

The Critical Role of Temporal Synchrony in the Salience of Intersensory Redundancy During Prenatal Development

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We explored the amount and timing of temporal synchrony necessary to facilitate prenatal perceptual learning using an animal model, the bobwhite quail. Quail embryos were exposed to various audiovisual combinations of a bobwhite maternal call paired with patterned light during the late stages of prenatal development and were tested postnatally for evidence of prenatal auditory learning of the familiarized call. Results revealed that a maternal call paired with a single pulse of light synchronized with one note of the five note call was sufficient to facilitate embryos' prenatal perceptual learning of the entire call. A synchronous note occurring at the onset of the call burst was most effective at facilitating learning. These findings highlight quail embryos' remarkable sensitivity to temporal synchrony and indicate its role in promoting learning of redundantly specified stimulus properties during prenatal development.

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This research was supported by grants NICHD RO1 HD048423, RO1 HD053776, and NIGMS R25 GM61347. Data collection was conducted at the Developmental Psychobiology Lab at Florida International University.

Intersensory redundancy is a particularly important and salient form of sensory stimulation and in our view plays a foundational role in early perceptual and cognitive development (Bahrlick & Lickliter, 2002). Intersensory redundancy refers to a particular type of multimodal stimulation in which the same information is presented simultaneously and in a spatially coordinated manner across two or more sensory modalities. For the auditory-visual domain, redundancy entails the temporally synchronous alignment of the information available to each modality. Thus, the sights and sounds of hands clapping or faces talking provide intersensory redundancy in that they are temporally synchronous, spatially co-located, and convey the same rhythm, tempo, and intensity patterns across vision and audition. Our research with nonhuman animal infants and human infants indicates that different properties of stimuli are attended when redundant multimodal stimulation is made available as compared with unimodal stimulation from the same events (Bahrlick, 2002; Bahrlick & Lickliter, 2000; Bahrlick, Lickliter, & Flom, 2006; Lickliter, Bahrlick, & Honeycutt, 2002, 2004).

We have proposed an “intersensory redundancy hypothesis” (IRH) to account for how this engagement of selective attention occurs in early development (Bahrlick & Lickliter, 2000, 2002; Bahrlick, Lickliter, & Flom, 2004). As attentional capacity is limited and the world provides our senses with far more stimulation than can be attended or processed at any given time, perceivers must selectively attend to some aspects of stimulation at the expense of other aspects. The IRH proposes that in early development, information that is simultaneously available across two or more senses (properties such as tempo, rhythm, duration, and intensity) is highly salient and is therefore more likely to be attended, learned, and remembered than when the same information is presented to only one modality. Conversely, when nonredundant, modality specific properties of objects and events (such as pitch, color, or orientation) are presented to a single sensory modality, they are attended, processed, and remembered better than when the same properties are presented in the context of redundant bimodal stimulation (Bahrlick & Lickliter, 2002; Bahrlick et al., 2004). Intersensory redundancy has a large influence on early perceptual development because most objects and events are multimodal and this gives an initial selective advantage for processing amodal over modality specific patterns of stimulation in early development when attention is most limited.

An unresolved topic of interest is what features of intersensory redundancy are most effective at “grabbing” the young infant’s attention and facilitating perceptual processing and perceptual learning. Research with neonates and young infants indicates that temporal synchrony is one of the earliest and most important perceptual attributes used to detect the unity

of multimodal stimulation (e.g., Bahrick, 1988, 2001; Lewkowicz, 1986; Morrongiello, Fenwick, & Chance, 1998; Slater, Quinn, Brown, & Hayes, 1999). Temporal synchrony has also been proposed by several investigators to be a critical basis for the salience of intersensory redundancy during early development (Bahrick & Lickliter, 2000; Lewkowicz & Kraebel, 2004; Prince & Hollich, 2005). This idea has received empirical support, in that previous research with both nonhuman animal and human infants has shown that the concurrent but *asynchronous* presentation of auditory and visual stimulation does not promote the perceptual learning of amodal properties in bimodal stimulation (e.g., Bahrick & Lickliter, 2000; Lickliter et al., 2002). By contrast, the *synchronous* presentation of bimodally specified redundant information has consistently been shown to facilitate discrimination and learning of amodal properties when compared to the same information presented unimodally (e.g., Bahrick & Lickliter, 2000; Flom & Bahrick, 2007; Hollich, Newman, & Jusczyk, 2005; Lewkowicz, 2004).

For example, previous research with bobwhite quail embryos and neonates has shown that the synchronous, redundant audiovisual presentation of an individual bobwhite maternal call enhances prenatal learning of that call when compared to a unimodal auditory presentation (Lickliter et al., 2002). Embryos receiving a maternal call paired with a synchronous pulsed light that matched the onset and offset of the notes of the call preferred the familiarized call over a novel maternal call in postnatal choice tests conducted one day after hatching. Furthermore, this was accomplished using only one fourth of the exposure time required to foster learning during unimodal auditory presentation of the same call. Quail embryos thus learned an individual maternal call four times faster when exposed to a temporally synchronous bimodally specified version of that call than when exposed to the same maternal call unimodally. By contrast, quail embryos receiving the redundant but asynchronous auditory and visual stimulation did not prefer the familiarized call over the novel call in postnatal tests, regardless of amount of exposure provided (Lickliter et al., 2002).

We have only begun to understand the origins of these early patterns of selective attention and perceptual learning and how they might influence subsequent perceptual and cognitive development. Although little human-based research on these issues is available, the fetus likely experiences intersensory redundancy across auditory, vestibular, and tactile stimulation in utero. For example, the mother's speech sounds, laughter, or sounds of breathing can create tactile and vestibular stimulation that share the temporal patterning of the sounds as a result of changes in the musculature involved in producing the sounds. Additionally, the mother can respond with temporally coordinated movements to externally generated sounds. She may dance or exercise to music, startle to a loud sound, or engage in

conversation that has a distinctive turn-taking structure—all of which produce movements that have tactile and vestibular correlates that share intensity and temporal patterning with the sounds. Thus, the fetus likely has ample opportunity to detect and become familiar with redundant sensory stimulation during the late stages of prenatal development. Here we assess what constitutes “effective” redundancy by manipulating the amount and timing of temporal synchrony and assessing its effects on prenatal perceptual learning, using an animal model, the bobwhite quail. Precocial birds like quail are particularly well suited for this type of research as they develop in an egg, allowing easy access to the developing embryo during the late prenatal period, have all sensory systems functional by the time of hatching, and can respond in behavioral tests within hours after hatching.

In the current study, quail embryos were exposed to various audiovisual combinations of an individual bobwhite maternal call paired with a pulsed light during the late stages of prenatal development and tested 24 hr after hatching for evidence of prenatal auditory learning. Two hypotheses were examined: (1) temporal synchrony of bimodal stimulation is critical to facilitate prenatal perceptual learning with respect to unimodal stimulation, and (2) facilitation of perceptual learning will be influenced by the timing and amount of prenatal temporal synchrony available to embryos.

GENERAL METHOD

Certain features of the experimental design were common to all experiments. These details are described first before describing the particular details of each experiment.

Subjects

Subjects were incubator reared bobwhite quail chicks (*Colinus virginianus*). Fertilized unincubated eggs were received weekly from a commercial supplier and set in a BSS-160 Grumbach Incubator (Grumbach, Germany) maintained at 75–80% relative humidity and 37.5 °C. Embryonic age was calculated on the basis of the first day of incubation being Day 0, the second day of incubation as Day 1, and so on. To control for possible variations in developmental age, only birds that hatched on Day 23 were used as subjects. Embryos for each condition were drawn from two or more different batches of eggs to control for possible between-batch variation in behavior. Following hatching, groups of 15–20 chicks were socially reared in large plastic tubs in a Nuaire Model NU-605-500 Animal Isolator (Plymouth, MN), which provided continuous filtered air. Chicks had continuous access to

food and water. Ambient air temperature was maintained at approximately 30 °C.

Apparatus

Approximately one day prior to hatching, embryos were transferred to a sound attenuated room and placed in a Model 1602N Hova-bator (Savannah, GA) portable incubator, maintained at approximately 37.5 °C and 80% relative humidity. This incubator allowed embryos to receive audiovisual stimulation via a transparent plastic window located on the top of the incubator directly above the embryos. Audiovisual stimulus presentations (described below) were delivered via a custom designed software program running a flat screen video monitor located 22 cm directly above the incubator window and a speaker placed on top of a small hole located on the top of the incubator, immediately adjacent to the window.

Postnatal behavioral tests took place in an arena 130 cm in diameter, surrounded by a wall 60 cm in height. The arena surface was painted flat black and the walls of the testing arena were insulated with a special layer of foam to attenuate reverberation. The arena walls were covered with an opaque black curtain. Two separate opposing approach areas each comprising approximately 5% of the total area of the testing arena, and directly opposite to one another (see Figure 1), contained a 4-inch speaker mounted to the arena wall and hidden behind the black curtain. Both speakers were powered by separate Yamaha SA-155 (Hamamatsu, Shizuoka, Japan) integrated stereo amplifiers and the auditory stimuli were played by two Sony CDP-XE370 (Minato, Tokyo, Japan) compact disk players. The ambient temperature in the testing room was maintained between 29–32 °C.

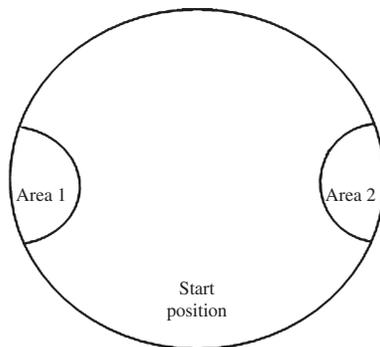


FIGURE 1 Top view of behavioral testing arena. Approach areas “1” and “2” contained either the familiar call or nonfamiliar call. Familiar call location was counterbalanced during testing.

Procedure

Approximately 24–36 hr prior to hatching, the bobwhite quail embryo moves its head into the airspace located at the large end of the egg, producing a visible indentation (a “pip”) on the surface of the egg shell. Approximately 24 hr prior to hatching, a group of pipped eggs were relocated to the portable incubator and placed in a light and sound attenuated prenatal stimulation room. Various sensory stimulation regimes were presented to embryos in the different experimental conditions across the hours prior to hatching. The auditory stimuli used were two individual variants of a species-typical bobwhite maternal assembly call (Call A and Call B). Both maternal calls were recorded in the field (Heaton, Miller, & Goodwin, 1978) and share similar phrasing, repetition rates, and frequency modulation. They vary primarily in minor peaks of dominant frequency and the temporal microstructure of rhythm and duration (see Figure 2).

For each condition, half of the embryos received prenatal exposure to Call A, the other half received prenatal exposure to Call B. The bobwhite maternal calls consist of a burst of five notes with a complex rhythmic pattern. The duration of the call is approximately 3 sec (the rate of the notes average 1.7/sec) and was followed by an intercall interval of 2 sec. The notes of the call vary in duration, intensity, and fundamental frequency. Various audiovisual configurations of the maternal call were achieved by presenting a pulsed light either in synchrony or in sequential order with the notes of the maternal call. A customized version of Javascript was used to create these patterns of stimulation.

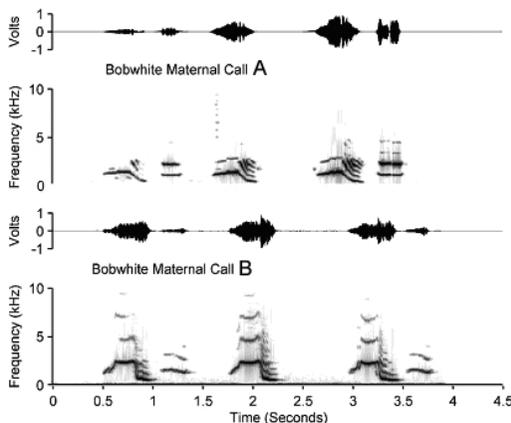


FIGURE 2 Spectrograms of the bobwhite maternal calls (Call A and Call B).

Testing

Postnatal testing was conducted 24 hr following the completion of prenatal stimulation and consisted of a 5 min (300 sec) simultaneous-choice test between the familiar bobwhite call presented during prenatal stimulus exposure and a novel maternal call (either Call A or Call B). The sound intensity of each call was adjusted to peak at 65 dB, measured from the start position where each chick was introduced into the arena. The locations of the calls presented during testing were counterbalanced across individual trials to prevent a possible side bias from affecting results. Each chick was tested only once. Chicks were scored on both their latency of approach and the duration of time they spent in each of the approach areas. Latency was defined as the amount of time (in seconds) that elapsed from the onset of the trial until the chick entered an approach area. However, these data were highly variable across subjects and thus will not be discussed further in the context of the individual experiments. Duration was defined as the cumulative amount of time (in seconds) the chick remained in an approach area. Any chick that did not enter an approach area received a score of 300 sec for latency (i.e., the length of the trial) and 0 sec for duration and was considered a non-responder. These chicks were excluded from subsequent analyses. Up to four observers blind to the experimental condition collected data in each experiment. We used an intraclass correlation coefficient (ICC) reliability analysis to assess interobserver agreement among three observers for total duration time spent in the approach areas for a videotaped subject (ICC = 1.00).

A custom computer program written with Visual Basic allowed for semi-automated collection of latency and duration of response to the test stimuli. During scoring, an observer viewed two separate monitors. One monitor displayed a live video feed from a camera mounted directly above the arena. The two semicircular approach areas, each comprising approximately 5% of the total area of the testing arena and directly opposite to one another, were demarcated on the monitor. This allowed for remote observation of chicks entering an approach area during testing. A second monitor was used for data scoring. This monitor displayed two boxes, one corresponding to each approach area. Each time a chick entered an approach area, the observer depressed a button for the duration of time that the chick remained in the approach area. The software program summarized the latency to approach and the duration of time spent in each approach area for each chick.

Data analysis

The primary data of interest were measures of duration (in seconds) in proximity to the auditory stimuli presented during the test trials. Several

analyses were performed on this interval data. First, a proportion of total duration time (PTDT) was calculated from the time chicks spent in the approach area containing the familiar maternal call relative to the total duration time spent in both familiar and novel approach areas. A proportion of .50 reflects chance responding, whereas a proportion $> .50$ reflects a majority of time spent in the approach area containing the familiar call. Proportions $< .50$ reflect a majority of time spent in the approach area containing the novel call. One sample *t*-tests, with Bonferroni corrections, were used to evaluate whether the PTDT spent in the approach area containing the familiar call was significantly $>$ chance. In addition, between groups comparisons of mean duration of time in proximity to the familiar call were evaluated with a one-way analysis of variance (ANOVA) and a post hoc multiple comparisons procedure (Tukey's honestly significant difference [HSD] test). All tests were two-tailed and alpha level was set at .05. Effect sizes were calculated using eta-squared (η^2) for ANOVA and Cohen's *d* for one sample *t*-tests and post hoc tests.

EXPERIMENT 1: TEMPORAL SYNCHRONY IS NECESSARY FOR THE FACILITATION OF PRENATAL PERCEPTUAL LEARNING

There is some evidence in the comparative developmental literature indicating animal infants differ in how simultaneous versus sequential events are learned. For example, infant rats 8–16 days of age outperform older pups at learning an association between preconditioned and neutral olfactory stimuli, but only with simultaneous presentations of the odors; sequential presentation of the preconditioned and neutral odors eliminates or reverses the infant rats' advantage (Chen, Lariviere, Heyser, Spear, & Spear, 1991). These results suggest that there is weak differentiation among synchronous stimuli in very young organisms that may promote something similar to perceptual configuration or "unitization" of stimuli presented simultaneously.

In this light, Lewkowicz and Kraebel (2004) have questioned whether young infants can perceive intersensory equivalence of amodal invariants in the absence of intersensory temporal synchrony. To explore this question, one could separate synchronous and redundant audiovisual information and present the audible and visible information sequentially to preserve the equivalent temporal patterning of the bimodal event, but eliminate temporal synchrony. Thus, the same temporal properties (rhythm, tempo, and duration) would be available to both the auditory and visual modalities, but would occur in a sequentially alternating pattern. This configuration would allow for assessing the role of bimodal temporal equivalence for facilitating perceptual learning in the absence of temporal synchrony. From here on, we

refer to this sequential form of audiovisual stimulation as *bimodal sequential temporal equivalence* (BSTE).

To date no studies have explored whether BSTE is sufficient for facilitating perceptual learning. Temporal synchrony and bimodal temporal equivalence have been confounded in most studies of early perceptual development, as temporally synchronous audiovisual stimulation is also typically temporally equivalent. This experiment unpacks these variables to independently assess the importance of temporal synchrony in early perceptual learning.

Given our research question, it is important to define what we mean by “facilitating” prenatal perceptual learning. Facilitation here refers to the enhancement of learning in redundant bimodal stimulation with respect to nonredundant unimodal or asynchronous bimodal stimulation. Our previous research has demonstrated that quail embryos or hatchlings provided unimodal auditory exposure to an individual maternal call require at least 240 min of exposure to prefer that familiar call in postnatal testing conducted one day following hatching (Lickliter & Hellewell, 1992; Lickliter et al., 2002). By contrast, embryos receiving redundant bimodal (audiovisual) exposure to the same maternal call have shown a significant preference for the familiarized call following 120 min or in some cases 60 min of prenatal exposure (Lickliter et al., 2002). Thus, we define “facilitation” as a significant preference for the familiar maternal call following <240 min of exposure.

Method

In this experiment, bobwhite quail embryos were assigned to one of four groups: (1) A *Unimodal* group ($n = 57$) which received auditory exposure to an individual variant of the bobwhite maternal call (Figure 3a). This group served to demonstrate the overall amount of unimodal stimulation needed to foster prenatal perceptual learning and was divided into two subgroups of different exposure amounts. Group A ($n = 29$) received exposure to the individual maternal call for 10 min/hr for the 24 hr prior to hatching (240 min of total stimulation). Group B ($n = 28$) received exposure for 10 min/hr for 12 hr prior to hatching (120 min of total stimulation). (2) A *Synchrony* group ($n = 60$) received bimodally redundant exposure to a maternal call such that the temporal patterning of all the notes of the call was recreated in a pulsed light. Thus, the amodal properties of rhythm, rate, and duration were redundant and temporally synchronized across the auditory and visual modalities (Figure 3b). As with the Unimodal group, this group also divided into two subgroups to assess the amount of bimodal synchronous stimulation necessary for prenatal perceptual learning. Group

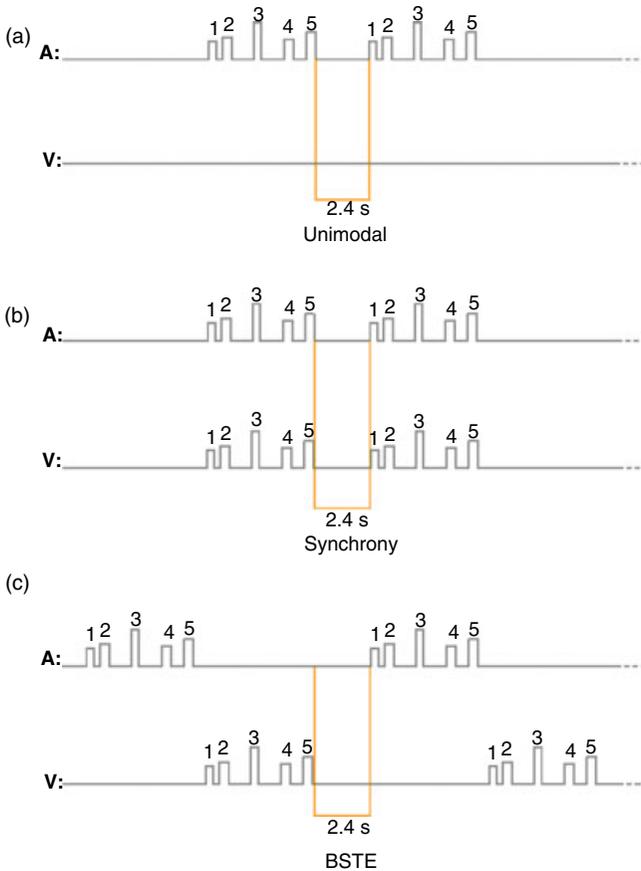


FIGURE 3 (a–c) Schematic representation of the various audiovisual configurations of the maternal call used in Experiment 1. The letters A and V represent the auditory and visual modalities, respectively. The numbers represent each note in the five note call burst. The duration of each call burst is 3 sec and the duration of the interburst interval is 2.4 sec.

A ($n = 30$) received exposure for 10 min/hr for 12 hr (120 min of total stimulation) and Group B ($n = 30$) received exposure for 10 min/hr for 6 hr (60 min of total stimulation). (3) A *BSTE* group ($n = 30$) received exposure to an individual variant of the bobwhite maternal call immediately followed by a pulsing light containing the same temporal properties (rhythm, rate, duration) of the call. The interstimulus interval between each sequence of call and light was 2.4 sec (Figure 3c). To determine whether this *BSTE* would facilitate prenatal perceptual learning, this group received

exposure for 10 min/hr for 12 hr (120 min of total stimulation) prior to hatching. (4) A *Control* group ($n = 31$) did not receive any supplemental prenatal stimulation prior to hatching.

Testing for all groups was conducted at 24 hr following hatching in a 5 min simultaneous-choice test between the familiarized maternal call and a novel variant of the bobwhite maternal call. The two maternal calls were presented unimodally at testing (i.e., were not paired with the pulsed light presented prenatally). As previously discussed, given that a minimum of 240 min of unimodal auditory exposure has been shown to be necessary for learning an individual maternal call in previous studies, we assessed the facilitative effects of prenatal exposure to audiovisual synchrony and BSTE by providing embryos with reduced amounts of prenatal exposure (120 min or 60 min of exposure).

Results and discussion

The results for the various conditions are summarized in Table 1. One sample *t*-tests were performed on the PTDT spent in the approach area with the familiar call against the chance value of .50. Results of the Unimodal condition revealed that embryos receiving 240 min of unimodal auditory exposure to the maternal call showed a significant PTDT to the familiar call at testing, $t(28) = 3.13$, $p = .004$, $d = .6$, whereas those receiving 120 min of unimodal auditory exposure did not show a significant PTDT to the familiar call at testing. This replicates our prior studies demonstrating that 240 min of unimodal auditory exposure is sufficient for prenatal learning (Lickliter & Hellewell, 1992; Lickliter et al., 2002).

TABLE 1
Mean Proportion of Total Duration Time (PTDT) and Mean Durations (in seconds) for the Familiar Call in Experiment 1

<i>Prenatal Condition</i>	<i>Mean PTDT (Standard Deviation)</i>	<i>Mean Duration to Familiar Call (Standard Deviation)</i>
Unimodal (240 min)	.68* (.31)	76.94 (58.72)
Unimodal (120 min)	.54 (.34)	67.71 (61.76)
Synchrony (120 min)	.81* (.25)	102.46 (67.15)
Synchrony (60 min)	.66* (.29)	74.54 (55.22)
BSTE (120 min)	.62 (.37)	47.97 (56.11)
Controls (Call A)	.44 (.33)	24.16 (38.19)
Controls (Call B)	.56 (.33)	29.47 (30.44)

* $p < .05$ (*t*-test).

Embryos from the 120 min and 60 min Synchrony groups both showed a significant PTD for the familiar call, $t(29) = 6.73$, $p = .000$, $d = 1.2$ and $t(29) = 2.91$, $p = .007$, $d = .2$, respectively (Table 1), indicating facilitated learning with respect to unimodal stimulation. By contrast, embryos receiving 120 min of exposure to BSTE did not prefer the familiarized maternal call over the novel call at testing. Because the chicks from the Control group could not respond to a familiarized call, two separate t -tests were performed; one for Call A and one for Call B. As expected, naïve control chicks did not show a significant PTD for either maternal Call A or Call B during testing (Table 1).

The experimental groups can also be compared in terms of their total amount of stimulation (summed across sensory streams). From this perspective, the 60 min audiovisual Synchrony group could be considered to provide the equivalent of 120 min of sensory stimulation, the same as the Unimodal 120 min and BSTE 120 min groups. Our results show a significant preference for the familiarized call in the Synchrony group, but not the Unimodal or BSTE groups, demonstrating that synchrony facilitates learning not because it provides a greater amount of overall stimulation than unimodal or sequential conditions, but because it provides a different type of stimulation. Moreover, the fact that a significant preference for the familiar maternal call was seen in the Synchrony groups (both 120 min and 60 min), but not the BSTE group (120 min), suggests that temporal synchrony plays a key role in facilitating prenatal perceptual learning

A one-way ANOVA compared the mean durations for the familiar call across groups. As previously discussed, naïve chicks from the Control group could not respond to a familiar call. Thus, in order to make a comparison with the Control group we designated Call B for the analysis. This test was found to be statistically significant at an alpha level of .05, $F(5, 172) = 5.954$, $p < .001$, $\eta^2 = .2$. A Tukey's HSD test indicated that the mean duration for the familiar call for the Synchrony 120 min group ($M = 102.46$) was significantly greater than the mean duration for the familiar call for the BSTE group ($M = 47.97$) and the Control group ($M = 29.47$), $d = 1.3$ and $d = 1.9$, respectively. In addition, the mean durations for the familiar call for the Synchrony 60 min group ($M = 72.54$) and the Unimodal 240 min group ($M = 76.94$) were significantly greater than the Control group, $d = 1.3$ and $d = .8$, respectively (Table 1). Overall, these results converge with our previous findings demonstrating intersensory facilitation of prenatal perceptual learning in bobwhite quail (Honeycutt & Lickliter, 2001; Lickliter & Hellewell, 1992; Sleight, Columbus, & Lickliter, 1998). Further, our results demonstrate that prenatal exposure to a bimodally synchronous maternal call for 120 min or even 60 min results in facilitated postnatal discrimination of the familiarized call from a novel maternal call

(replicating the results of Lickliter et al., 2002). By contrast, providing amodal equivalence across audition and vision in the absence of temporal synchrony (BSTE) was not sufficient to facilitate prenatal learning.

EXPERIMENT 2: EXPLORING THE AMOUNT AND TIMING OF SYNCHRONY NECESSARY FOR THE FACILITATION OF PRENATAL PERCEPTUAL LEARNING

In Experiment 1, the Synchrony group demonstrated facilitated perceptual learning on the basis of exposure to audiovisual temporal synchrony available across the entire maternal call. In other words, a temporally synchronous flash occurred on every note of each call burst (e.g., all five notes). This raises the question as to whether consistent and distributed synchrony is necessary to foster prenatal learning, or is it possible that intermittent synchrony could be sufficient for facilitated learning? Given the sequestered nature of the prenatal environment (both in ovo and in utero), it is likely the case that synchrony across sensory systems is not always consistent or continuous in a given exposure. The goal of this experiment was thus to determine whether reduced amounts of distributed temporal synchrony could facilitate prenatal perceptual learning with respect to unimodal stimulation. We exposed quail embryos to one temporally synchronous note within each five note call burst. Embryos were thus exposed to prenatal stimulation that contained a temporally synchronous note only 20% of the total exposure time (as compared with 100% in the Synchrony groups of Experiment 1) in several conditions, including the first, middle, or last note of the maternal call.

Previous research has demonstrated that young infants are sensitive to the temporal patterning of visual stimulation (Lewkowicz, 1985). Thus, we also independently varied synchrony and temporal pattern (periodicity) to assess the possibility that periodicity, instead of synchrony, could facilitate prenatal learning. Together, these manipulations were designed to provide more detail about the amount and timing of audiovisual synchrony necessary for the facilitation of prenatal perceptual learning.

Method

Each experimental group of embryos was exposed to the same individual maternal call variants (Call A and Call B, Figure 2) used in the previous experiment. There were six experimental groups: (1) An *Asynchronous Flash* group ($n = 30$) received a flash of light .9 sec prior to the onset of a maternal call burst (Figure 4a) and served as a control for the possibility that a

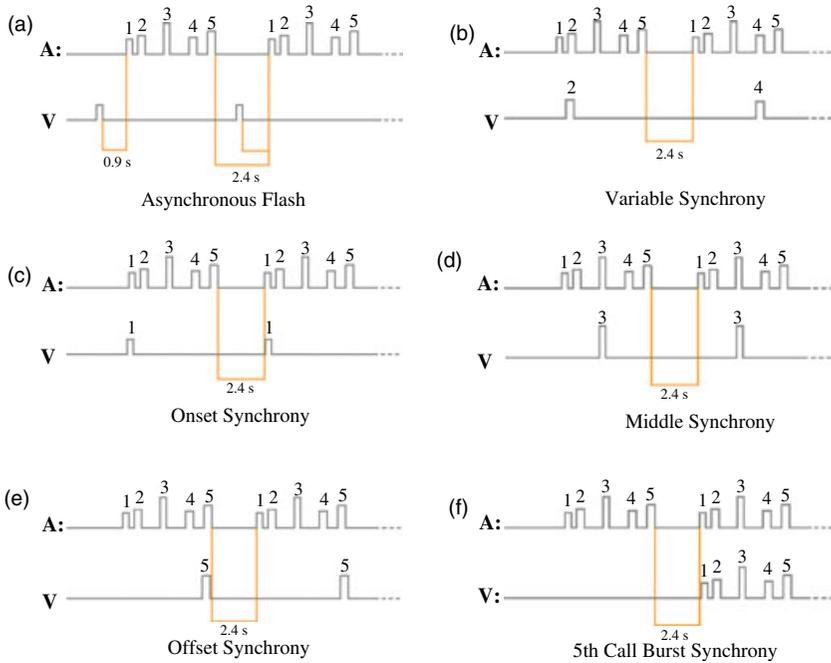


FIGURE 4 (a–f) Schematic representation of the various audiovisual configurations of the maternal call used in Experiment 2. The letters A and V represent the auditory and visual modalities, respectively. The numbers represent each note in the five note call burst. The duration of each call burst is 3 sec and the duration of the interburst interval is 2.4 sec.

flash of light (prior to the call burst), independent of its synchrony relative to the notes of the call, could produce an exogenous shift of attention to the maternal call. (2) A *Variable Synchrony* group ($n = 29$) received exposure to a maternal call paired with a single flash of light randomly synchronized to any of the five notes of the call burst (Figure 4b). Embryos in this condition received auditory exposure to the entire maternal call, with a single temporally synchronous visual burst of light occurring randomly with one of the five notes of the maternal call. (3) An *Onset Synchrony* group ($n = 31$) received a single burst of light synchronized to the onset (first note) of the call (Figure 4c). (4) A *Middle Synchrony* group ($n = 30$) received a single burst of light synchronized with the middle (third note) of the call (Figure 4d). (5) An *Offset Synchrony* group ($n = 32$) received a single burst of light synchronized with the offset (last note) of each call (Figure 4e). (6) A *5th Call Burst Synchrony* group ($n = 29$) received unimodal auditory exposure to the maternal call for four consecutive call bursts and every fifth call

burst was fully synchronized with the light (Figure 4f). These embryos thus also received 20% of their total prenatal exposure to the call synchronized with light, but distributed less frequently than in Experiment 1 to equate for the reduced amount of synchrony available in the other experimental conditions of this experiment. The interburst interval for all prenatal stimulation conditions was 2.4 sec. To test for facilitated prenatal learning, embryos from all groups received 120 min (10 min/hr for 12 hr) of exposure prior to hatching and were then subsequently tested at 24 hr following hatching in a 5 min simultaneous-choice test between the familiarized maternal call and the novel variant of the bobwhite maternal call.

Results and discussion

Results are summarized in Table 2. One-sample *t*-tests were performed on the PTDT spent in the approach area with the familiar call against the chance value of .50. Embryos from the Variable Synchrony, Onset Synchrony, and Middle Synchrony groups showed significantly greater PTDT for the familiar call at testing, $t(28) = 4.62, p < .01, d = .9$; $t(29) = 3.40, p < .01, d = .6$; and $t(30) = 2.08, p < .05, d = .4$, respectively. The Offset Synchrony group, the Asynchronous Flash group, and the 5th Call Burst Synchrony group did not show significant PTDT for the familiar call at testing, even though the proportion of temporally synchronous notes made available prenatally was the same across all experimental groups.

A one-way ANOVA compared the mean duration for the familiar call of all the experimental groups and the Control group from Experiment 1. This test was found to be statistically significant at an alpha level of .05, $F(6, 211) = 7.81, p < .01, \eta^2 = .2$. A Tukey's HSD test indicated that the mean duration for proximity to the familiar call for the Variable Synchrony

TABLE 2
Mean Proportion of Total Duration Time (PTDT) and Mean Durations (in seconds) for the Familiar Call in Experiment 2

<i>Prenatal Condition</i>	<i>Mean PTDT</i> (<i>Standard Deviation</i>)	<i>Mean Duration to Familiar Call</i> (<i>Standard Deviation</i>)
Asynchronous Flash	.56 (.36)	101.81 (94.22)
Variable Synchrony	.78* (.32)	123.74 (76.14)
Onset Synchrony	.64* (.37)	65.22 (47.86)
Middle Synchrony	.70* (.33)	85.43 (60.66)
Offset Synchrony	.62 (.36)	57.01 (48.77)
5 th Call Burst Synchrony	.59 (.34)	61.58 (46.16)

* $p < .05$ (*t*-test).

group ($M = 123.74$) was significantly greater than the mean duration for the familiar call of the Onset Synchrony group ($M = 65.22$), the Offset Synchrony group ($M = 57.01$), the 5th Call Burst Synchrony group ($M = 61.58$), and Control group ($M = 29.59$), $d = 1.3$, $d = 1.5$, $d = 1.4$, and $d = 2.2$, respectively. In addition, the mean duration for proximity to the familiar call of the Asynchronous Flash group ($M = 101.81$) and the Middle Synchrony group ($M = 85.43$) were significantly greater than the Control group, $d = 2.6$ and $d = 1.6$, respectively (Table 2).

These results suggest that the temporal distribution of temporally synchronous notes is an important feature of stimulation in facilitating prenatal learning. When in the stimulus event is temporal synchrony most effective at facilitating prenatal learning? Because the Variable Synchrony, Onset Synchrony, and Middle Synchrony exposure groups all showed facilitated prenatal perceptual learning of the familiar call following 120 min of prenatal exposure (based on their PTDT scores), little can be said from the results of the current experiment as to *when* the occurrence of a synchronous note might be most effective. However, analysis of the mean duration scores across the experimental groups suggested that the Variable Synchrony condition was more effective than other synchrony conditions in facilitating learning.

EXPERIMENT 3: EXPLORING THE FACILITATION OF PRENATAL PERCEPTUAL LEARNING: TIMING OF TEMPORAL SYNCHRONY

This experiment further explored the role that timing of a single temporally synchronous event plays in the facilitation of prenatal perceptual learning. In a previous investigation of prenatal learning with quail embryos, Lickliter, Bahrack, and Markham (2006) demonstrated that embryos' attention can be educated to the amodal stimulus properties of a unimodal maternal call when a relatively brief exposure to the same bimodally redundant call precedes it (6 hr of a temporally synchronous call followed by 18 hr of a unimodal call). However, when the stimulation was presented to embryos in the reverse order (18 hr of a unimodal call followed by 6 hr of a temporally synchronous call) facilitated prenatal learning did not occur. Thus, the initial presentation of temporally synchronous audiovisual stimulation can enhance attention to subsequent unimodal auditory stimulation. Based on these previous findings, it seemed plausible that a synchronous note would be most effective at facilitating prenatal perceptual learning when it occurred at the onset of the call, thereby educating attention to the amodal properties of the entire call that followed. To explore this possibility and to further assess the somewhat ambiguous results from Experiment 2, the learning task

was made more difficult than that of Experiment 2 by reducing the overall amount of prenatal exposure to the maternal call provided embryos.

Method

Only those experimental groups that showed facilitated prenatal perceptual learning in Experiment 2 were included in this experiment. However, in this experiment embryos received exposure to the maternal call for 10 min/hr for 6 hr (60 min total stimulation time) prior to hatching, rather than the 120 min of exposure provided in Experiment 2, thereby increasing the difficulty of the learning task. There were three experimental groups: (1) a Variable Synchrony group ($n = 28$), (2) an Onset Synchrony group ($n = 28$), and (3) a Middle Synchrony group ($n = 29$). As in the previous experiments, all chicks were tested individually at 24 hr following hatching in a 5 min simultaneous-choice test between the familiarized maternal call and a novel variant of the bobwhite maternal call.

Results and discussion

Results are summarized in Table 3. One-sample t -tests were performed on the PTDT spent in the approach area with the familiar call against the chance value of .50. Results revealed that only embryos from the Onset Synchrony (60 min) group showed a significantly greater PTDT for the familiar call at testing, $t(27) = 3.52$, $p < .01$, $d = .7$. The Variable Synchrony and Middle Synchrony groups did not show facilitated learning following 60 min of prenatal exposure.

A one-way ANOVA compared the mean durations for the familiar call for all experimental groups and the control group. This test was found to be statistically significant at an alpha level of .05, $F(3, 115) = 5.59$, $p < .01$, $\eta^2 = .1$. A post hoc (Tukey's HSD test) procedure indicated that the mean duration for the familiar call for the Onset 60 min ($M = 88.83$) and Middle

TABLE 3
Mean Proportion of Total Duration Time (PTDT) and Mean Durations (in seconds) for the Familiar Call in Experiment 3

<i>Prenatal Condition</i>	<i>Mean PTDT (Standard Deviation)</i>	<i>Mean Duration to Familiar Call (Standard Deviation)</i>
Variable Synchrony	.55 (.32)	68.27 (49.36)
Onset Synchrony	.73* (.34)	88.83 (63.88)
Middle Synchrony	.57 (.45)	90.40 (97.51)

* $p < .05$ (t -test).

60 min Synchrony ($M = 90.40$) groups were significantly greater than the Control group, $d = 1.6$ and $d = 1.2$, respectively. However, the mean duration for the familiar call for the Variable 60 min, Onset 60 min, and Middle 60 min Synchrony groups did not significantly differ from each other (Table 3).

Taken together these results suggest that under conditions of limited exposure, the most effective position for a temporally synchronous note to occur for the transfer of prenatal training to postnatal perceptual discrimination is at the onset of the call. When our prenatal learning task was made more difficult by reducing the familiarization time from 120 min to 60 min, only the Onset Synchrony group showed significantly greater PTDT to the familiar call during testing. This is in contrast with the results from Experiment 2, in which 120 min of Variable Synchrony appeared to have an advantage in facilitating prenatal perceptual learning. These differences suggest that the total amount of prenatal exposure to the maternal call can influence the effectiveness of the timing of synchrony. Limited exposure appears to favor onset synchrony, but greater exposure time allows other synchrony configurations to also be effective. Further research is needed to assess this possibility. In any case, the results of Experiment 2 and Experiment 3 highlight the important role that timing can play in the effectiveness of bimodally redundant stimulation for prenatal perceptual learning (see also Honeycutt & Lickliter, 2001; Lickliter et al., 2006).

GENERAL DISCUSSION

Synchrony detection has been argued to be foundational for infants' early perceptual development and organization (e.g., Bahrack, 1992; Lewkowicz, 2000; Prince & Hollich, 2005). Temporal synchrony has been found to facilitate learning of redundant audiovisual events (Bahrack & Lickliter, 2000; Lewkowicz, 2004; Lickliter et al., 2002), contribute to the learning of arbitrary intermodal relations (Bahrack, 2001; Slater et al., 1999), contribute to learning related to the self (Bahrack & Watson, 1985; Rochat & Striano, 2000), and facilitate early word learning (Gogate & Bahrack, 1998). In this study, we found that prenatal perceptual learning was facilitated by providing bobwhite quail embryos synchronous bimodal exposure to an individual maternal call. We also found that manipulating the amount and timing of temporal synchrony between redundant auditory and visual information modified learning during the prenatal period.

Previous studies of the effects of intersensory redundancy on the detection of amodal stimulus properties have confounded temporal synchrony and bimodal temporal equivalence, in that temporally synchronous audiovisual

stimulation is typically also temporally equivalent. In Experiment 1, we used BSTE to unpack temporal synchrony from intersensory temporal equivalence by sequentially alternating the same amodal properties across the auditory and visual modalities. Our findings indicated that providing embryos with BSTE was not sufficient to facilitate prenatal perceptual learning. This result suggests that temporal synchrony is a powerful feature in the deployment of attention and the facilitation of perceptual learning and supports our hypothesis that temporal synchrony of bimodal stimulation is necessary for the facilitation of prenatal perceptual learning with respect to unimodal stimulation. Human infant studies on sensitivity to intersensory equivalence indicate that this ability emerges between 4 and 8 months of age (e.g., Allen, Walker, Symonds, & Marcell, 1977; Mendelson & Ferland, 1982; Lewkowicz, 1992), suggesting the successful detection of intersensory sequential temporal equivalence is likely dependent upon experience accrued over the first several months following birth.

Our results from Experiment 2 suggested that prenatal exposure to variable synchrony was most effective at facilitating prenatal perceptual learning. However, when the total exposure time provided embryos was reduced by half (Experiment 3), variable synchrony was no longer effective at facilitating prenatal perceptual learning. These patterns of results suggest that the effectiveness of bimodally synchronous stimulation in facilitating prenatal perceptual learning of an individual maternal call depends on both the distribution of synchronous notes and the total duration of exposure time to the stimulus. Previous comparative work has provided initial evidence that the temporal configuration of multisensory stimulation can modulate bobwhite quail embryos' level of arousal (Reynolds & Lickliter, 2002). It is possible that different temporal configurations of synchrony affect arousal (and in turn selective attention) differently as a function of overall exposure time. A more fine grained analysis of the effects of episodic temporal synchrony could shed light on the interplay between attention and arousal involved in the facilitation of prenatal learning.

In any case, several temporal factors involved in the facilitation of prenatal perceptual learning were identified in this study: (1) For the facilitation of prenatal perceptual learning to occur with respect to unimodal stimulation, the temporally synchronous overlap of auditory and visual information appears necessary. Sequential presentation of redundant audiovisual information did not facilitate prenatal learning (Experiment 1). (2) For the facilitation of prenatal perceptual learning to occur with respect to unimodal stimulation, a single temporally synchronous note within a five note maternal call appears sufficient to learn the entire call (Experiment 2). (3) When embryos are provided with reduced exposure, a single temporally synchronous note is most effective when it occurs at the onset of the stimulus

event (Experiment 3). Indeed, under the condition of reduced exposure time onset synchrony was just as effective as full synchrony in facilitating prenatal learning. Taken together, our data indicate that precocial avian embryos are remarkably sensitive to the presence, amount, and timing of temporal synchrony during the late stages of prenatal development. Consistent with the human infant literature (see Lewkowicz, 2000), the present study provides evidence that temporal synchrony is one of the most salient stimulus properties for the facilitation of selective attention and perceptual learning during early development.

What might be the basis for the effectiveness of temporal synchrony in facilitating prenatal perceptual learning? It is possible that the effectiveness of synchrony in promoting learning is in part a result of neural enhancement created by the so-called “multiplicative effect” of temporally synchronous multimodal stimulation. Research investigating the response of multisensory neurons in the superior colliculus of cats and monkeys has provided evidence that temporally synchronous audiovisual stimulation produces enhanced neural responses that are greater than the sum of each individual unimodal component (for a review see Stein & Meredith, 1993). This “multiplicative” or “superadditive” effect has been demonstrated across a variety of extracellular recording techniques; including response reliability, decreased reaction time, impulses evoked, peak impulse frequency, and duration of discharge (Stein & Meredith, 1993; Stein, Meredith, & Wallace, 1993). This enhancement of neural responsiveness to temporal synchrony could potentially underlie the facilitation of learning observed in the present study. However, it is important to note that little is known about the neural aspects of attention during prenatal development and additional research is needed to better understand the prenatal links between the salience of temporal synchrony and neural responsiveness.

In the more general sense, our results suggest that the role of prenatal experience in shaping and guiding young infants’ selective attention and perceptual processing should not be overlooked. The infant has already had a great deal of prenatal sensory experience at the time of birth and the nature and type of this prenatal experience undoubtedly plays an important role in later perceptual functioning (e.g., Fifer & Moon, 1995; Moon & Fifer, 2000; Slater et al., 1999).

ACKNOWLEDGMENTS

This research was supported by grants NICHD RO1 HD048423 and NIGMS R25 GM61347. Data collection was conducted at the Developmental Psychobiology Lab at Florida International University.

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