specific effects on both the visual modality and other earlier developing sensory modalities (e.g. audition).

Along with the timing of stimulation, another potential factor that may influence early perceptual development is the amount of stimulation that the organism encounters during its development. Radell & Gottlieb (1992) suggest that the amount of stimulation that young organisms normally encounter within specific modalities may be optimal for normal perceptual development. Slight alterations within this range (such as that employed in Lickliter 1990a) may accelerate or facilitate perceptual development. However, changes in the amount of sensory stimulation provided that exceed the optimal range can result in functional deficits in normal patterns of perceptual development (Radell & Gottlieb, 1992; Sleigh & Lickliter, 1995,1996, 1997).

For example, Lickliter and Stoumbos (1991) examined the effects of providing augmented prenatal auditory stimulation on postnatal auditory and visual functioning in bobwhite quail chicks. This procedure, called experiential enhancement, provides the embryo more stimulation within a currently developing modality than the organism normally receives. Lickliter and Stoumbos found that presenting augmented species-typical auditory stimulation prenatally to bobwhite quail chicks results in no apparent differences in auditory responsiveness at 12 and 24 hours post-hatching. Chicks exposed to this augmented auditory stimulation did, however, exhibit an accelerated pattern of visual responsiveness, again highlighting the strong linkage within the modalities and the dynamic nature of intersensory perception.

Sleigh and Lickliter (1997) examined the effects of substantially augmented prenatal auditory stimulation on the auditory and visual functioning of bobwhite quail chicks. In contrast to the accelerative effects of slightly augmented auditory stimulation found in Lickliter and Stoumbos, Sleigh and Lickliter (1995, 1997) found that by greatly increasing the overall amount of prenatal visual/auditory stimulation (regardless of type of stimulus), chicks exhibit interference in the emergence of normal intersensory perceptual development. Specifically, embryos exposed to greatly augmented prenatal auditory stimulation (40 min/hr) continued to respond to maternal auditory cues into later stages of postnatal development and failed to respond to maternal visual cues at the ages when normally reared chicks typically do. In addition, these chicks exhibited an overall higher level of arousal and higher mortality rates than chicks prenatally exposed to either no or slightly (10 min/hr) augmented auditory stimulation. Sleigh and Lickliter (1995) found similar effects with augmented prenatal visual stimulation, suggesting that substantially augmented amounts of stimulation (regardless of type) can lead to overall interference in normal patterns of intersensory functioning.

V. Effects of Sensory Stimulation: Human Research

Overall, the data from comparative work suggests that the early sensory environment, specifically visual and auditory stimulation, affects species typical behavioral organization. The implication of this line of research suggests that the sensory environment of the preterm infant might affect behavioral and physiological organization as well. Given that the auditory and visual systems of human infants are at sensitive periods of development during the last trimester of pregnancy, altered sensory stimulation patterns might have an impact on developing physiological and behavioral organization.

Researchers examining the issue of sensory stimulation and the human preterm infant have relied upon a number of different measures to determine the impact of sensory stimulation on physiological or behavioral organization. Some studies have focused on overall outcome measures, such as weight gain (e.g Miller, White, Whitman, O'Callaghan, & Maxwell, 1995) or performance on infant adjustment scales (e.g Segall, 1970). Other studies have examined physiological measures such as heart rate or oxygen saturation as indicators of physiological and neurological organization (e.g. Miller et al., 1995; Standley & Moore, 1995). Finally, a number of studies have focused on behavioral organization, with measures such as infant state and behaviors indicative of stress and self regulation.

According to Ingersoll and Thoman (1999), an infants' state is a reflection of the degree to which the central nervous system is organized, and is the context in which other behavioral displays are manifest. Therefore, state both influences and reflects behavioral organization, and is reflective of physiological organization as well. According to Stratton (1982, pg 18), "…almost every neonatal function is influenced in its form, strength, and frequency, by the state during which it is examined". State as a construct encapsulates sleep/wake patterns of activity, and these sleep/wake patterns reflect activity in several areas of the central nervous system. State measures are thought to tap into arousal modulation, behavioral display, attention and orientation to stimulation, and systemwide coping of stress (Als, 1995b; Ingersoll & Thoman, 1999).

Measures of infant state are commonly used in the assessment of preterm infant physiological organization for both clinical and research purposes. For example Als (1995b) has helped develop specific assessment tools based largely on the assessment of infant state, as well as indications of physiological functioning such as heart rate and respiration, and behavioral displays of stress and self regulation. These tools provide an overall assessment of the quality of system organization, rather than any specific quantity of behaviors or amount of specific states displayed. Therefore, these measures can be used clinically to determine overall what the infant's strengths and challenges are in terms of coping with their environment (these tools will be discussed in greater detail forthcoming). Given this discussion, it is important to consider the methods used in assessing the impact of visual and auditory stimulation on preterm infants. While treated as independent, each method used attempts to provide some indication of physiological and behavioral organization.

VI. Visual and Auditory Stimulation in the Neonatal Intensive Care Unit

The concept and impact of visual stimulation on the preterm infant has been approached in several different ways. One approach has been to examine the effects of presentation of specific patterned stimulation on preterm infants (e.g. Gardner & Turkewiz, 1982; Marshall-Baker, Lickliter, & Cooper, 1998). Studies like these have typically focused on factors such as the impact of visual frequency and contrast on preterm infants' visual attention, arousal, behavioral organization, or other outcomes, and questions have focused on how infants react to the presentation of specific *types* of visual stimulation. Another approach has looked at the impact that overall *illumination* has on preterm infants. The focus of this line of research has not been on the supplemental presentation of *specific* visual stimulation, but rather what is the impact of the presence or absence of light (illumination level) in the environment on the preterm infant. This approach is typically more concerned with the impact of the opportunity for visual processing on factors such behavioral organization, establishment of sleep-wake states, and processing of other stimulation while in lighted conditions (e.g Blackburn & Patternson, 1991; Glotzbach, Rowlett, Edgar, Moffat, & Ariagno, 1993). For purposes of this literature review, visual stimulation was examined from the perspective of the overall level or amount of light (illumination).

A. Lighting in the NICU

Recent work has begun to analyze the preterm infants' visual experience in the NICU, and has focused on how different types and amounts of visual stimulation affect their overall functioning. Lotas (1992) notes that standard lighting in the NICU can range from 24-90 foot-candles with 60 foot-candles is considered appropriate for most medical procedures, and Brandon (2000) cites references from multiple studies which report light levels between 240-1400 lux. A footcandle is a unit of illumination equal to one lumen per square foot, and is measured off of reflective surfaces. Lux is a metric unit of illumination equal to one lumen per square meter, and is a measurement of ambient illumination.

According to Lotas (1992), these light levels tend to be continuously high, with the highest levels of light used with the youngest and sickest infants, such as during phototherapy (300-400 footcandles). In the late 1970's and throughout the 1980's, concerns about the effects of light stimulation in the NICU grew (Lotas, 1992). As a result, many nurseries began to dim lights, provide covers for incubators to shield preterm infants from the lights, and explored the use of cycled lighting on preterm infants' outcomes and state regulation. Earlier animal work had implicated the presence of high levels of light (400-550 footcandles) on the development of retinopathy of prematurity (ROP) and other retinal damage (Sykes, Robinson, Waxler & Kuwahara, 1981). Work with human preterm infants suggests that high lighting may be implicated in ROP, but the effects of more moderate lighting levels remains unclear (Glass, Avery, & Kolinjavadi, 1985; Lotas, 1992). Several studies suggest that continuous lighting patterns can have an aversive effect on sleep/awake states and circadian rhythms (Glotzbach,et al., 1993; Lotas, 1992). For example, Glotzbach and colleagues (1993) examined the light levels and changes in those levels at the bedsides of preterm infants. The ambient lighting at these sites did not exhibit any rhythmic variation, and seldom changed unless it grew higher due to additional lighting being added for medical tasks. These consistently high levels of ambient lighting found around preterm infants can potentially place them at risk for difficulties establishing appropriate and responsive circadian rhythms (Glotzbach et al., 1993). The stress associated with high lighting may result in the interruption of sleep states, and these infants may also experience difficulty attaining quiet sleep. Quiet sleep is typically thought of as being indicative of better neurological and physiological organization (Als, 1982)

Research by Blackburn and Patterson (1991) and Miller, White, Whitman, O'Callaghan, and Maxwell (1995) suggest that use of cycled lighting may have some positive effects on the preterm infant. Blackburn and Patterson (1991) examined the effect of cycled lighting in the NICU. They dimmed light levels for an average of 10 hours in the evening and night hours (no reported measure of lux or footcandle levels provided for either condition), and examined the activity levels, heart rate and respiratory rates of the preterm infants in the cycled versus noncycled lighting conditions. Preterm infants (29-33 gestational age) in the cycled light condition had more stable heart rates and activity levels during the evening/night hours, and exhibited overall stablized (slightly lowered and more regular) heart rates than the noncycled group. More stabilized, slightly lowered heart rates and organized activity levels indicates an overall decreased level of arousal, greater stability in physiological and behavioral subsystems, and potentially an initial establishment of circadian rhythms (Blackburn & Patterson, 1991). Indeed, Als (1982, 1983) suggests that regular, slightly lowered heart rates and organized patterns of activity indicates a more adapted organization and coordination of systems and subsystems. Blackburn and Patterson also noted that cycled lighting had another unforeseen effect: noise levels in the NICU decreased. Dimming lights resulted in lower conversational levels between staff, and a decrease in overall NICU activity. The suggestion is that lowered lighting not only decreases the visual stimulation presented to preterm infants, but also results in a by-product of lowered sound stimulation, which may also have an effect on preterm infants' state organization.

Miller and colleagues (1995) also examined the effects of using cycled light with preterm infants (less than 37 weeks gestational age, 28 weeks ± 2). They found that infants in the cycled light condition exhibited significantly more weight gain, spent fewer days on the ventilator, exhibited greater motor coordination, and were better able to handle oral feedings. They suggested that cycled lighting might assist infants to regulate state and stress levels. This suggests that the occurrence of early and augmented exposure to visual stimulation may interfere with optimal behavioral and physiological organization. By decreasing the demands on the infant to self-regulate behavior and state through lowering and cycling light levels, the infant might conserve energy and direct resources to other regulatory tasks. A brief discussion of infant state, self regulation and stress reactivity is forthcoming.

B. Sound in the NICU

Sound levels in the NICU have been found to range from 50 -90, and sometimes as high as120, decibels (dbs) (Lotas, 1992). Decibels are the measuring unit for sound energy. To put these measures in perspective, normal adult conversation is around 60 decibels, a busy street corner is around 80 decibels, and loud thunder around 120 decibels. Much of this ambient noise is associated with the equipment used in the NICU, voices of staff, and alarms of equipment. In addition, this noise is often constant, with little variation or fluctuation (except for the unpredictability of alarms), and not contingent with any other sensory stimulation. In addition, the ambient decibel level of many incubators can be around 60 dbs. (Philbin, 2000). Unlike the low-intensity, low frequency sound that is present *in utero*, preterm infants are exposed to mechanical and speech sounds which are much more variable in pitch, intensity, complexity and regularity (Philbin, 2000; Philbin, Balweg & Gray, 1994). According to Lotas (1992), the NICU sound environment can potentially lead to hearing loss, difficulties in processing auditory input, difficulty in sleep and state rhythmically, and can influence and disrupt physiological systems, resulting in problems such as apnea and bradycardia. It could also be the case that these atypical, altered auditory experiences might lead to deficits in overall intersensory development.

VII. Differential Effects: Human Research

Given the findings from comparative research, it seems important to consider the effect of different types and amounts of sensory stimulation on the developing preterm infant's ability to organize physiological and behavioral subsystems. One important area to examine is the auditory environment of the preterm infant. The sound environment of the NICU is atypical and potentially noxious for the preterm infant, and it is also limited in the typical maternal vocal input normally experienced by the fetus. Maternal visits to their preterm infants can be brief, and in some cases, uncommon. If fetal exposure to maternal voice assists the full term infant in fostering the capacity to regulate attention and arousal states, then reducing the amount of prenatal exposure to maternal vocal stimulation might influence early behavioral and physiological organization. Thus, a preterm infant might benefit from the presentation of vocal stimulation, since it is believed that prenatal vocal stimulation is influential in the organization of infant auditory perception and in the infant's behavioral regulation of attention and arousal. In addition, given the comparative evidence suggesting that atypical (or early) exposure to different types and amounts of sensory stimulation results in altered behavioral responses, it is important to explore some of the current literature on preterm infants' behavioral and state organization to supplemental sensory stimulation, especially the effects of auditory stimulation and the influence of the relative amount of visual stimulation on preterm infants' arousal and behavioral responses.

A. Arousal and Attention in Infancy

Arousal and attention are typically viewed as differentiated processes, and this differentiation appears to take place during early infancy. For full term neonates, the link between arousal states and attentional states is not yet clearly differentiated at birth; rather, arousal and attention appear to work interdependently and go through a developmental process of differentiation (Gardner & Karmel, 1983, 1984, 1995; Karmel, Gardner, & Magnano, 1991; Turkewitz, Lewkowicz, & Gardner, 1983). According to Karmel, Gardner, and Magnano (1991), the relationship between arousal and attention in full term neonates is a single, organized homeostatic system. This system is comprised of internal (endogenous) and external (exogenous) factors that influence arousal-modulated attention. As such, when a neonate is more internally stimulated, their responsiveness to external stimuli decreases and behaviorally they show signs of withdrawal (i.e. gaze aversion, negative emotional responsiveness). In contrast, when a neonate is less internally stimulated, their responsiveness to external stimulation may increase and/or they may exhibit preferences for more complex types of stimulation (i.e. increase looking and visual searching, positive emotional responsiveness to external stimulation).

For example, Gardner and Karmel (1984) examined full term neonates' responsiveness to several visual temporal frequencies when they were either more internally aroused (unswaddled before feeding) or less internally aroused (swaddled after feeding). They found that the level of internal arousal (low versus high) appeared to have a direct effect on the amount of looking the infant directed to the different stimuli. Infants looked longer at faster temporal frequencies when they were less internally aroused, and looked longer at slower frequencies when they were more internally aroused. These effects were replicated and extended by Gardner and Karmel (1995), who found the same pattern of responsiveness for-one month-olds, but a different pattern for four-month-olds. At four months, the infant's level of arousal did not seem to influence their patterns of looking; all infants preferred the faster temporal frequencies. Gardner and Karmel speculate that this difference in the effect of arousal on visual attention may be the result of the development of cortical systems that modify the subcortical influences of arousal on the behavioral responses of attention (i.e., looking). Thus, the relationship between arousal and attention appears to go through a process of differentiation that occurs in the first several months after birth.

In preterm infants, the homeostatic processes between arousal and attention may take on an altered developmental pattern. While Gardner and Karmel (1981, 1993) found the same direction of effects for preterm infants (tested near term age) as that of Gardner and Karmel (1984, 1995), the effects were not as robust and there was a great deal of variability within the groups of preterms. Preterm infants were more likely to become overaroused, fussing or exhibiting "shut down" behavior (e.g. gaze aversion, flaccidity).

In addition, these preterm infants did not exhibit the typical 'locking on' behavior seen in normal newborn infants (Karmel, Gardner, & Magnano, 1991). Thus, even though these infants were tested near term age, something about their experience as a preterm infant appeared to affect the way they respond to stimulation.

B. Social Stimulation and Arousal in Preterm Infants

One way in which infants' arousal, attention and affect are regulated is via interactions with caregivers. Infants directly impact their arousal states via actions such as gaze behavior, head orientation, and other attentional behaviors, and they indirectly regulate their arousal by the signals they emit to their social partners via facial expressions or vocalizations (Ekerman, Hsu, Molitor, Leung, & Goldstein, 1999). Additionally, caregivers influence infant's arousal through their own gaze behaviors, vocalizations, facial expressions, imitative behaviors, contingent responsiveness, and their ability to read and respond to the infant's behavioral cues (Gable & Isabella, 1992; Tronick and Giaino, 1986). Mastery of this mutual regulation is a shared task between the infant and the caregiver.

Preterm infants appear to be differentially responsive to social stimuli and exhibit unclear behavioral cues in social interactions. Research has suggested that behaviors such as infant smiling, mutual gazing, affectionate displays, and other social interaction behaviors are predictive of outcomes at two years on the Bayley Mental Scale and Gesell scores for preterm infants (Beckwith & Cohen, 1980). Often, preterm infants tend to exhibit less positive interaction behaviors (smiling, gaze holding) and exhibit more aversive behaviors (gaze aversion, fussing) to normal interaction attempts (Field, 1979,1980). In addition, behaviors of the mothers of preterm infants tend to be almost excessively interactive, more verbal, exhibit less positive affect, and more fretting behaviors (Field, 1979, 1980; Goldberg, Brachfeld, & DiVitto, 1980).

Eckerman, Hsu, Molitor, Leung, and Goldstein (1999) explored preterm infants' mastery of arousal regulation during social stimulation via *en face* exchanges with parents and in controlled stranger interactions. Eckerman and colleagues suggest that preterm infants' mastery of arousal regulation is complicated by the difference in the overall degree of arousal experienced in response to social stimulation, the clarity of the signals and behavioral cues that preterm infants exhibit, and how parents and caretakers perceive and interpret the infant's cues and how they then respond to those cues. Using the *en face* exchange to examine the effect of social stimulation on arousal and attention behaviors, these researchers focused on four-month-old full-term and preterm infants (high and low risk; corrected age) responsiveness to a standardized protocol game of peek-a-boo. Eckerman and colleagues found no difference in visual attention between these three groups, but significantly less positive arousal, significantly more negative arousal, and more variability in the clarity of cues that the preterm infants displayed. There were no significant differences between the high and low risk preterm infants. While a small portion of the preterm infants exhibited the typical full term infant pattern for the clarity of cue displayed (strong positive cues and few negative cues), most of the preterm infants exhibited patterns of strong positive/negative cues, strong negative cues only, or no strong cues at all.

The finding of no difference in visual attention between the full term and preterm infants supports Gardner and Karmel's (1995) contention that the process of differentiation between attention and arousal is occurring during the first months after birth. Additionally, because Eckerman and colleagues (1999) did not find any difference between the high and low risk preterm infants on attention and/arousal, the implication is that the differences in arousal reactivity between the full term and preterm infants were due to the impact that social stimulation (i.e. the external stimuli) had, rather than any internally-driven arousal (Gardner & Karmel, 1995). Thus, social or external stimulation appears to differentially affect preterm infants' arousal modulation and reactivity.

Als (1982, 1983) suggests that because preterm infants are more poorly organized, more sensitive and reactive to social/environmental stimulation, and more generally stressed and overstimulated, any interaction with preterm infants should be supportive of their ability to deal with sensory/social stimulation. However, since the cues that preterm infants display indicating that they are overstimulated or stressed may be more subtle or unclear, it may be difficult for most parents to discern these signals (Ekerman et al., 1999). Nevertheless, given these infants' poor self-regulation and state control abilities, it may be vitally important to provide support in dealing with postnatal social stimulation.

Overall experience with vocal stimulation may play a key role in how preterm infants develop responsiveness to social stimulation. In addition, overall sensory experience may influence the impact that vocal stimulation has on a preterm infant's behavioral organization. For example, Ekerman, Oehler, Medvin, and Hannan (1994) looked at preterm newborns as social partners in three different and progressive types of social interaction. These very low birth weight infants were placed into one of three protocols of social interaction: a quiet face/ *en face* situation with a female experimenter, an initial *en face* which then transitioned to quiet infant directed (ID) speech, and finally an *en face* to quiet ID speech accompanied by touch. This progressive interaction was used to examine the relationship between the amount and nature of social stimulation, the infant's state, and his/her ability to attend to and maintain social responsiveness. Ekerman et al. (1994) found that preterm infants responded to the infant-directed speech with more visual attention, while they responded to the combination of speech and touch with decreased visual attention and increased signs of distress and avoidance. These results suggest that preterm infants not only respond to infant-directed speech, but also respond in ways that can potentially help the infant maintain state and attention to social situations. In addition, it also suggests that multiple sources of stimulation can be overwhelming for the preterm infant to regulate, and may lead to increased stress reactions.

C. Maternal Vocal Stimulation and Preterm Infants

A limited number of studies have examined how auditory stimulation (specifically vocal stimulation) affects the physiological and behavioral organization of preterm infants, with mixed results regarding the positive or negative affects of vocal stimulation on preterm infants. However, some of these studies have a few methodological or conceptual concerns, or are preliminary projects that need additional empirical follow-up. Therefore, it is important to present a careful examination of both the data and methods, the conflicting results, and the implications of these results

A few studies have suggested that vocal stimulation (specifically maternal) has a limited or little impact on either immediate or long term physiological or behavioral organization. For example, Malloy (1979) attempted to examine the effects of supplemental maternal vocal stimulation or musical stimulation on the short-term and long-term physiological and behavioral outcomes of these infants. All infants were evaluated at discharge using Grahm's Behavioral Examination of the Neonate (called a baseline measure by Malloy), and with the Bayley Scales of Infant Development at 9 months. Infants in the experimental groups were exposed to either 1) tapes of their mother's voice or, 2) recordings of Brahms Lullaby, for 5 minutes, 6 times a day, from 5 days after birth until the infant weighed 2000 gm. No assessment of the infants gestational age at birth or health status was noted. Compared to controls, neither experimental group exhibited any immediate or long-term gains. The only significant result reported was that both the music and maternal voice groups had earlier discharges than controls. However, Malloy notes that the study was flawed by the use of Grahm's Behavioral Examination. The infant examined were a "rather mature" population for this exam at the time of initial testing, resulting in a ceiling effect of scores (Malloy, p.84, 1979). In addition, the test was not able to account for the infant's state, hunger, or other environmental factors (light, noise, etc.). Thus, Malloy suggests that this measure was an inappropriate baseline measure that did not reliably measure the immediate effects of the auditory input.

Malloy's (1979) use of the outcome measures of discharge date and assessment scores suggested that maternal voice should affect physiological and behavioral organization in such as way to lead to more energy conservation, more efficient growth rates, and overall better development. Indeed, this study did find that exposure to maternal voice was related to earlier discharge rates. However, since there were no differences between groups at 9 months, this study provided no conclusive evidence that exposure to maternal vocal stimulation resulted in long term changes in infant development

Another study suggesting negligible effects of vocal stimulation on preterm infants ability to exhibit better organization was conducted by Standley and Moore (1995). They examined the positive effects of either music or maternal vocal input on the oxygen saturation level of preterm infants. Standley and Moore hypothesized that exposure to music rather than vocal stimulation should have a greater organizing effect on preterm infants, resulting in higher levels of O_2 saturation. Twenty preterm infant (10 per group, GA unspecified) in the NICU were exposed to 20 minutes of either lullaby or recording of their mother's voice speaking to them for three consecutive days, and measurements of their oxygen (O2) saturation levels were taken every two minutes before (10 minute baseline), during (20 minutes), and after (10 minutes) the stimulus presentation. Results revealed that the presentation of music significantly and immediately increased and stabilized O_2 saturation levels in infants during the presentation of the music stimulus, but this difference was only found on the first day. There were no significant differences between the two groups on day two or day three. In addition, while infants exhibited increased or stabilized O_2 saturation levels during the presentation of musical stimulation, infants in the music group also exhibited significantly depressed O_2 saturation levels during the period of silence after termination of the stimulus on both the second and third day. Although the infants in the maternal voice group did not exhibit the immediate increase in O_2 saturation levels, Standley and Moore found that by day three, these infants did show a moderate increase in O_2 saturation. Additionally, they did not exhibit the depressed O_2 saturation once the auditory stimulation was terminated. This is an interesting effect, suggesting that vocal stimulation may not have an immediate effect on organizational patterns, but rather has a gradual impact as more exposure is provided to the infant. It may be interesting to explore more prolonged exposure (over several days) to see if this trend continues. Nevertheless, while there does appear to be immediate physiological regulatory effects of musical stimulation, the disruptive effect to autonomic regulation by terminating the musical stimulus suggests that music may not be the most appropriate stimulus to help infants learn to self-organize behavioral and physiological systems. While Standley and Moore focused O_2 saturation as a measure of physiological organization, it would also be interesting to examine the relationship of the infant's state and the efforts the infant makes in regard to behavioral displays of stress and self-regulatory efforts to vocal stimulation.

Finally, a poster presentation by Norwik-Stern, Clarkson, Morris, Shabazz and Stephens (1996) examined the preferences of preterm infants to different types of vocal stimulation. This study was concerned with the impact of early birth on species typical preference in attention and orientation to speech stimuli. They found that four-month-old preterm infants (corrected age) exhibited a preference for infant-directed over adult-directed speech, like their full-term counterparts. This suggests that the altered auditory experiences of the preterm infant did not appear to result in a change in speech typed preferences. However, unlike full-term infants, preterm infants exhibited more variability in their preferences, and did not show positive affect to either infant-directed or adult-directed speech. This could suggest that the change in experience might have more subtle effects on displays of preference, and overall behavioral organization. In a follow-up study, Norwik-Stern, Clarkson, Morris, and Bakeman (1998) examined the effect of preterm infants' speech preferences on the mother-infant face-to-face interaction patterns. While no effect of actual speech preferences was found, preterm infants did gaze at their mothers longer when the mothers used infant-directed speech. However, preterm infants still showed predominantly neutral affect during these face-to-face interactions, with overall less smiling or positive facial expressions during interaction. This suggests that preterm infants may not use maternal vocal input to regulate their emotional and social state interactions in the same way as full-term infants. However, to this date no other empirical presentation of this data has been identified.

Other studies lend more support for the contention that vocal stimuli are organizing for preterm infants. For example, Segall (1972) examined the effect of vocal stimulation to preterm infants' physiological organization as indicated by heart rates. Again, the infant's ability to regulate stable heart rate patterns is commonly used as an indication of physiological organization. Segall (1972) examined the relationship between the presentation of maternal voice and heart rate responses in preterm infants. Specifically, this study appears to be concerned with the differential effects of auditory stimulation on the preterm infant's ability to attain or maintain optimal organization patterns. Preterm infants in the experimental group (28-32 weeks gestational age) were exposed to a tape recording of their mother's voice every day for 30 minutes until 36 weeks gestational age. When the infants reached 36 weeks gestational age, one testing session was conducted in which the infants were exposed to a variety of auditory stimuli while heart rate measurements were taken. Infants were tested during two different states of wakefulness: quiescence and arousal. Quiescence was defined as a state of near sleep, with eyes half shut, little body movement, and shallow respiration rate. Arousal was defined as a state of crying. When infants were in a state of quiescence, they were presented with 10 seconds of white noise. When the infants were in a state of arousal, they were presented with a recording of their own mother's voice and another woman's voice. Recordings of the infant's heart rate were taken during the presentation of these different stimuli. Infants in the experimental group exhibited greater overall heart rate accelerations to the white noise than the infants in the control group. In addition, experimental infants also exhibited greater heart rate deceleration to both the presentation of the mother's voice and the other female voice compared to baseline heart rate. Infants also exhibited significantly greater heart rate deceleration to the mother's vocal stimulation that to the unfamiliar female voice.

Segall suggests that these differential responses to auditory stimuli possibly reflect an increase in the infants' receptive response to auditory stimuli. In other words, by exposing infants to recordings of their mother's voice, infants learned to be more responsive to auditory stimuli. Thus, these infants respond to a noxious stimulus with greater heart rate accelerations, indicating an avoidance or withdrawal response (Lacey, 1967; Segall, 1972), while the decelerative heart rate response to voices indicates greater orientation or attention to the stimulus (Richards & Casey, 1992; Segall, 1972). This study lends support to Als and others contention that the preterm infant becomes oriented to and affected by the type of stimulus presented to the infant, and that differential levels of organizational responsiveness can be seen depending on the type of stimulation presented.

However, the presentation of vocal stimulation may be influenced by whether the stimulation is presented unimodally or accompanied by other types of stimulation. White-Traut, Nelson, Silvestri, Cunningham, and Patel (1997) examined the immediate physiological and behavioral responses of preterm infants to unimodal and multimodal sensory stimulation, and found differential effects of the auditory stimulation (female voice) dependent on whether the stimulation was in isolation (unimodal) or in combination with other stimulation (multimodal). White-Traut and colleagues exposed infants to either a) auditory, b) tactile, c) auditory-tactile-visual (ATV), and d) auditory-tactile-visual-vestibular (ATVV) stimulation and recorded behavioral state and physiological responses to this stimulation. Infants (32-33 weeks GA) were assigned into one of five groups (4 stimulation conditions and a control) and assessed before, during and after the stimulation procedure.

White-Traut and colleagues (1997) found that when infants were exposed to only auditory stimulation they exhibited significantly lower pulse rate during the post-stimulation measures, and significantly more quiet sleep than any other group. They suggest that increased quiet sleep and more restful states indicate positive outcomes for preterm infants. These states reduce energy expenditures and allow preterm infants to gain more weight, and organize sleep-wake states more effectively. In addition, as seen in Segall (1972), increased behavioral quieting appeared to lead to an increased ability to direct or sustain attention. Finally, White-Traut and colleagues (1997) found that infants in the ATV and the ATVV groups exhibited increased alertness, increased pulse rate, lower respiration, and overall more quiet alert states. In contrast, the tactile only group exhibited the highest levels of alertness, highest pulse rate, and highest respiration rates. This suggests that these infants may have been exhibiting signs of overstimulation. White-Traut and colleagues note that the best organization appeared to come from the ATVV group, and suggest that the arousing elements of the tactile stimulation, along with the soothing elements of the auditory and vestibular stimulation, allowed the infants to maintain a moderate state and exert greater control over their behavioral organization. From this perspective, the results of this study further support the role that auditory stimulation may have in infant's ability to develop self-regulatory abilities.

Finally, an recent unpublished dissertation by Bozzette (1999) examined the behavioral (facial, motor, activity, visceral, state and attending) and physiological (heart rate, respiratory rate, and oxygen saturation) responses of preterm infants (GA 31 to 34 weeks) to presentation of taped maternal voice, four times a day for three consecutive days. Infants were observed every ten seconds for three minutes pre-stimulus (baseline), three minute during presentation of the taped maternal voice, and for three minutes post stimulus (recovery). Bozzette (1999) found that there were no differences in physiological responses, but the preterm infants did show significant changes in behavioral responding. Preterm infants attending, "quiet alert" behaviors (stilling, bright eyes) significantly increased from baseline to the tape segment, and attending and stability behaviors (controlled movement and tone) were significantly higher during the vocal stimulation that during the post-stimulus segment. Infants tended to show overall less activity and more wakefulness during the maternal vocal stimulation. These results again suggest that maternal vocal input may allow infants to organize their state and attentional systems and may help facilitate social responsiveness.

Overall, the results of Segall (1971), White-Traut et al. (1997), Ekerman, et al. (1994) and Bozzette (1999) suggest that vocal stimulation could be an organizing element for preterm infants. However, data from Malloy (1979) and Stanley and Moore (1995) do not support these interpretations, suggesting that exposure to increased vocal stimulation may not lead to important differences in physiological organization or later developmental outcomes. In addition, the effect of multiple types and amounts of stimulation for preterm infants appears to result in contradictory outcomes, as seen in the differential effects found by White-Traut and colleagues (1997) and Ekerman and colleagues (1994, 1999).

These contrasting views make it difficult to judge whether the reported positive effects of vocal stimulation on infant state and behavioral organization are reliable. In addition, it is still unclear if the effectiveness of vocal stimulation is influenced by other types of stimulation (like light), which may interfere with these positive effects. Indeed it is unclear what the light levels were during exposure of stimulation for these studies.

White-Traut and colleagues (1997) suggest the presentation of concurrent visual stimulation with auditory stimulation influences organization of infant responses, resulting in greater organization of states and behaviors. It could be that the relative level of illumination during exposure to the vocal stimuli had a moderating effect on the impact of the vocal stimulus. Since there does not appear to be a clear sense from these studies about the organizational impact of vocal stimulation, and because data from comparative work suggests that presentation of stimulation from later developing sensory systems can interfere with the development of currently developing process, it might be interesting to examine the moderating effect that light level has on responsiveness to vocal stimulation. Finally, it is unclear whether the vocal stimulation needs to be maternal, or if any female voice could be used. As noted previously, most of the current research in this area is poorly designed and controlled, with little random assignment or consistency within the subject populations. The relative uncertainty of the data, the contradicting evidence, the lack of specification about the effects of vocal stimulation on behavioral and state organization, and the questions regarding unimodal versus multimodal stimulation all suggest that further examination of the use of supplemental vocal stimulation with preterm infants is warranted.

Hypotheses and Predictions

Given the evidence that early sensory stimulation can affect preterm infants' perceptual and behavioral development, it seems important to examine the effects of different amounts of auditory and visual stimulation on the developing preterm infants' ability to organize different behaviors that indicate adaptive responsiveness or stress. In this light, the present study sought to examine: (1) whether exposure to taped female voices would affect the behavioral and state organization of preterm infants, (2) whether the overall amount of exposure to this vocal stimulus would influence these effects and (3) whether concurrent visual stimulation moderated the infants' responsiveness to the auditory stimulation.

Hypothesis I

It was hypothesized that preterm infants would exhibit more organized state and behavior patterns during and after exposure to vocal stimulation, and that the magnitude of these differences would be affected by the amount of exposure to the vocal stimulus. Preterm infants would exhibit changes in responsiveness as they had more exposure across the five day period, and preterm infants who had even more exposure (three times a day versus once a day) would exhibit even more behavioral organization.

Hypothesis II

It was hypothesized that the main effect of vocal stimulation would be modified by the relative amount of light (illumination) in the environment, so that infants who we exposed to the vocal stimulus during relatively lowered illumination conditions would exhibited more organized state and behavior patterns (lowered stress and greater self regulation behaviors), whereas infants who were exposed to the vocal stimulus in typical NICU illumination conditions would exhibit less organized state and behavior patterns.

Chapter 2

Methods

Participants

Forty preterm infants, between the ages of 31-34 weeks gestation, and their mothers were asked to participate in this study. Infants were chosen from this age range for a number of reasons. In general, infants in the previous studies citied were between the ages of 29-36 weeks gestation, with a majority of them tested between 32-34 weeks GA. By testing infants at a similar age, there will exist some parity between results, a least as far as concerning the relative developmental level of the infants. In addition, while it may be interesting to look at very preterm infants (>28 weeks gestation), the relative fragility of that sample makes it programmatically difficult to attain an appropriate sample. Nonetheless, future work may wish to examine differential effects found in a younger population.

All participants were recruited from the Neonatal Intensive Care Units (NICUs) of the Medical College of Virginia Hospital in Richmond Virginia (6 subjects) and from Carilion Community Hospital of Roanoke in Roanoke Virginia (34) subjects. Of these subjects, four had to be excluded due to incomplete data sets. Given the great variability in the subject population, there were several key criteria used to establish a more homogeneous subject pool. These criteria were collected from a chart review of every infant who fell within the age range needed for the study, and were used as guideline for recruiting subjects for participation. Infants with a history of congenital abnormalities, major neurological or physiological problems (including Grade 4 interventricular (IV) bleeds, hydrocephalus, hyperbilirubinemia, and other serious medical concerns), any confirmed hearing loss, and/or a reported history of maternal diabetes or drug use were not admitted into the study. In addition, infants who were currently on sedatives such as Fentanyl or Versed were withheld from participating until such time as they were not on the medication. If, at any time during the course of the study, an infant experienced any significant medical problems, they were released from the study per their physician's recommendations. Due to significant shifts in neurological organization that occur during the 27th and 28th week of gestation, all infants selected for the study were born after the 28th week (GA), as determined by maternal report or Ballard score (Als, personal communication, 1999). All infants were at least 10 days old at time of testing, off ventilator support, and either on room air or minor oxygen support. In addition to these selection criteria, additional information such as birth weight, birth length, head circumference, and current weight at testing were collected (Table 1). No significant differences were found between these groups of subjects on measures of birth weight, gestation age at birth, or gestational age at testing.

Table 1

Infant Characteristics by Group

Group	Mean Birthweight	Mean Length	Mean H.C.*	Mode GA @ Birth **	Mode GA @ Test
Standard Illumination/ High Voice	1666g	43.8 in	29.9 in	32 weeks Range 32-33 wks	34 weeks
Standard Illumination / Low Voice	1596g	40.7 in	28.6 in	32 weeks Range 29-32 wks	34 weeks
Decreased Illumination / High Voice	1621 g	41.78 in	28.3 in	31 weeks Range 29-32 wks	33 weeks
Decreased Illumination / Low Voice	1697 g	41.8 in	28.7 in	32 weeks Range 30-33 wks	34 weeks

Materials

A recording of a female reading a bedtime story (*Mama Loves* by Molly Goode) was created using an Audio Technica ATR35s lapel microphone and a SonyTCM-435 V-O-R tape recorder. This recording was looped to allow for a 10-minute sample of uninterrupted vocalization, and presented to the infant using the Sony TCM-435 V-O-R tape player and a speaker that was positioned approximately 4-6 inches from the top of the infant's head in the incubator. Volume was set at approximately 50-60 decibels, as used by Standley and Moore (1995). The vocal stimulus was recorded using an adult-directed vocal inflection. While full-term infants respond more favorably to infant-directed speech, this mode of auditory stimulation may be too arousing for preterm infants at this gestational age. Additionally, the vocal stimulation that fetuses are exposed to during gestation is more commonly adult-directed in nature, thus making it a more naturally occurring stimulus.

For purposes of this study, measures of light were taken in lux. Lux represents a more representative measure of overall illumination. Light levels within the NICU vary depending on location and amount of light on. The light levels with all overhead lights on was between 300-400 lux, with measurements of over 1000 lux from the high intensity bedside lights that were typically used only for precise medical procedures. However, light levels in the incubator were manipulated using a large incubator cover that went over the incubator and video camera. Two types of covers were utilized: a "typical" cover and a 'low' light cover. The 'typical' cover was a small, thin baby blanket commonly used as a "temporary" incubator covers in NICUs. These covers allow an illumination at about 20 lux. These covers allow light to come in on the top and side, but only diffusely through the top of the incubator. The 'low' light cover was made of a black fabric with illumination levels in the incubator at about 3 lux. The "low" light cover was only placed on the incubator during the vocal stimulus presentation. During all pre- and post- stimulus conditions, the "typical" cover was used. A Sony Nightshot 8MM video camera was positioned on a tripod on one end of the incubator to record the infant's behavior before, during, and after stimulus presentation. This camera is able to record at 0 lux conditions.

Data Coding

As outlined previously, physiological and behavioral organization can be examined in multiple ways: with outcome measures, with physiological measures, or with state and behavioral measures. Als (1982, 1983, 1995b) developed assessments such as the Assessment of Preterm Infants' Behavior (APIB) and the Newborn Individualized Developmental Care and Assessment Program (NIDCAP) to assess the organization of several subsystems by examining overall state and organized behaviors. These tests provide a clinical tool for assessing current state and provide an avenue for outcome measures. However, tools like the ABIB require extensive training to be reliable, and tools like the NIDCAP do not lend themselves well as research measures. However, both assessments identify sets of behaviors and states that are indicative of overall organization. Als proposes a set of behaviors she considers 'self-regulatory', or indicative of the preterm infant's attempts to maintain state and adapt to environmental stimulation. Additionally, Als proposes a set of behaviors she suggests are indicative of 'stress reactive', behaviors that the preterm infant exhibits when his/her coping skills are challenged. Therefore, in order to examine the relationship between vocal/illumination level and preterm infants' organizational patterns, behavioral organization will be examined via assessment of states before, during, and after stimulus presentation, and by measuring the frequency of specific behaviors that are indicative of self regulation and stress reactivity.

In order to examine these infant's stress reactivity and self-regulation abilities in response to the stimuli, the infants' motor and state behaviors were examined. Als (1995b) has illustrated how specific sets of motor behaviors, as well as particular states, can be indications of overall system stress and ability to cope and display self-regulation. As stated previously, the NIDCAP is a clinical tool that systematically outlines motor, state, and attentional behaviors exhibited by preterm infants over time and allows clinicians to make overall judgments about the infants strengths and struggles in self-regulation efforts and levels of overall stress (Als, 1995b). Other assessments such as the

ABIB are more formalized tools that allow subjects to receive an overall 'score' after completing a series of tasks to test competencies (Als, 1995b). All of these assessment tools focus on a similar set of motor behaviors and states. The motor subsystem is assessed by observing the infant's posture, movements, tones, and the amount/degree of activity. Sets of specific motor behaviors can indicate stress reactivity or self-regulation. State organization is manifest by various configurations of behaviors involving eye movement and opening, facial expressions, gross body movements and tone, and respiration efforts as they are indicative of consciousness.

For the purposes of this study, specific motor behaviors were selected from these tools (see Table 2 for details of specific stress reactivity and self regulatory behaviors measured and Appendix 2 and 3 for their definitions). These behaviors were chosen for their relative frequency of occurrence in most preterm populations, for the ease in which they can be observed via videotape, and for their classification as indicating stress behaviors or self-regulatory behaviors. Frequency counts of the specific motor behaviors were taken during each 10-minute epoch (baseline, stimulus presentation, and recovery periods). In addition, measures of overall quality of state were recorded in 15 second increments over each 10 minute epoch into one of the following five categories: Sleep, Drowsy, Quiet Alert, Awake Alert, or Aroused. While these states can be identified as 'A' and 'B' states, videotaped recordings made it difficult to accurately identify these different deviations in state. In addition, Deep Sleep and Light Sleep were also consolidated into one overarching "sleep" category, again due to difficulty in making accurate estimates of the quality of sleep via videotape. These motor behaviors and states were coded from videotapes made of each epoch.

Table 2 : Experimental Design

	Decreased Illumination Condition	Standard Illumination Condition
Low Voice Condition	N = Stimulation once a day with dark cover	N = Stimulation once a day with no change in light
High Voice Condition	N = Stimulation three times a day with dark cover	N = Stimulation three times a day with no change in light

Procedures

A 2 x 2 factorial design was used, with two levels of vocal stimulation (High and Low) and two levels of lighting (Standard Illumination and Decreased Illumination) (see Table 3). Each infant was randomly assigned into one of the four groups (High Voice, Decreased Illumination (HVDI); High Voice, Standard Illumination (HVSI); Low Voice, Decreased Illumination (LVDI); Low Voice, Standard Illumination (LVSI)), and each subject served as his/her own control. All videotape recordings were made during the afternoon session. Prior to baseline, all infants (regardless of group) were diapered and repositioned on their back. At this time, the speaker was positioned in the incubator.

In the High Voice conditions, infants received the taped vocal stimulus for 10 minutes, three times a day, for five continuous days. Each stimulus session was spaced approximately 3 hours apart. For example, an infant might have received stimulus presentation at 9:00 AM, 12:00 PM, and at 3:00 PM. Measurement of infant state and behavior was recorded only during the last session of the day. In the Low Voice conditions, the infants received the taped vocal stimulus for 10 minutes a day, once a day, for five consecutive days. Measurement of infant state and behavior was made during a single (afternoon) session of the day.

In the Standard Illumination conditions, the "typical" cover was used during the pre stimulus, stimulus presentation, and post stimulus period. In other words, there was no change in relative amount of light in the incubator before, during, or after the voice was presented. During the Decreased Illumination conditions, a "typical" cover was used during the pre-stimulus and post-stimulus conditions. However, the low light cover was placed over the incubator and video camera during the 10-minute voice presentation session. Upon completion of the 10-minute stimulation period, the vocal stimulus was turned off and the cover removed. The experimenter was available and observing all trials to monitor all activity during these sessions.

Each subject's name was coded as a number and all of the research records and videotapes were referred to via this number. Videotapes were coded by the experimenter, with interrater reliability coding completed by a NIDCAP trainer. Ten percent of the original forty subjects were randomly selected for interrater reliability coding (4 videotapes; total of 10 hours).

Data Analysis

In order to examine the effects of the various levels of vocal stimulation and lighting conditions on the infant's stress reactions and self-regulatory abilities, infant's behaviors were coded for the frequency of exhibiting specific behaviors related to either stress reactivity or self-regulation (see Table 3 for behaviors and Appendix 1 for definitions). These frequency counts were taken during the baseline, stimulus presentation, and recovery periods, totaled for each condition, and then Z score standardized. This allowed each behavior to be equated with the other behaviors within the category. The z-score values for each category were then added together to create a total Stress Reactivity score and a Self-regulation score for each day and condition. A repeated measures 2 x 2 x 5 x 3 analysis of variance (ANOVA) was used to analyze the relationship between types of stimulation (amount of vocal stimulation and illumination level) and the day (5 days) and condition in which the behaviors were observed.

In addition, measures of infant's behavioral states were assessed. Certain states may be indicative of self-regulation whereas other states are typically associated with stress. For example, the occurrence of quiet alert states is most frequently associated with better organization and self-regulation, whereas active alert and aroused states are most frequently associated with greater levels of stress. States like sleep or drowsiness are typically viewed as more regulated. However, infants under stress will commonly 'escape' into sleep states in response to overstimulation. This "escaping" into a sleep state is a stress reaction. Therefore, while certain states (i.e. quiet alert) clearly indicate self-regulation or stress reactivity, other states do not clearly indicate either. Infant states were rated every 15 seconds, with the overarching quality of the infant's state determining what rating was marked. These ratings within each state were then summed for each condition (baseline, stimulus, and recovery). A repeated measures ANOVA was calculated to examine the relationship between the type of stimulation (vocal stimulation and light level) and the state, day, and condition for each subject. Initial analysis on the state data revealed that several of the variables (state/day/condition scores) violated appropriate expectations of skewness and kurtosis, and thus a natural log transformation was performed on the data to correct for a normal distribution

Table 3
Observed Behaviors

	Stress Reactivity Behaviors	Self-Regulatory Behaviors
Motor Subsystem		
	Squirming	Smiling
	Extending Leg	Leg bracing
	Extending Arms	Frowning
	Yawning	Mouthing
	Grimacing	Suck search
	Finger splaying	Sucking
	Airplaning	Foot clasp
	Saluting	Hand clasp
	Sitting on air	Grasping
	Fisting	
	Sneezing	
	Fussing	

Chapter 3

Results

Stress Motor Data

In order to test the effects of female vocal stimulation on stress reactivity (Hypothesis I) and the effects of multimodal stimulation (lighting level and amount of vocal stimulation) on stress reactivity in preterm infants (Hypothesis II), a repeated measures ANOVA was conducted on the Stress Reactivity Behavior scores. This repeated measures ANOVA resulted in a three-way interaction between Day, Condition, and Illumination level (see Table 4). In an effort to isolate this effect, post hoc analysis of the data was conducted, in which day was held constant and the relationship between condition and light was examined for each day.

Table 4

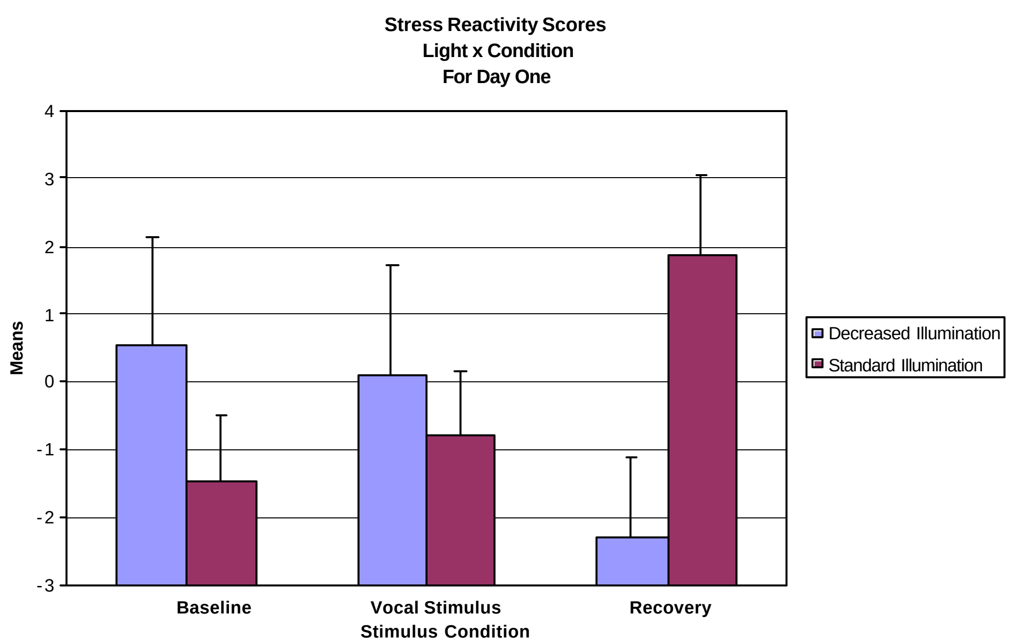
Summary of ANOVA significant F values for Stress Scores, Self-Regulation Scores, and Infant State

	Df	F	p-value
Stress Scores			
Day X Condition X Light	8, 25	2.577	$p = .03$
Self- Regulation Scores			
Day X Light	4, 29	3.747	$p = .01$
Condition X Voice	2, 31	3.508	$p = .04$
Day X Voice X Light	4, 29	5.028	$p = .00$
Infant State			
State	4, 29	188.748	$p < .00$
State X Light	4, 29	4.191	$p = .00$
State X Condition	8, 25	8.453	$p < .00$
State X Condition X Light	8, 25	2.429	$p = .04$
State X Condition X Sound	8, 25	2.527	$p = .03$

<u>Effect of Light and Condition by Day</u>

This analysis revealed only an interaction between illumination level and condition for day 1 ($F(8,25) = 2.58$, $p = .033$). Subsequent analysis found that there were no overall differences in stress levels for either levels of light during either baseline or during stimulus presentation, but there was a difference in stress reactivity during the recovery period. Infants who were in the standard illumination condition exhibited more stress reactivity in the recovery period after the vocal stimulus was turned off than the infants in the decreased illumination condition. In other words, infants who were in the darker conditions decreased in their overall stress reactivity behaviors after the vocal stimulation, whereas infants in the standard illumination exhibited significantly more stress reactivity behaviors after hearing the voice (see Figure 1). This effect was only found for the first day of exposure, however (see Appendix 4 for graphs of non-significant illumination condition x day data). There was no effect of the amount of vocal stimulation on stress reactivity behavior. However, since there is a pattern of significant increase or decrease in stress reactivity (depending on light condition) *after* vocal stimulation, being merely exposed to the vocal stimulus may play a part in the overall stress reactivity behaviors. Since we do not have a "no vocal" stimulation condition, this effect remains unclear.

Figure1



- Y bars denote standard error

Self-Regulation data

In order to test the effect of vocal stimulation on self-regulatory behavior (Hypothesis 1) and the modulation of light stimulation (varying illumination conditions) on preterm infants' self-regulation behaviors (Hypothesis 2), a repeated measures ANOVA was conducted on the Self-Regulation Behavior data (see Table 4). Several significant effects were found in the self-regulation data. A repeated measures ANOVA revealed a three way interaction between Day x Illumination Condition x Voice, Day x Illumination Condition interaction, and a Condition x Voice interaction (see Table 5). Since the Day x Illumination Condition x Voice effect supercedes the Day x Illumination Condition effect, further analyses were conducted, in which day was held constant and the relationship between light and sound was examined. Effects of vocal stimulation and illumination condition were found for day one, and an illumination condition effect was found for day four (see Table 5).

Table 5

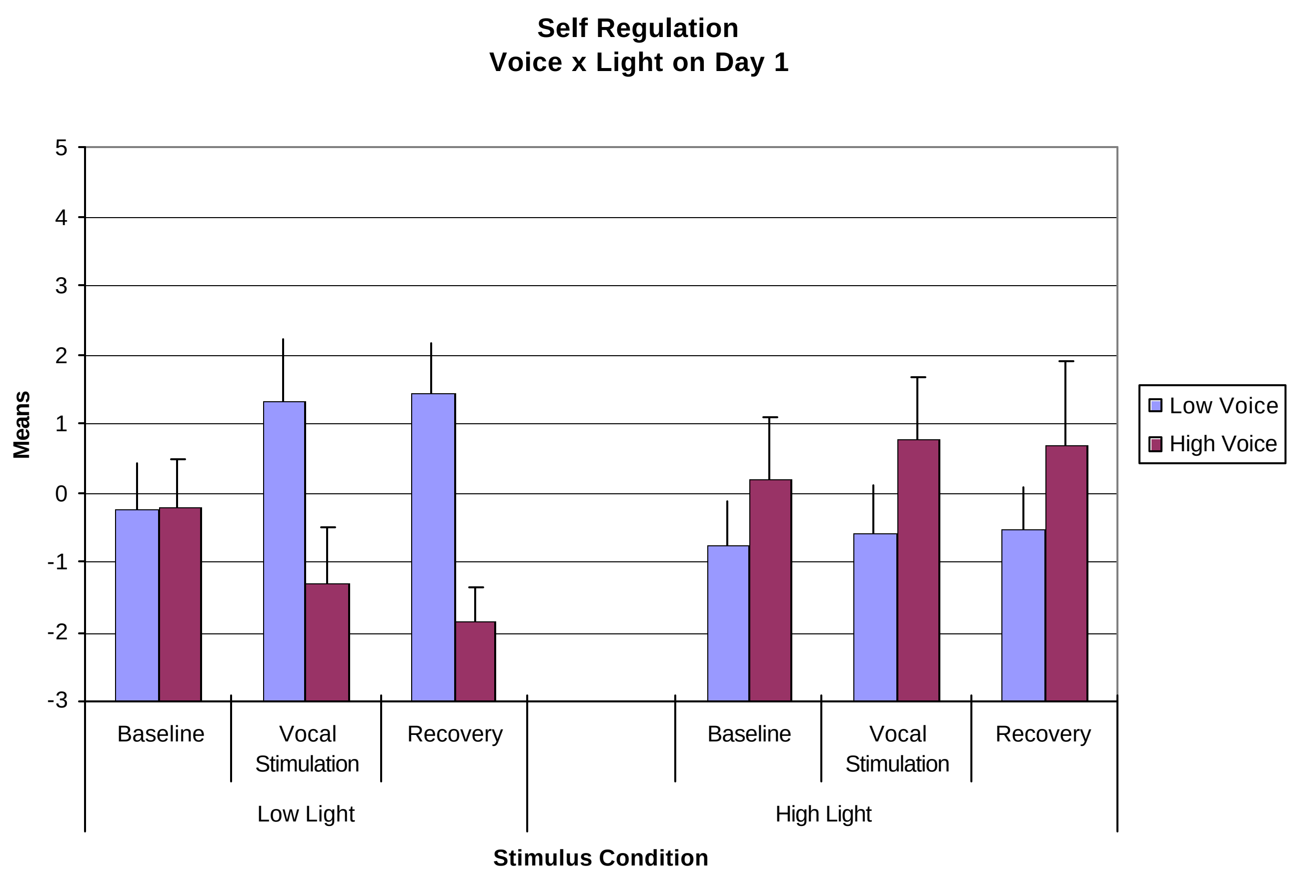
Summary of significant F values for post hoc ANOVA of Stress Reactivity Scores and Self-Regulation Scores

	Df	F	p-value
Stress Reactivity (Day x Condition x Light)			
Day One			
Condition x Light	2, 33	10.314	.00
Self-Regulation (Day x Voice x Light)			
Day One			
Voice x Light	1, 32	5.207	.02
Day Four			
Light	1, 32	12.64	.00
Self-Regulation (Condition x Voice)			
Condition X Voice	2, 31	3.508	.04

Effect of Light and Voice for Day One

This analysis revealed an interaction between voice stimulation and lighting condition on day one, in which the Standard Illumination/High Voice group and the Decreased Illumination/Low Voice group both exhibited significantly more self-regulation behaviors than either the Standard Illumination /Low Voice group or the Decreased Illumination / High Voice group (F (1,32) = 5.21, p = .029; See Figure 2). This is a confusing result, suggesting that either very little stimulation (decreased illumination conditions and little exposure to the vocal stimulus) or that very high stimulation levels (high lighting conditions and frequent exposure to the vocal stimulus) led to increased amounts of self-regulation behaviors.

Figure 2

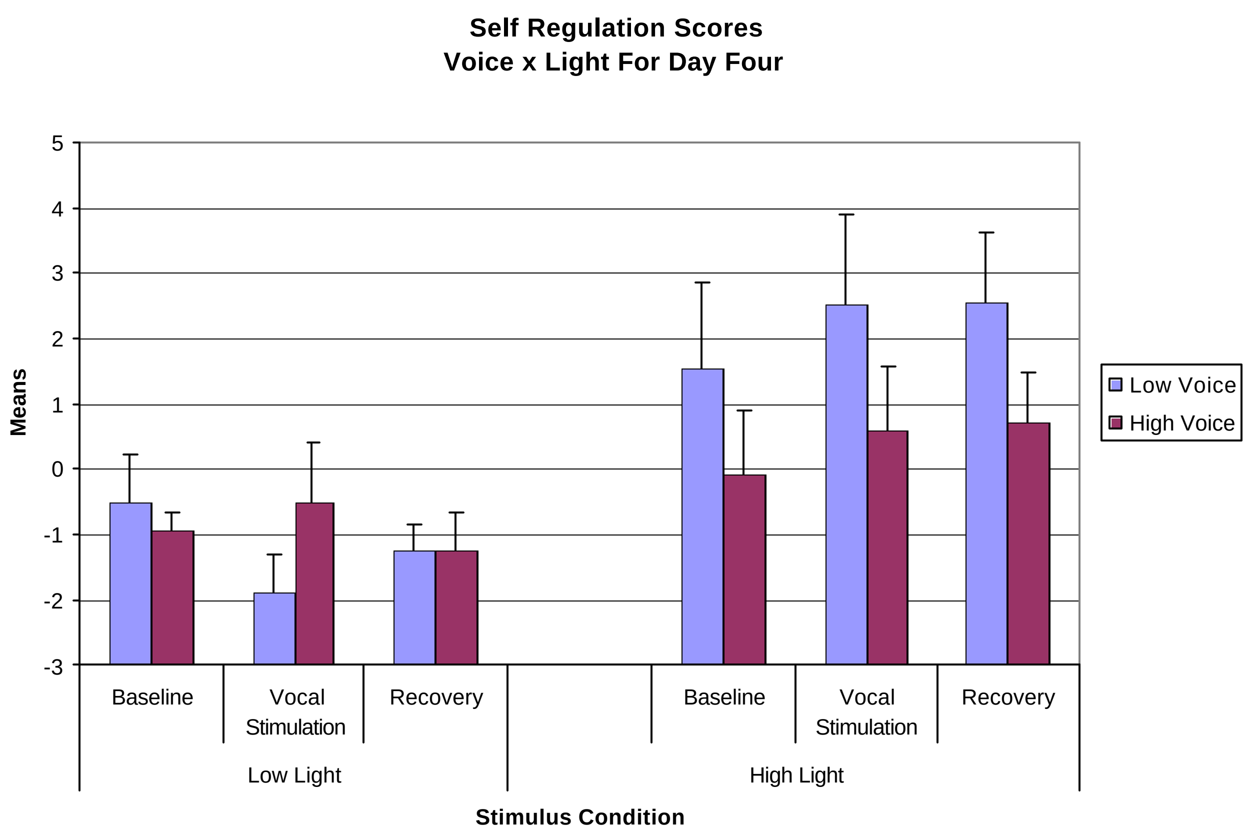


* Y bars denote standard error

Effect of Light for Day Four

In addition to the significant effect for voice and illumination on day one, there was an effect of illumination condition for day four, in which infants in the standard illumination groups also exhibited more self-regulation behaviors than the decrease illumination group, (F (1, 32) = 12.644, p = .001, see Figure 3). Again, this is unusual, since self-regulation behaviors were thought to be indicative of better organization and less overall stress. Non significant effects for day 2, 3 and 5 are represented graphically in Appendix 5.

Figure 3

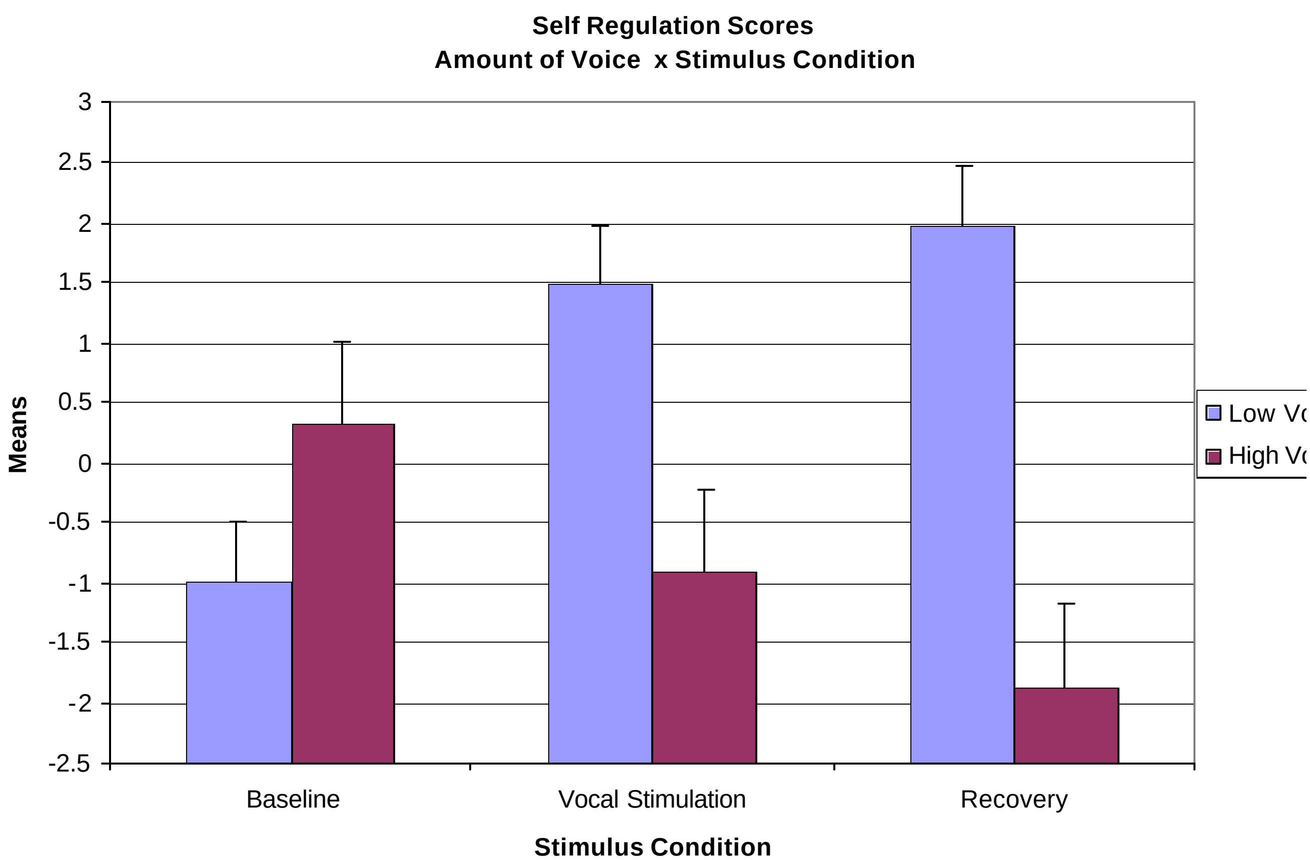


* Y bars denote standard error

Effect of Voice and Condition

The issue becomes more complicated when the condition x voice effect is examined (see Table 3). The repeated measures ANOVA revealed a condition by voice effect (F (2, 31) = 3.5 , p = .042), in which the low vocal stimulation group appeared to increase the overall self-regulation behaviors across the three conditions, where the high vocal stimulation group decreases the overall amount of use of self-regulation behaviors across the three conditions (see Table 5, Figure 4). However, no significant effects were found at any of the three conditions.

Figure 4



- Y bars denote standard error

Supplemental Data Analysis

An additional data analysis was conducted to determine if any of the effects found in the previous analyses were largely influenced by the measures at baseline. An analysis of covariance (ANCOVA) was conducted for the stress reactivity and self-regulation by day. No changes were found for any of the effects previously discussed here. Table 6 displays the significant effects found by the ANCOVA, which correspond to the effects found in the previous analysis.

Given all of these conflicting results, it is unclear the role that multimodal stimulation plays in the self-regulatory behavior of preterm infants. It may be that the composite measure that was used for self-regulatory behaviors is tapping into two separate factors of self-regulation. Self-regulation can be viewed as the infant's ability to organize and maintain adaptive behaviors in response to stimulation. These behaviors could be present both in response to high stress (in order to cope) or in times of low stress (as an indication or organization and allocation of attention to the environment). Therefore, self-regulation behaviors might be used by an infant attempting to *maintain* organization in the face of stressful events. In contrast, we may see self-regulation behaviors in infants who are not experiencing high levels of stress.

Table 6

Summary of significant F values for post hoc ANCOVA of Stress Reactivity Scores and Self-Regulation Scores by Day

	Df	F	p-value
Stress Reactivity (Day x Condition x Light)			
Day One			
Condition x Light	1, 31	14.288	.001
Self-Regulation (Day x Voice x Light)			
Day One			
Voice x Light	1, 31	8.205	.007
Day Four			
Light	1, 31	6.804	.01

State data

In order to test the overall effects of vocal stimulation on self regulation and stress reactivity (Hypothesis I) and the modifying effects of light stimulation (amount of vocal stimulation in various illumination levels) on those variables (Hypothesis II), a repeated measures ANOVA was completed on the two levels of voice and two levels of illumination, as well as the within subjects variables of state, day, and stimulus condition (baseline, stimulus, or recovery). Several effects were found when examining infant states. The repeated measures ANOVA revealed a main effect of State, an interaction of State x Illumination, an interaction of State x Condition, and two three-way interactions: State x Condition x Illumination and State x Condition x Voice (Table 5). Since the three way interactions supercede the two-way interactions and the main effect of state, further ANOVA analyses were conducted, in which the levels of state were held constant and the relationship between condition and illumination, and condition and sound, were explored.

Effects of Light and Condition for States

By isolating each level of state, significant illumination by condition interactions were found for infants in sleep states, drowsy states, and quiet alert states (Table 7).

Table 7

Summary of F values for post hoc ANOVA on Infant State Scores

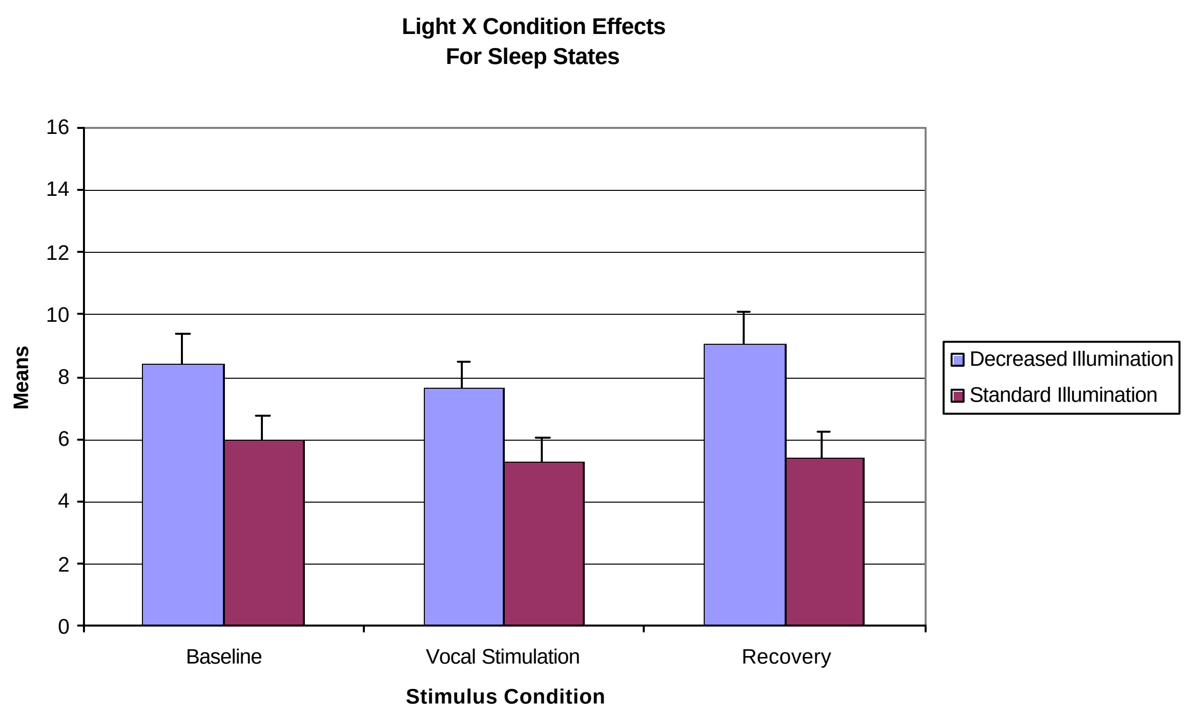
	Df	F	p-value
State x Condition x Light			
Sleep			
Condition	2, 33	4.271	.02 *
Condition x Light		3.30	.05 *
Drowsy			
Condition	2, 33	13.300	.00 *
Condition x Light		2.22	.12
Quiet Alert			
Condition	2,.33	13.327	.00 *
Condition x Light		2.53	.09
Active Alert			
Condition	2, 33	2.321	.11
Condition x Light		2.048	.14
Aroused			
Condition	2, 33	2.118	.13
Condition x Light		.104	.90
State x Condition x Voice			
Sleep			
Condition	2, 33	4.650	.01 *
Condition x Voice		3.405	.04 *
Drowsy			
Condition	2, 33	16.363	.00 *
Condition x Voice		4.36	.02 *
Quiet Alert			
Condition	2,.33	12.441	.00 *
Condition x Voice		.562	.57
Active Alert			
Condition	2, 33	2.244	.12
Condition x Voice		1.397	.26
Aroused			
Condition	2, 33	2.164	.13
Condition x Voice		2.195	.12

* $p < .05$

Sleep States

When looking at the effects of illumination and condition for sleep states, the analysis revealed a within subjects effect of condition and a light x condition interaction (see Table 6). These effects can be seen in Figure 5. As can be seen in the graph, there more overall sleep states for the infants in the decreased illumination condition than for the infants in the standard illumination condition. In addition, there was an increase in sleeping states during the recovery period (post voice) for the infants in the decreased illumination condition. Thus, even though the lighting condition returned to 'normal' during the recovery period, infants still remained in or increased the amount of sleeping they exhibited. While sleeping can be viewed as a response to high stress ("escaping"), sleeping is generally considered a positive state (indicative of energy conservation and greater self-regulation) (Chuman, 1983; White-Traut, et al., 1997). Given the increase in stress reactivity behaviors for the high light condition exhibited in earlier analyses, I would argue that this overall increase in sleep states for the decreased illumination groups is indicative of greater organization and self-regulation.

Figure 5

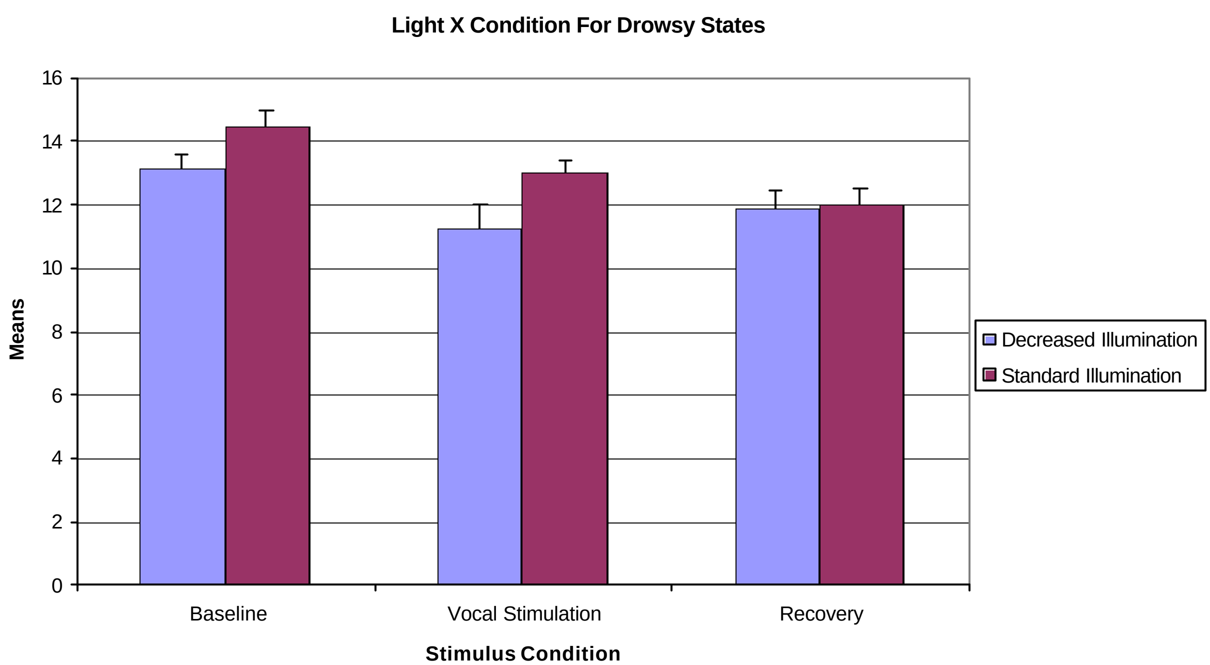


* Y bars denote standard error

Drowsy States

When looking at the effects of lighting level and condition on drowsy states, the ANOVA analysis revealed a within subjects effect of condition (see Table 7). In this case, the occurrence of drowsy states appears to be much greater during the baseline condition than during the stimulus period and the recovery period, regardless of lighting (Figure 6). Considering the sleep state data and the quiet alert data, this is not unexpected (see following discussion on quiet alert data).

Figure 6

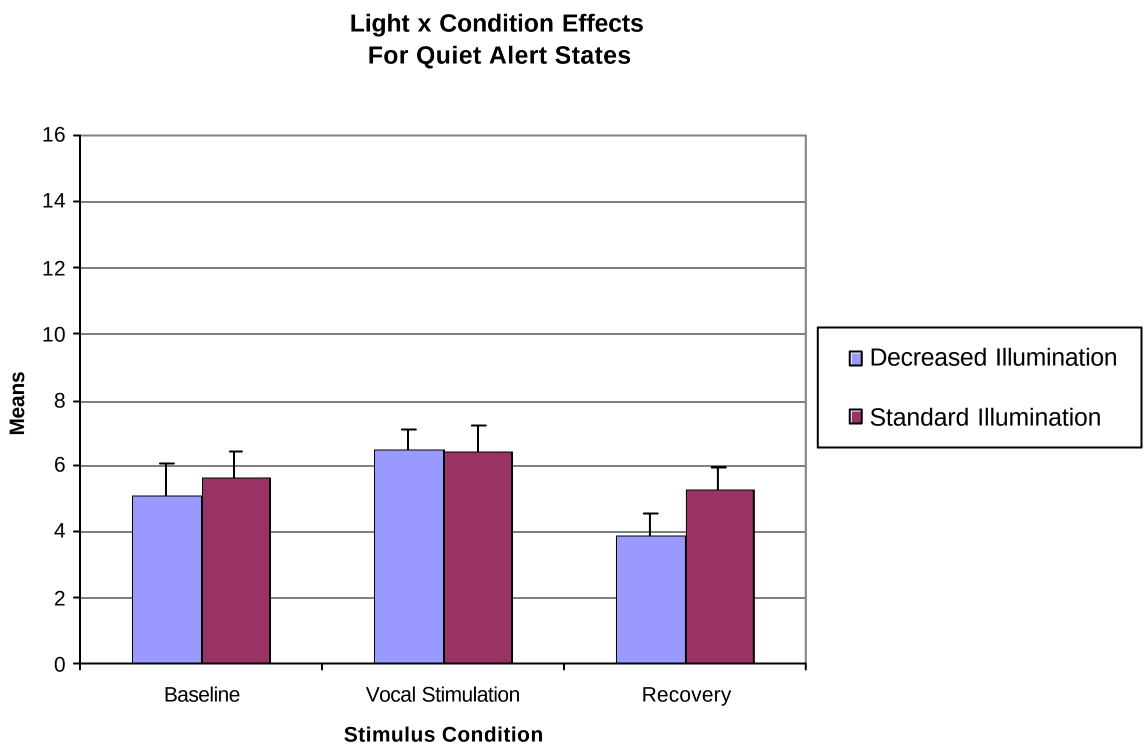


- Y bars denote standard error

Quiet Alert States

The ANOVA on the effects of illumination level and stimulus condition on quiet alert states revealed an effect of condition (see Table 7). As seen in Figure 7, infants exhibited an overall increase in quiet alert states during the vocal stimulation than during baseline or recovery conditions. Quiet alert states are generally considered states that are indicative of greater organization and self-regulation. This suggests that, at least while being exposed to the vocal stimulation, all infants exhibited greater levels of state organization and self-regulation. When you pair this data with the sleep state data (see Figure 5), an interesting pattern develops. Infants in both the decreased and standard illumination conditions exhibit comparable levels of quiet alert states during the vocal stimulation (increased from baseline as well), but they exhibit differences in quiet alert states during recovery. During recovery infants in the decreased illumination condition exhibit much less quiet alert states and exhibit an increase sleep states. In contrast, infants in the standard illumination condition show more occurrences of quiet alert and also exhibit more active alert states as well. While there were no significant effects of either active alert states ($p = .11$) or aroused states ($p =.136$), this does suggest an interesting trend in which decreased illumination coupled with vocal stimulation lead to greater organization and greater energy conserving states, whereas standard lighting coupled with vocal stimulation leads to greater organization during the stimulus period, but possibly greater costs in terms of overall stress on the system during recovery. In essence, the ability of the infants in the standard illumination groups to remain organized and calm appeared somewhat compromised.

Figure 7



- Y bars denote standard error

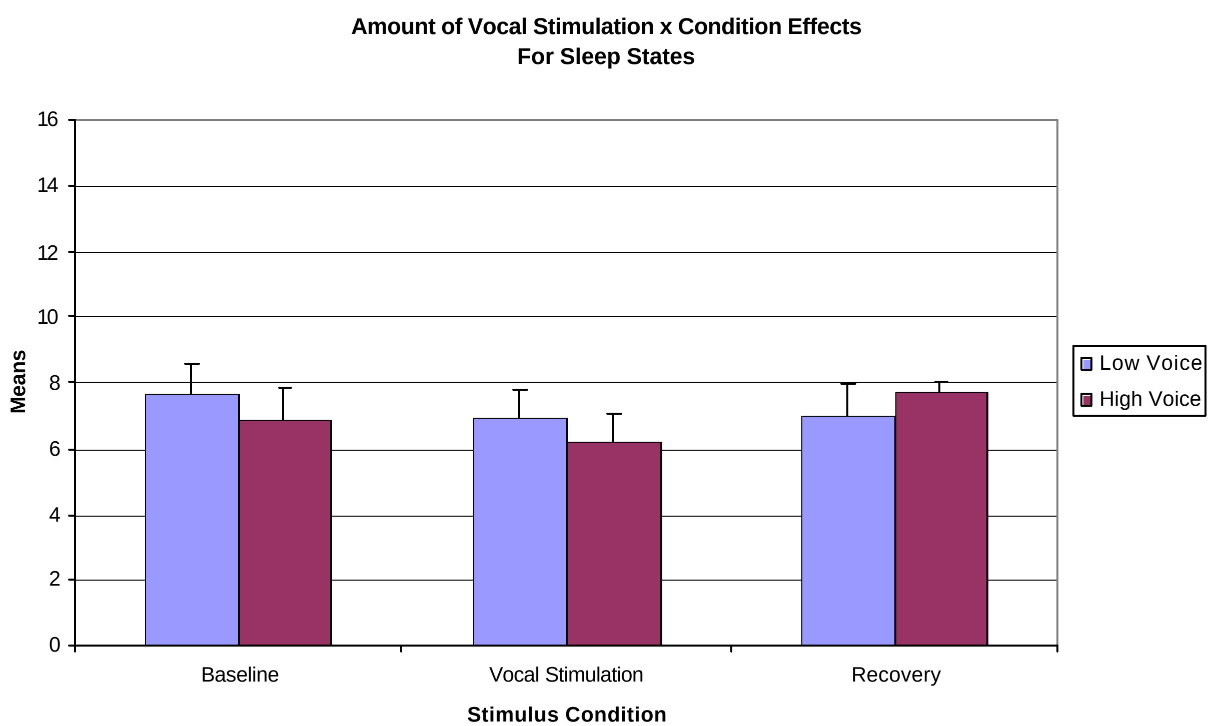
Effects of Amount of Vocal Stimulation and Condition on States

While this data suggests that the vocal stimulation does play a role in the organization of infants self-regulatory and stress reactivity (Hypothesis 1), the effect of the relative *amount* of vocal stimulation remains unclear. Thus, further analysis on the State x Condition x Voice interaction was conducted, in which the levels of state were held constant and the relationship between stimulus condition and voice were explored (see Table 7). An ANOVA revealed significant effects for sleep states, drowsy states, and quiet alert states. No significant effects for either active alert or aroused states were found.

Sleep States

When isolating the occurrence of sleep states, there was a within subjects effect of stimulus condition and a condition x voice interaction (see Table 7). Seen in Figure 8, infants in the low vocal stimulation group exhibited more overall sleep during the baseline period and vocal stimulation period than the high vocal stimulation group. However, this pattern changed during the recovery period, when the infants who received more vocal stimulation exhibited a greater increase in sleeping than infants who received less overall vocal stimulation. This effect complements the results found in the analysis of drowsy states (see Table 7), in which there was a significant within subject's effect of stimulus condition and a significant condition x voice interaction.

Figure 8

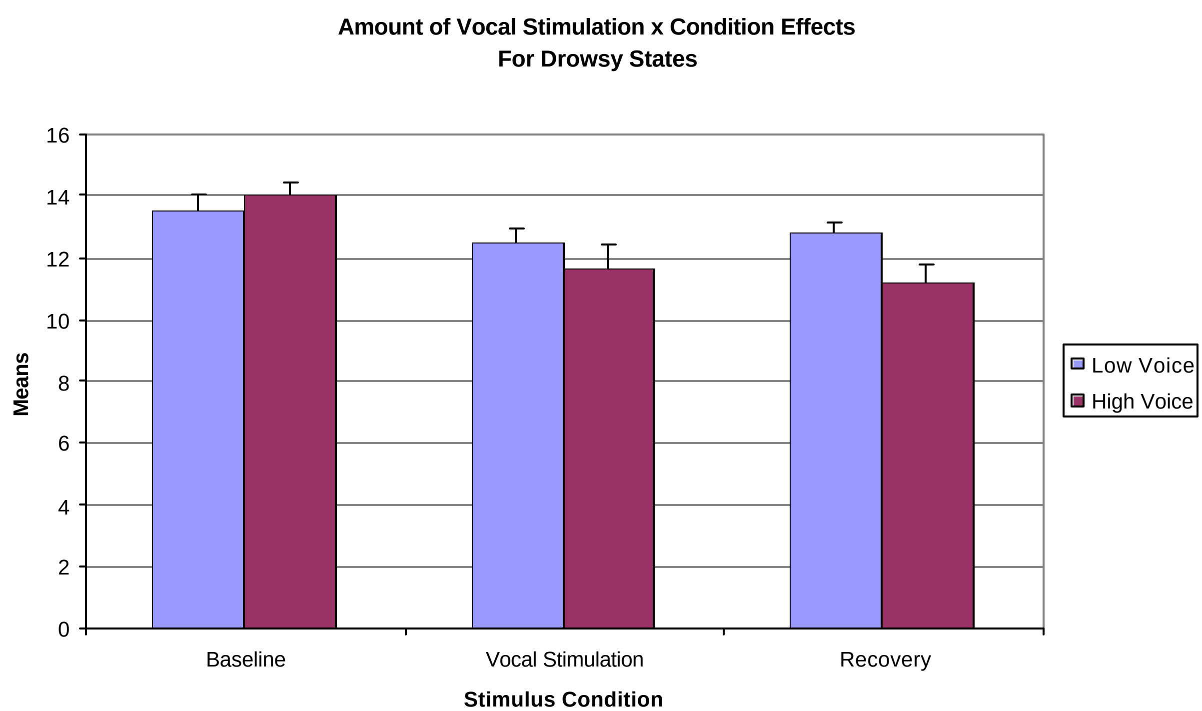


- Y bars denote standard error

<u>Drowsy States</u>

As seen in Figure 9, infants in both low and high vocal stimulation groups exhibited higher levels of overall drowsiness during baseline than during the other two periods. More importantly, infants in the high vocal stimulation group exhibited a decrease in the overall amount of drowsiness they exhibited during the recovery period. This may be because they exhibited more sleep states. If we accept that greater amounts of sleep states are representative of greater organization and self-regulation, the implications suggest that infants who were exposed to more vocal stimulation were better able to achieve restful sleep states. However, one could also take the view that greater amounts of vocal stimulation over time (i.e. three times a day vs. one a day) may have lead to overall *overstimulation,* resulting in infants retreating into sleep states after the exposure period.

Figure 9

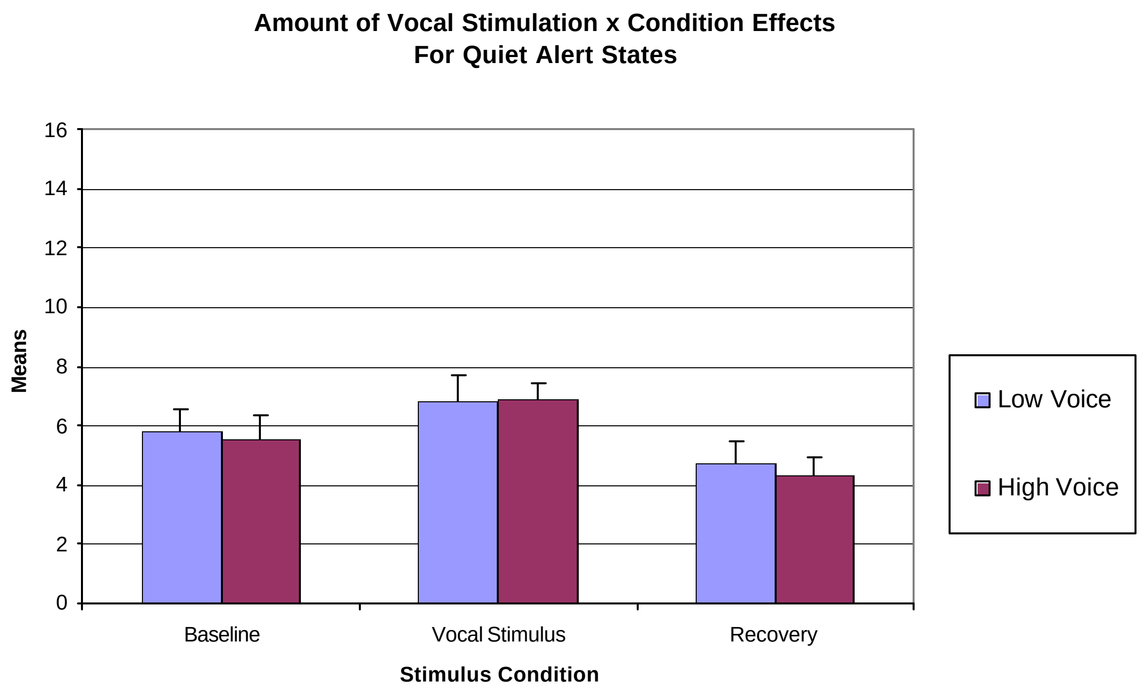


* Y bars denote standard error

<u>Quiet Alert States</u>

Finally, an ANOVA was conducted to explore the effects of different amounts of vocal stimulation and the stimulus condition on the occurrence of quiet alert states. Results of this analysis found a significant effect of stimulation condition (see Table 6). As seen in Figure 10, infants exhibited an overall increase in quiet alert states during the vocal stimulation when compared to the baseline or recovery conditions, regardless of voice group. Again, quiet alert states are generally considered states that are indicative of greater organization and self-regulation. This suggests that, at least while being exposed to the vocal stimulation, infants exhibited greater levels of state organization and self-regulation. These results complement the results found in the analysis of condition x light discussed earlier.

Figure 10



- Y bars denote standard error

Supplemental Data Analysis

An additional data analysis was conducted to determine if any of the effects found in the previous state analyses were largely influenced by the measures at baseline. An analysis of covariance (ANCOVA) was conducted for the state data. Results of this analysis can be found on Table 8. There were no significant effects of the covariance of baseline with any of the other conditions. However, these analyses did alter some of the significant effects. These changes were consistent with the conclusions drawn from the original data analysis. With the sleep data, when baseline was taken out of the analysis, the condition effect disappears from both the Illumination and the Vocal results (Table 8). However, effects for the both the Light x Condition and the Voice x Condition are significant. With the drowsy state data, the condition effect also disappeared after co-varying with baseline. This is not surprising, given that it appears the differences between the baseline condition and the other two conditions were driving the effect. Finally, covarying baseline out of the quiet alert state data did not have any effect on both the significant condition(s), and resulted in a significant condition x light effect. That is, when you look at just the vocal stimulation and the recovery conditions, there is a interaction between illumination level and condition, in which decreased illumination levels appear to result in a greater change in amount of quiet sleep from the stimulation condition to the recovery period, compared to the standard illumination (Figure 7). This is not unexpected given the change in illumination level that accompanies the shift between the stimulation condition and the recovery period, in which both the presence of vocal stimulation and the level of illumination changes between these two conditions for the decreased illumination group.

Table 8

Summary of F values for post hoc ANCOVA on Infant State Scores

	Df	F	p-value
State x Condition x Light			
Sleep			
Condition	1,31	.004	.95
Condition x Light		4.181	.05*
Drowsy			
Condition	1, 31	2.687	.11
Condition x Light		1.89	.17
Quiet Alert			
Condition	1, 31	7.769	.00 *
Condition x Light		2.53	.03 *
State x Condition x Voice			
Sleep			
Condition	1,31	.0041	.95
Condition x Voice		9.411	.004*
Drowsy			
Condition	1,31	2.678	.11
Condition x Voice		.530	.47
Quiet Alert			
Condition	1,31	7.769	.00 *
Condition x Voice		1.17	.287

* $p < .05$

Chapter 4

Discussion

The purpose of this study was to examine the effect that vocal stimulation has on preterm infants' behavioral and state organization and to explore the possible effects that concurrent light stimulation have on the organizing effects of vocal stimulation. When taken together, the results from this study suggest that the presence of female vocal stimulation may provide opportunities for preterm infants to exhibit more organized behavioral and state patterns, conserving energy and allowing opportunity for interaction. However, the evidence also suggests that these effects can be mediated by the relative amount of visual (light) stimulation available while the vocal stimulation is being presented. When listening to the vocal stimulation, the infants in the present study all exhibited quiet alert states. However, the overall light level appeared to mediate the effect that this vocal stimulation had on the states and behavior during the recovery period. Specifically, infants in higher levels of light exhibited more wakefulness after the vocal stimulation was turned off, whereas infants in the low lighting condition exhibited more sleep states.

The increase in quiet alert states during vocal stimulation found in the present study supports Bozzette's (1999) findings, in which preterm infants exhibited active stilling, bright eyes, and controlled motor tone during exposure to the vocal stimulation. These behaviors suggest that infants may have been in a quiet alert state during the presentation of vocal stimulation. In the present study, many of the infants appeared to spend relatively more time in either drowsy or sleeping states during vocal presentation. Once the vocal stimulus was presented, however, the infants exhibited a quiet alert state that would allow them to respond to the stimulus in an organized way. Bozzette (1999) found that the infants in her study decreased the amount of the behaviors which suggested a 'quiet alert' state during the post stimulus period, but did not report significant differences in either behavior or state during this period. It is unclear from Bozzette's study what these specific post stimulus behaviors were. Infants in the present study who had more exposure to the female voice exhibited more sleeping states during the recovery period compared to the low vocal stimulation infants. In contrast, infants who had less exposure to the vocal stimulus exhibited more drowsy states then the high vocal stimulation infants.

Sleep states are associated with less energy expenditure and greater behavioral and physiological organization (Chuman, 1983; White-Traut, et al., 1997). Indeed, regardless of vocal or light condition, infants exhibited overall more sleep and drowsy states, compared to all other states. However, one limitation of the present study was that the preterm infants' sleep states could not be broken down into either 'A' or 'B' sleep states. This is important because a 'B' sleep state is considered to represent more organized functioning. Unfortunately, due to limitations in the videotape recording procedure, factors involved in making determinations between 'A' and 'B' states (such as respiration rates or infant color) could not be made. However, since sleep and drowsy states were much more prevalent than aroused states, it can be said that infants exhibited more overall energy conservation. Indeed, there was no significant increase in the amount of active alert or arousal states for any of the infants across conditions. Since quiet alert states did increase in occurrence during the presentation of vocal stimulation, the fact that infants in the high voice condition experienced more sleep states during the recovery period suggests that these infants transitioned from quiet alert states to sleep states more easily. It is possible that the more repeated exposure to the vocal stimulus allowed them to more readily respond the vocal stimulus to organize their behavior. In addition, this effect might be the result of adaptation to the stimulus. That is, infants who had more exposure to the female voice may have been accustomed the stimulation and were thus less aroused when the stimulus was turned off.

The infants in the low vocal stimulation group displayed similar relative amounts of time in quiet alert states during the vocal stimulation, but displayed significantly less time in sleep states during the recovery period. This group also spent more time in drowsy states during the recovery period. This may help explain why infants in the low vocal stimulation group appeared to exhibit more self-regulation motor behaviors during the recovery period as well. Since the infants in the high vocal stimulation group were more likely to be in a sleep state, they would exhibit less overall motor behavior while in that state. However, since the infants in the low vocal stimulation group were less likely to be sleeping and more likely to be awake (even diffusely), they may have exhibited more self-regulation motor behaviors as a result. Since the Bozzette study (1999) did not vary the amount of exposure to vocal stimulation (her study utilized exposure comparable to the high voice condition of this study), the results of the present study can be seen as a first step in clarifying the differential effect of the amount of vocal stimulation on preterm infants' behavioral organization.

None of the previous studies (Bozzette, 1999; Malloy, 1979; Segall, 1972; Standley & Moore, 1995, or White-Traut, et al., 1997) on vocal stimulation of preterm infants examined the effect that lighting might have on overall responsiveness to vocal stimulation. Previous research does suggest that visual stimulation (whether alone or coupled with other stimulation) can effect the organization of preterm infants (White-Traut, et al., 1997). The results of this study suggest that the effects of vocal stimulation on behavioral and state organization can be somewhat mediated by the presence or absence of light during the vocal stimulus. Recall, light levels were only manipulated during the presentation of the vocal stimulus. Regardless of lighting condition, all infants displayed more quiet alert states during presentation of the vocal stimulation than before or after. However, infants in the low light conditions also exhibited more sleep states that the infants in the high light condition. Presentation of vocal stimulation with decreased light levels appeared to allow better state organization.

In contrast, higher levels of light appeared to increase the amount of stress behaviors that infants displayed. Infants in the high light condition exhibited significantly more stress motor behaviors during recovery on the first day, and (while not significant) exhibited a trend for greater amounts of active alert states during the recovery period. Infants in the high light conditions also exhibited more self-regulatory motor behaviors on the fourth day. These results are not surprising, given the results from comparative work which suggest that unusually early visual stimulation can alter early perceptual and behavioral organization (Lickliter, 2000). White-Traut, et al.(1997) found that the more stimulation provided to preterm infants (across modalities) resulted in more alert/awake states as well. It is unclear whether the effects found in this study were the result of the unique combination of vocal and light stimulation, or whether other multimodal stimulation would result in similar effects. It would be interesting to explore factors such as lighting level during tactile or vestibular stimulation, both of which are available earlier in prenatal development and may not be as influenced by the amount of visual (light) stimulation in the environment.

The vocal stimulation used in this study was an 'adult-directed', unfamiliar female voice. The reasoning behind using adult-directed speech was that the acoustic nature of infant-directed speech (with its greater pitch variability and higher frequency) might be overstimulating for preterm infants. However, there is no empirical evidence to indicate that this is indeed the case. The acoustic nature of vocal stimulation in previous studies was not reported, so it remains unclear as to the effects of either the familiarity or acoustic nature of the vocal stimulation. While this study found some effects of vocal stimulation with the use of an unfamiliar female voice, it would be interesting to know whether these effects would be more pronounced if maternal vocal stimulation was provided. Previous research by Bozzette (1999), Segall (1972) and others suggests that maternal vocal stimulation does have positive organizational effects. Since maternal vocal stimulation is a naturally occurring stimulus in the womb, and has been implicated in later behavioral responsivity, it would be interesting to compare these two types of vocal stimulation and see if, at this level of development, there are any immediate differences in the effects of these stimuli. Additionally, it would be interesting to determine if the relative familiarity of the vocal stimulus would affect the behavioral responses given concurrent stimulation from other modalities. That is, does the relative type of vocal stimulation (maternal voice versus familiar nursing staff versus other female vocal stimulation) influence how visual (light) stimulation influences the state and behavioral organization of the preterm infant?

The infants in this study were exposed to non-contingent vocal stimulation. However, preterm infants might display better organization when the vocal stimulation is presented in dyadic way, with the stimulation presented in response to the infant's behavioral displays. Further while this study showed short-term effects of vocal stimulation and light level on preterm infants' behavioral and state organization, it is unclear whether these effects translate into any long term differences in physiological or behavioral organization in the days and weeks following exposure.

Given the results of this and the other studies, a few tentative recommendations can be made regarding the presentation of vocal stimulation in the NICU. While vocal stimulation appears to lead to increases in quiet alert states and does not appear to result in significant increases in stress behaviors, this can apparently be somewhat mediated by the presence of light stimulation in the environment. Decreased levels of light appear to result in decreased stress and overall better organization. This fact is supported by the research on the effects of light levels in the NICU. Previous research suggests that lowered lighting decreases stress and energy consumption, and those elements assist the preterm infant in physiological organization. This current study also suggests that positive effects of supplemental vocal stimulation may be fostered by the use of lowered lighting. Additionally, the amount of vocal stimulation needs to be carefully monitored. Preterm infants often exhibit a delay in the display the effects of stimulation and other stressful events (Als, 1983, 1995b). This delay in behavioral stress displays and physiological changes (such as oxygen desaturation or heart rate changes) can sometimes be exhibited long after the stressful event has occurred. Since this study (and many others) did not examine the effects of stimulation beyond a 10 minute recovery period, it would be appropriate to be cautious about implementing a 'stimulation regime' to supplement the preterm infants' available stimulation. Results from this study suggest that more stimulation might provide some benefits, however further research is needed to determine the exact amounts (exposure time, frequency) and the types of stimuli to be used (maternal voice versus other vocal stimulation). It seems important for staff and families who use vocal stimulation with their preterm infants to do so within the context of careful monitoring and observation of the infant's responses to the stimulation both during and after exposure. Since preterm infants will often display a delay in responsiveness, it is important to look well beyond the immediate effects of stimulation and observe the infant's behavioral and physiological states for longer periods of time than reflected in the present study.

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Appendix 1: Als NIDCAP Manual Motor Behaviors
From Als, 1995b, Manual of Naturalistic Observation of Newborn Behavior (NIDCAP Manual), pp. 7-14.

Selected Motor/Attentional System Behaviors

1. **Extend arm** - refers to the active extension/maintenance of arm(s) in extension either in mid air or on a surface.
2. **Extend leg** - refers to the active extension/maintenance of legs(s) in extension either in mid air or on a surface.
3. **Squirm**- Refers to small, writhing, wriggling motions of the trunk, often with accompanying movement of the extremities, yet not showing the labored stretching, struggling patterns of stretch/drown.
4. **Leg brace**: The infant extends leg(s) and/or feet towards the edge of or wall of incubator, crib, foot roll, and/or caregiver's hand or body, as if in an effort to stabilize, brace, and gain boundary and inhibition to extensor movement or posture. Once touching, the infant may flex the legs and relax while maintaining the bracing, or may restart the active bracing efforts. Even if no surface is available against which the bracing is successful, efforts at apparently seeking such a surface are also marked in this category. The infant may be activity pressing one or both feet against the mattress or a blanket roll, etc.
5. **Grimace**: This is a facial extension configuration often accompanied by lip retraction and facial retraction and distortion. Eyebrow knitting or frowning is not a part of this configuration, since these represent facial flexion rather than facial extension.
6. **Smile**: Smiling requires facial relaxation with flaccidity and is formed by an at least slightly upward curving of the corner(s) of the mouth, often accompanied by a momentary or prolonged softening of the cheeks.
7. **Mouthing**: The infant makes one or several repetitive lip and/or jaw opening and closing movements. These are distinguished from suck searching. In mouthing, the lips usually soft and relaxed and are not directly forward.
8. **Suck Search**: The infant actively extends the lips forward or sideways and/or opens the mouth in a searching rooting fashion; the infant often moves the head while doing so, as if seeking something to suck on.
9. **Sucking**: The infant sucks on hand or fingers, on clothing, bedding, the caregiver's finger or mother's breast, a pacifier or other object that the infant has either obtained or that the caregiver has inserted into the infant's mouth.
10. **Finger Splay**: The infant's hand(s) open and the fingers are extended and separated from each other.
11. **Airplane**: The infant's arm(s) are either fully extended out to the side at approximately shoulder level or upper and lower arm are at an angle and are extended out at the shoulder.
12. **Salute**: The infant's arm(s) are fully extended into mid air in front of the infant, either singly or simultaneously. This is often but not necessarily accompanied by finger splaying.
13. **Sitting on air**: The infant's legs are extended into mid air either singly or simultaneously.
14. **Hand clasp**: The infant grasps one hand with the other or clutches the hands in midline to the body. Each hand may be closed, yet they hold onto each other or actively press against each other. Interdigitation of fingers of one hand with those of the other hand is a subcategory of hand clasp.
15. **Foot clasp**: The infant positions one foot against the other, either foot sole to foot sole or one foot sole against the other ankle or leg, or the infant folds the legs in a crossed position with feet grasping the legs resting against them.
16. **Hand to mouth**: The infant attempts to bring one or both hands to the mouth in an apparent attempt to suck on them. The effort does not need to be successful to be marked.
17. **Grasping**: The infant makes grasping movements with the hands, either directed at the face or body, or in midair, or to the caregiver's hands or fingers or body, the infant's own tubing or bedding, etc.
18. **Fisting**: The infant appears to hold on to the own hand by flexing the fingers and forming a fist, occasionally observed with an object in the hand (e.g., edge of a blanket).
19. **Fuss**: While fussing is often a component of State 5 behavior, this is not necessarily so. At times, fussing occurs in State 3 or even in State 2. Fussing is an audible vocal expression of discomfort, uneasiness, unhappiness, upset, and/or disorganization.
20. **Yawn**: The infant opens the mouth widely, usually with a deep inspiration.

21. **Sneeze**: The infant expels air forcibly from the mouth and nose in an explosive, spasmodic action.
22. **Frown**: The infant knits the eyebrows or darkens the eyes by contracting the periocular musculature, engaging in a flexion of the upper face.

Appendix 2: Als NIDCAP Manual States
From Als, 1995b, Manual of Naturalistic Observation of Newborn Behavior (NIDCAP Manual), pp. 7-14.
State-Related Behaviors

I. Sleep States

State 1: Deep Sleep

- 1A: Diffuse deep sleep with obligatory regular breathing or breathing in synchrony with only the respirator, eyes closed, no eye movements under closed lids; quiet facial expression; no spontaneous activity; typically poor color.
- 1B: Robust deep sleep with predominantly modulated regular breathing; eyes closed, no eye movements under closed lids, relaxed facial expression; no spontaneous activity; typically poor color.

State 2: Light Sleep

- 2A: Diffuse light sleep with eyes closed, rapid eye movements may be observed under closed lids; low amplitude activity level with diffuse and disorganized movements; respirations are irregular and there are many sucking and mouthing movements, whimpers; facial, body, and extremity twitchings, much grimacing; the impression of a diffuse state is given. Color is typically poor.
- 2B: Robust light sleep with eyes closed; rapid eye movements may be observed under closed lids; low activity level with movements and dampened startles; movement are likely to be lower amplitude and more monitored than in State 1; the infants responds to various internal stimuli with dampened startle. Respirations are more regular, mild sucking and mouthing movements may occur, as well as infrequent sighs or smiles.

II. Transitional States

State 3: Drowsy

- 3A: Diffusely drowsy, semi-awake or semi-asleep; eyes may be open or closed, eyelids fluttering or blinking very exaggerated; if eyes are open, they may have a glassy veiled look; activity level is variable, with or without interspersed, startles from time to time; diffuse movement; fussing and/or much discharge of vocalization whimpers, facial grimacing, etc.
- 3B: Robustly drowsy, as above yet with little discharge of vocalization, whimpers, facial grimacing, etc.

III. Awake States

State 4: Quietly awake or alert

- 4A : Diffusely awake. Two types:
    - L Low keyed, lidded, diffuse awakeness; quiet minimal motor activity, eyes half open or open with glazed, dull or pained look, giving the impression of little energy; or focused yet strained alertness, appearing to look through, rather than at, an object or the caregiver.
    - M Hyperalert; eyes wide open, giving the impression of panic, fear, or overwhelmedness; appearing to be hooked by the stimulus; has difficulty breaking gaze away from stimuli; The infants seems to have difficulty in modulating or breaking the intensity of the fixation to an object or the caregiver, or other stimulus.
- 4B: Robustly alert with bright shiny eyes, animated facial expression; the infant appears to focus attention on a source of stimulation or a person and appears to process information actively and with modulation; motor activity is at a minimum.

State 5: Actively awake and aroused

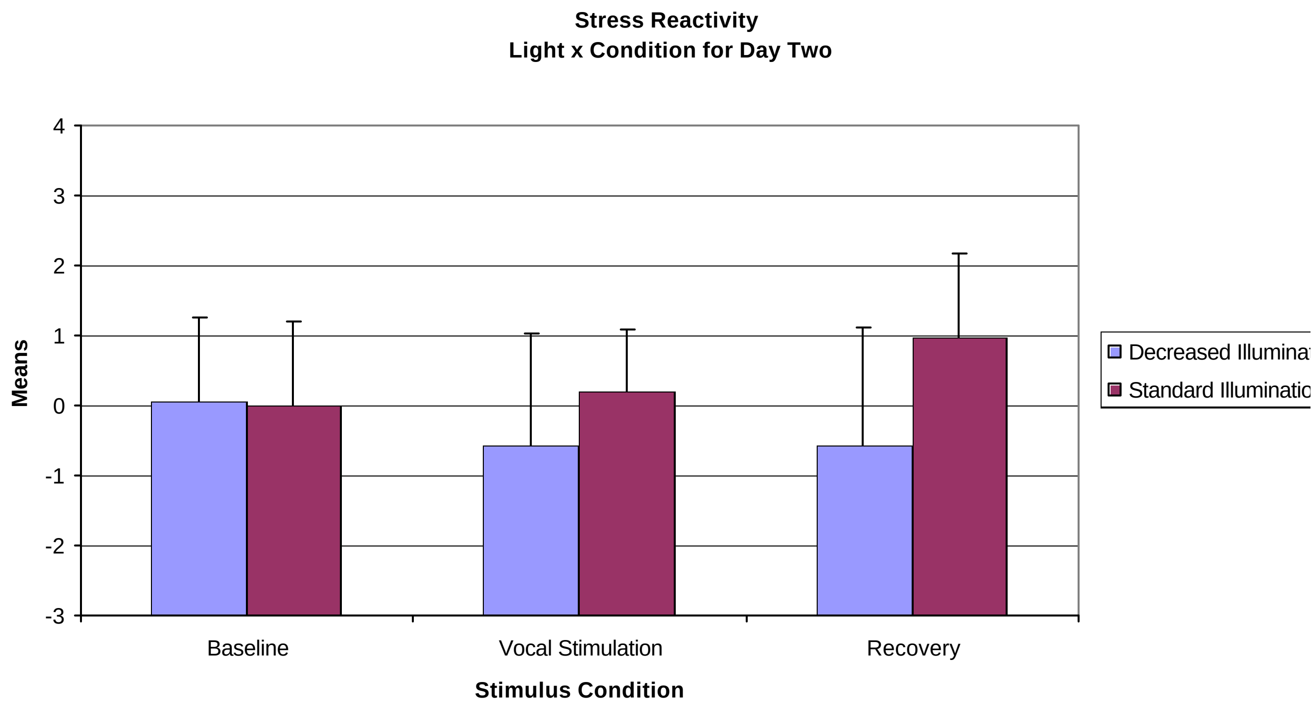
- 5A: Diffusely actively aroused; eyes may or may not be open; the infant is clearly awake and aroused, as indicated by motor arousal, tonus, and distressed facial expression, grimacing, or other signs of discomfort. Vocal fussing, if present, may be diffuse or strained.
- 5B: Robustly actively aroused; eyes may or may not be open; infant is clearly awake and aroused, with considerable, yet well-defined motor activity. The infant may also be clearly fussing without crying robustly.

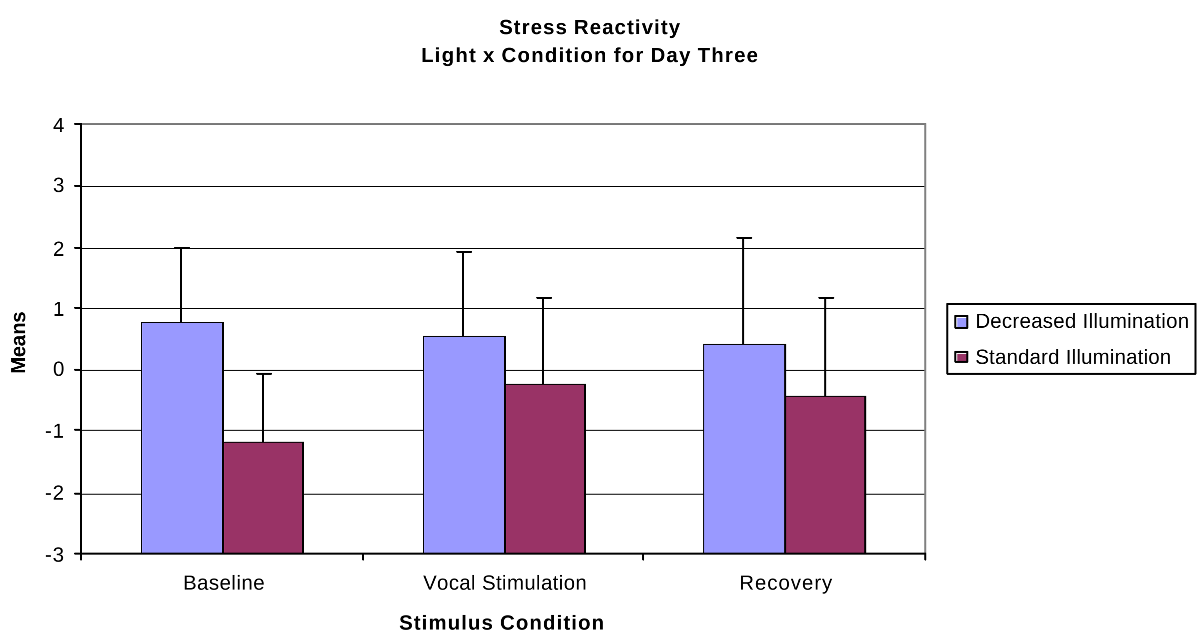
State 6: Highly aroused, agitated, upset, and/or crying.

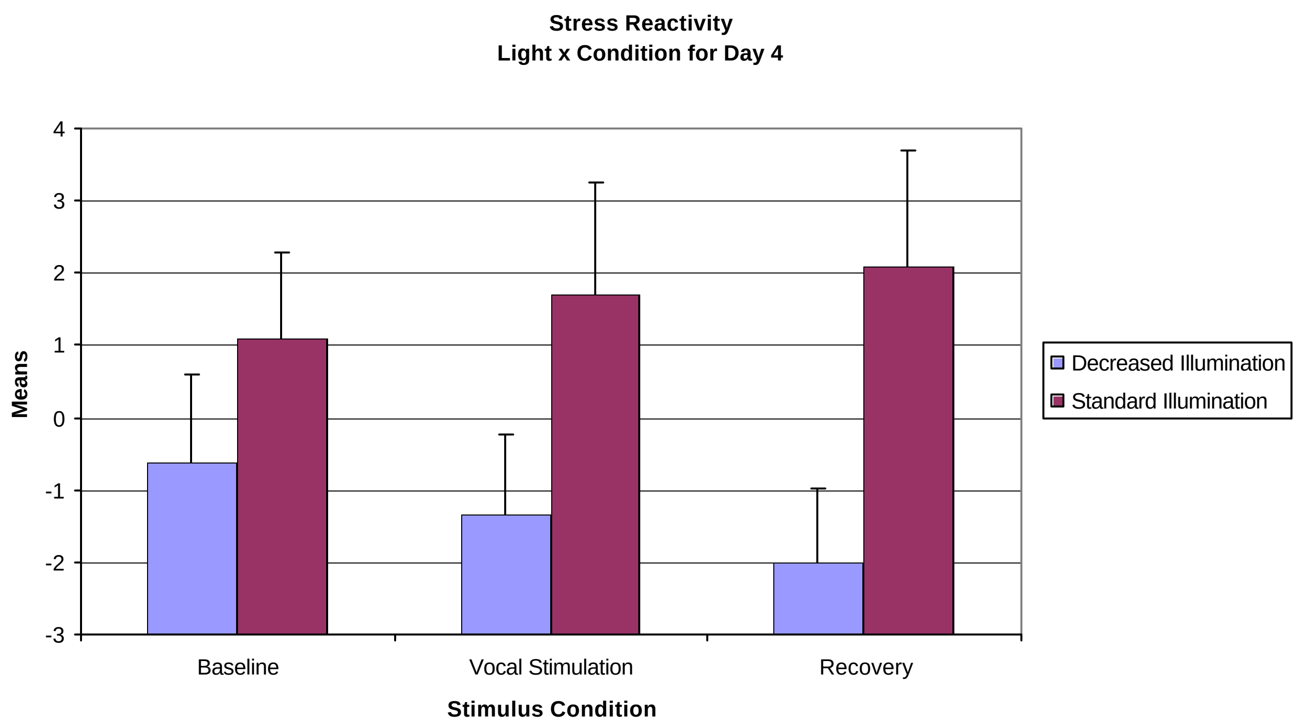
- 6A: Diffusely highly aroused with intense upset, as indicated by intense grimace and cry face, yet cry sound may be very strained, weak, or absent; intensity of upset is very high.
- 6B: Robustly highly aroused with rhythmic, intense, lusty crying which is robust and vigorous in sound.

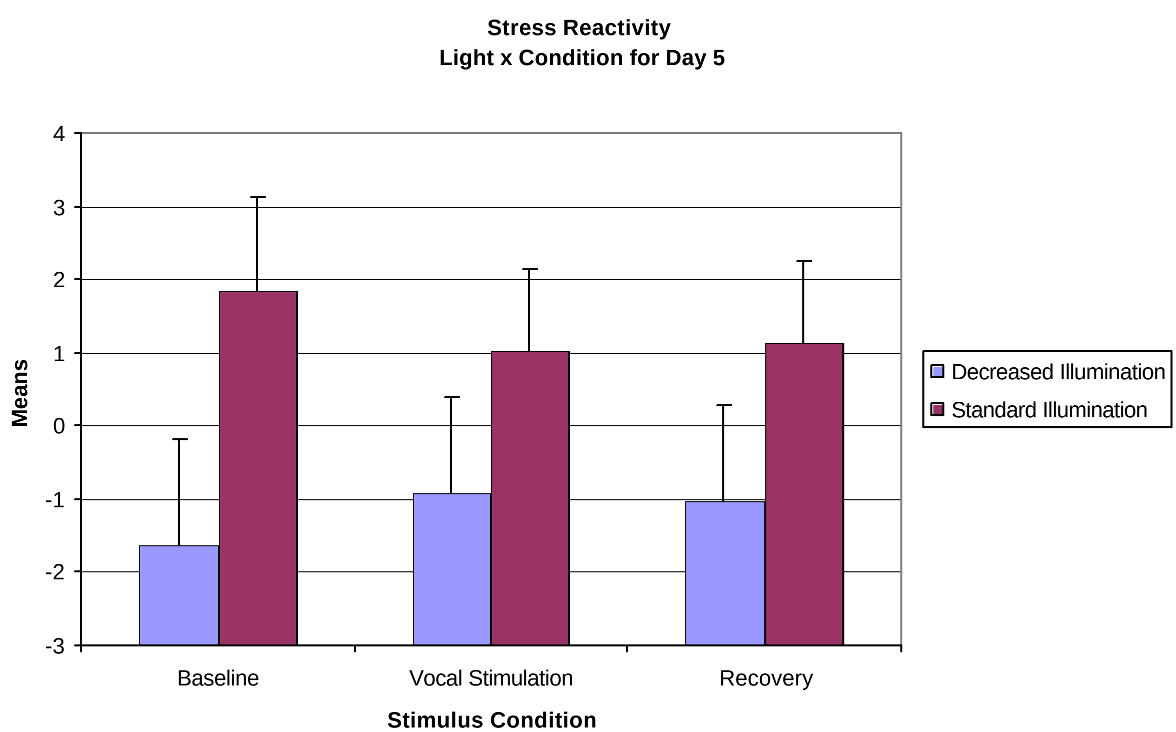
Appendix 3 Supplemental Figures: Stress Reactivity

Graphs of non-significant effects for Stress Reactivity Scores for Light x Condition
Day Two through Day Five



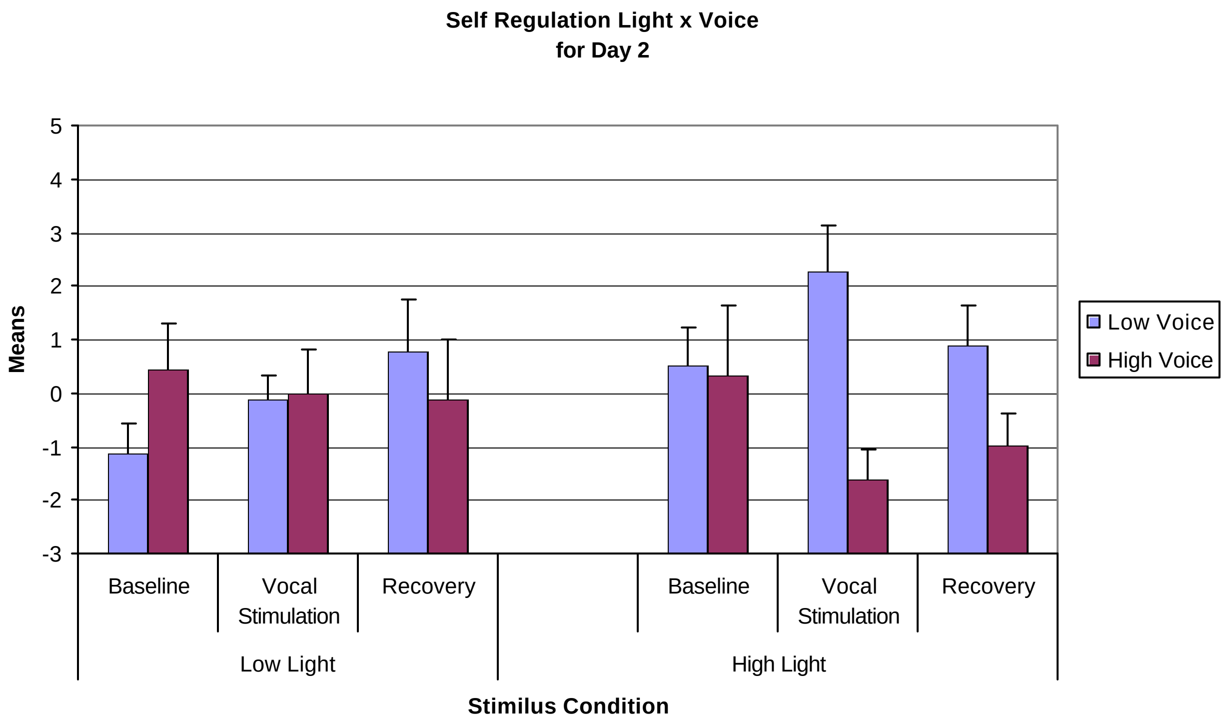


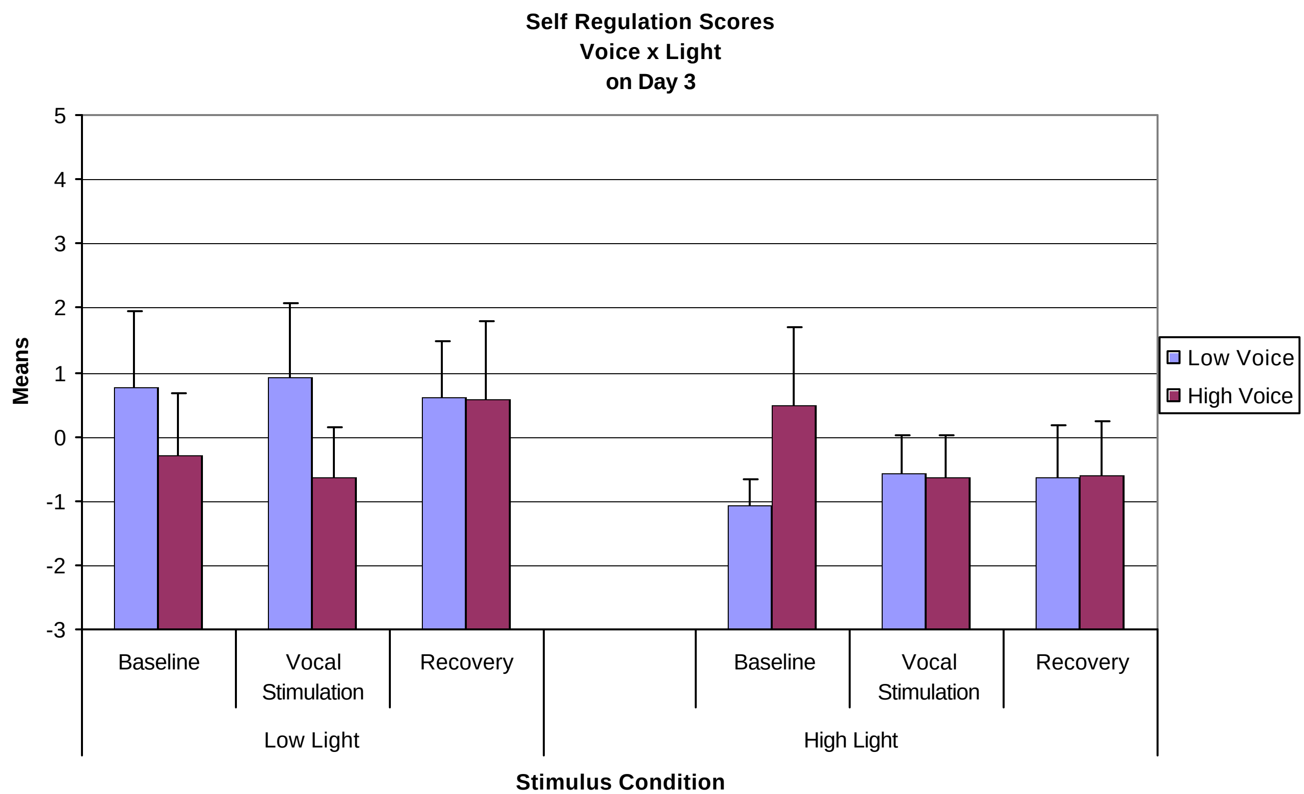

Stress Reactivity
Light x Condition for Day 4
Means
4
3
2
1
0
-1
-2
-3
Baseline
Vocal Stimulation
Recovery
Stimulus Condition
Decreased Illumination
Standard Illumination

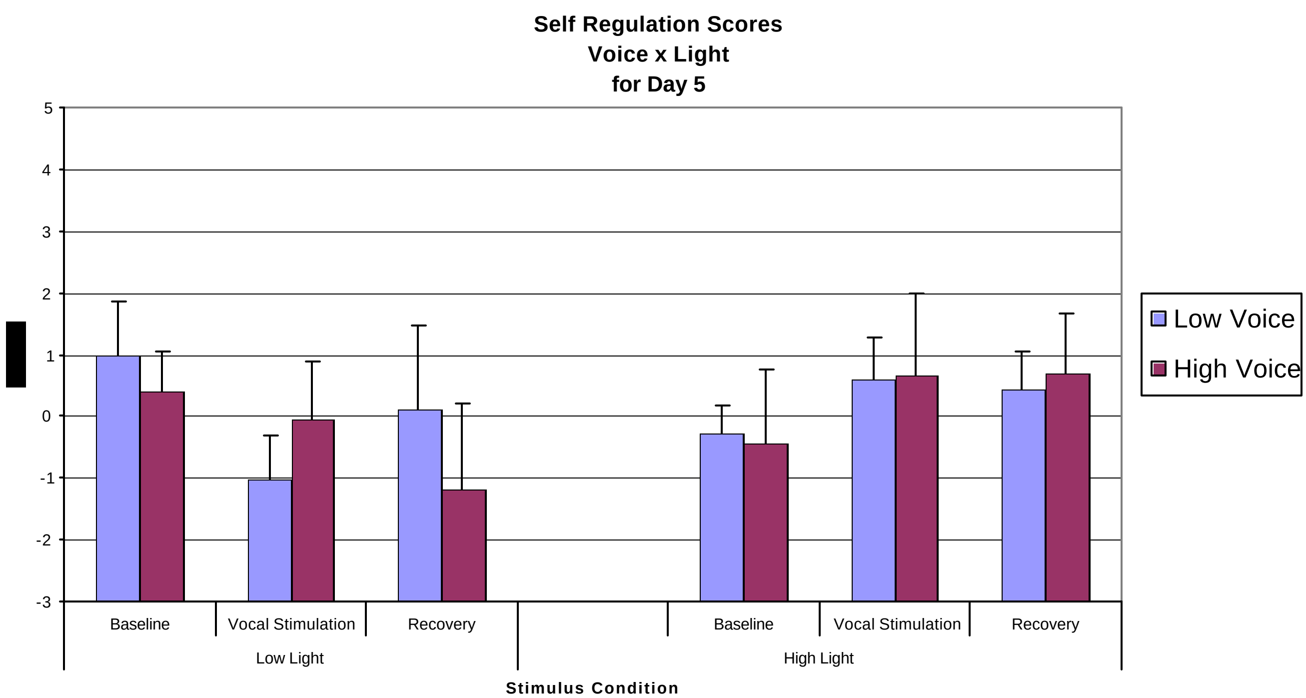

Stress Reactivity
Light x Condition for Day 5
Means
4
3
2
1
0
-1
-2
-3
Baseline
Vocal Stimulation
Recovery
Stimulus Condition
Decreased Illumination
Standard Illumination

Appendix 4: Supplemental Figures: Self Regulation

Graphs of non-significant effects for Self Regulation Scores for Voice x Light
Day Two, Three, and Five




Self Regulation Scores
Voice x Light
on Day 3
Means
5
4
3
2
1
0
-1
-2
-3
Low Voice
High Voice
Baseline
Vocal Stimulation
Recovery
Baseline
Vocal Stimulation
Recovery
Low Light
High Light
Stimulus Condition


Self Regulation Scores
Voice x Light
for Day 5
[redacted]
5
4
3
2
1
0
-1
-2
-3
Low Voice
High Voice
Baseline
Vocal Stimulation
Recovery
Baseline
Vocal Stimulation
Recovery
Low Light
High Light
Stimulus Condition

Appendix 5

Consent Form

Title

Preterm infants' responsiveness to differential amounts of taped female speech in different lighting conditions.

Introduction

This study is being conducted by Pia Strunk, M.S., a doctoral student at Virginia Polytechnic Institute and State University under the direction of Rita H. Pickler, PhD., R.N., Associate Professor in Maternal Child Nursing at Virginia Commonwealth University and Robert Lickliter, Ph.D., Full Professor at Virginia Polytechnic Institute and State University. You and your infant are being asked to participate in a study on the effects of vocal stimulation on preterm infants. The main purpose of this study is to see how preterm infants respond to audiotaped recordings of a female voice, and to see if those responses change over time. In addition, we are also looking at whether the amount of light has an effect on the infants' responsiveness to female voices. This study will be completed in the MCV Hospitals Neonatal Intensive and Intermediate Care Nurseries, and will enroll 40 infants.

You infant will hear a taped female voice telling a story. Every 'listening session' that the infant has will be 10 minutes in length. Your infant will be randomly assigned (like the flip of a coin) to one of four groups. The infants in Group 1 will listen to the mother's voice tape 3 times a day for 5 days with a 'typical' NICU blanket cover on their incubator. The infants in Group 2 will also listen to the mother's voice tape for 3 times a day for 5 days, but with a very dark incubator cover on their incubator. The infants in Group 3 will hear the mother's voice tape once a day for five days with a 'typical' NICU blanket cover on their incubator. Finally, the infants in Group 4 will also listen to their mother's voice tape once a day, but with a very dark incubator cover on their incubator. Groups 1 and 2 will be videotaped and observed during the last listening session for each of the 5 days, while Groups 3 and 4 will be videotaped and observed during their once a day 'listening session'.

Benefits

The results of this study may help us determine if playing speech to infants help them develop. Your infant may find this to be a pleasant experience. However, there is no guarantee of benefit to you or your infant

Alternative Therapy

This is not a therapeutic study. You have the alternative not to participate.

Risks, Inconveniences, Discomforts

Participation in this study does not mean you cannot visit your infant; visitation is encouraged. During the actual running of the voice sessions, however, we ask parents not touch, hold, or talk to the infant. However, parents are welcome to observe any of the voice sessions, and would be free to ask questions of the investigators at these times.

Your infant may experience a little discomfort to hearing the vocal stimulus. If the infant becomes very upset, the tape will be turned off and the infant soothed. There may be other risks that are not yet identified. You will be notified if any additional information is discovered that may affect your willingness to allow your child to participate in this study.

Cost

There will be no cost to you/your child for participation in this study.

Research Related Injury

There is no anticipated injury related to this study. You and your infant's physician can withdraw your infant from this study at any time if there is the suspicion of potential injury occurring.

In the event of physical and/or mental injury resulting from your participation in this research project, Virginia Commonwealth University/MCV Hospitals will not provide compensation. If injury occurs, medical treatment will be available at the MCV Hospitals. Fees for such treatment will be billed to you or appropriate third party insurance.

Confidentiality of Records

Your child's identity will be treated with professional standards of confidentiality. His/her name will be coded as a number and all of the research records, audiotapes, and videotapes will be referred to by this number. These records and tapes will be housed in a locked cabinet and be released for view only to the investigators and research associates. Videotapes may be seen by one outside observer for assessment/data analysis purposes only. Information gained from this study that can be identified with your child will be released only to the investigators and, if appropriate, to your child's physician. This information will be used only for the purposes of research and will not be used for any purpose other than this study without your express written consent. If this study is published, the results will be reported in such a way that your child cannot be identified. All identifying records will be destroyed 5 years after completion of the study.

Withdrawal

Participation in this study is voluntary. The investigators will answer any questions you may have about the study. You are free to withdraw your consent and discontinue participate at any time. If you decide to withdraw your infant from this study, you should contact Rita Pickler, Ph.D. or Pia Strunk, M.S. Discontinuation will in no way affect or jeopardize the quality of care you and your infant receive now or in the future at this institution. Your infant's doctor may also withdraw your infant for medical or administrative reasons. Any significant new findings which develop during the course of the research study which in the opinion of the investigator may affect your willingness to continue to participate will be provided to you as soon as possible.

Current Telephone Numbers

The investigators may be contacted at any time at the following numbers:

Principal Investigators:	Pia Strunk, M.A.	(804)828-4725 (daytime) (804)364-2743 (evening) e-mail: pstrunk@vt.edu
Advisor(s)	Rita Pickler, Ph.D.	(804) 828 -0721 (voicemail) e-mail: rpickler@hsc.vcu.edu
	Robert Lickliter, Ph.D	(540) 231-6581 (daytime) e-mail: duckling@vt.edu

If you have any questions concerning your child's rights as a research subject, you may contact the Committee on the Conduct of Human Research (804- 828-0568) for information or assistance. You will receive a copy of this consent form to take home with you.

	Signature	Printed Name	Date
Parent or Guardian	________________________	_____________________	_________
Investigator	________________________	_____________________	_________
Witness	________________________	_____________________	_________

Appendix 6

Interrater Reliability Protocol

State Data Analysis:

In order to measure state, the infants will be rated on their overall state every 15 seconds. This measure will reflect the quality of overall state during this time period. Overarching criteria for each state is located in the NIDCAP training manual. No assessment of “A” and “B” states will be used. Please use the state forms to complete these state assessments for each day. Mark the box corresponding to the state for each 15 second period. Be sure and not the time of start and stop.

Motor Behavioral Data Analysis

In order to measure behaviors, frequency counts will be taken of the following behaviors:

Squirming	Smiling
Extending Leg	Leg bracing
Extending Arms	Frowning
Yawning	Mouthing
Grimacing	Suck search
Finger splaying	Sucking
Airplaning	Foot clasp
Saluting	Hand clasp
Sitting on air	Grasping
Fisting	
Sneezing	
Fussing	

Measures of occurrence will be marked on the Motor Behaviors form, and are broken down for every two minutes to help you keep track of your time. The definitions and characteristics of each motor behavior to be assessed can be found in the NIDCAP training manual. Please note the beginning time and ending time of assessment. Some behaviors needed further clarification (other than is in the NIDCAP Training manual) and are included below.

Extend Leg: Any movement in which the leg extends outward or upward, unless both legs extend upward and the rear is slightly raised (this is a sitting on air). Toes can be pointed or flexed. Each extension is counted. Movements back into flexion and then reextended are counted, but flex position must last at least one second. If extension turns into a leg brace, only the leg brace will be counted.

Extend Arm: Any movement in which the arm extends outward along the side/front of body; unless hand is raised in an obvious salute (in the front of the body); or both arms exten outward from the shoulders in an obvious airplane. If any extension turns into a salute or an airplane, it will not be counted as an extension, but rather as one of the latter.

Squirm: any squirming movement (arms, legs, trunk). A squirm must last at least 3 secs, and be broken up by a pause of at least 3 secs to be counted.

Leg Brace: extend leg in which the infant makes contact with the bed, blanket roll, etc and secures self. One or both feet can be used.

Smooth arm/leg: movements that are fluid, slow and controlled. Not jerky or flayling. A 5 sec pause between stop/start of movement required to be counted as a new movement. Arms, legs, or both are counted.

Tuck trunk: infant must pull legs into tucked position and maintain for at least 5 secs to be counted. Each effort counted.

Finger splay: action of all fingers on one hand spreading out and hyperextending (slightly). Must relax for at least 5 secs before re-extending before a new splay can occur. Count