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Development of Heart Inter-beat Interval Variability in Preweanling Rats: Effects of Exposure to Alcohol and Hypoxia

SANDRA J. KELLY [1] AND JOHN E. RICHARDS

Department of Psychology, University of South Carolina, Columbia, SC 29208 USA

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KELLY, S. J. AND J. E. RICHARDS. *Development of heart inter-beat interval variability in preweanling rats: Effects of exposure to alcohol and hypoxia.* PHYSIOL BEHAV **61**(2) 231–241, 1997.—The effect of alcohol exposure and hypoxia on the development of heart rate and heart inter-beat interval (IBI) variability was studied in preweanling rats. Rats were artificially reared from postnatal day (PD) 4 through 12 and either exposed to alcohol (5 g/kg/day) or hypoxia (2 15-min episodes/day) from PD 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 for 20-min sessions every other day from PD 5 through 21. Inter-beat intervals and measures of their variability caused by respiratory sinus arrhythmia (RSA) were computed from the recordings. There was a steady decline in average IBI across this age range. There was little change in RSA from PD 5 to 15, followed by a large increase in RSA level from PD 15 to 21. The alcohol- and hypoxia-exposed rats showed significantly less increase in RSA level on PD 19 and 21. Large bradycardias occurred in all groups on PD 5, 9, and 17, and were more prevalent in rats exposed to alcohol or hypoxia. These data suggest that neural control of the chronotropic functions of the heart undergoes major changes in the late preweanling stage, and the changes in neural control are slowed by hypoxia or alcohol exposure during the early postnatal period. *Copyright © 1997 Elsevier Science Inc.*

Respiratory sinus arrhythmia Heart rate Alcohol Hypoxia Fetal alcohol syndrome Attention Respiratory distress syndrome Teratology

RESPIRATORY sinus arrhythmia (RSA) is an acceleration of heart rate shortly after the beginning of inspiration and a deceleration shortly after the beginning of expiration. This measure, which is in part controlled by the parasympathetic nervous system via the vagus nerve (1–4,10,29), contributes to the heart's inter-beat interval (IBI) variability. In addition to being a measure sensitive to vagal control of the heart (57), RSA is an extremely good predictor of the capability to sustain attention in infants (42,43,45). At about 2 months of age, the heart rate response of infants to novel stimuli changes from an acceleration to a deceleration (5,18). From 2 to 6 months, both heart rate and behavioral indices of sustained attention increase in magnitude (45). In conjunction with these measures of attention, there are striking increases in RSA from 2 to 6 months (e.g., 21,23,28,54). RSA has been hypothesized to be correlated with sustained attention because it indirectly indexes brainstem integrity necessary for coherent attentional responses (43), or because it is an index of vagal parasympathetic tone, which is important for control of attention (38). The increases in RSA found in the first few months of life are thought to underlie the increases in sustained attention over this age range (46).

RSA may be used to index developmental disorders. Porges et al. (40) have argued that populations such as hyperactive, autistic, and retarded children manifest irregular patterns of RSA. Preterm infants with likely central-nervous-system damage (22,50) or respiratory distress syndrome (RDS) (15,43) show immature patterns of heart rate responding compared to healthy preterm or full-term infants. Of importance, preterm infants with RDS also exhibit low RSA (33,48) in conjunction with irregular behavioral patterns of attention (14,43,47). Infants with signs of atypical fetal growth also show signs of irregular heart rate cycles (58).

There have been few studies examining heart rate and variability in heart rate in rat models of developmental disorders. Two rat models of interest are those of fetal alcohol effects and RDS because both syndromes are associated with attentional deficits. As described above, infants with RDS show a low level of RSA and concomitant changes in attention. An animal model of this syndrome utilizing hypoxia might mimic the changes in RSA, which would allow further investigation into the neural bases of these changes. Most rat models of hypoxia utilize one prolonged hypoxic episode at birth, modelling birth trauma (35–37). However, infants with RDS are premature and have repeated episodes of hypoxia during the period that would normally be the third trimester of prenatal development. An appropriate rat model of RDS would entail repeated hypoxic periods through-

[1] To whom requests for reprints should be addressed. E-mail: kelly@garnet.cla.sc.edu

out the early postnatal period, a period equivalent to the human third trimester (11,12).

Children with fetal alcohol syndrome have a constellation of symptoms consisting of retarded growth, facial abnormalities, and central-nervous-system dysfunction (26,27), including attention deficits (51,52). There have been no studies examining variability in RSA or heart rate in children with fetal alcohol syndrome. However, there have been two studies examining heart rate in animal models of fetal alcohol effects. Caul et al. (8) found no changes in baseline heart rate, heart rate response to a novel auditory stimulus, or a conditioned heart rate response in 56–82-day-old rats exposed to alcohol during the prenatal period. Hayne et al. (24) found that, although prenatal alcohol exposure did have an effect on basal heart rate on postnatal days 1 and 6, there was no effect of prenatal alcohol exposure on postnatal day 12 on basal heart rate or the heart rate response and habituation of that response to a novel olfactory stimulus. The studies by Caul et al. (8) and Hayne et al. (24) used prenatal alcohol exposure. Alcohol exposure during the early postnatal period, a period in the rat equivalent to the third trimester of prenatal development in humans with respect to brain growth (11,12), has been shown to have stronger effects on the nervous system than prenatal exposure (19,49). It may be that this period is critical to the development of the neural control of heart rate. Furthermore, neither study examined RSA or the period in which the parasympathetic system begins to have tonic control of heart rate (around 16 days of age). The RSA measure may be likely to show teratogenic effects in the developmental period at and after the onset of tonic control of the heart rate, rather than prior to onset.

The purpose of this study was to examine the development of baseline heart rate, heart rate variability, and RSA during normal rat development and in rat models of fetal alcohol effects and RDS. Both of the rat models entail artificial rearing of the rats to control for any nutritional effects induced by the hypoxia or alcohol treatments. The artificial rearing is a modified procedure (31) that allows the rat pups to have some contact with each other and a simulated dam; nevertheless, a group of animals reared by dams is included to control for artificial rearing effects. It was hypothesized that untreated rats will show low levels of RSA (as computed by both time-domain and frequency-domain methods) that will increase over the preweanling period. Alcohol exposure and hypoxia during the early postnatal period are hypothesized to disrupt the development of RSA.

METHODS

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.

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. The offspring were assigned in a quasi-random manner to an alcohol group, hypoxia group, gastrostomy control (GC) group, and a suckle control (SC) group. The assignment to a group was, in part, determined by past mortality. The first 3 groups were artificially reared from PD 4 to PD 12. The SC group was reared normally by dams. All rats were weighed from PD 4 through 12 and on PD 21. Litter size was maintained at 10 pups by the addition and/or removal of nonexperimental rats.

Two cohorts were done. In the first cohort, rats were assigned to alcohol, GC, or SC groups until 10 rat pups in the alcohol condition had recordings from PD 5 through 21; there were totals of 6 GC and 8 SC rats in this cohort. In the second cohort of rats, rats were assigned to the hypoxia, GC, or SC groups until 10 rat pups in the hypoxia condition had recordings from PD 5 through 21; there were totals of 7 GC and 8 SC rats in this cohort. The number of litters used in the alcohol, hypoxia, GC, and SC groups were 7, 9, 13, and 16, respectively. Thus, the GC and SC groups had only 1 animal from a particular litter assigned to them. In the alcohol group, there were 3 litters from which 2 animals of opposite sex were taken. In the hypoxia group, there was 1 litter from which 2 female animals were taken. In the analyses of mean IBI, standard deviation of IBI and the 2 measures of RSA, 10 rats were chosen from the 13 GC rats and 10 rats from the 16 SC rats. The rats chosen were from the same litters as the alcohol or hypoxia rats, so that any litter effects were distributed across treatment groups.

Artificial Rearing Protocol

On PD 4, the pups that were to be artificially reared were anesthetized with halothane and implanted with gastrostomy tubes (32,49). 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 covered 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, South Nateck, MA, Model 935) administered 20-min milk feedings (56) through the gastrostomy tubes every 2 h. An amount of milk (ml) totalling 33% of the pups' mean body weight (g) 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 4 times daily facilitated excretion. Alcohol and hypoxia treatments (see below) occurred from PD 4 to 10. All feedings were milk solution alone throughout PD 10 and 11. The mortality rate during the artificial rearing and treatment procedures was 20%. On the morning of PD 12, all pups were paw-marked with permanent ink for later identification (16) and returned to a lactating dam. To facilitate the dams' acceptance of the pups, they were covered in a slurry of the dam's feces.

Alcohol and Hypoxia Exposure

On PD 4 to 10, the alcohol group received 5 g/kg of ethanol condensed into the 4 feedings that occurred during the light phase of the light-dark cycle. Both the GC group and hypoxia group had maltose dextrin added to these 4 feedings, so that the milk solution was isocaloric with the alcohol-containing milk solution. The 8 remaining daily feedings for all 3 groups were of milk solution alone.

Peak blood alcohol concentrations were determined on PD 6, 70 min after the end of the last alcohol-containing feeding (30); 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, Palo Alto, CA) 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 (13).

Hypoxia was induced in the hypoxia group twice a day from PD 4 to 10. The hypoxic episodes occurred just prior to 1100 h and just after 1720 h. The animals were put on a warmed pad in a dessicator cabinet (Plas-Labs, Lansing, MI). The dessicator cabinet was then filled with 5% oxygen/95% argon (an innert noble gas), so that the oxygen content of the cabinet was below 5.5% as measured by an oxygen monitor (Instrumentation Laboratory, Lexington, MA; 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. The animals were then returned to the artificial rearing apparatus. During the hypoxic episodes, all animals showed a distinct change in skin color, from pink to blue, evident over the whole body in young pups and in the paws and nose of the older pups; this change indicated that hypoxia treatment was effective.

Recording of ECG and Respiration

A 20-min recording of the electrocardiogram and respiration was done on PD 5, 7, 9, 11, 13, 15, 17, 19, and 21. Testing always occurred between 0930 and 1045 h. The timing was such that the alcohol group had not received alcohol for 16 h and, thus, had no alcohol in their bloodstream (30), and that the hypoxia group had not received a hypoxic episode for 16 h and had normal color. Thus, regardless of the age, the rat pups were always tested in a state that was free of acute influences of treatment.

For testing, the rat was transferred from the artificial rearing environment (PD 5 to 11) or its cage (PD 13 to 21) to a small container placed on an electrically controlled warm pad in a quiet environment. A flexible stainless-steel wire was inserted SC through the dorsal surface of the neck and another wire was inserted SC through the right surface above the hind leg. One end of each flexible wire was linked to form a closed small loop. The other end of each wire was attached to the recording apparatus. The wires were long enough to allow movement. The rats were not habituated prior to the beginning of recording, nor were they restrained during recording. If the rat appeared distressed by the recording electrode placements, the placement was changed so that the rat appeared comfortable. Intervals of heart rate and RSA measures were only used when the recording was noise-free. Noise in the recording occurred in intervals when the rat was distressed or exploring the environment, and were typically due to electrode movement. There were no systematic differences in the amount of time for noise-free data across the 4 testing groups. The intervals analyzed were those taken when the animal was in a quiescent state.

The electrocardiogram (ECG) was recorded directly from the wires and was amplified with a Layfayette Instruments polygraph (Lafayette, IN). Respiration was transduced with UFI Impedance Pneumograph and was amplified with a Layfayette Instruments polygraph. ECG and respiration were recorded with a Vetter FM recorder (Rebersburg, PA), and they were played back into a computer that digitized each signal at 1000 Hz (each ms). From the 20-min recording, 30-s intervals of ECG and respiration were selected. Intervals with excessive noise in either the ECG or respiration were eliminated. This procedure resulted in a variable number of epochs for an animal on any given day.

Quantification of Inter-beat Interval Variability

A computer algorithm identified the R-waves in the ECG, and inter-beat interval (IBI) was defined as the duration between successive R-waves in the ECG. Artifact correction of the IBIs was done using the Cheung (9) and Berntson et al. (7) algorithms, along with visual inspection of suspect beats.

A preliminary inspection of the data showed large IBI increases over a few beats (i.e., bradycardia). The IBI variability estimates were inflated artificially in the intervals with these bradycardias. The bradycardias were removed from the IBI record with the following procedure. A difference IBI series was modeled with an adaptive forward-autoregressive model based on 50 beats. When the next IBI difference deviated from the predicted value by at least 2 standard deviations (SD), the total period of the deviations was identified. The bradycardia duration was defined as all beats that deviated, and each identified epoch was visually inspected for accuracy. The IBI value preceding the bradycardia, the maximum IBI value during the bradycardia, and the number of beats of the bradycardia were recorded. The beats during the bradycardia were replaced with the average of the predictions from the forward-autoregressive model, the predictions from a backward-autoregressive model that began with the last beat of the bradycardia, and a linear interpolation from the first to the last beat of the bradycardia.

Four variables were quantified for each 30-s interval. The inter-beat intervals (artifact- and bradycardia-corrected) were proportionally assigned to 100-ms intervals (see 17). The inter-beat interval (IBI) mean and the standard deviation of the inter-beat intervals were computed for each animal and analyzed as 2 of the 4 dependent variables. The IBI mean is a measure of heart rate and the standard deviation is a measure of inter-beat interval variability.

The third dependent variable was a time-domain quantification of respiratory sinus arrhythmia. The standard deviation of the IBI series transformed by a band-pass filter was computed using methods described by Porges (39). The filtering was done on 100 ms interval IBIs with a polynomial coefficient 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 power spectrum of the respiration signal (see later paragraph) ranged from 0.937 to 4.176 Hz (Mean = 2.43, SD = 0.485). The peak of the respiration power spectrum for the 30-s epochs was less than 2.6 Hz for 70% of the epochs, and less than 3.22 Hz for 93% of the epochs. Thus, the variability in this bandpass filter RSA measure included most of the respiratory frequencies of the rats. This measure represents a time-domain quantification of variability in IBI values at the same frequency as respiration (i.e., respiratory sinus arrhythmia). This time domain RSA measure has been advocated as a simple quantitative measure of RSA, and has been shown to

be closely related to attentional and socioemotional measures in young infants (38).

Two other time-domain RSA measures were computed. One was based on the same procedure described by Porges (39), but used trigonometric coefficients for the moving average low-pass filter (0.50 Hz) and a low-pass filter based on similar coefficients in the second step to provide the high-frequency filter (2.5 Hz; see 44). The other RSA measure was based on filtering the data with a Kaiser-Bessel (6) bandpass algorithm with a larger band (0.50 to 4.0 Hz). For these measures, the standard deviations of the filtered series were calculated. These measures were highly correlated with the Porges measure and were affected similarly by the experimental conditions.

The respiration frequencies were analyzed with a Days (9) × Group (4) ANOVA. There were no significant effects involving the group factor, and a slight decrease in respiration frequencies over the testing days. The mean respiration frequency were 2.39 (0.453), 2.44 (0.496), 2.44 (0.453), and 2.45 (0.494) for the alcohol, hypoxia, GC, and SC groups, respectively. Because the respiration frequency was not affected by the group factor, it was acceptable to apply a common frequency band for all 4 groups for the time-defined variable. The frequency-domain quantification picked the power estimate at the peak of the respiration power spectrum for that 30-s epoch, so that any subject differences in respiratory frequency would be controlled for in that measure.

The fourth dependent variable was a quantification of IBI variability with spectral analysis (44). 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 spectral analysis respiratory sinus arrhythmia measure was defined as the natural logarithm of the IBI 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. The metric for the spectral analysis variable is the natural logarithm of the root-mean-squared variation of the IBI values at the respiration frequency ranges. This measure represents a frequency-domain quantification of respiratory sinus arrhythmia, and has been used extensively in the study of rat RSA (34,57) and RSA in human infants (38,43,44).

RESULTS

Body Weights and Blood Alcohol Concentrations

Analyses of the body weights indicated that the artificially reared animals weighed less than the SC animals on PD 12 and 21 (see Table 1), and that the hypoxia animals weighed less than the SC animals on PD 10 and 11. There were no differences between genders. Body weights were analyzed with a Group (4; alcohol, hypoxia, GC, SC) × Gender (2) × Days (10) analysis of variance (ANOVA). A significant effect of Days, $F(9,369) = 951.75$, $p < 0.0001$, and a significant interaction between Days and Group, $F(27,369) = 9.51$, $p < 0.0001$, were found. Analysis of simple main effects indicated that there was a significant effect of Group on PD 10, $F(3,45) = 3.27$, $p < 0.05$; PD 11, $F(3,45) = 4.14$, $p < 0.05$; PD 12, $F(3,45) = 5.91$, $p < 0.01$; and PD 21, $F(3,45) = 11.05$, $p < 0.00001$. There were no effects of Gender nor interactions with Gender. Duncan's post hoc tests indicated that, on PD 10 and 11, the SC group weighed significantly more than the hypoxia group, ps < 0.01, and that there were no other significant differences among groups. On PD 12 and 21, Duncan's post hoc tests indicated that the SC group weighed significantly more than the artificially reared groups, ps < 0.05, which did not differ from each other. The lack of a main effect of Gender (with males weighing more than females) is unusual. However, in the Alcohol, GC, and SC groups, males do consistently weigh more than females (see Table 1). In contrast, within the hypoxia group, the usual Gender effect is not present on PD 12 and PD 21 (see Table 1), and this lack is enough to prevent the usual finding of a main effect of Gender.

TABLE 1

BODY WEIGHTS OF TREATMENT GROUPS (MEANS ± SEM)

Treatment Group	Gender	Body Weight at PD 4	Body Weight at PD 12	Body Weight at PD 21
Alcohol	Female	12.75 ± 0.49	23.37 ± 0.50	43.75 ± 2.92
	Male	13.43 ± 0.54	25.58 ± 0.77	45.70 ± 2.00
Hypoxia	Female	12.4 ± 0.41	23.34 ± 1.06	44.09 ± 1.44
	Male	13.74 ± 0.75	23.02 ± 1.82	40.04 ± 1.76
Gastrostomy	Female	11.23 ± 0.34	24.47 ± 0.87	38.22 ± 2.80
	Male	12.19 ± 0.40	25.63 ± 1.59	42.09 ± 2.05
Suckle	Female	11.94 ± 0.36	26.76 ± 0.72	51.79 ± 2.44
	Male	12.91 ± 0.33	29.27 ± 0.76	53.20 ± 1.61

In the alcohol group, a one-way ANOVA indicated that there were no differences in blood alcohol concentration between the genders. The blood alcohol concentration (M and standard error of the mean, SEM) was 367.8 ± 33.0 mg/dl.

Inter-beat Intervals and Respiratory Sinus Arrhythmia

The mean of the 0.1-s by 0.1-s inter-beat interval values, the standard deviation of those values, the time domain RSA measure, and the frequency domain RSA measure were analyzed with a Days (9) × Group (4; alcohol, hypoxia, GC, SC) ANOVA. We analyzed these variables separately to compare our results to prior studies using these different variability quantification techniques (e.g., 37,41,46,47,50). There was a statistically reliable effect of Days on all 4 variables; mean IBI, $F(8,285) = 26.06$, $p < 0.0001$; standard deviation of IBI, $F(8,285) = 9.11$, $p < 0.0001$; time-domain RSA, $F(8,285) = 39.29$, $p < 0.0001$; frequency domain RSA, $F(8,285) = 44.19$, $p < 0.0001$. There were no main effects nor interactions involving the Group factor.

Figure 1a, b, c, and d) shows the IBI mean, standard deviation, time-domain RSA, and frequency-domain RSA measures over the testing sessions for the 4 groups. The mean IBI values show a decrease in level from PD 5 through 13, and then little change through PD 21 (see Fig. 1a). The standard deviation of the IBI values has a curvilinear relation to the rat pup age, with an initial decrease in variability from PD 5 to 9, and an increase in variability from PD 11 through 17 (see Fig. 1b). The 2 RSA measures showed little change for the first 13 days, a slight increase on PD 15, then a dramatic increase on PD 17 to 21 (see Fig. 1c, d). Most of the RSA change occurred between PD 15 and 17.

Figure 1c and d shows that the RSA levels for the alcohol and hypoxia groups were lower than those of the control animals on the last 2 recording days. A planned comparison was done to test the treatment Group effect for the days prior to the hypothesized onset of parasympathetic control of tonic heart rate (PD 5 to 15) and after the hypothesized onset of parasympathetic control (PD 17 to 21). The Scheffe control procedure for post hoc tests was used with the error term coming from the omnibus Days × Group

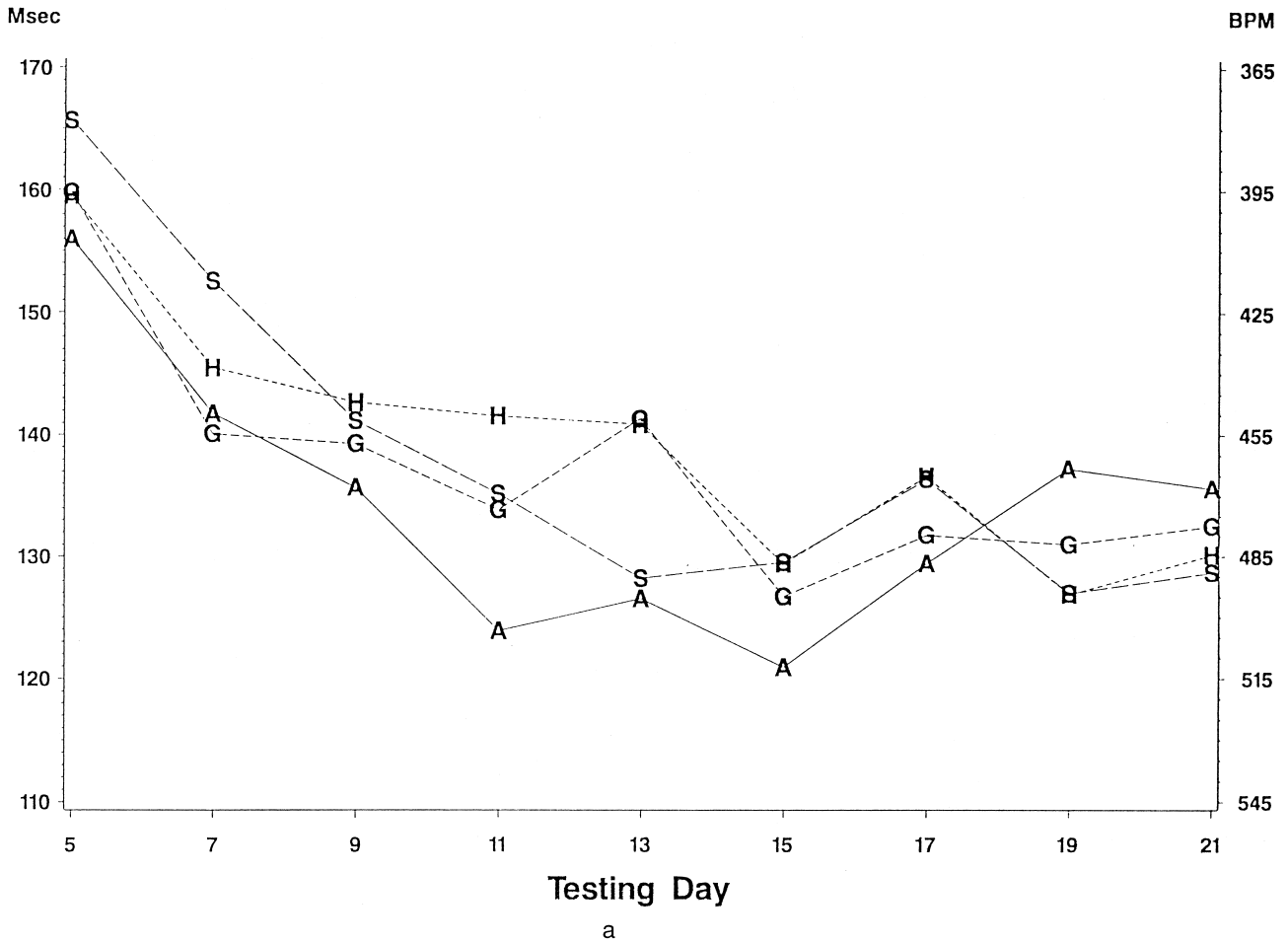

FIG. 1. The mean inter-beat interval (or beats/min, BPM)(a), standard deviation of the inter-beat intervals (b), the time-domain respiratory sinus arrhythmia measure (c), and the frequency-domain respiratory sinus arrhythmia measure (d) across the testing days. The alcohol, hypoxia, GC, and SC groups are represented by the letters A, H, G, and S, respectively.

interaction. The groups did not differ in RSA level for PD 5 to 15, but they did differ for the test of PD 17 to 21 ($p < 0.05$). Both the time-domain and frequency-domain RSA measures had higher RSA for the 2 control groups than the alcohol and hypoxia groups, with the difference primarily coming on the last 2 recording days.

The 4 dependent variables (IBI mean, standard deviation of IBI, time-domain RSA, and frequency-domain RSA) were analyzed for gender and cohort effects. The variables were analyzed with a Gender (2) × Days (9) × Group (4) ANOVA. There were no main effects nor interactions involving the Gender variable. Cohort effects in the control group animals were examined by separating the control groups (GC, SC) into the alcohol and hypoxia rat cohorts from all control rats that were recorded ($n = 27$). The 4 variables were analyzed again with a Cohort (2: alcohol, hypoxia) × Days (9) × Group (2: gastrostomy, suckle) ANOVA. There was a main effect of Cohort on mean IBI, $F(1,25) = 6.27$, $p < 0.05$ and on the standard deviation of IBI, $F(1,25) = 4.93$, $p < 0.05$. The mean IBI level was smaller in the control groups of the alcohol cohort than in the control groups of the hypoxia cohort, and the SD of the IBI values was smaller. The Cohort effect did not interact with Day or Group. Thus, although there were overall differences, these did not differentially affect animals in the GC and SC groups.

Inter-beat Intervals/RSA Correlations

The large increases in the time-domain and frequency-domain measures of RSA suggest that parasympathetic cardiac control becomes active between PD 15 and 17. Because the parasympathetic control of the heart has a tonic effect on mean inter-beat interval level, as well as the rhythmic variability quantified in RSA, a correlation between mean IBI level and RSA level may be expected when the parasympathetic control system affects the chronotropic heart functions. To examine this, we computed the correlations between mean IBI level and the 2 RSA variables. Table 2 presents those correlations. Both RSA variables were significantly correlated with mean IBI level on PD 5 and on PD 15 through 21. They were not significantly correlated with mean IBI level on PD 7 through 13. For the frequency-domain RSA variable, there was a steady increase in the correlation with mean IBI from PD 15 to 21. The time-domain RSA measure was significantly correlated with the SD of the IBI values on all testing days; the frequency-domain RSA measure was significantly correlated to the SD of the IBI values on PD 7 and PD 13 through PD 21 only. Table 2 also contains the correlations between the time- and frequency-domain RSA measures. There was a highly significant correlation between these measures across all ages.

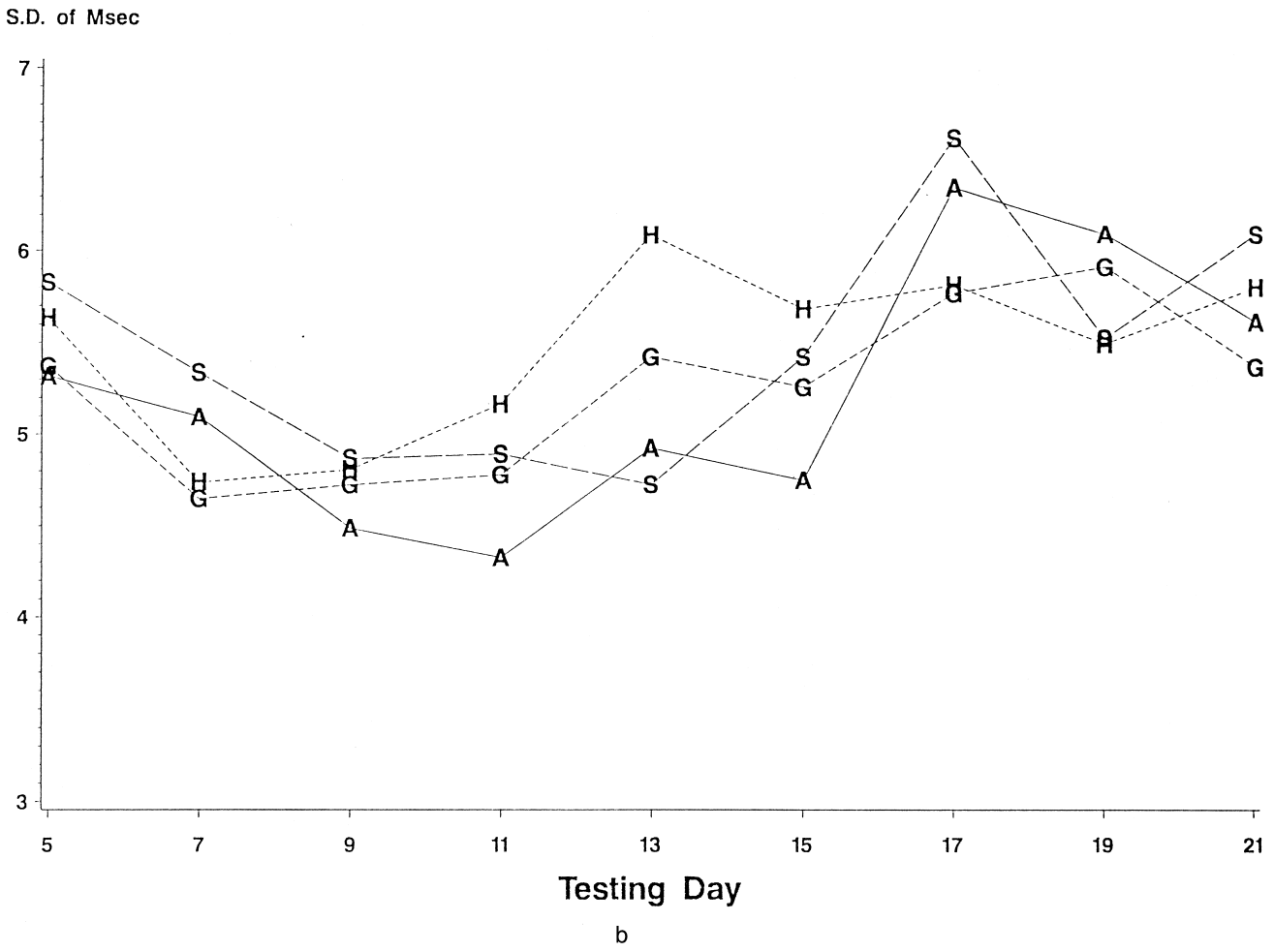

FIG. 1. Continued

Bradycardias

The extremely large increases in inter-beat interval length during the recording were removed from the IBI record. The number of bradycardias in the 4 groups differed. The hypoxia exposure group had the most (38.5 per animal over all days), the alcohol group the next (30.5 per animal over all days), and the 2 control groups the least (25.3 and 25.1 per animal for the gastrostomy and suckle control groups, respectively). The size (in ms) of the IBI change and the average change in ms per beat for the bradycardias was calculated for all rats. The size of the detected bradycardias ranged from 11 to 134 ms (M = 37.95 ms, Median = 33 ms), and the number of beats in the bradycardias ranged from 3 to 26 (M = 7.50, Median = 6 beats).

The size and change per beat of the bradycardias were analyzed with a Days (9) × Group (4) ANOVA. Bradycardia size was affected by the Days factor, $F(8,125) = 7.48$, $p < 0.0001$, and an interaction between Days and Group, $F(8,125) = 2.50$, $p < 0.001$. The change per beat of the bradycardias was affected only by the Group factor, $F(3,44) = 3.24$, $p < 0.05$. Figure 2 shows that there was an overall decline in the size of the bradycardias over the recording days. Post hoc tests showed significant Group and Group × Days effects on PD 5 through 11 (ps < 0.05) and no Group and Group × Day effects on PD 13 through 21. There was no systematic pattern of bradycardia change for PD 5 through 11 for the different groups, although it can be seen in Fig. 2 that the hypoxia group had large mean bradycardias on PD 5 and 11, and the alcohol group had large mean bradycardia size on PD 9 and 11. The Group effect in the average change per beat was due to rapid IBI change in the hypoxia group, particularly on PD 5, 11, 17, and 21. The average change per beat in ms/beat for the alcohol, hypoxia, GC, and SC groups were M = 8.96, M = 10.67, M = 8.40, and M = 9.21, respectively.

There were no gender effects in the bradycardias. The frequency of bradycardias may have been influenced by litter effects. The number of bradycardias each animal had over the course of recording, and the days in which a significant number of bradycardias occurred, were examined. Four of the animals in the alcohol-exposure group with the most bradycardias each had an animal from the same litter in one of the control groups that also had a high frequency of bradycardias, and was among the animals with the most bradycardias in its group. Similarly, 3 animals in the hypoxia group with large numbers of bradycardias each had an animal (from the same litter) in a control group with a high frequency of bradycardias, that was among the animals

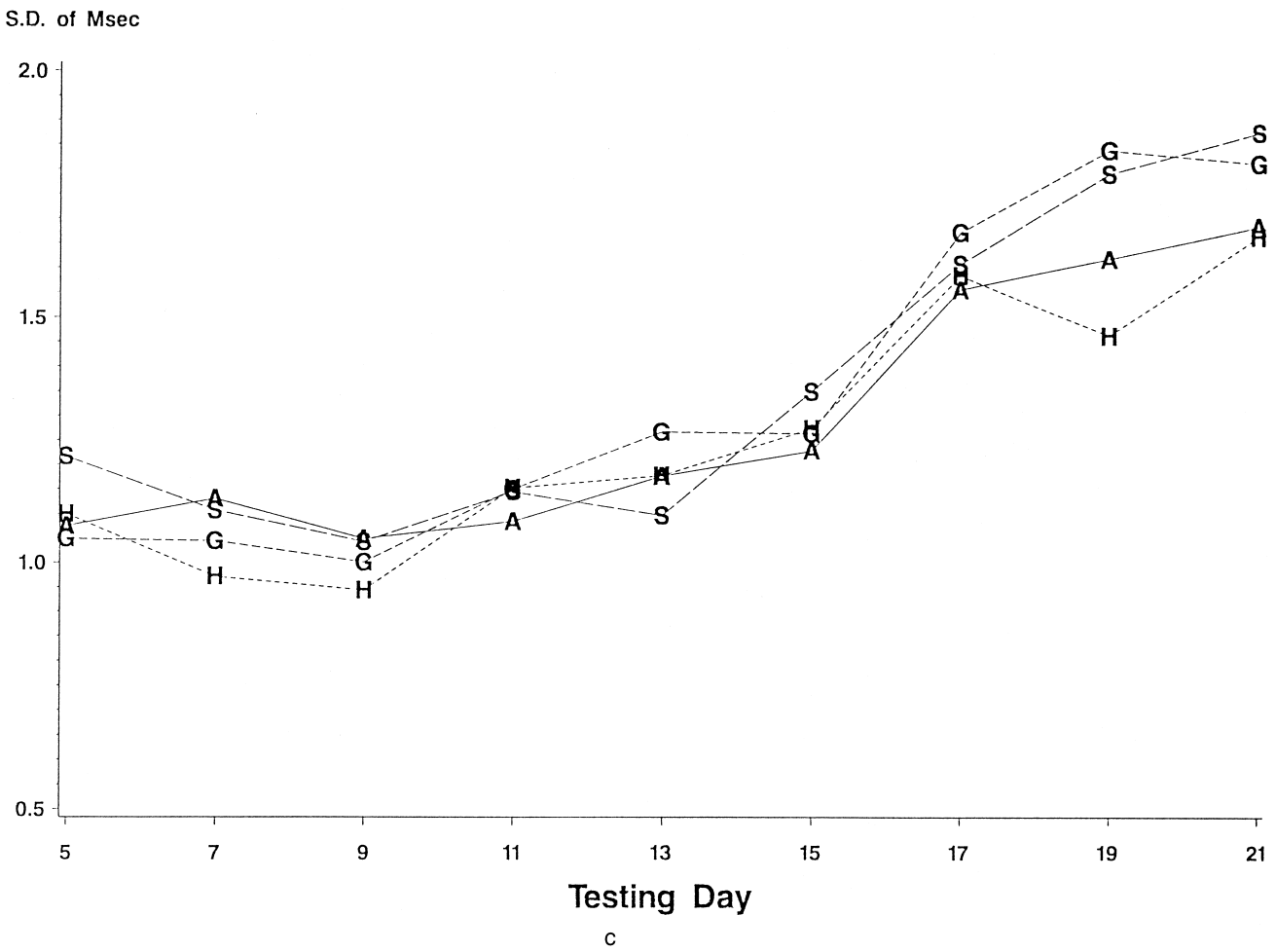

FIG. 1. Continued

with the most bradycardias in its group. Striking in these correspondences was the similarity between animals from the same litter in the pattern of recording days on which significant numbers of bradycardias occurred, even if the animals were assigned to different groups.

DISCUSSION

The present study shows that normal development of heart rate and heart rate variability in the rat has a distinct pattern from PD 5 to 21. The IBI in the rat declines from PD 5 through 13 and then is stable until PD 21. The variability in the IBI as measured by standard deviation initially decreases from PD 5 to 9 and then increases until it stabilizes around PD 17. The variability in IBI as a function of RSA shows the most dramatic developmental time-course in normal animals. RSA is low and relatively stable until PD 15, at which time it increases dramatically until PD 21. Neither gender nor artificial rearing altered the developmental time-course of control of heart rate. There were no differences between the 2 measures of RSA.

The slow decrease in IBI over PD 5 to 13 is similar to that described by Hayne et al. (25). However, Larson and Porges (34) found no change in IBI over this time period; reasons for this discrepancy might include the small number of litters used in their study. Adolph (2,3) found very little decrease in IBI during the period from PD 5 to 13, but it is unclear how many days were tested during this period in Adolph's studies. The increase in IBI variability over days 15 and 17 is in contradiction to the finding of a decrease by Larson and Porges (34); once again, this discrepancy might be due to litter effects. Consistent with Larson and Porges (34), the correlation of both RSA measures with IBI variability is significant throughout most of the postnatal period; this correlation was expected given that the IBI variability includes variability due to RSA within it.

The timing of the dramatic increase in RSA between PD 15 and 17 in the present study corresponds to the peak RSA on PD 16 found by Larson and Porges (34). There is a divergence in the starting point of the increase in RSA; the increase in RSA in the present study did not occur until PD 15, whereas that of Larson and Porges (34) began at PD 9. Nevertheless, both studies indicate that the period around PD 16 is when RSA becomes quite robust. In addition, the finding that levels of RSA begin to correlate significantly with the IBI after PD 15 is strongly suggestive that there is a change in neural control of heart rate at this point. Sympathetic control of the heart is present very early in the rat (2,3,20). Although artificial activation of the parasympathetic system via the vagus nerve can decrease heart rate quite early, blocking vagal activity with cholinergic antagonists does not increase heart rate until either late in the second or in the

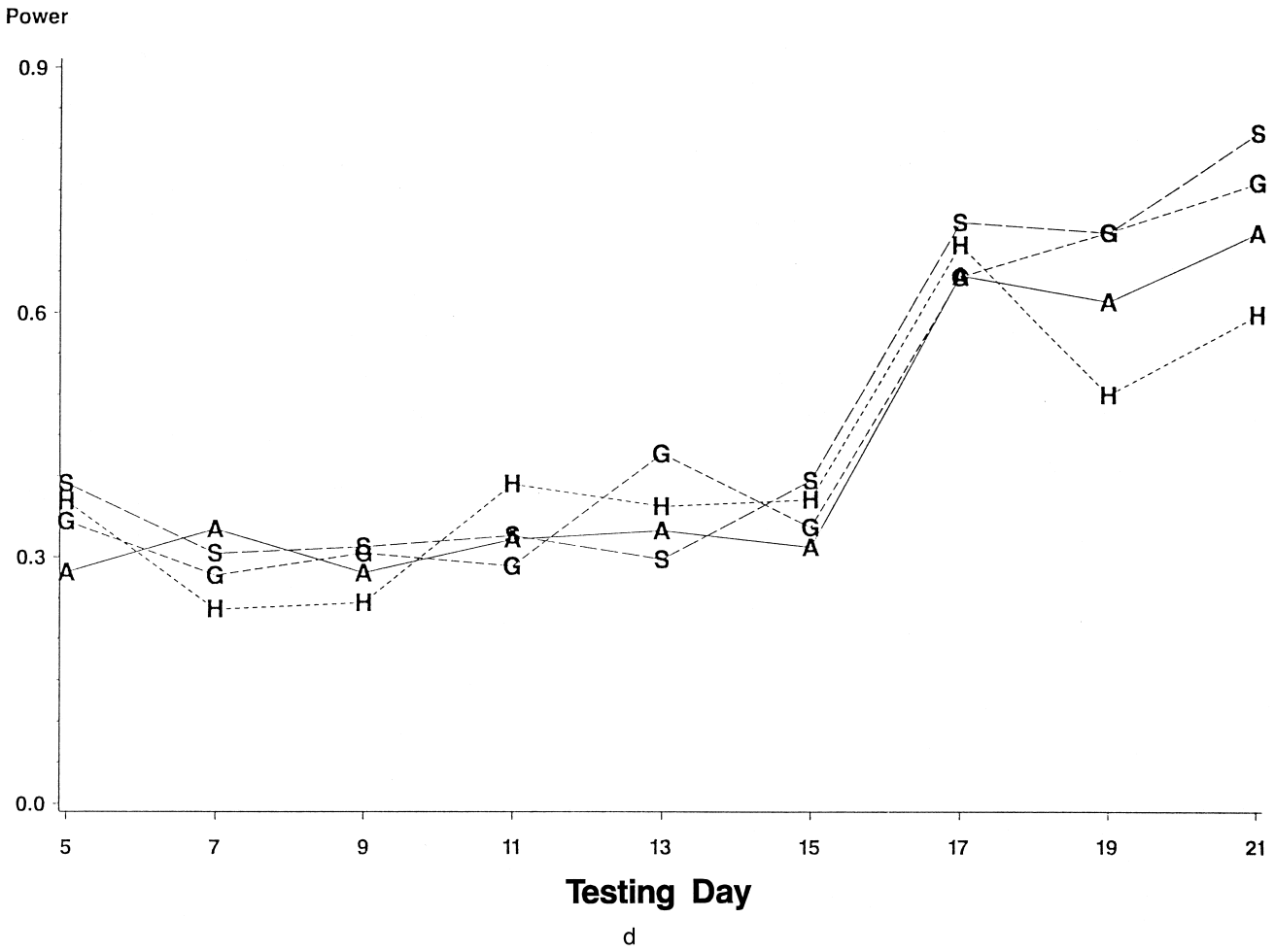

FIG. 1. Continued

third week of development in the rat, indicating the onset of tonic parasympathetic control (2,3,53,55). The timing of the onset of parasympathetic control corresponds to the increase in RSA levels and the significant correlation of RSA to IBI. RSA in the adult rat is considered to be primarily under tonic control of the parasympathetic nervous system (57) and, thus, an increase in RSA at the point where this tonic control becomes functional is not surprising.

The possibility that the changes in IBI and RSA observed in the present study reflect effects of repeated testing, and not mat-

TABLE 2

CORRELATIONS BETWEEN MEAN INTER-BEAT INTERVAL, THE STANDARD DEVIATION OF THE INTER-BEAT INTERVALS, AND THE TIME-DOMAIN AND FREQUENCY -DOMAIN MEASURES OF RESPIRATORY SINUS ARRHYTHMIA

	Recording Age (Days)								
	5	7	9	11	13	15	17	19	21
Mean inter-beat interval									
w/Time-domain RSA	0.374*	−.070	−0.091	0.241	0.227	0.382*	0.353*	0.104	0.363*
w/Freq-domain RSA	0.293*	0.019	−0.052	0.074	0.057	0.349*	0.430*	0.490*	0.548*
SD inter-beat interval									
w/Time-domain RSA	0.410*	0.408*	0.316*	0.378*	0.471*	0.692*	0.626*	0.577*	0.567*
w/Freq-domain RSA	0.233	0.283*	0.042	0.132	0.281*	0.461*	0.423*	0.502*	0.364*
Time-domain RSA									
w/Freq-domain RSA	0.924*	0.916*	0.818*	0.885*	0.920*	0.891*	0.821*	0.736*	0.794*

* $p < 0.05$.

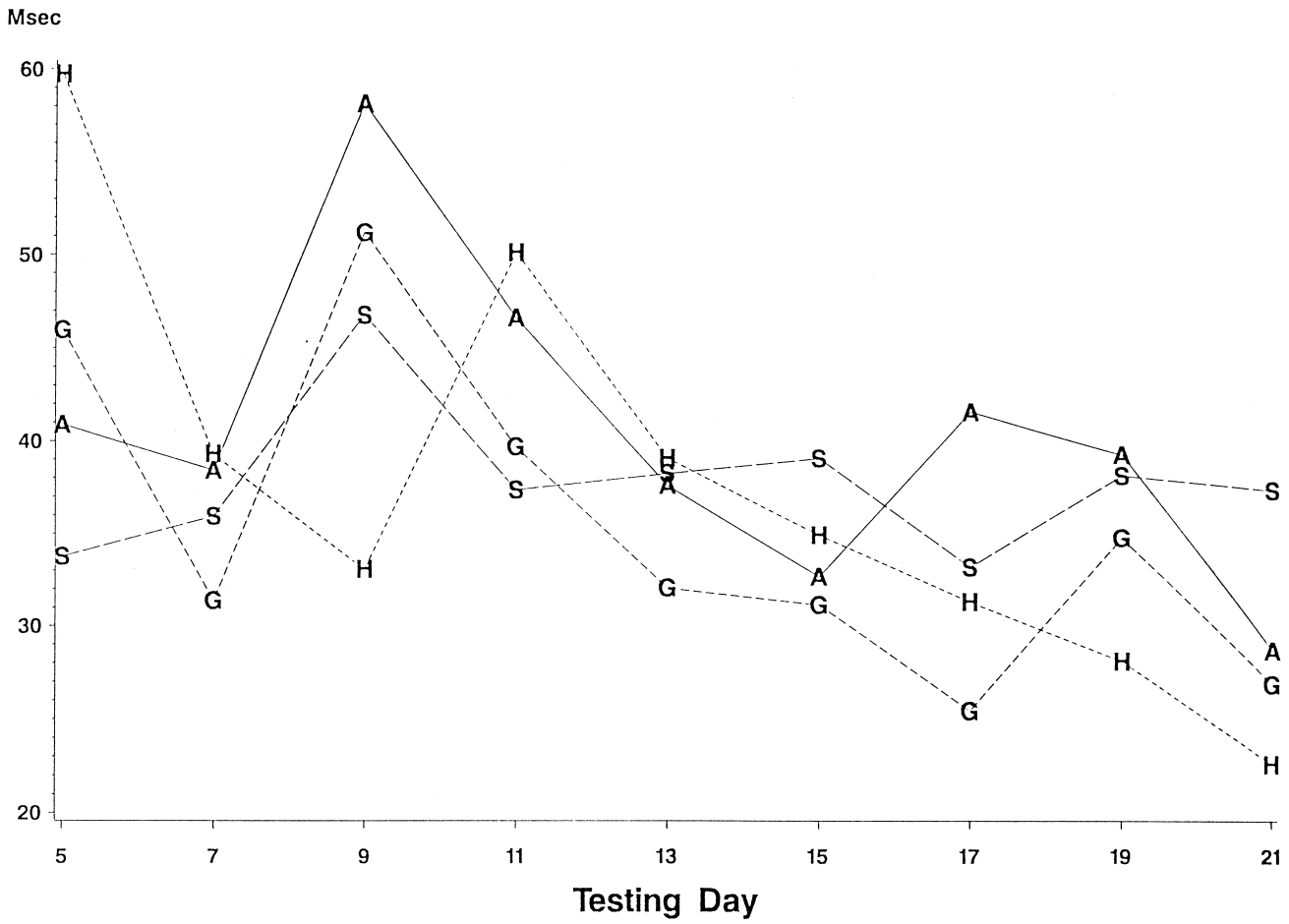

FIG. 2. The mean inter-beat interval size for bradycardias across testing days. The alcohol, hypoxia, GC, and SC groups are represented by the letters A, H, G, and S, respectively.

uration, is unlikely for the following reasons. First, the changes in IBI which occur from PD 5 to 13 are very similar to those described by Hayne et al. (25) and these researchers used independent groups at each time-point. Second, we have run another cohort of animals that were only tested from PD 13 to 21 (manuscript in preparation) and the IBI and RSA data on day 13 in this cohort were identical with the present data. Third, the timing of the dramatic change in RSA in the present study corresponds to the timing of the onset of parasympathetic control of heart rate, which was determined using independent groups (1,3).

Both alcohol exposure and hypoxia during the early postnatal period in the rat moderate the amount of increase in RSA beginning with PD 19. The effect is the same in both genders and in both treatment groups. Interestingly, the RSA is not affected during the period of hypoxia or alcohol exposure (PD 5 through 9), but is affected by both treatments at the point which tonic parasympathetic control of heart rate begins (1,3). It is not possible to tell whether the effects of hypoxia and alcohol during the early postnatal period are permanent or whether they simply impose a delay on the development of RSA. However, it is clear that the development of this index of attentional processes is disrupted by teratogenic insults. Furthermore, the use of intermittent hypoxia during the early postnatal period in the rat induces changes in RSA that are similar to those seen in infants with RDS.

Hayne et al. (24) found that prenatal alcohol exposure slowed heart rate on PD 1 and 6; this study found that early postnatal alcohol exposure had no effect on heart rate from PD 5 through 21. The main difference between these two studies is the period of alcohol exposure; it may be that prenatal alcohol exposure slows down the development of the sympathetic control of heart rate, whereas postnatal alcohol exposure is too late to affect the development of this system (2,3). Neither Hayne et al. (24) nor Caul et al. (8) examined RSA or heart rate during the period in which tonic neural control of heart rate by the parasympathetic system occurs. This study suggests that RSA may be particularly susceptible to disruption by teratogens and that the third week after birth in the rat is a period during which deficits can be observed. Given the results using the animal model of fetal alcohol effects, research utilizing RSA as an index of attention in children with fetal alcohol syndrome might be of interest.

The mechanism(s) by which alcohol and hypoxia disrupt the development of RSA is not clear. An explanation based on a reduction in body weight by the alcohol or hypoxia treatments cannot be accepted because the GC group showed a similar reduction in body weight at PD 21 and did not show reduced levels of RSA. One obvious mechanism is that alcohol and hypoxia disrupt the maturation of vagal control of the heart; this hypothesis could be examined using a psychopharmacological ap-

proach. The finding of an increase in the frequency of large bradycardias after alcohol or hypoxia exposure, particularly early in development, supports the possibility that there is a defect in cardiac control in both groups, although the specifics of the defect may differ between the 2 groups. However, the differences among groups were only observed prior to PD 13, so bradycardia cannot be the explanation for the differences in RSA observed on PD 12 and 21 in the alcohol and hypoxia animals. Although clearly dependent upon peripheral mechanisms, RSA has also been shown to be strongly correlated to heart rate and behavioral indices of attention (42,43,45). Therefore, it is also possible that the teratogenic treatments alter more central neural structures involved in attentional processes that also have direct or indirect effects on RSA (10,38,43). If this is the case, the possibility that measures of sustained attention as measured by the heart rate-orienting response might detect the teratogenic effects of alcohol and hypoxia is fairly high.

In summary, RSA shows a clear developmental time-course with a dramatic increase after PD 15 that is correlated with changes in IBI. To the extent that RSA is correlated to attention, these data imply that hypoxia or alcohol exposure during the period equivalent to the third trimester of prenatal development in humans may induce attentional deficits. Most importantly, the effects of hypoxia or alcohol exposure on RSA and the frequency of strong bradycardias imply that these developmental insults seriously disrupt cardiac control to the point where the disruptions may even be life-threatening.

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REFERENCES

1. Adolph, E. F. Capacities for regulation of heart rate in fetal, infant and adult rats. Am. J. Physiol. 209:1095–1105; 1965.
2. Adolph, E. F. Ranges of heart rate and their regulation at various ages. Am. J. Physiol. 212:595–602; 1967.
3. Adolph, E. F. Ontogeny of heart rate controls in hamster, rat, and guinea pig. Am. J. Physiol. 220:1896–1901; 1971.
4. Anrep, G. W.; Pascual, W.; Rossler, R. Respiratory variations of the heart rate. I. The reflex mechanism of the respiratory arrhythmia. Roy. Soc. London Proc. Series B 119:191–217; 1935.
5. Berg, W. K.; Berg, K. M. Psychophysiological development in infancy: State, startle and attention. In: Osofosky, J. D., ed. Handbook of infant development. New York: Wiley; 1987:238–317.
6. Bernadin, C. P. C/Math toolchest for engineering and scientific applications. Englewood Cliffs, NJ: Prentice Hall; 1993.
7. Bernston, G. G.; Quigley, K. S.; Jany, J. F.; Boysen, S. T. An approach to artifact identification:Application to heart period data. Psychophysiology 27:586–598; 1990.
8. Caul, W. F.; Fernandez, K.; Michaelis, R. C. Effects of prenatal ethanol exposure on heart rate, activity, and response suppression. Neurotoxicol. Teratol. 5:461–464; 1983.
9. Cheung, M. N. Detection and recovery from errors in cardiac interbeat intervals. Psychophysiology 18:341–346; 1981.
10. Daly, M. D. B. Interactions between respiration and circulation. In: Cherniack, N. S.; Widdicomb, J. G., eds. Integrative functions of the autonomic nervous system. New York: Elsevier/North Holland Biomedical Press; 1986:283–392.
11. Dobbing, J. The later development of the brain and its vulnerability. In: Davis, J. A.; Dobbing, J., eds. Scientific foundation of pediatrics. London: William Heinemann Medical Books; 1974:744–759.
12. Dobbing, J.; Sands, J. Comparative aspects of the brain growth spurt. Early Hum. Develop. 3:79–83; 1979.
13. Dudek, B. C.; Abbott, M. E. A biometrical genetic analysis of ethanol response in selectively bred long-sleep and short-sleep mice. Behav. Genetics 14:1–19; 1984.
14. Field, T. M.; Dempsey, J. R.; Hatch, J.; Ting, G.; Clifton, R. K. Cardiac and behavioral responses to repeated tactile and auditory stimulation by preterm and term neonates. Dev. Psychol. 15:406–416; 1979.
15. Fox, N. A.; Lewis, M. Cardiac response to speech sounds in preterm infants: Effects of postnatal illness at 3 months. Psychophysiology 20:481–488; 1983.
16. Geller, L. M.; Geller, E. H. A simple technique for the permanent marking of newborn albino rats. Physiol. Rep. 18:29–46; 1969.
17. Graham, F. K. Constraints on measuring heart rate and period sequentially through real and cardiac time. Psychophysiology 15:492–495; 1978.
18. Graham, F. K.; Jackson, J. C. Arousal systems and infant heart rate responses. In: Lipsett, L. P.; Reese, R. P., eds. Advances in child development and behavior. Vol. 6. New York: Academic Press; 1970:59–117.
19. Hamre, K. M.; West, J. R. The effects of the timing of ethanol exposure during the brain growth spurt on the number of cerebellar Purkinje and granule cell nuclear profiles. Alcohol. Clin. Exp. Res. 17:610–623; 1993.
20. Haroutunian, V.; Campbell, B. A. Development and habituation of the heart rate orienting response to auditory and visual stimuli in the rat. J. Comp. Physiol. Psychol. 95:166–174; 1981.
21. Harper, R. M.; Hoppenbrouwers, T.; Sterman, M. B.; McGinty, D. J.; Hodgman, J. Polygraphic studies of normal infants during the first six months of life. I. Heart rate and variability as a function of state. Pediatr. Res. 10:945–961; 1976.
22. Harper, R. M.; Leake, B.; Hodgman, J. E.; Hoppenbrouwers, T. Developmental patterns of heart rate and heart rate variability during sleep and waking in normal infants and infants at risk for the sudden infant death syndrome. Sleep 5:28–38; 1982.
23. Harper, R. M.; Walter, D. O.; Leake, B.; Hoffman, H. J.; Sieck, G. C.; Sterman, M. B.; Hoppenbrouwers, T.; Hodgman, J. Development of sinus arrhythmia during sleeping and waking states in normal infants. Sleep 1:33–48; 1978.
24. Hayne, H.; Hess, M.; Campbell, B. A. The effect of prenatal alcohol exposure on attention in the rat. Neurotoxicol. Teratol. 14:393–398; 1992.
25. Hayne, H.; Richardson, R.; Campbell, B. Developmental constraints on the expression of behavioral and heart-rate orienting responses: I. The role of cardiosomatic coupling. Dev. Psychobiol. 24:1–18; 1991.
26. Jones, K. L.; Smith, D. W. Recognition of the fetal alcohol syndrome is early infancy. Lancet 2:999–1001; 1973.
27. Jones, K. L.; Smith, D. W.; Ulleland, C. N.; Streissguth, A. P. Pattern of malformation in offspring in chronic alcoholic mothers. Lancet 2:1267–1271; 1973.
28. Katona, P. G.; Frasz, A.; Egbert, J. R. Maturation of cardiac control in full-term and preterm infants during sleep. Early Human Dev. 4:145–159; 1980.
29. Katona, P. G.; Jih, F. Respiratory sinus arrhythmia: Noninvasive measure of parasympathetic control. J. Appl. Physiol. 39:801–805; 1975.
30. Kelly, S. J.; Bonthius, D. J.; West, J. R. Developmental changes in alcohol pharmacokinetics in rats. Alcohol. Clin. Exp. Res. 11:281–286; 1987.
31. Kelly, S. J.; Dillingham, R. J. Social behavior and the amygdala region are altered by perinatal alcohol exposure. Neurotoxicol. Teratol. 16:377–384; 1994.
32. Kelly, S. J.; Pierce, D. R.; West, J. R. Microencephaly and hyperactivity in adult rats can be induced by neonatal exposure to high blood alcohol concentrations. Exp. Neurol. 96:580–593; 1987.

33. Kero, P. Heart rate variation in infants with the Respiratory Distress Syndrome. Acta Paediatr. Scand. (suppl. 250); 1973.
34. Larson, S. K.; Porges, S. W. The ontogeny of heart period patterning in the rat. Dev. Psychobiology 15:519–528; 1982.
35. Mactutus, C. F.; Fechter, L. D. Prenatal exposure to carbon monoxide: Learning and memory deficits. Science 223:409–411; 1984.
36. Mactutus, C. F.; Fechter, L. D. Moderate carbon monoxide exposure produces persistent, and apparently permanent, memory deficits in rats. Teratology 31:1–12; 1985.
37. Mactutus, C. F.; Fechter, L. D. Perinatal hypoxia. Implications for mammalian development. In: Riley, E. P.; Vorhees, C. V., eds. Handbook of behavioral teratology. New York: Plenum Press; 1986:427–470.
38. Porges, S. W. Autonomic regulation of attention. In: Campbell, B. A.; Hayne, H.; Richardson, R., eds. Attention and information processing in infants and adults. Hillsdale, NJ: Lawrence Erlbaum; 1992:201–223.
39. Porges, S. W. Method and apparatus for evaluating rhythm in oscillations in physiological response systems. United States Patent no. 4, 510, 944; 1985.
40. Porges, S. W.; McCabe, P. M.; Yongue, B. G. Respiratory-heart rate interactions: Psychophysiological implications for pathophysiology and behavior. In: Caccioppo, J.; Petty, R., eds. Perspectives in cardiovascular psychophysiology. New York: Guilford; 1982:223–264.
41. Richards, J. E. Power spectral analysis quantification of respiratory sinus arrhythmia. Psychophysiology 23:414; 1986.
42. Richards, J. E. Infant visual sustained attention and respiratory sinus arrhythmia. Child Develop. 58:488–496; 1987.
43. Richards, J. E. Baseline respiratory sinus arrhythmia and heart rate responses during sustained visual attention in preterm infants from 3 to 6 months of age. Psychophysiology 31:235–243; 1994.
44. Richards, J. E. Reliability of respiratory sinus arrhythmia in 14, 20, and 26 week old infants. Infant Behav. Develop. 18:155–161; 1995.
45. Richards, J. E.; Casey, B. J. Heart rate variability during attention phases in young infants. Psychophysiology 28:43–53; 1991.
46. Richards, J. E.; Casey, B. J. Development of sustained visual attention in the human infant. In: Campbell, B. A.; Hayne, H.; Richardson, R., eds. Attention and information processing in infants and adults. Hillsdale, NJ: Lawrence Erlbaum; 1992:30–60.
47. Rose, S. A.; Feldman, J. F.; McCarton, C. M.; Wolfson, J. Information processing in seven-month-old infants as a function of risk status. Child Develop. 59:589–603; 1988.
48. Rother, M.; Zwiener, U.; Eiselt, M.; Witte, H.; Zwacka, G.; Frenzel, T. Differentiation of healthy newborns and newborns-at-risk by spectral analysis of heart rate fluctuations and respiratory movements. Early Human Develop. 15:349–363; 1987.
49. Samson, H. H.; Diaz, J. Effects of neonatal ethanol exposure on brain development in rodents. In: Abel, E. L., ed. Fetal alcohol syndrome, vol. 3. Animal studies. Boca Raton, FL: CRC Press; 1982:131–150.
50. Schulman, C. A. Effects of auditory stimulation on heart rate in premature infants as a function of level of arousal, probability of CNS damage, and conceptual age. Dev. Psychobiology 2:172–1983; 1970.
51. Streissguth, A. P.; Barr, H. M.; Sampson, P. D.; Parrish-Johnson, J. C.; Kirchner, G. L.; Martin, D. C. Attention, distraction and reaction time at age 7 years and prenatal alcohol exposure. Neurobehav. Toxicol. Teratol. 8:717–725; 1986.
52. Streissguth, A. P.; Martin, D. C.; Barr, H. M.; Sandman, B. M.; Kirchner, G. L.; Darby, B. L. Intrauterine alcohol exposure and attentional decrements in 4-year-old children. Dev. Psychology 20:533–541; 1984.
53. Tucker, D. C.; Johnson, A. K. Development of autonomic control of heart rate in genetically hypertensive and normotensive rats. Am. J. Physiol. 246:R570–R577; 1984.
54. Watanabe, K.; Iwase, K.; Hara, K. Heart rate variability during sleep and wakefulness in low-birthweight infants. Biol. Neonate 22:87–98; 1973.
55. Wekstein, D. R. Heart rate of the preweanling rat and its autonomic control. Am. J. Physiol. 208:1259–1262; 1965.
56. West, J. R.; Hamre, K. M.; Pierce, D. R. Delay in brain growth induced by alcohol in artificially reared rat pups. Alcohol 1:83–95; 1984.
57. Yongue, B. G.; McCabe, P. M.; Porges, S. W.; Rivera, M.; Kelley, S. L.; Ackles, P. K. The effects of pharmacological manipulations that influence vagal control of the heart on heart period, heart-period variability and respiration in rats. Psychophysiology 19:426–432; 1982.
58. Zeskind, P. S.; Goff, D. M.; Marshall, T. R. Rhythmic organization of neonatal heart rate and its relation to atypical fetal growth. Dev. Psychobiol. 24:413–429; 1991.