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Heart Rate Variability during Respiratory Pauses in Puppies and Dogs

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ABSTRACT. We studied the time course and change in heart rate during respiratory pauses in puppies (3–4 wk) and young adult dogs. We measured ventilation and ventilatory pattern using barometric plethysmography and recorded the respiratory rate (RR) interval using a preprocessor with an accuracy of 0.2 ms. During tidal breathing, the fluctuations in RR interval were an order of magnitude smaller in the puppy than in the dog. During respiratory pauses in dogs, the RR interval increased sharply, stabilized around the level of expiration of previous breaths, and dropped immediately with the subsequent inspiratory effort. The time course of the change in heart rate was different in the puppy: there was a gradual increase in the RR interval during the entire course of the pause and the maximum RR interval reached was substantially higher than during expiration of previous breaths. Our results suggest that 1) the change in heart rate at the outset of respiratory pauses is too fast to be related to blood gas changes in both puppies and dogs and 2) the mechanisms responsible for the vagal gating of heart rate during tidal breathing and during respiratory pauses are not well developed in early life in the puppy. (*Pediatr Res* 22: 306–311, 1987)

Abbreviations

- **HRV, heart rate variability**
- **RR, respiratory rate**
- **ANS, autonomic nervous system**
- **EEG, electroencephalogram**
- **REM, rapid eye movement**
- **Vt, tidal volume**
- **Ti, inspiratory time**
- **Te, expiratory time**
- **Ttot, total respiratory**
- **max(RR), maximum RR interval within a respiratory cycle**
- **min(RR), minimum RR interval within a respiratory cycle**
- **range(RR), difference between min(RR) and max(RR)**
- **NTS, nucleus tractus solitarius**
- **NA, nucleus ambiguus**
- **VMN, vagal motoneurons**

The importance of the study of heart rate and HRV stems from the fact that abnormal HRV has been associated with certain clinical conditions (1–3). For instance, fetal heart rate has been used clinically to diagnose fetal distress during labor for a number of years (3). We have demonstrated previously that HRV is abnormal in certain groups of infants who are at risk of sudden death (4, 5) and more recently, it as been shown that HRV can be used to estimate cardiac age in adult humans (6, 7).

We (8) and others (9–12) have previously examined the relation between breathing and heart rate and found that HRV is closely tied to breathing in mature subjects. However, HRV is not determined only by breathing, especially in early life (8, 12). For instance, during apnea or lack of breathing, heart rate varies, albeit much less than during breathing. These heart rate changes during respiratory pauses have not been studied in detail. For example, we do not know the time course of the change in RR interval during pauses in early life and how these changes compare with those in the mature subject. In order to understand further the mechanisms that control heart rate and HRV, we examined herein the time course and the change in heart rate during respiratory pauses and compared them to those during breathing. Since the ANS is not mature early in life (8, 12–14) and since the ANS plays a major role in HRV (10), we studied the heart rate response to a respiratory pause in early life (puppies) and in an adult mature animal (dog).

METHODS

Animal population. Seven puppies from two litters (four puppies from one litter and three from another) and five young adult dogs (2–6 yr of age) were used. Postnatally, all puppies were nursed by the bitch through the experimental age but supplemental nutrition was sometimes required after the age of 1 wk to keep the weight gain of puppies at >20 g/day. Adult dogs weighed between 8–12 kg.

Physiological experiments. Experiments on puppies were performed at 3–4 wk of age. This age was chosen because maturational changes of the nervous system in the puppy occur for the most part after 2 wk of age (15). Also, sleep states could not be determined from neurophysiologic criteria before 2 wk of age (8). Experiments were started within 1 h after feeding. A more detailed description of these experiments on puppies is given in a previous publication (8). In brief, experiments were performed at a room temperature of 26–30° C. The lights were dimmed in the room and, for consistency, reflected light from the experimental chamber was kept below 5 ft-cd in all experiments. Auditory noise was also measured and kept at less than 40 dB. Experiments were performed on healthy young adult dogs between 1100 and 1600 h.

Neurophysiological measurements. Sleep staging in puppies and state consciousness in adult dogs were determined from polygraphic records with the aid of bipolar EEG and behavioral criteria (8). To record the EEG, coated steel wires (0.003 inch diameter) were inserted subcutaneously in the temporoparietal region before each study using a modified Basmajian technique

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Table 1. *Duration of respiratory pauses in puppies and adult dogs**

Puppy	Ttot (s)	State of consciousness	Dog	Ttot (s)	State of consciousness
1	5.7	Q	1	14.04	Q
2	8.88	Q		18.16	Q
	5.40	R	2	20.60	A
	9.72	Q	3	14.60	Q
	6.64	R		15.52	A
	10.08	R		13.52	A
3	7.48	Q		12.44	Q
	5.40	Q		13.48	A
	6.16	Q		12.68	Q
	5.04	Q		13.40	Q
	7.96	R		13.92	Q
4	7.22	Q	4	17.96	Q
	7.60	R		15.84	A
5	7.84	Q		19.00	A
6	5.88	R		22.52	A
	5.92	R		26.12	A
	9.28	Q		18.96	A
	9.40	Q		18.40	A
7	7.04	R		18.92	A
	7.44	R		16.80	A
	6.60	R	5	27.24	R
	6.04	R		25.92	R
	7.20	R			
	7.04	Q			
	6.92	R			

* State of consciousness is denoted by R, REM sleep; Q, quiet sleep; A, wakefulness.

(8). While dogs were studied during wakefulness and during sleep (REM and quiet sleep), puppies could only be studied in sleep. Generally, while awake, puppies moved considerably, thus preventing the acquisition of a sufficiently long period of artifact-free ECG for the analysis of RR oscillations.

Measurement of RR interval. To record the RR interval, electrodes were placed subcutaneously on the anterior chest using the same techniques as for the recording of EEG. To measure the RR interval, a preprocessor with an accuracy of 0.2 ms was used. The method used for detection of the RR interval has been previously described (16). Each RR interval when determined by the preprocessor, was transferred to a microcomputer using a parallel I/O port, and stored on disk. Artifactual RR intervals were identified using automatic screening and examination of the original polygraphic records. Periods used for analysis did not contain any of the artifactual RR intervals.

Respiratory measurements. Dogs and puppies were studied noninvasively using barometric plethysmography. The main advantages of this method are: 1) animals are freely moving during experiments and 2) no devices (*e.g.* mouthpiece, mask), which are known to influence breathing are needed. Ventilation, *i.e.* Vt, Ti, Te, and Ttot were obtained.

Data analysis. A sigh is defined as a breath with a Vt that is larger than twice the mean Vt of baseline. All the RR intervals analyzed occur within a respiratory cycle which includes inspiration and expiration. The first RR interval considered as part of the respiratory pause coincides in general with the first RR interval during expiration or during inspiratory-expiratory transition.

Herein, we studied the changes in the RR interval during apnea in puppies and dogs. We examined the direction of change in the RR interval and applied the sign test in both groups of

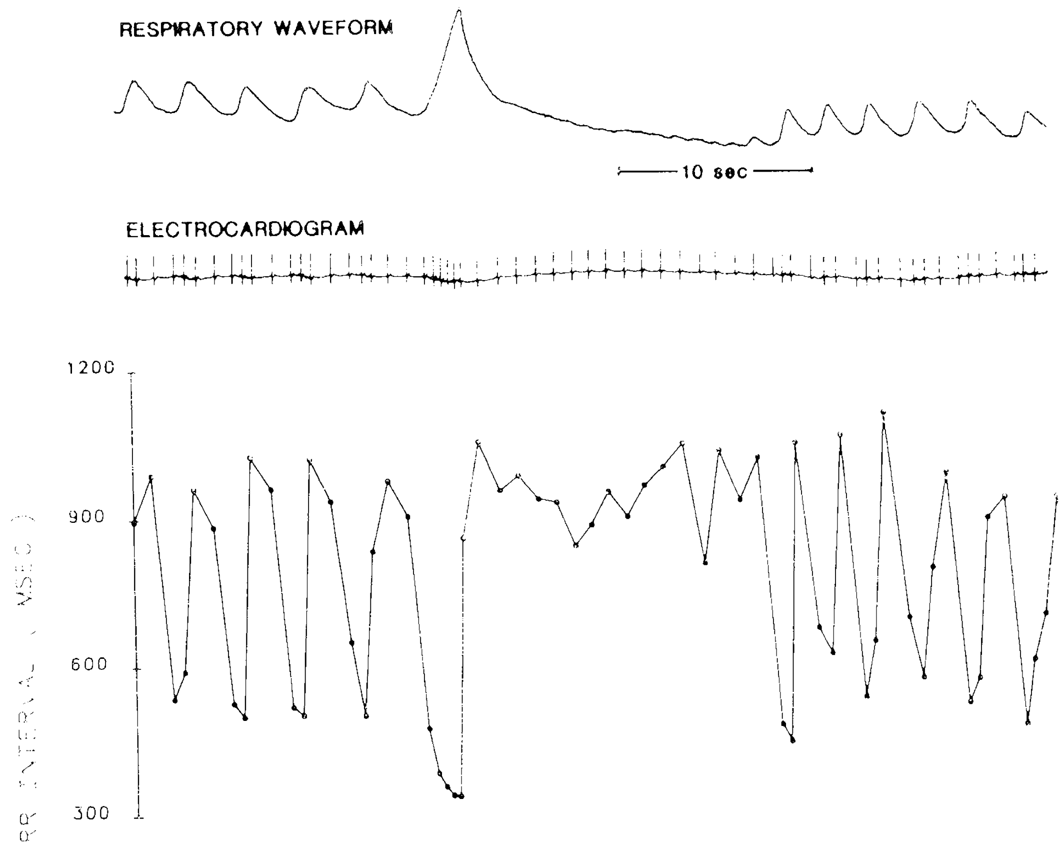

Fig. 1. Respiratory waveform (*top*), electrocardiogram (*middle*), and RR interval (*bottom*) in one dog during wakefulness. Note the near cyclic fluctuations in RR interval during breathing and the lack thereof during the respiratory pause following the sigh (6th breath from left of top trace). Note also that the RR interval stays high during the respiratory pause.

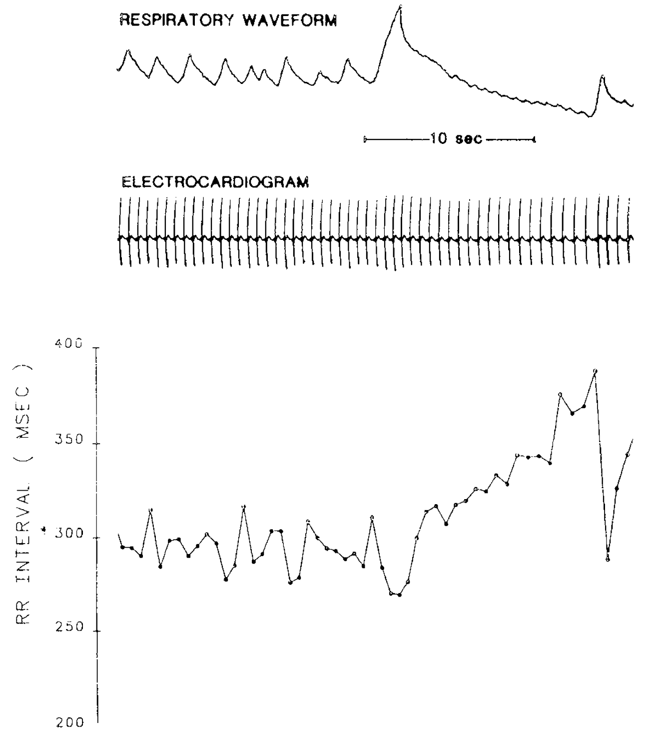

Fig. 2. Respiratory waveform (*top*), electrocardiogram (*middle*) and RR interval (*bottom*) in one puppy during REM sleep. Note that the fluctuations in RR interval during breathing are small when compared to those in the dog (Fig. 1). The RR interval increases continuously during the respiratory pause.

animals. Standard linear regression was performed in puppies because of a continual linear trend within the pause (see "Results"), while two-phase regression was performed in dogs where there was an initial sharp rise in the RR interval followed by a subsequent flat or decreasing trend within the pause. Two-phase regression (17) uses the method of least squares to determine the "change-point" x and to fit the lines $b_1 (x - x_0)$ for $x < x_0$ and $b_2 (x - x_0)$ for $x > x_0$. We also used the *t* test and the Kolmogorov-Smirnov two-sample test (18) to study the change in maximum RR interval (see "Results") from baseline during a respiratory pause in puppies and dogs and determine whether this change in puppies is significantly different from that in dogs.

RESULTS

Table 1 shows the duration of all the respiratory pauses studied in puppies and dogs. Note that these pauses occurred in both REM and quite sleep in the puppy and in wakefulness or sleep (REM or quiet) in the adult dog. In this section, we categorize each respiratory pause by the state it occurs in. Our assumption was that a pause occurring, say, in REM sleep may show a different heart response from that occurring in quiet sleep in dogs and puppies. However, our analysis shows that heart rate response during pauses depends on maturation, *i.e.* on whether it occurs in dogs or puppies more than on any other factor including state of consciousness. Actually, changes in RR during pauses in wakefulness are often superimposable on those from REM or quite sleep. Figures 1 to 5 still show the relation between pauses and state of consciousness.

Figure 1 gives the plots of the breathing waveform, the ECG, and the magnitude of the RR interval in an adult dog before, during, and after a respiratory pause in quiet sleep. Prior to the respiratory pause, the RR interval exhibits cyclical variations

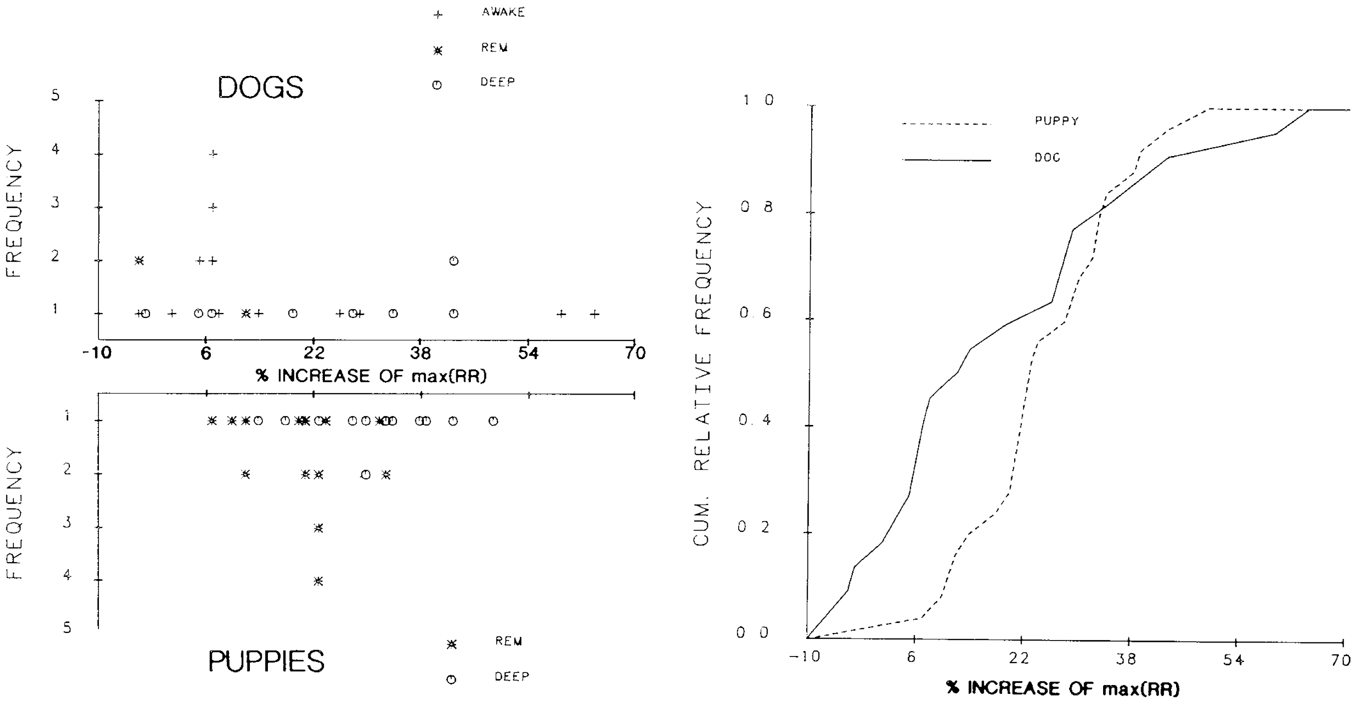

Fig. 3. *Left panel*, frequency distribution of the percent increase of max(RR) in puppies and dogs in wakefulness and sleep. Although the distribution overlaps, the median percent increase in max(RR) is higher in puppies than in dogs. *Right panel*, cumulative relative frequency of the percent increase of max(RR). About half of the percent increase in dogs are below 8% whereas a much smaller proportion of values are below this level in puppies.

with breathing; in each breath, the minimum RR interval occurs during inspiration and the maximum RR interval during expiration. Note that the pause occurs immediately after a sigh. During the inspiratory phase of the sigh, the RR interval decreases and reaches a minimum that is lower than the minima of previous breaths. During the respiratory pause, the RR interval increases sharply, fluctuates much less than during breathing and reaches a level that is similar to the maxima of previous breaths. It then stabilizes around this level for the rest of the respiratory pause. The initiation of the subsequent inspiratory effort coincides with an immediate drop in RR interval.

Figure 2 shows the same signals as in Figure 1 for a puppy at 3 wk of age in quiet sleep. Note that in the puppy: 1) the fluctuations in RR interval occurring prior to the respiratory pause are much smaller than those in the dog, 2) there is a gradual increase in the RR interval during the entire course of the pause, and 3) the maximum RR interval reached during the pause is much higher than the RR maxima of previous and subsequent breaths.

As shown in both Figures 1 and 2, the RR interval undergoes cyclical variations with respect to respiratory cycles and is much more clearly seen in the dog than in the puppy. Max(RR) occurs during the expiratory phase or pause of the cycle. The mean of max(RR) during five consecutive baseline breaths, denoted by max$_0$(RR) was computed and compared to the max(RR) during respiratory pauses denoted by max$_1$(RR), for dogs and puppies. Max$_1$(RR) was higher than max$_0$(RR) in each puppy, and the majority (19/22) of max$_1$(RR) was also higher than max$_0$(RR) in adult dogs. Therefore max$_1$(RR) is significantly higher than max$_0$(RR) in dogs ($p < 0.01$) and in puppies ($p < 0.0001$) by the sign test. The percent increase in max(RR) (from baseline) during respiratory pauses is reported in Figure 3 for both puppies and dogs. Cumulative distribution functions show that the percent increase in max(RR) tends to be considerably higher in puppies than in dogs, and the Kolmogorov-Smirnov two-sample test shows that this difference between puppies and dogs is statistically significant ($p < 0.01$).

Figure 2 shows that min(RR) differs little from max(RR) in the puppy during baseline. This contrasts remarkably with the big difference between min(RR) and max(RR) in the adult dog as shown in Figure 1. The mean of range(RR) during five consecutive baseline breaths, denoted by range$_0$(RR) was found to be lower than range$_1$(RR) [=range(RR) during respiratory pause] for all respiratory pauses in all dogs and puppies. The percent increase in range(RR) (from baseline) during respiratory pauses is summarized in Table 2 using the 5-number summary (19). This shows that each of the summary values is an order of magnitude larger in the puppy than in the dog. Thus the percent increase in range(RR) is significantly higher in puppies than in dogs ($p < 0.01$, t and Kolmogorov-Smirnov tests). As shown in Figure 2 for one puppy, the RR interval gradually increases during a respiratory pause. The pattern of increase in RR interval for other respiratory pauses and for all puppies is summarized in Figure 4 which shows the means of RR intervals of three equal periods which represent the initial, middle, and latter thirds of each respiratory pause in puppies. The individual lines joining individual means of RR intervals describe an increasing trend in 23 of 25 respiratory pauses. The increasing trend is therefore statistically significant ($p < 0.01$, sign test). Regression analysis of individual RR intervals during the course of a respiratory pause shows that all slopes are positive and range from 0.7 to 11.5 ms per interval (mean slope = 5.6 ms per RR interval).

Figure 1, which shows the RR interval in time during a respiratory pause, suggests that there is an abrupt change in the magnitude of the RR interval shortly after the pause starts. We therefore applied a two-phase regression (see "Methods") and Figure 5 shows the result of such an analysis for all pauses in dogs. Unlike puppies, dogs have a biphasic pattern: they show a sharp initial increase in their RR interval but subsequently the RR interval decreases or changes little. The slopes of the first phase range between 125 and 679 ms/RR interval in all dogs (mean slope = 323 ms/RR interval) whereas the slopes of the second phase range from −29 to 18 ms/RR interval (mean slope = −6.3 ms/RR interval). The change-point occurs at about one to four RR intervals (mean = two RR intervals) after the start of the respiratory pause.

DISCUSSION

Although the relation between heart rate, HRV, and respiration has been studied in animals and humans (8–13), to our

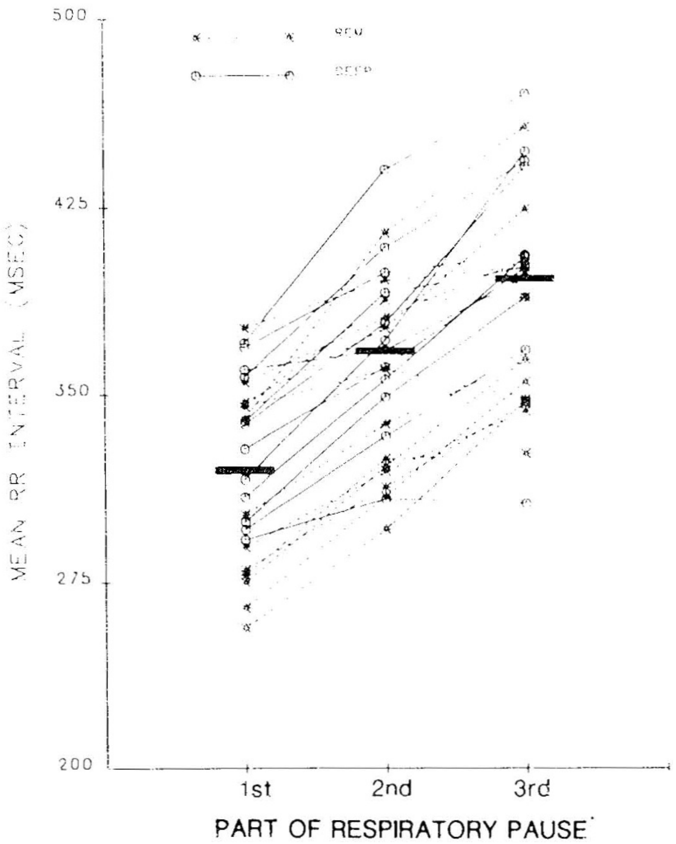

Fig. 4. Means of RR interval of three equal periods which represent the initial, middle, and latter thirds of each respiratory pause in puppies. Note the increasing trend in the RR interval in the majority of (23 of 25) respiratory pauses.

Table 2. *Percent increase in range (RR) (from baseline) during respiratory pauses in puppies and dogs: summary statistics*

	5-number summary (of % increase)						
	Smallest value	25th percentile	Median	75th percentile	Largest value	Mean % increase	SD of % increase
Puppies	131	218	344	688	1050	499	237
Dogs	4	27	46	124	189	70	57

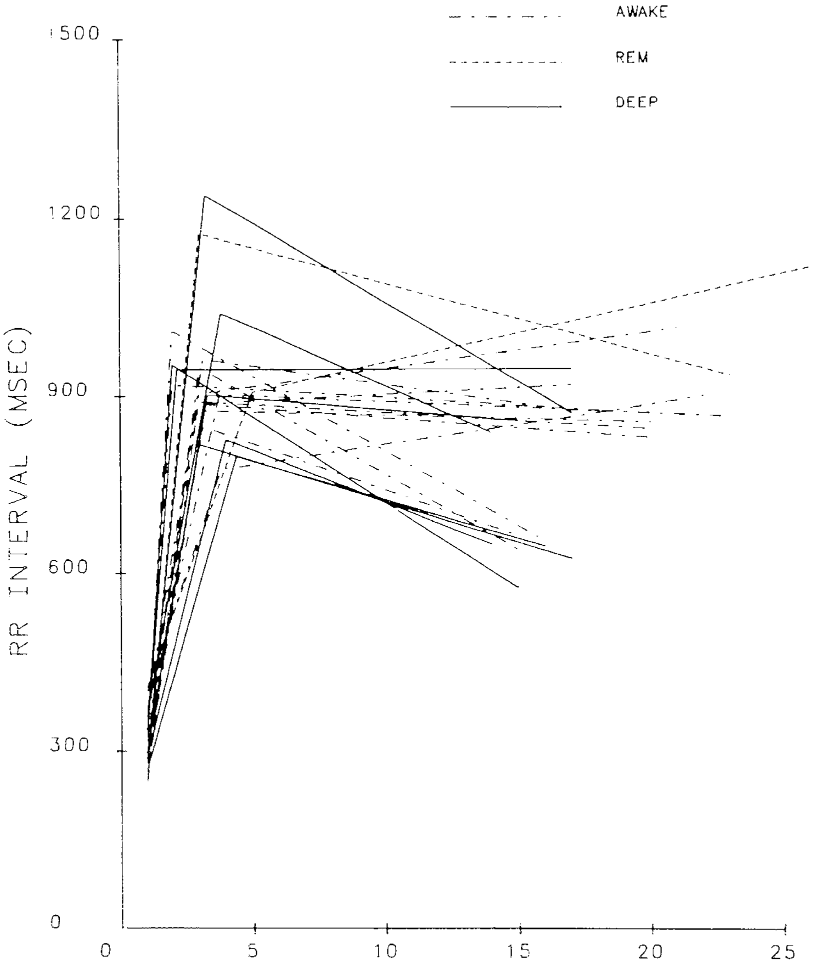

Fig. 5. Two-phase regression line of the RR intervals for each respiratory pause in adult dogs. The biphasic pattern shown a sharp increase initially followed by a decreasing or a steady RR interval. The mean initial slope is 323 ms/RR interval and the mean slope of the second phase −6.3 ms/RR interval.

knowledge, this is the first report that details the changes in heart rate during respiratory pauses in a young, relatively immature animal such as the puppy and in mature dogs. These studies were performed in the dog and puppy for at least two reasons. First, the dog is fairly well studied from the point of view of brain development (15) and this is helpful for the interpretation of results. Second, since the puppy is immature at birth, developmental trends in this species would be more readily apparent than in other more mature animals.

There are two main findings herein. The first is that during respiratory pauses the RR interval increases and remains high in both puppies and dogs. However, the pattern of increase in the puppy is very different from that in the dog. The second is that the RR interval starts to increase within a very short time after the onset of the respiratory pause in both the dog and puppy.

Figures 1 and 2 as well as our analysis on dogs and puppies indicate that whereas the increase in RR interval during respiratory pauses is gradual in puppies, it is very abrupt and reaches maximum or near maximum after the second RR interval in the adult dog. Although the pauses in the puppies are shorter than those in the dog (Table 1), the maximum RR interval that is reached in the puppy during respiratory pauses occurs at a much later time than in the dog. The reason for this difference is not clear. We believe that the understanding of the changes in heart rate during breathing and respiratory pauses requires understanding of some of the peripheral and central mechanisms that control vagal tone to the heart.

In the mature adult subject, we known that there are several components of the respiratory and cardiovascular control systems that are essential for the expression of heart rate changes during breathing and respiratory pauses. For example, it has been shown that there are at least three central nuclei that are important for the decrease in heart rate during apnea or for its acceleration during tidal inspiration. These are the NTS, NA, and the VMN (20–23). Information via the carotid sinus, baroreceptor, vagal, and possibly intercostal afferents have also been considered critical components in this system (20, 21, 24, 25). These nuclei and pathways form a "gating" network (21) which controls the passage of impulses and thereby modulates heart rate and heart variability during breathing and apnea. These previous studies have suggested that a basal vagal (or slow) heart rate exists and that this basal level is interrupted by phasic accelerations during tidal breathing (21). Hence, in the mature animal, as in our dogs, the basal level is apparent when breathing is absent. In this situation and using the gating model, the absence of lung inflation is tantamount to dysfacilitation of the NTS which, in turn, ceases to inhibit the NA and VMN. Therefore the gate is open and complete vagal discharge is let pass. The opposite occurs with lung inflation.

This scheme is based on the assumption that the neuronal circuitry (structure and function) that is responsible for the changes in heart rate is present and well developed. This assumption may not be valid for the puppy. Our previous data (8) and those of others (12, 13) support the idea that the parasympathetic system is not well developed in early life postnatally and the predominance of the vagal system for heart rate control takes several weeks to mature in young mammals (13). Additionally, since there is no *a priori* reason to assume that the development of peripheral afferents, central circuitry, and efferent limb of the control system proceeds at the same rate, it is likely that some of the componemts of this control system mature at a faster rate than others. On the basis of the preceding analysis and published data, we speculate that this differential maturation can create differences in time constants of responses to natural stimuli. Hence, it is not surprising that although the RR interval increases in both puppies and dogs, the time course of this increase is different: the RR interval reaches maximum within two RR intervals while the RR interval in the puppy has a much more progressive course as detailed in our results.

Since the respiratory pauses in the adult dog are rather lengthy (>10 s) and since the young have a high O_2 consumption when related to body weight and O_2 stores, one might ask whether the mechanism that triggered the increase in RR interval, (or the drop in heart rate) that is associated with respiratory pauses is related to O_2 desaturation in both puppy and dog. Our current data and analysis suggest that this mechanism is not related to O_2 desaturation. Our regression analysis shows that in the dogs, maximum RR interval is reached within an average of two RR intervals, with some cases reaching maximum within one RR interval and all cases within four RR intervals. Assuming that, on average, an RR interval is about 600–700 ms, this analysis indicates that within 2.5–3 s of the start of the respiratory pauses and in all pauses in the dog, the RR interval increases to about its highest value for the rest of the respiratory pause duration. Although the time course is not as brisk in puppies, the start of the increase in RR interval takes place also within few seconds after the start of the respiratory pause. Therefore, we do not believe that O_2 desaturation triggered the increase in RR interval in the dog or puppy.

It is possible that hypoxia may develop during the course of the respiratory pause, especially during the more lengthy ones and may have an effect on the change in heart rate (26). The magnitude of O_2 desaturation and its effects will clearly depend on a number of factors including the size of O_2 stores, O_2 consumption, state of consciousness, and the length of the respiratory pause. Previous studies from our laboratory in the dog (27) have shown that PaO_2 does not drop below 50–55 mm Hg in adult dogs after pauses of 15 to 20 s duration. Also, since most pauses do not generally exceed 10 s in the puppy, we do not expect major falls in PaO_2. Although O_2 desaturation can start after a few seconds of apnea in the young mammal, decreases in desaturation reaching levels of about 60–70% occur only after about 30 s of apnea (28). Therefore, herein we doubt that hypoxia plays an important role in modulating heart rate during respiratory pauses since the carotid bodies, the major hypoxia sensors, are minimally stimulated by PaO_2 levels that are higher than 45–50 mm Hg.

In summary, we have described and quantitated the changes in heart rate during respiratory pauses in both puppies and dogs. Although the RR interval increases (bradycardia) during pauses in both puppies and dogs, the pattern of change in the RR interval in the puppy is different from that in the dog. These changes are not considered to be related to hypoxia.

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