Effects of Redundant and Nonredundant Bimodal Sensory Stimulation on Heart Rate in Bobwhite Quail Embryos

ABSTRACT: Research with both animal embryos and human infants has provided evidence that information presented redundantly and in temporal synchrony across sensory modalities (intersensory redundancy) can guide selective attention, perceptual learning, and memory during early development. How this facilitation is achieved remains relatively unexamined. This study examined the effects of redundant versus nonredundant bimodal stimulation on a measure of physiological arousal (heart rate) in bobwhite quail embryos. Results show that quail embryos exposed to concurrent but nonredundant auditory and visual stimulation during the late stages of incubation exhibit significantly elevated heart rates following stimulus exposure and during stimulus reexposure when compared to embryos exposed to redundant and synchronous audiovisual stimulation, unimodal auditory stimulation, or no supplemental prenatal sensory stimulation. These findings indicate a functional distinction between redundant and nonredundant bimodal stimulation during early development and suggest that nonredundant bimodal stimulation during the prenatal period can raise arousal levels, thereby potentially interfering with the attentional capacities and perceptual learning of bobwhite quail. In contrast, intersensory redundancy appears to foster arousal levels that facilitate selective attention and perceptual learning during prenatal development.

Keywords: perceptual development; intersensory redundancy; prenatal heart rate

Based on his work with domestic chicks, Gray (1990) argued that responsiveness to experimental manipulations during early development is mediated in large part by the arousal level of the young organism. Excessive or reduced arousal levels are generally associated with poor performance whereas moderate levels of arousal are associated with peak performance (see also Ashton, 1971; Williams & Golenski, 1979). A large body of evidence indicates that the amount or type of available sensory stimulation can directly affect young organisms’ arousal level, thereby serving to increase or decrease an infant’s attention to the specific features of sensory stimulation present in the perinatal environment (for examples, see Gardner & Karmel, 1995; Richards, 2000; Ruff & Rothbart, 1996). The tight link between arousal and attention in early development likely plays an important role in young organisms’ perceptual learning and development.

In keeping with this view, studies of precocial avian species have provided converging evidence that embryos provided unimodal auditory exposure to an individual species-typical maternal call in the days prior to hatching subsequently prefer this familiar maternal call over a novel maternal call in postnatal choice tests (Gottlieb, 1988; Gottlieb, Tomlinson, & Radell, 1989; Honeycutt & Lickliter, 2001; Lickliter & Hellewell, 1992). In contrast, embryos exposed to a maternal call concurrently with
visual stimulation (nonsynchronized patterned light) do not demonstrate a preference for the familiar maternal call in postnatal testing (Gottlieb et al., 1989; Lickliter & Hellewell, 1992), suggesting that concurrent stimulation of the visual modality somehow interferes with prenatal auditory learning. Parallel results also have been found using concurrent stimulation of the vestibular system (Radell & Gottlieb, 1992). Several researchers working in this area have proposed that concurrent bimodal stimulation likely alters embryos’ arousal levels in such a way as to interfere with or disrupt emerging attentional capabilities, thereby rendering embryos unable to successfully process the distinguishing acoustic features of the individual maternal call. In contrast, unimodal stimulation is thought to foster or maintain arousal levels that can support selective attention and promote perceptual learning of the maternal call (for further discussion, see Honeycutt & Lickliter, 2002; Lickliter & Lewkowitz, 1995; Radell & Gottlieb, 1992).

Few studies have, however, directly focused on how embryos or fetuses respond in the presence of different amounts or types of prenatal sensory stimulation (but see Gottlieb, 1971; Robinson & Smotherman, 1992; Ronca & Alberts, 1990, 1994; Ockeford & Vince, 1977; Vince & Cheng, 1970, for examples from birds and mammals). As a result, we know relatively little about the immediate behavioral or physiological effects of unimodal versus bimodal sensory stimulation during the prenatal period. To begin to address this issue, Reynolds and Lickliter (2002) recently examined the immediate effects of various types of prenatal unimodal and bimodal sensory stimulation on behavioral and physiological arousal in bobwhite quail embryos. Embryos’ behavior was videotaped, and their heart rate was monitored during exposure to (a) no supplemental sensory stimulation, (b) unimodal auditory stimulation (an individual bobwhite maternal call), (c) unimodal visual stimulation (patterned light), (d) two sources of concurrent auditory stimulation (different bobwhite maternal calls), or (e) concurrent bimodal audiovisual stimulation (the maternal call paired with patterned light). Results indicated that quail embryos’ activity levels and heart rate can be significantly affected by the type of prenatal sensory stimulation provided during the period prior to hatching. In particular, concurrent (but asynchronous) bimodal stimulation was found to significantly increase both behavioral activity levels and heart rate when compared to control and unimodal stimulation conditions. This increase in behavioral and physiological arousal during exposure to prenatal bimodal stimulation is consistent with the notion that concurrent bimodal stimulation elicits increased arousal levels that can potentially exceed an optimal range for supporting selective attention and early perceptual learning (Radell & Gottlieb, 1992).

Importantly, recent behavioral evidence indicates that not all bimodal sensory stimulation is equivalent during early development. Research with both animal embryos and human infants has provided support for the view that redundant bimodal stimulation can facilitate selective attention and perceptual learning during both the prenatal and postnatal periods (Bahrick & Lickliter, 2002). Redundant bimodal stimulation refers to the spatially coordinated and temporally synchronous presentation of the same information (e.g., tempo, rhythm, duration, intensity) across two or more sensory modalities. Recent findings from human infants indicate that they can discriminate changes in tempo (at 3 months) and changes in rhythm (at 5 months) following redundant bimodal (audiovisual) presentation, but do not discriminate these temporal changes under conditions of unimodal presentation or concurrent but asynchronous presentation (Bahrick, Flom, & Lickliter, 2002; Bahrick & Lickliter, 2000).

Similarly, bobwhite quail embryos have been shown to learn an individual maternal call presented concurrently with synchronized patterned light (redundant bimodal presentation) four times faster than embryos receiving unimodal presentation of the same maternal call. In this case, synchronous audiovisual stimulation provided embryos redundant information about several temporal properties of the maternal call including rate, rhythm, and duration. In contrast, embryos exposed to concurrent but asynchronous (and thus nonredundant) presentation of the maternal call and patterned light did not demonstrate prenatal auditory learning (Lickliter, Bahrick, & Honeycutt, 2002). A related study found that intersensory redundancy also can facilitate memory for the familiar call. Chicks receiving redundant bimodal familiarization as embryos remembered and preferred a familiar maternal call up to 4 days following hatching. In contrast, chicks that received only unimodal familiarization during the prenatal period failed to remember the familiar call beyond 24 hr following exposure (Lickliter, Bahrick, & Honeycutt, in press).

What remains to be explored is how redundant versus nonredundant bimodal stimulation affects the state of the young organism at the time of stimulation. Exposure to concurrent but nonredundant audiovisual stimulation appears to significantly increase the arousal level of embryos in the period prior to hatching (Reynolds & Lickliter, 2002), but how redundant bimodal sensory stimulation affects the embryo’s arousal level is not known. The purpose of this study was to assess the relative effects of redundant (synchronous) bimodal sensory stimulation versus nonredundant (asynchronous) bimodal stimulation on heart rate in bobwhite quail embryos. Given previous behavioral findings demonstrating the facilitative effect of intersensory redundancy on selective attention, learning,
and memory during early development, we predicted that quail embryos provided redundant audiovisual stimulation would show lower arousal levels (as indicated by heart rate) both during and after stimulus exposure as compared to embryos receiving concurrent but nonredundant bimodal stimulation.

METHODS

Subjects

Subjects were 68 incubator-reared bobwhite quail embryos (Colinus virginianus). Fertile, unincubated eggs were received from a commercial supplier and set in a Petersime Model I incubator, maintained at 37.5°C. The humidity inside the incubator was maintained at 80 to 85%. The possible influence of between-batch variation was controlled by drawing subjects for each experimental group from three or more batches (weeks) of eggs. As a result of incubator rearing, the only sounds that the embryos were exposed to until the time of experimental manipulation were their own embryonic vocalizations (and broodmates’ vocalizations) and the low-frequency background noise of the incubator.

Procedure

The bobwhite quail embryo’s bill normally penetrates the air space at the large end of the egg approximately 24 to 36 hr prior to hatching. The penetration of the shell produces a visible indentation (or “pip”) on the outer shell of the egg. At this time, the embryo begins to respire and vocalize (Freeman & Vince, 1974). Eggs showing a single pip during the first half of Day 21 of incubations were placed in a portable Hovi-bator incubator located in a darkened room. The incubator was manufactured with a Plexiglas top, allowing for observation and stimulation of the embryos within.

Following relocation to the portable incubator, embryos received either (a) unimodal (auditory) stimulation; (b) concurrent but unsynchronized, nonredundant bimodal (audiovisual) stimulation; (c) temporally synchronized, redundant bimodal (audiovisual) stimulation; or (d) no supplemental stimulation (controls). There were 17 embryos in each exposure group (N = 68). All groups (with the exception of the control group) received 10 min/hr of stimulus exposure for 6 hr on the day prior to hatching. The auditory stimulation used during the experiment was a looped recording of an individual variant of the species-typical bobwhite maternal call. All subjects in each experimental group received exposure to this same individual maternal call. The sound level of the individual call was adjusted to a peak intensity of 65 dB (A scale, fast response) inside the incubator, as measured by a Bruel and Kjaer Model 2232 sound-level meter. The maternal call was broadcast with a Sony portable CD player through a speaker located above the portable incubator. The maternal call consisted of five notes that repeated at a rate of 1.7 notes/s. The synchronized bimodal stimulation was comprised of the maternal call and a temporally patterned light set to flash at the same rhythm, rate, and duration as the maternal call.

The intensity of the light did not vary from onset to offset. The call and light were synchronized by capturing the call’s audio track with Apple’s Quick Time Movie Player, saving the visual image in waveform and then converting the height of each peak into a numerical value, thus providing time interval and height value for each peak. The final output was a Windows file that would play the call and present a flash through a projector that coincided with the call when it exceeded a threshold amplitude. The light persisted until the call’s amplitude dropped below the threshold. A Proxima 2810 desktop projector connected to a Dell computer was used to deliver the light. The projector was situated directly above the Plexiglas top of the portable incubator. Particular care was taken to insure that the presence of the light did not alter the temperature or humidity of the incubator. The nonsynchronous bimodal stimulation was identical to the synchronous bimodal stimulation, with the exception that the maternal call and patterned light were temporally misaligned. In other words, the onset of the light was presented out of phase with the onset of the call, thereby eliminating intersensory redundancy for rhythm, rate, and duration.

Testing

Following the 6-hr stimulus-exposure period, embryos were individually placed in a portable incubator to allow for the measurement of their heart rate. Two small holes were gently scraped in the outer egg shell without penetrating the inner-shell membrane. Heart rate was recorded using two Biopac 18-gauge unipolar needle electrodes inserted through the holes in the outer egg shell and making contact with the inner-shell membrane. The area surrounding the electrodes was covered with a light coat of Vaseline to preserve the integrity of the membrane. Before determining placement of the electrodes, the embryo’s position inside the shell was observed by candling the egg. This insured that all subjects were positioned such that the electrodes were placed away from the embryo’s head, which was typically located in the large end of the egg. The R-spikes were amplified using a Biopac MP100 amplifier and converted to bpm through the use of Acqknowledge software. The embryo’s confinement in its egg shell kept movement artifacts to a minimum.

Approximately 30 min following the offset of stimulation, each embryo’s heart rate was measured during an initial 1-min period of no supplemental stimulation followed by a 3-min stimulus-reexposure period. The stimulus used during this reexposure period was identical to the stimulus the embryo received during the initial 6-hr exposure period. Heart rate was measured during a 4-min period of no supplemental stimulation for the control group.

Data Analysis

The mean bpm were calculated for every 15-s interval of the 4 min of the recording period. Baseline heart rate was defined as the average of the subject’s mean bpm for the first and last 15-s intervals of the initial 1-min period of no supplemental stimulation. Heart rate during the twelve 15-s intervals following initial stimulus onset was expressed as a change score. Group differences were analyzed using a two-way mixed ANOVA with group
serving as a between-subjects factor and exposure period serving as a within-subjects factor. A one-way ANOVA was used to analyze group differences in baseline heart rate. Subgroup comparisons were made using the Tukey HSD post hoc test. An alpha level of .05 was used to determine significance.

RESULTS

Baseline Heart Rate

As illustrated in Figure 1, a one-way ANOVA on baseline heart rate following stimulus exposure was significant, $F(3, 64) = 38.77$, $p < .001$. Following the initial 6-hr exposure period, the nonredundant bimodal exposure group displayed significantly higher heart rate ($M = 299.68$, $SE = 2.84$) than the redundant audiovisual group ($M = 271.58$, $SE = .97$), the unimodal auditory exposure group ($M = 264.75$, $SE = 4.04$), and the control group ($M = 265.53$, $SE = 1.65$).

The two-way mixed-design ANOVA on raw bpm revealed a between-subjects main effect for group, $F(3, 64) = 95.55$, $p < .001$. The nonredundant group’s heart rates remained significantly higher ($M = 309.31$, $SE = 1.24$) than the redundant ($M = 272.69$, $SE = 1.09$), unimodal auditory ($M = 263.78$, $SE = 3.51$), and control ($M = 265.41$, $SE = 2.01$) groups’ heart rates for the duration of the stimulus reexposure period ($p < .001$ in all cases, see Figure 2). The redundant group ($M = 272.69$) also maintained significantly higher heart rates ($p = .027$) than the unimodal auditory group ($M = 263.78$) during the reexposure period, but not significantly higher than that of control embryos. After adjusting the degrees of freedom with the Greenhouse–Geisser correction, no significant within-subjects main effect for blocks of time was found, $F(3.078) = 2.00$, $p = .114$. However, there was a significant interaction between blocks of time and group, $F(9.23) = 2.06$, $p = .033$, with the nonredundant group’s heart rates significantly greater than baseline for all blocks of time during the stimulus reexposure period.

Change Scores

The two-way mixed-design ANOVA on change scores from baseline heart rate during stimulus reexposure was significant on the between-subjects factor of group, $F(3, 64) = 7.19$, $p < .001$. Once again, there was a main effect for group, with the nonredundant group showing greater changes from baseline during stimulus reexposure ($M = 9.62$, $SE = 3.13$) than the redundant group ($M = 1.11$, $SE = .61$), the unimodal auditory ($M = -0.97$, $SE = 1.54$), and the control ($M = -0.12$, $SE = .85$) groups (see Figure 3).

GENERAL DISCUSSION

The main purpose of this study was to examine the effects of redundant versus nonredundant bimodal sensory stimulation on a physiological measure of arousal in bobwhite quail embryos. Results supported our prediction that quail embryos would show higher levels of arousal (as indexed...
by heart rate) following nonredundant audiovisual stimulation than embryos receiving redundant audiovisual stimulation in the period prior to hatching. More specifically, we found that embryos exposed to nonredundant stimulation exhibited significantly higher heart rates following stimulus exposure and during stimulus reexposure than embryos exposed to (a) redundant audiovisual stimulation, (b) unimodal auditory stimulation, or (c) no supplemental prenatal sensory stimulation.

These results replicate and extend the findings of Reynolds and Lickliter (2002), which demonstrated that bobwhite quail embryos show significant increases in heart rate during exposure to asynchronous (nonredundant) bimodal stimulation when compared to embryos receiving unimodal auditory, unimodal visual, or no supplemental stimulation. The present results extend this finding and indicate that nonredundant bimodal stimulation can have an enduring effect upon the physiological state of bobwhite quail embryos. Embryos exposed to nonredundant bimodal stimulation demonstrated significantly higher baseline heart rate than did the other exposure groups (synchronous audiovisual and unimodal auditory) following 6 hr of intermittent stimulus exposure (Figure 1) and also demonstrated a significant increase from baseline during a brief stimulus reexposure period (Figures 2 and 3).

The present findings indicate that not all types of concurrent bimodal stimulation result in higher levels of heart rate than typically seen in unstimulated control embryos. Although the synchronous bimodal group did display significantly higher heart rate than the unimodal auditory group during stimulus reexposure, no significant differences in change scores were found between the synchronous bimodal, unimodal auditory, and control groups (Figure 3). Furthermore, the nonredundant group displayed significantly higher baseline heart rate and greater change scores from baseline than the redundant bimodal exposure group. These results provide the first evidence of the differential effects of intersensory redundancy on physiological arousal during early development. Redundant bimodal stimulation appears to foster only a moderate increase in heart rate following intermittent prenatal exposure. Unlike the dramatic increases in heart rate found following exposure or during reexposure to nonredundant bimodal stimulation, this moderate increase likely falls within a range of arousal level that fosters prenatal perceptual learning (see Lickliter, Bahrnick, & Honeycutt, 2002, in press, for behavioral evidence supporting this view). Given the tight link between arousal and attention in early development (e.g., Gardner & Karmel, 1995), this facilitation is likely mediated by changes in the selective attention of embryos during the prenatal period.

The present results also inform previous behavioral studies on developmental intersensory interference in precocial birds. Results from research with both ducklings and quail chicks have indicated that concurrent (but nonredundant) bimodal stimulation can interfere with prenatal perceptual learning (Gottlieb et al., 1989; Honeycutt & Lickliter, 2001; Lickliter & Hellewell, 1992; Radell & Gottlieb, 1992). For example, Gottlieb et al. (1989) found that if duck embryos were exposed to visual stimulation during prenatal exposure to an individual maternal call, they appeared unable to learn the individually distinctive auditory features of that call. However, embryos unimodally exposed to an individual maternal call were capable of learning the call, as evidenced by their preference for the familiar call over an unfamiliar maternal call in postnatal testing. Importantly, intersensory interference with perceptual learning occurred only when the two sensory systems (auditory and visual) were stimulated concurrently (but asynchronously) and was not seen when the maternal call was presented unimodally. Parallel results have been obtained in studies of bobwhite quail embryos, who likewise fail to learn an individual maternal call when it is presented concurrently with nonredundant visual stimulation (Honeycutt & Lickliter, 2001; Lickliter & Hellewell, 1992). However, it is important to note that none of these studies of prenatal perceptual learning provided subjects redundant, synchronous bimodal stimulation. In all cases, embryos were exposed to concurrent but nonredundant bimodal stimulation. The present results suggest that this type of prenatal bimodal stimulation fosters higher arousal levels in embryos than does redundant or unimodal stimulation, likely contributing to the intersensory interference effect reported in earlier studies.
In contrast, synchronous bimodal stimulation makes overlapping temporally coordinated information available to different senses and appears to foster arousal levels that promote selective attention, perceptual learning, and memory for redundant stimulus properties common across modalities (see Bahrick & Lickliter, 2000, 2002, for further discussion). For example, synchronous audio-visual stimulation (intersensory redundancy) has been found to promote auditory learning in quail embryos at a rate that was four times that of embryos receiving unimodal auditory stimulation (Lickliter et al., 2002).

We know from recent findings from neural and behavioral studies that perceptual responsiveness to the presence of one stimulus can be significantly altered by the presence of a stimulus presented to another modality during early development (Calvert, Spence, & Stein, in press). The present study indicates that these alterations also are evident at the level of physiological responsiveness. It is interesting to note that much of what we know regarding infant arousal, attention, perceptual responsiveness, and information processing is based on unimodal research designs. These unimodal studies provide important information about the perception of modality-specific stimulus properties, but reveal little about the role of multimodal stimulation in early development (Lickliter & Bahrick, 2001). The natural environment provides young organisms an array of dimensions of stimulation, including unimodal-multimodal, moving-static, and social-nonsocial. Which aspects of these dimensions will be differentially perceived, processed, and learned at different points in development remain poorly understood, and additional research is needed to better define the various processes and determinants that guide and constrain infant responsiveness.

NOTES

This research was supported by NIMH Grant RO1-MH 62225 awarded to the second author. Portions of this data were presented at the 2002 annual meeting of the International Conference on Infancy Studies, Toronto.

REFERENCES


