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Heart Rate Orienting and Respiratory Sinus Arrhythmia Development in Rats Exposed to Alcohol or Hypoxia

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KELLY, S. J. AND J. E. RICHARDS. *Heart rate orienting and respiratory sinus arrhythmia development in rats exposed to alcohol or hypoxia*. NEUROTOXICOL TERATOL **20**(2) 193–202, 1998.—The effect of alcohol exposure and hypoxia on the heart rate orienting response and RSA development was studied in preweanling rats. Rats were artificially reared from postnatal days 4 through 12 and either exposed to alcohol (5 g/kg/day) or hypoxia (two 15-min episodes/day) from postnatal days 4 to 10. Control groups consisted of artificially reared and normally reared rats not exposed to alcohol or hypoxia. The heart rate and respiration was recorded at baseline and during repeated exposures to auditory and visual stimuli every other day from postnatal day 13 through 21. The hypoxia group showed an enhanced heart rate orienting response to the auditory stimuli on postnatal days 17 and 19 compared to the other three groups, which did not differ from each other. The baseline interbeat interval increased over this period of time and there was a large increase in respiratory sinus arrhythmia from postnatal day 15 to 21. The alcohol and hypoxia rats showed significantly less of an increase in respiratory sinus arrhythmia on postnatal days 19 and 21. All rats showed a greater response to the auditory stimuli than to the visual stimuli on postnatal days 17 and 19 and all groups showed equivalent habituation to both stimuli within a session. The results suggest that respiratory sinus arrhythmia and the heart rate response to stimuli may not be strongly related during this developmental stage in the rat and that hypoxia but not alcohol exposure alters attentional processes for auditory stimuli as measured by the heart rate orienting response. © 1998 Elsevier Science Inc.

Fetal Alcohol Syndrome Hypoxia Respiratory sinus arrhythmia Heart rate orienting response

ALTERATIONS of attentional processes are commonly reported effects of developmental insults; indeed, attention deficit hyperactivity disorder (ADHD) has been speculated to be the result of exposure to developmental insults, such as alcohol and perinatal trauma (29). Both alcohol exposure (47,48) and hypoxia induced by respiratory distress (13,44) during development cause deficits in some attention tasks. However, recently it has been shown that the observed changes in attention subprocesses in children with Fetal Alcohol Syndrome (FAS) is not the same as that seen in children with ADHD. Children with ADHD have difficulty in focusing and sustaining attention whereas children with FAS have deficits in encoding and retrieval aspects of attention and no deficits in sustaining attention (10). In contrast, it has been suggested that children with Respiratory Distress Syndrome (RDS) have difficulty in sustaining and shifting attention (44). Although considerable progress is being made in the understanding of the neural bases of attention [e.g., see (38) for a review], the neural bases of the changes in attention in these developmental disorders is not known.

To investigate the specific neural alterations underlying the changes in attention in FAS and RDS, animal models of these syndromes need to demonstrate changes in attention. The heart rate change in response to a stimulus is a useful measure of attention because it can be used throughout development in both humans and animals. The response of a newborn infant to a stimulus is an acceleration of heart rate, whereas by 2 months this response changes to a deceleration (5,19,46). The period during which the heart rate is lowered represents a period of sustained attention, as indicated by correlations to behavioral measures of sustained attention, and this period increases in length from 2 to 6 months of age (40,43). Similarly, rats show an acceleratory heart rate response to neutral auditory (but not visual) stimuli on postna-

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tal day 15 and this changes to a deceleration at 16 days of age (20). The change from acceleration to deceleration at 16 days of age in the rat approximates the point when the parasympathetic control of the heart in the rat becomes mature (1–3,49,50,52). The deceleration of heart rate to neutral stimuli at 16 days of age in rats is proposed to be the result of an increase in parasympathetic activity and a concomitant decrease in sympathetic activity (22) and has been shown to be under the control of a variety of brain stem and more rostral neural structures (39).

With respect to heart rate measures of attention, it would be predicted that hypoxia during development would result in a deficit in the magnitude of the heart rate orienting response, reflecting a deficit in sustained attention, and exposure to alcohol during development would alter habituation of the heart rate orienting response, reflecting a deficit in encoding and retrieval. Indeed, preterm infants with RDS show immature patterns of heart rate responding compared to healthy preterm or full-term infants (14,41) and lower levels of respiratory sinus arrhythmia, a measure of heart rate variability that is related to respiration and highly correlated to the heart rate orienting response (41,42). Although there have been no studies investigating heart rate responding in an animal model of RDS, performance in learning and memory tasks is disrupted by hypoxia and these disruptions may be mediated by attentional deficits (30–32). In contrast to RDS, there have been no studies examining the heart rate orienting response in children with FAS but there have been some studies using animal models of FAS that examined heart rate. Caul, Fernandez, and Michaelis (8) found prenatal alcohol exposure produced no changes in baseline heart rate, heart rate response to a novel auditory stimulus, or conditioned heart rate response in 56–82-day-old rats. Hayne, Hess, and Campbell (21) found that whereas prenatal alcohol exposure did have an effect on basal heart rate on postnatal days 1 and 6, there was no effect on the heart rate response and more importantly, habituation of that response, to a novel olfactory stimulus on postnatal day 12. Both of these studies used prenatal alcohol exposure, which is a period equivalent to only the first and part of the second trimester in humans with respect to brain growth (4); it may be that the early postnatal period in the rat is the critical period for alcohol-induced attention deficits and concomitant changes in heart rate responses. In both rats exposed to alcohol and rats exposed to hypoxia during development, respiratory sinus arrhythmia has been shown to be reduced on postnatal days 19 and 21 (27); because this measure has been shown to be strongly correlated to both the heart rate measures and behavioral measures of sustained attention (40–42), this finding suggests that both animal models should reveal deficits in the magnitude of the heart rate orienting response.

The purpose of this study was to examine the utility of using the heart rate orienting response to auditory and visual stimuli to examine attentional processes in rat models of FAS and RDS, with exposure to the insults during the early postnatal period. In addition, measures of respiratory sinus arrhythmia were taken. The period in which the heart rate measures were taken was from postnatal day (PD) 13 through 21. Ear and eye opening occur in the strain of rats used in this study by PD 14 (personal observation), and it has been shown that a heart rate response occurs to auditory and visual stimuli by PD 14 in albino rats (20). Because RDS in humans has been shown to alter sustained attention and animals exposed to hypoxia during the early postnatal period have reduces respiratory sinus arrhythmia, it was hypothesized that animals exposed to hypoxia during the early postnatal period would exhibit impairments in the heart rate orienting response. Animals exposed to alcohol during the postnatal period were hypothesized to show a deficit in habituation of orienting to stimuli because of deficits in encoding and retrieval; a prediction with respect to the magnitude of the heart rate orienting response in animals exposed to alcohol is difficult to make with confidence because the suggestions from the human literature (10) and the animal literature on respiratory sinus arrhythmia (27) are contradictory.

METHOD

Subjects

All rats, except when artificially reared, were housed in the animal quarters in the Department of Psychology at the University of South Carolina and had free access to food and water. Room temperature was 23°C, with 50% humidity. The schedule of lighting was maintained on a 12:12 h light/dark cycle, with the light phase beginning at 0700 h.

The Long–Evans rats used in this experiment were bred in the Department of Psychology. The morning on which a vaginal smear was positive for sperm was considered to be gestational day 0. At 26 days postconception (usually postnatal day 4), the offspring in each litter were assigned to a treatment group such that there was only one rat from the same litter in each treatment group. Each treatment group consisted of nine rats of both sexes; there was never more than five rats of one sex in a treatment group. Litter size was maintained at 10 pups by the addition and/or removal of nonexperimental rats. The groups consisted of an alcohol group, hypoxia group, gastrostomy control group, and a suckle control group. The first three groups were artificially reared from postnatal days 4 to 12. The suckle control group was reared normally by dams in litters maintained at 10 rats by the addition of same-age nonexperimental rats. All rats were weighed from postnatal days 4 through 12 and on postnatal day 21.

Artificial Rearing Protocol

On postnatal day 4, the pups that were to be artificially reared were anesthetized with halothane and implanted with gastrostomy tubes (17,26,45). The artificial rearing apparatus was housed in a room on the same light/dark cycle as the animal quarters. The artificial rearing apparatus was made of clear Plexiglas and measured 40 cm (width) × 35 cm (length) × 15 cm (height). The apparatus was divided into 10 compartments by cotton netting each measuring 8 cm × 17.5 cm × 15 cm. Running through each compartment along the middle wall was a cotton cylinder that contained a rubber tube (1.5 cm in diameter). The netting that divided the compartments was sewn around the cotton cylinder to keep the pups in their individual compartments, but the rubber tube could pass freely through the cylinder. The cotton cylinder was approximately 5 cm from the floor of the apparatus; within each compartment the cotton cylinder was covered by fake fur (a cotton/polyester blend with a fur thickness of 0.5 cm obtained from a local fabric store). The fur was wrapped around the cotton cylinder and held in place by flexible wire. In this manner, the fur could be changed easily when it was flattened by pup activity. A water pump submerged in 58°C water was placed at one end of the tube; the water was circulated continuously through the tube. The temperature under the fur-covered tube was 30°C and the temperature in other areas of the compartment was 22°C. The floor of the apparatus was cov-

ered by wood chips (2 cm deep). The circulating water warmed the fur and caused a slight pulsing movement of the fur—characteristics that were designed to simulate a dam. The artificially reared rats were put in the individual compartments separated by the netting, which allowed indirect tactile and olfactory contact with other rat pups.

A timer-controlled infusion pump (Harvard Apparatus, Model 935) administered 20-min milk feedings (51) through the gastrostomy tubes every 2 h; the milk solution was made from a condensed milk base with additions such that it resembled milk from a rat dam (51). An amount of milk (milliliters) totaling 33% of the pups' mean body weight (grams) was evenly distributed among 12 feedings in a 24-h period. Each morning, the pups were weighed, bathed, and given 0.1 ml distilled water through their gastrostomy tubes. Stimulation of the pups' anogenital region facilitated excretion. Alcohol and hypoxia treatments (see below) occurred from postnatal days 4 through 9. All feedings were milk solution alone throughout postnatal days 10 and 11 to allow the alcohol-exposed rats to withdraw. On the morning of postnatal day 12, all pups were paw-marked with permanent ink for later identification (15) and returned to a lactating dam. To encourage the dam to accept the pups, they were covered in a slurry of the dam's feces.

Alcohol and Hypoxia Exposure

On postnatal days 4 through 9, the alcohol group received 5 g/kg of ethanol condensed into the four feedings that occurred during the light phase of the light/dark cycle. Both the gastrostomy control group and hypoxia group had maltose dextrin added to these four feedings such that the milk solution was isocaloric with the alcohol-containing milk solution. The eight remaining daily feedings for all three groups were of milk solution alone.

Peak blood alcohol concentrations were determined on postnatal day 6, 70 min after the end of the last alcohol-containing feeding (23); blood samples were taken from all artificially reared animals. The tip of the animal's tail was cut, and 10 μl of blood was drawn into a capillary tube. The blood was then placed in 190 μl of 0.52 N perchloric acid and neutralized with 200 μl of 0.30 M potassium carbonate. The resulting solution was centrifuged (Beckman Microfuge E) on high for 15 min. All samples were refrigerated (4°C). The following day, all samples and ethanol-containing standards were analyzed for alcohol content using an enzymatic procedure (12).

Hypoxia was induced in the hypoxia group twice a day from postnatal days 4 through 9 at 0930 and 1730 h. This regimen was chosen to mimic the repeated hypoxic episodes during the third trimester that children with RDS experience. The animals were put on a warmed pad in a desiccator cabinet (Plas-Labs). The desiccator cabinet was then filled with a 5% oxygen/95% argon (an innert noble gas) such that the oxygen content of the cabinet was below 5.5% as measured by an oxygen monitor (Instrumentation Laboratory; Model 408). The reduction in oxygen content took approximately 3 min and then the animals were left in the low oxygen environment for 15 min. During this period, the pups showed clear evidence of hypoxia because of their blue color; normal color returned within a few minutes after being removed from the hypoxia chamber. The animals were returned to the artificial rearing apparatus after the hypoxia episode.

Baseline Recording of the Electrocardiogram and Respiration

A 10-min baseline recording was done on postnatal days 13, 15, 17, 19, and 21 after the rat pups became quiescent. The rat was transferred from its cage to a separate room into a sound-attenuated chamber and placed in a small container on a warmed pad. The container contained clean shavings and the rat pup was isolated during testing. Stainless steel wires were inserted SC in the dorsal surface of the neck and in the right side above the hind leg. The wires were very thin, pierced the skin of the unanesthetized pup easily, and left no detectable scar tissue over days. The electrocardiogram was recorded directly from the wires and was amplified with a Layfayette Instruments polygraph. Respiration was transduced with an UFI Impedance Pneumograph and was amplified with a Layfayette Instruments polygraph. The electrocardiogram and respiration were recorded with a Vetter FM recorder, and were played back into a computer that digitized each signal at 1000 Hz (each millisecond). From the 10-min recording, 30-s intervals of the electrocardiogram and respiration were selected. Intervals with excessive noise in either the electrocardiogram or respiration were eliminated. The noise was due to excessive animal movement and/or poor wire insertions and resulted in the removal of 28.8% of the recordings. This resulted in a variable number of epochs for an animal on any given day.

Stimulus Exposure Recording of Electrocardiogram and Respiration

Immediately following the 10-min recording, the rat pups were exposed to auditory and visual stimuli. The auditory stimulus consisted of a 80 dB 1600 KHz pure tone, pulsed on and off at 250-ms intervals (250 ms on, 250 ms off). The visual stimulus was a 60-W bulb, pulsed on and off at 250-ms intervals. The auditory and visual stimuli were presented separately. The presentations consisted of a 20-s exposure followed by a interstimulus interval that randomly varied from 30 to 60 s. In each two-trial block the auditory and visual stimulus were presented, and order within the block was random. Ten presentations of each stimulus were given. The electrocardiogram and respiration were recorded continuously, and 30-s intervals were selected starting at least 5 s before the stimulus and through the entire stimulus period. Intervals with excessive noise in either the electrocardiogram or respiration (a total of 28.8%) were eliminated.

Quantification of Interbeat Interval

A computer algorithm identified the QRS complex in the electrocardiogram and interbeat interval was defined as the duration between successive R-waves in the electrocardiogram. Artifact correction of the interbeat intervals was done using the Cheung (9) and Berntson et al. (6) algorithms along with visual inspection of suspect beats. Four variables were quantified for each 30-s interval of the baseline recording; these were the interbeat interval, the standard deviation of the interbeat interval, and time–domain and frequency–domain quantifications of respiratory sinus arrhythmia. The interbeat intervals (artifact corrected) were proportionally assigned to 100-ms intervals [see (18,19)]. The interbeat interval average and the standard deviation of those values were computed.

A time–domain quantification of respiratory sinus arrhythmia was calculated for the baseline recording (36). A bandpass filter was applied to the interbeat intervals with high- and low-pass settings at approximately the respiratory frequency of the rat pups. The standard deviation of the filtered interbeat interval series represents a time–domain quantification of the variability in interbeat values at the same frequency as

respiration (i.e., respiratory sinus arrhythmia). The filtering was done on 100-ms interbeat intervals with a polynomial coefficients moving average low-pass filter (0.60 Hz), a subtraction resulting in a high-pass series and a subsequent bandpass filter with weights to eliminate higher frequencies (50% amplitude = 2.60 Hz; 1% amplitude = 3.22 Hz). The resulting series had variability in a fixed frequency interval. The peak of the respiration power spectrum for the 30-s epochs was less than 2.6 Hz for 90% of the epochs. Thus, the standard deviation of this filtered series was a measure of respiratory sinus arrhythmia that included most of the respiratory frequency of the rats.

A frequency–domain measure of respiratory sinus arrhythmia was computed by spectral analysis. The spectral analysis

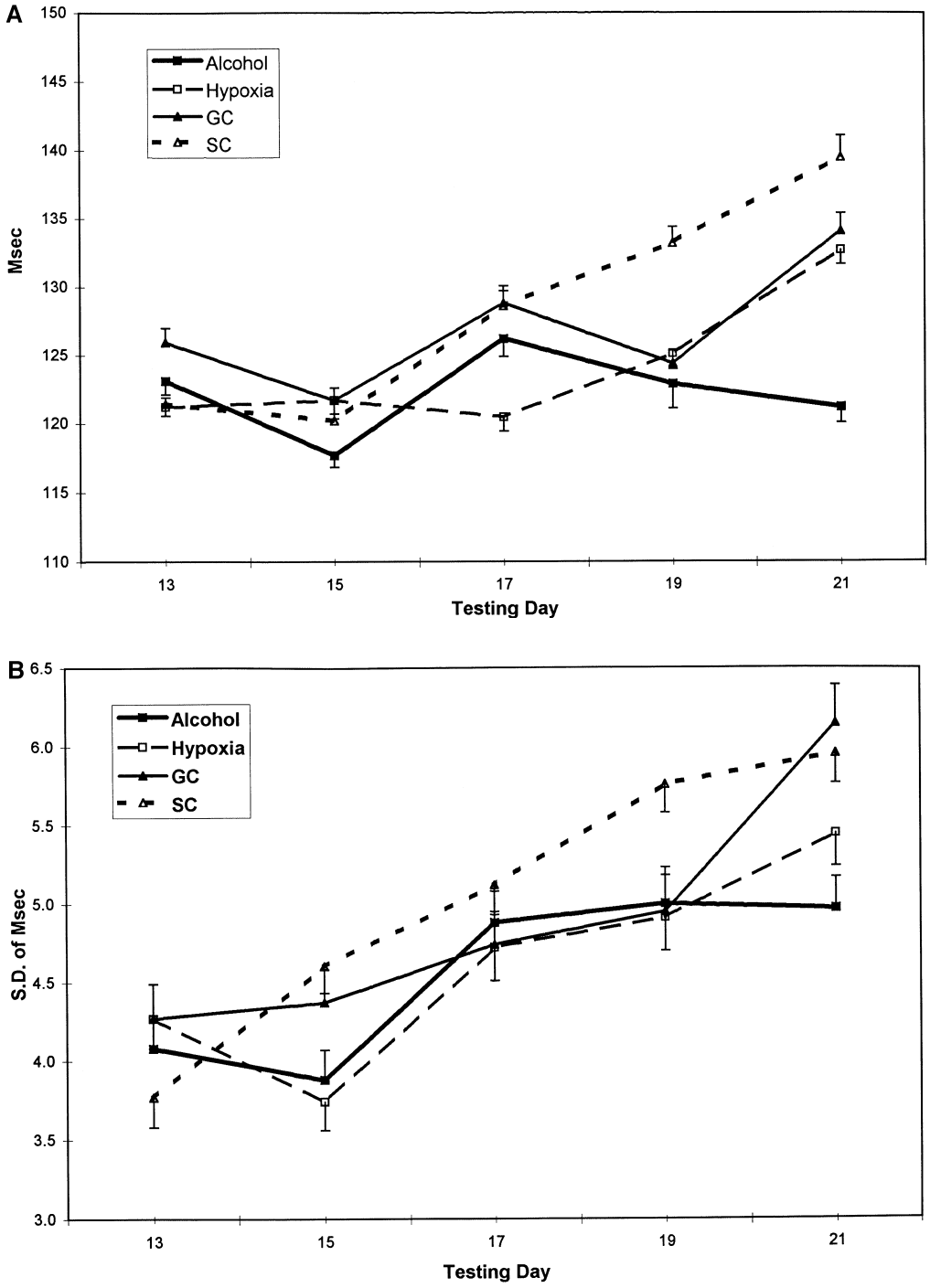

FIG. 1. Mean interbeat interval (A), standard deviation of the interbeat intervals (B), and the time–domain (C) and frequency–domain (D) respiratory sinus arrhythmia measures across the recording days, separately for the four experimental groups. Error bars represent standard error of the mean (SE).

measure quantifies the power spectrum of the interbeat intervals. The amplitude of the power spectrum at the respiration frequency of the 30-s epochs represents the variability of the interbeat intervals occurring at the frequency of respiration (i.e., respiratory sinus arrhythmia). The periodogram was computed with the Fast Fourier Transform from values assigned to the first 256 0.1-s intervals of each of the 30-s epochs (cosine tapered), giving a frequency resolution of 0.01953 Hz. A modified Daniell smoothing algorithm was applied to the periodogram to obtain the power spectrum. A spectral analysis was also done of the digitized respiration recording. The frequency–domain respiratory sinus arrhythmia measure was defined as the natural logarithm of the interbeat interval power estimates summed over 0.1953 Hz (11.71 breaths per minute) and centered at the peak of the power spectrum of the respiration signal for that 30-s epoch. Thus, the interbeat

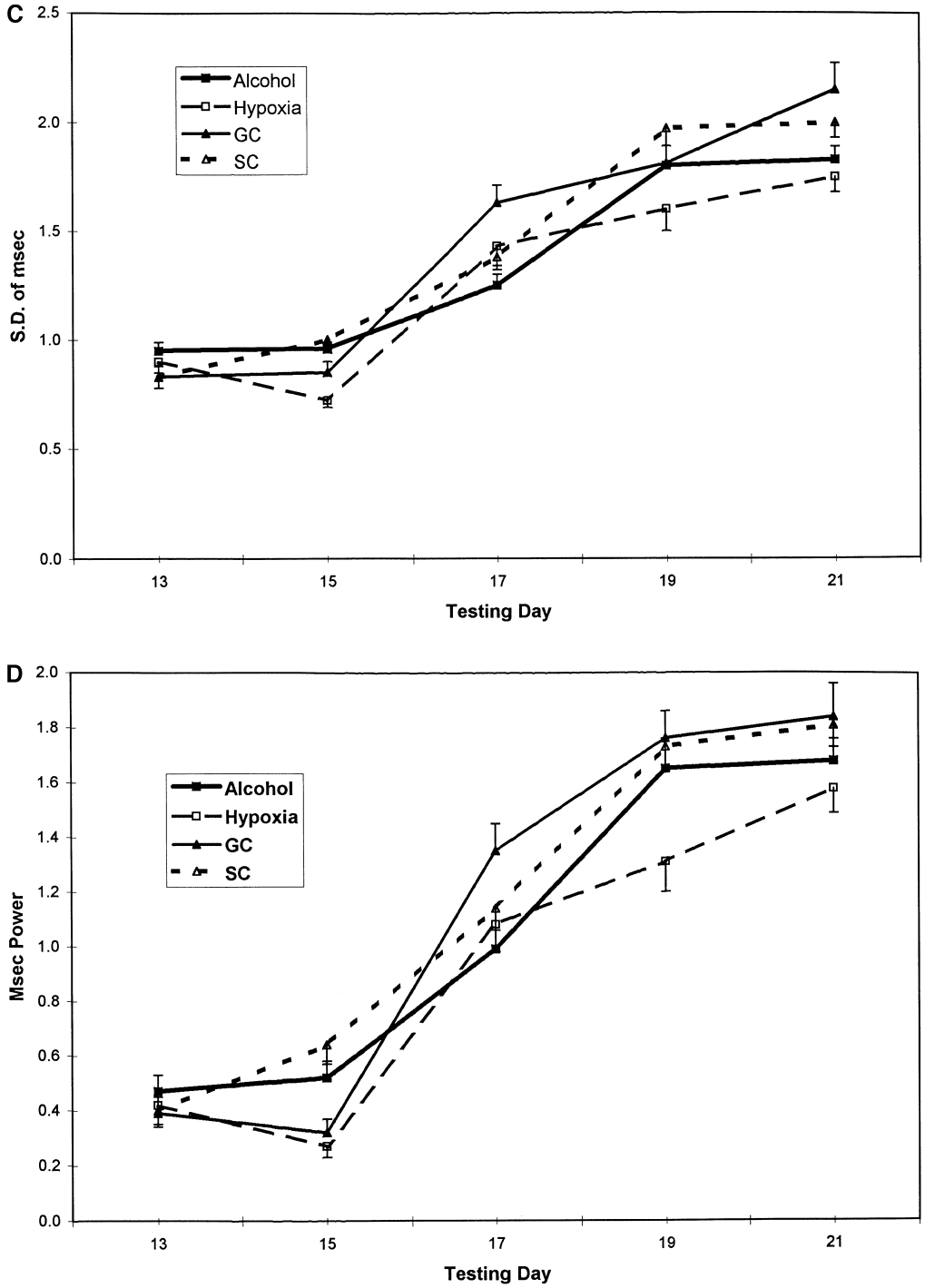

FIG. 1. Continued

power spectrum measure was taken at the respiratory frequency for that individual rat during the 30-s epoch.

The interbeat intervals were quantified from the stimulus exposure periods. The interbeat intervals were proportionally assigned to 100-ms intervals. The interbeat interval average was computed for the 5 s immediately prior to the stimulus presentations, and for the four 5-s epochs of the 20-s stimulus exposure.

RESULTS

Body Weights and Blood Alcohol Concentrations

Analyses of the body weights indicated that the artificially reared animals weighed less than the suckle control animals on PD 21. Body weights were analyzed with a Group (4; alcohol, hypoxia, gastrostomy control, suckle control) × Sex (2) × Days (10) analysis of variance (ANOVA). A significant effect of Days, $F(9, 252) = 481.72$, $p < 0.0001$, $\epsilon = 0.3821$, and a significant interaction between Days and Group, $F(27, 252) = 3.04$, $p < 0.01$, $\epsilon = 0.3821$, were found. Analysis of simple main effects indicated that there was a significant effect of group only on PD 21, $F(3, 32) = 4.67$, $p < 0.005$, and not on PD 4 through 12. There were no effects of nor interactions with sex. Scheffe's post hoc tests indicated that on PD 21, the SC group weighed significantly more than the artificially reared groups (ps < 0.01) and there were no other significant differences among groups.

In the alcohol group, a t-test indicated that there were no differences in blood alcohol concentration between the sexes. The blood alcohol concentration was 239.3 ± 10.6 mg/dl (mean and SE).

Baseline Interbeat Intervals and Respiratory Sinus Arrhythmia

The mean of the 0.1-s by 0.1-s interbeat interval values, the standard deviation of those values, the time domain respiratory sinus arrhythmia, and frequency domain respiratory sinus arrhythmia measures were analyzed with a Days (5) × Group (4; alcohol, hypoxia, gastrostomy control, suckle control) ANOVA. The data were collapsed across sex because the only significant effect or interaction of sex was an interaction between sex and day of testing on the standard deviation of the interbeat interval, $F(4, 79) = 2.83$, $p < 0.05$, $\epsilon = 0.7993$. This interaction occurred because females had a slightly larger standard deviation of the interbeat interval on postnatal day 17 only.

There was a statistically reliable effect of Days on all four variables: mean IBI, $F(4, 111) = 3.55$, $p < 0.05$, $\epsilon = 0.8181$; standard deviation of interbeat interval, $F(4, 111) = 13.74$, $p < 0.0001$, $\epsilon = 0.8462$; time–domain respiratory sinus arrhythmia, $F(4, 111) = 22.34$, $p < 0.0001$, $\epsilon = 0.7824$; frequency–domain respiratory sinus arrhythmia, $F(4, 111) = 17.34$, $p < 0.0001$, $\epsilon = 0.7299$. There was a statistically reliable interaction of Days × Group on the frequency-domain respiratory sinus arrhythmia, $F(12, 111) = 2.00$, $p = 0.0512$, $\epsilon = 0.7299$. Post hoc analyses were done using Scheffe's multiple comparison procedure.

Figure 1 shows the interbeat interval mean, standard deviation, time–domain respiratory sinus arrhythmia. and frequency–domain respiratory sinus arrhythmia measures over the recording sessions for the four groups. The mean interbeat interval values were not significantly different from days 13 to 19 and were slightly longer on day 21 ($p < 0.05$) (see Fig. 1a). The three variability measures were not significantly different on days 13 and 15 and increased significantly from days 17 to 21 (ps < 0.05) (see Fig. 1b–d). The Days × Group interaction effect on the frequency–domain respiratory sinus arrhythmia measure was due to significant group differences occurring on days 19 and 21 and no group differences on the other days (see Fig. 1d). The combined experimental groups had lower respiratory sinus arrhythmia compared to the two control groups (ps < 0.05) on days 19 and 21. Similarly, although the overall Days × Group interaction was not significant for the time–domain respiratory sinus arrhythmia measure, the two exposure groups had significantly lower respiratory sinus arrhythmia than the control groups on postnatal day 21 (ps < 0.05) and the hypoxia group had lower respiratory sinus arrhythmia on postnatal day 19 also ($p < 0.05$) (see Fig. 1c).

Interbeat Intervals/Respiratory Sinus Arrhythmia Correlations

The large increase in the time–domain and frequency–domain measures of respiratory sinus arrhythmia suggest that parasympathetic cardiac control becomes active around postnatal day 17. Because the parasympathetic control of the heart has a tonic effect on mean interbeat interval level as well as the rhythmic variability quantified in RSA, a correlation between mean interbeat interval level and respiratory sinus arrhythmia may be expected when the parasympathetic control system affects the chronotropic heart functions. To examine this, the correlations between mean interbeat interval and the two respiratory sinus arrhythmia variables were computed. Table 1 has those correlations. Both respiratory sinus arrhythmia measures were significantly correlated with mean interbeat interval and standard deviation of interbeat interval on all testing days. However, there was a large increase in the correlation on day 17 between mean interbeat interval and the respiratory sinus arrhythmia variables. The correlations between the standard deviation of interbeat intervals and the respiratory sinus arrhythmia measures increased steadily across the testing days.

Heart Rate Orienting Response

The interbeat interval values from the stimulus exposure conditions were analyzed. In addition to the Days (5) and Group (4; alcohol, hypoxia, gastrostomy control, suckle control) factors, we included Stimulus Type (2; visual, auditory) and Epochs (4; 5-s intervals of 20-s exposure to the stimulus) factors. A Trial Block (2; first 4 presentations, second 4 presentations) factor was included to examine habituation effects. The data were collapsed across sex because there were no significant effects of nor interactions with sex. Only those effects interacting with the Epochs factor are reported, because the Epochs factor represents the interbeat interval change in response to the stimulus.

There were several main effects and interactions, including Epoch, $F(3, 96) = 61.84$, $p < 0.001$, $\epsilon = 0.7513$; Day × Epoch, $F(12, 384) = 3.82$, $p < 0.001$, $\epsilon = 0.7507$; Day × Stimulus Type × Epoch, $F(12, 384) = 3.82$, $p < 0.001$, $\epsilon = 0.7507$; Day × Stimulus Type × Epoch, $F(12, 384) = 1.85$, $p = .0563$, $\epsilon = 0.7881$. There were four effects involving Trial Blocks, including Trial Block × Epoch, $F(3, 96) = 8.82$, $p < 0.01$, $\epsilon = 0.7522$; Trial Block × Stimulus Type × Epoch, $F(3, 96) = 3.94$, $p < 0.05$, $\epsilon = 1.000$; Trial block × Days × Epoch, $F(12, 384) = 5.40$, $p < 0.001$, $\epsilon = 0.9271$; and Trial Block × Stimulus Type × Day × Epoch, $F(12, 384) = 4.03$, $p < 0.001$, $\epsilon = 0.8082$. There was one interaction involving the Group factor, Group × Trial Block × Day × Epoch, $F(36, 384) = 1.66$, $p < 0.05$, $\epsilon = 0.9271$. Because of the large number of significant effects and

TABLE 1

CORRELATIONS BETWEEN MEAN INTERBEAT INTERVAL, THE STANDARD DEVIATION OF THE INTERBEAT INTERVALS, WITH THE TIME–DOMAIN AND FREQUENCY–DOMAIN MEASURES OF RESPIRATORY SINUS ARRHYTHMIA

	Recording Age (Days)				
	13	15	17	19	21
Mean interbeat interval w/					
Time–domain RSA	0.155*	0.143*	0.453†	0.362†	0.461†
Frequency–domain RSA	0.144*	0.199*	0.451†	0.345†	0.401†
Standard deviation of interbeat interval w/					
Time–domain RSA	0.262†	0.345†	0.439†	0.501†	0.501†
Frequency–domain RSA	0.207†	0.314†	0.372†	0.440†	0.561†

*$p < 0.05$; †$p < 0.0001$.

the complexity of the effects, several Scheffe's post hoc analyses based on the significant omnibus tests were performed to detail the responses. The following represent these analyses.

There were differences between the mean interbeat interval response to the two stimulus types over the days (i.e., Days × Stimulus Type × Epochs). The response to the two stimuli was similar on postnatal days 13, 15, and 21. The heart rate response on those days was significant over epochs, but the responses were small (Fig. 2). In comparison, the response to the sound was much larger than the response to the light on both days 17 and 19 ($ps < 0.05$) (see Fig. 2). The interbeat interval changes on the first four stimulus presentations (i.e., Days × Stimulus Type × Trial Block × Epochs). On the second four presentations the responses were much smaller and were not significantly different for the stimulus types.

There were differences between the groups on the heart rate responses to the stimulus types over the testing days (i.e., Days × Stimulus Type × Groups × Epochs). There were no group differences in the interbeat interval change during stimulus exposure on days 13, 15, and 21. On postnatal days 17 and 19 there were Group × Epochs interactions for the mean interbeat interval change to the auditory but not the visual stimulus. Figure 3 illustrates the interbeat interval change for the four groups to the auditory stimulus on postnatal days 17 and 19. The hypoxia group had a much larger response to the sound on postnatal day 17 than the other groups, and had a

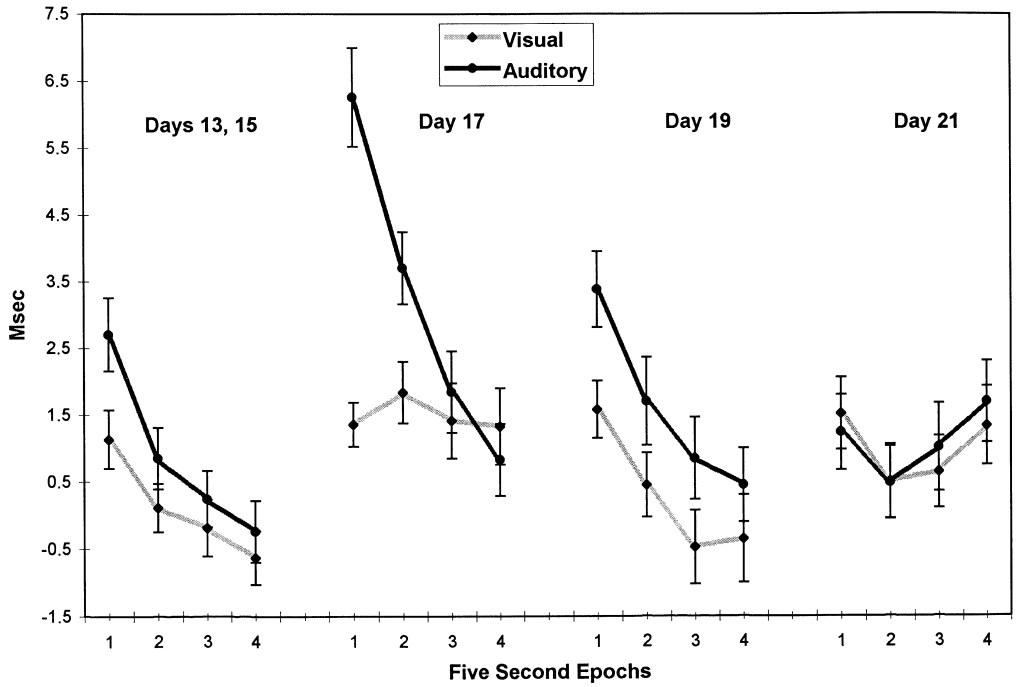

FIG. 2. Mean interbeat interval changes (5-s averages) to the visual and auditory stimuli over the testing days. The averages represent the data from the first four stimulus presentations of each stimulus type. Error bars represent SE.

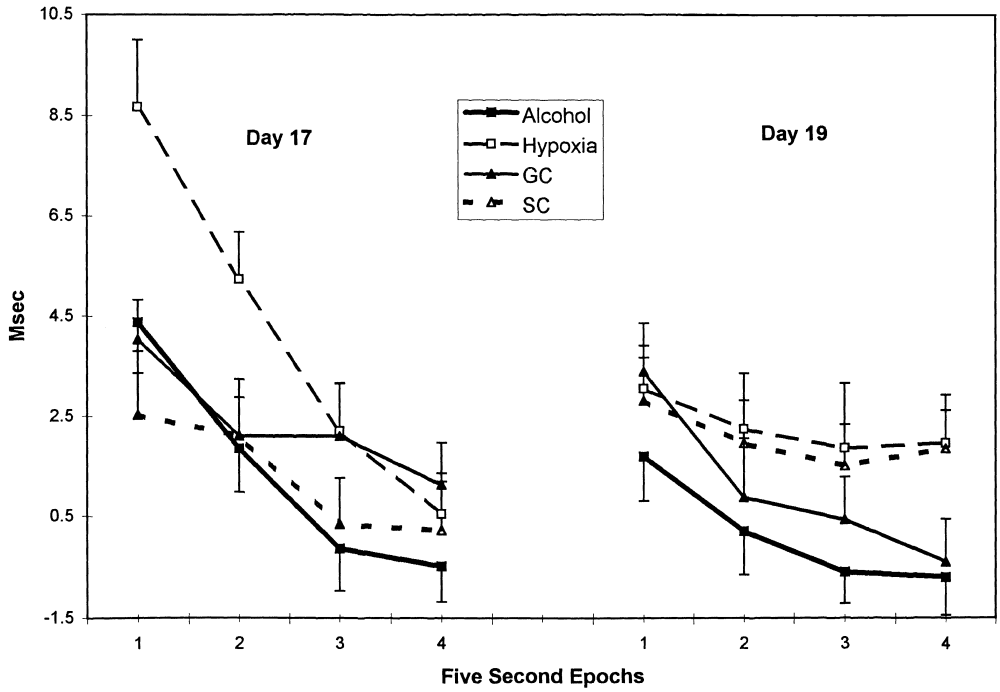

FIG. 3. Mean interbeat interval changes (5-s averages) to the auditory stimulus on days 17 and 19 for the four experimental groups. The averages represent the data from the first four stimulus presentations of each stimulus type. Error bars represent SE.

slightly larger response on postnatal day 19 ($ps < 0.05$). The other three groups (alcohol, gastrostomy control, suckle control) showed a significant interbeat interval change on those days ($ps < 0.05$) but were not significantly different from each other in their response. As with the overall response, this response difference was restricted to the first four stimulus presentations and did not occur on the later trials.

DISCUSSION

Although hypoxia and alcohol exposure during the early postnatal period dampen the increase in respiratory sinus arrhythmia on postnatal day 19 and 21, replicating previous results (27), the predictions that hypoxia exposure would result in a deficit in the magnitude of the heart rate orienting response and that alcohol exposure would result in a deficit of habituation of the response were not confirmed. In control rats and alcohol-exposed rats, the orienting response began as a slight deceleration to both stimuli on postnatal days 13 and 15, became a much more pronounced deceleration to the auditory stimulus on postnatal days 17 and 19, and then became a very small deceleration to both stimuli. Rats exposed to hypoxia during the early postnatal period had a similar pattern except that there was an enhanced heart rate orienting response to auditory stimuli on postnatal days 17 and 19 compared to the other groups. There were no differences in the rate of habituation to the stimuli among the groups.

The lack of effect of postnatal alcohol exposure on the heart rate orienting response and the habituation of this response is in agreement with prior studies using prenatal alcohol exposure (8,21). This lack of effect occurs in a variety of animal models, at different ages of testing, in different sensory modalities, and with either repeated or single tests. One possible explanation is that the isolation of the rat during testing could obscured any effects of alcohol on the response. This is an inherent problem with the heart rate measure in young animals and could only be overcome by the development of the ability to record heart rate using a SC implant of some kind—this would allow recording in the presence of the dam and siblings. However, a more likely explanation of the lack of effect of alcohol exposure is that the behavioral measures of attention disrupted in children with FAS do not correspond to the heart rate measure of attention used in this study—that is to say that the types of attention being measured are different. Coles et al. (10) suggest that FAS does not cause deficits in sustained attention, which would be reflected in the magnitude of the heart rate orienting response, and whereas habituation of the response may reflect encoding and retrieval, it is unlikely that this is a sensitive measure (29). Future studies utilizing animal models of FAS should use measures of attention more sensitive to encoding deficits [for a discussion of tests of animal attention, see (34)].

The hypoxia animals show a deficit in respiratory sinus arrhythmia yet an enhanced heart rate orienting response to the auditory stimulus. A possible explanation of these contradictory effects is that the relationship of these two measures in rats may not be the same as in humans. Another possibility is that the developmental period that is being examined in the rat is not equivalent to the periods that are used in the human. The respiratory sinus arrhythmia and the heart rate orienting response in humans does not become strongly correlated until 3 months of age (40). Recent evidence has suggested that postnatal days 16 through 19 in the rat correspond to only the first postnatal month in humans with respect to brain development (4). Thus, whereas the tonic parasympathetic control may occur at postnatal day 16, more central structures in-

volved in controlling heart rate and heart rate responses in the rat have not necessarily reached the same maturity as that seen in a 3-month-old child and, thus, the heart rate response is not correlated yet to respiratory sinus arrhythmia. An alternative explanation arises from findings in humans. Although children with RDS have been demonstrated to have decreased respiratory sinus arrhythmia, others have shown that 6- to 8-year-old children, with surgically repaired congenital heart malformations and thus hypoxic episodes during development, have an enhanced heart rate orienting response to vibrotactile stimuli but not to auditory stimuli (33). It is interesting to note that in both the rat and the human, the increase in the heart rate orienting response is modality specific. It could be hypothesized that hypoxia delays the onset of vagal tonic control and alters more central structures involved in the attentional processes of specific modalities of stimuli.

The ears and eyes of all of the groups of rats were opened by PD 14 and their orienting responses on both PD 13 and 15 were similar, suggesting that there were no initial differences in responsivity to auditory and visual stimuli. Alcohol exposure during the postnatal period does not delay ear and eye opening; indeed, there is a trend towards alcohol having an acceleratory effect (24). Despite this trend, there are no observable differences in how alcohol-exposed and control rats orient to visual and auditory stimuli. Even severe hypoxia during development does not alter the emergence of the developmental milestones of ear and eye opening (32). Thus, it is unlikely that the changes in the heart rate orienting response on PD 17 and 19 in the hypoxia group are the result of effects on the processing of auditory stimuli by the peripheral nervous system.

Given the dissociation between RSA levels and the heart rate orienting response, the suggestion that the decrease in RSA levels in the alcohol and hypoxia groups reflects deficits in attention (27) may be incorrect. Baseline RSA levels have also been hypothesized to reflect the stress vulnerability in mammals (28,35,37), with lower levels reflecting an increase in stress vulnerability. This interpretation of the current data is consistent with the findings that alcohol exposure during development does enhance the stress response (25) and increases vulnerability to stress as measured by the immune response in rats (16). The finding of the hypoxia-induced decrease in RSA level suggests that these animals may exhibit changes in stress vulnerability as well.

With respect to the heart rate orienting response, we did not observe an initial acceleratory response to stimuli as did Haroutunian and Campbell (20). However, in the latter study, the only significant acceleration was to an auditory stimulus on postnatal day 15; there were no significant accelerations on any other day or for the visual stimulus (20). A possible explanation is that our study tested animals repeatedly whereas the previous study used independent groups. It may be that the animals in the present study developed some long-term habituation on postnatal day 13 such that no acceleration was observed on postnatal day 15. Another difference is that our animals received a fair amount of handling during the developmental period prior to testing. All of the artificially reared animals are handled to conduct the artificial rearing properly. In addition, the normal control animals get weighed daily and are raised in litters that are disturbed on postnatal day 12 when the artificially reared animals are fostered back to the dams. It is known that neonatal handling can alter (usually by decreasing) the responsiveness of animals to novel stimuli (7,11) and this may account for some of the discrepancies. However, given that there were no differences between the artificially reared and normal control group, it is unlikely that differences in the amount of handling can account for the differences among the groups in the present study.

In conclusion, the findings suggest that the heart rate orienting response is not a sensitive measure of any attentional deficits that may be examined with animal models of FAS but that the heart rate orienting response may be useful in examining the effects of hypoxia during development. The interpretation of the enhanced heart rate orienting response seen in hypoxic animals remains to be determined. The idea that hypoxia enhances attentional responses seems unlikely (albeit possible). Hypoxia might result in a hypersensitive auditory system or a hyperreactive vagus nerve and not reflect attentional processes. The finding of a deficit in development of respiratory sinus arrhythmia induced by alcohol and hypoxia does suggest that these teratogens disrupt the development of cardiac control. Whether this disruption is related to disruptions in more central structures and a deficit in attention remains to be seen. Future work should focus on delineating the neural and neurochemical bases of the changes induced by alcohol and hypoxia during development and could begin by examining the relative involvement of peripheral and central alterations.

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