Mechanism of cardioventilatory coupling: insights from cardiac pacing, vagotomy, and sinoaortic denervation in the anesthetized rat

Y. C. Tzeng, P. D. Larsen, and D. C. Galletly

Department of Surgery and Anaesthesia, Wellington School of Medicine and Health Sciences, Wellington, New Zealand

Submitted 25 September 2006; accepted in final form 6 December 2006

Tzeng YC, Larsen PD, Galletly DC. Mechanism of cardioventilatory coupling: insights from cardiac pacing, vagotomy, and sinoaortic denervation in the anesthetized rat. Am J Physiol Heart Circ Physiol 292: H1967–H1977, 2007. First published December 15, 2006; doi:10.1152/ajpheart.01049.2006.—Cardioventilatory coupling (CVC), a temporal alignment between the heartbeat and inspiratory activity, is a major determinant of breath-to-breath variation in observed respiratory rate ($f_o$). The cardiac-trigger hypothesis attributes this to adjustments of respiratory timing by baroreceptor afferent impulses to the central respiratory pattern generator. A mathematical model of this hypothesis indicates that apparent CVC in graphical plots of ECG R wave vs. inspiratory time is dependent on the heart rate (HR), the rate of the intrinsic respiratory oscillator ($f_i$), and the strength of the hypothetical cardiovascular afferent impulse. Failure to account for HR and $f_i$ may explain the inconsistent results from previous attempts to identify the neural pathways involved in CVC. Cognizant of these interactions, we factored in the HR-to-$f_i$ ratio in our examination of the role of the vagus nerve and arterial baroreceptors in CVC by cardiac pacing 29 anesthetized Sprague-Dawley rats and incrementally changing the HR. With the assumption of a relatively constant $f_o$, CVC could be examined across a range of HR-to-$f_i$ ratios before and after vagotomy, sinoaortic denervation, and vagotomy + sinoaortic denervation. We confirmed the relation between CVC, HR-to-$f_i$ ratio, and breath-to-breath respiratory period variability and demonstrated the loss of these relations after baroreceptor elimination. Sham experiments ($n = 8$) showed that these changes were not due to surgical stress. Our data support the notion that inspiratory timing can be influenced by cardiac afferent activity. We conclude that the putative cardiovascular input arises from the arterial baroreceptors and that the vagus nerve is not critical for CVC.

Cardioventilatory coupling (CVC) is a temporal alignment between the heartbeat and inspiratory activity (11–14, 28–30, 34, 42). Clinical studies show that CVC is a major determinant of breath-to-breath respiratory period variability, and a “cardiac-trigger hypothesis” has been proposed to explain the experimental data. It has been hypothesized that CVC reflects breath-to-breath adjustments of respiratory timing by afferent impulses to the central respiratory pattern generator arising from the arterial baroreceptors (12, 13, 26). This hypothesis is supported by modeling studies (13, 26, 34), as well as experimental observations of CVC under conditions where heart rate (HR) is largely independent of efferent neural modulation, for example, during fixed-rate cardiac pacing (21), in the presence of an artificial pulsatile circulation (1), and during atrial fibrillation (28). It has also been shown that the most consistent relation between inspiration and the heartbeat is the interval between inspiratory onset (I) and the immediately preceding ECG R wave (RI$_1$ interval) (28, 42). Taken together, these observations suggest that CVC most likely reflects a “triggering” of inspiration by the heartbeat and accounts for the use of time domain plots of consecutive R wave to following I times (RI plot) to demonstrate CVC (28, 42).

In previous studies, examination of RI plots for horizontal bands, indicating a constant temporal alignment between R and I times, has led to the classification of CVC into patterns I, II, III, and IV on the basis of criteria related to the RI$_1$ interval and the ratio of HR to observed respiratory rate ($f_o$; Fig. 1). Pattern I coupling is associated with a constant RI$_1$ interval and HR-to-$f_i$ ratio. Pattern II coupling is also associated with a constant RI$_1$ interval; however, the HR-to-$f_i$ ratio varies from breath to breath. In contrast, the RI$_1$ interval and HR-to-$f_i$ ratio alternate between two values during pattern III coupling. Pattern IV coupling is characterized by alternation between a “coupled phase,” where the RI$_1$ interval is temporally aligned, and a “transitional phase,” where the RI$_1$ interval slowly increases (pattern IV$_a$) or decreases (pattern IV$_b$) from breath to breath (12). Plots of HR vs. $f_i$ for each coupling pattern showed that coupling patterns were associated with specific regions of the HR-to-$f_i$ map, suggesting that the CVC pattern might be causally related to the HR-to-$f_i$ ratio (10).

In addition, CVC is also a major determinant of breath-to-breath respiratory period variability (10). Analysis of consecutive differences in respiratory period (CDI-1) showed minimal variability during pattern I coupling and greater variability during pattern II, III, and IV coupling. In particular, pattern II was associated with breath-to-breath changes in respiratory period (quantal fluctuations) equivalent to one heart period, whereas quantal fluctuations during pattern III coupling were a little less than one heart period. Consideration of these fluctuations in the context of the cardiac-trigger hypothesis led to the development of a model of CVC (13). Contrary to classical cardiorespiratory models, where respiratory modulation of the heart is well recognized (respiratory sinus arrhythmia), our model incorporated a “cardiac burst” parameter that could advance inspiration, ahead of the slower rhythm of the intrinsic respiratory oscillator ($f_i$), on attainment of a hypothetical inspiratory threshold. With HR, $f_i$, and the cardiac burst as parameters, we have successfully replicated the four patterns of CVC and respiratory period variability observed in clinical data (13).

However, critical questions remain. What is the origin of the cardiovascular afferent signal implicated by the cardiac-trigger
hypothesis? Which neural pathways contribute to this process? A prime candidate for the putative cardiac afferent signal was thought to be the arterial baroreceptors, because baroreflex afferents are known to terminate in the nucleus tractus solitarius, with secondary connections to brain stem areas involved in respiratory rhythm generation (13, 26, 31–33, 40, 41, 43). Another important question relates to the role of vagal modulation of the heart in CVC, which has yet to be fully established (2, 3, 22, 24).

Unfortunately, interpretation of experimental data is challenging. In particular, modeling studies highlighted a critical distinction between observed CVC, indicated by the presence of horizontal bands on the RI plot, and the strength of central coupling between the cardiac afferent signal and the respiratory oscillator (13). According to the model framework, if HR or $f_i$ is altered, the presence of CVC may vary without a change in strength of the cardiac afferent input. However, because neither the strength of the cardiac signal nor $f_i$ can be easily measured in vivo, it would be necessary to examine the time interval between each R wave and the following I (RI intervals) relations across entire HR-to-$f_o$ ratio domains to reliably ascertain truly absent CVC (13). This was not well appreciated by early investigators and potentially explains why previous studies of the role of the vagus nerve in rabbits produced conflicting results, with CVC apparently “abolished” in one study (3), but not in another (24), after bilateral vagotomy.

One way to deal with this challenge may be to alter the HR by electrically pacing the heart and examining CVC and CDI-I across a range of HR-to-$f_o$ ratios. This approach would enable a more definitive examination of the role of the vagus nerve and arterial baroreceptors in CVC after selective nerve sectioning. We hypothesize that elimination of the relevant neural pathway(s) should completely abolish CVC, such that no CVC will be observed at any HR-to-$f_o$ ratio.

**METHODS**

After approval from the Wellington Animal Ethics Committee, the studies were performed on 37 adult Sprague-Dawley rats (350–400 g body wt). On the day of the experiment, animals were retrieved from the containment facility and placed in an induction chamber with 4% isoflurane in $O_2$. Adequate depth of anesthesia was confirmed by a negative limb-withdrawal test before commencement of surgery.

With the animal in a fixed supine position, a tracheostomy was performed and an endotracheal tube was inserted. The right internal jugular vein was dissected free of surrounding tissue, and a bipolar cardiac pacing wire was inserted and advanced toward the heart (23). The pacing wire was connected to a square-pulse stimulator (model S88, Grass Telefactor, W. Warwick, RI) in series with a stimulus isolation unit (model SIU7, Grass Telefactor) and left on standby. A femoral artery and vein, dissected free of surrounding tissue, were cannulated (24-gauge intravenous cannulas, Becton Dickinson) for continuous recording of blood pressure, intravenous fluid replacement, and administration of vasoactive drugs. All catheters were filled with heparinized saline and secured to the surrounding skin flap with surgical sutures. After cannulation, animals were left undisturbed for ≥30 min for hemodynamic stabilization. Anesthesia was thereafter maintained at adequate levels with isoflurane (2%) and supplemental $O_2$. Body temperature was monitored with a rectal probe and maintained at 38°C using a heat lamp.

During each experiment, ECG (lead CM5, Corometrics Neo-Trak 502), respiratory flow (Hans Rudolph heated Pneumotach 8420 A; Vacumed differential pressure transducer 4500), and continuous arterial blood pressure (P23 pressure transducer, Grass Instruments, Quincy, MA) were recorded. Data were sampled at 2,000 Hz per channel with a 16-bit input-output data acquisition board (PCI-6023E series, National Instruments) and streamed to a Macintosh G4 computer for storage and subsequent offline analysis.

**Data Analysis**

From the ECG and continuous blood pressure recordings, R wave times and beat-to-beat systolic blood pressures (SBP) were extracted. To ensure that heartbeats did not mechanically produce an artifact in respiratory airflow that would bias the assessment of I times, the raw airflow signal was low-pass filtered using a Butterworth filter. A known drawback of the Butterworth filter is that filtered series are phase shifted. This effect was minimized by extension of the original series at the beginning and end with its own reversed array and back-to-back filtering (25, 35). The filtered signal was superimposed on the raw signal and manually examined to ensure satisfactory removal of any signal artifacts. The I values were obtained by detection of the point of positive signal deflection following the point

---

**Fig. 1.** Stylized time domain plot of consecutive R wave to the following inspiratory onset (RI plots) showing examples of cardioventilatory coupling (CVC) according to classified patterns (I, II, III, IV$_{lo}$, and IV$_{hi}$). An uncoupled plot is shown for comparison.
of zero flow at end expiration. From these time series, the RI intervals were established and then plotted as a time series (RI plot). In the RI plot, horizontal banding indicates the presence of CVC, where R waves fall in constant timing relation with I. The interval between inspiration and the immediately preceding heartbeat is designated the RI-1 interval (28, 29). The RI plots were visually examined, in a blinded fashion without reference to the HR and fE time series or the study protocol, for patterns of CVC, as previously defined, on the basis of the RI-1 interval and the HR-to-fE ratio (Table 1) (28, 29). Episodes of CVC were then verified against the entropy index of CVC (see below) and confirmed only when pattern-consistent changes in the HR-to-fE ratio and CDI-I time series were also observed.

Consistency of the RI-1 interval was quantified by calculation of the proportional Shannon entropy (SHn) and the mean of the absolute values of consecutive breath-to-breath difference in RI-1 interval (CDRI-1). SHn, a measure of RI-1 interval dispersion, has previously been applied as an index of CVC (12, 42). This was achieved by passing an overlapping n-breath moving window over the data series and allocating the n RI-1 intervals within each window into a 10-bin histogram with outer limits set at a minimum of 0 and a maximum of the mean of the R-R intervals covered by n values of I. The 10-bin histogram was chosen to ensure comparability of results obtained with previous descriptions of CVC, whereas the choice of the value n varied depending on the total length of data being analyzed. On the basis of the histogram distribution of RI-1 intervals, SHn was calculated according to the following algorithm

\[
SH = \sum_{b=1}^{N} Pb \times \log(Pb) 
\]

(1)

\[
SH_{\text{max}} = - \log \left( \frac{1}{N} \right) 
\]

(2)

\[
SH_n = \frac{SH}{SH_{\text{max}}} 
\]

(3)

where \(P\) is the histogram bin probability, \(b\) is the bin number, and \(N\) is the total number of histogram bins. During perfect CVC, an R wave would fall at a constant time interval before each I and, therefore, give a minimum SHn of ~0; in the complete absence of CVC, RI-1 intervals would be uniformly distributed, giving a maximum SHn of ~1. In practice, neither single-bin distribution nor complete uniformity of the intervals across the range of histogram bins is likely to occur with small data sets. By nature of its construction, SHn is most sensitive in the detection of pattern I, II, and III coupling and less sensitive for pattern IV coupling, where the RI-1 interval alignment changes from breath to breath during the transitional phase. Therefore, to enhance the temporal resolution of the index, using an overlapping 40-point window, we plotted SHn as a time series for data epochs containing >40 RI-1 intervals (42). The 40-point moving window was chosen to maximize the sensitivity of the index. For data epochs containing ≤40 RI-1 intervals, we used a window of equivalent to the total number of RI-1 intervals in that epoch (n) to calculate SHn.

The statistical threshold for significant CVC at the 5% level was determined by applying the entropy algorithm described above to random number series. Since the distribution of SH values varies according to the total number of RI-1 intervals processed (n), threshold values were generated with random series that matched n. Thus we determined the threshold (SH) for the moving window SHn by calculating the SH for 1,000 random data sets, each representing 40 RI-1 intervals (n = 40) between 0 and 1. The resulting series of SHn values were ranked in ascending order, and the 50th number (0.90) was taken as SHI. Values on the SHI time series that fall below SHI are considered statistically significant. Individual SHI values were calculated for data epochs containing ≤40 RI-1 intervals. To achieve this, we calculated SHn using n random numbers between 0 and 1 matching the total number of RI-1 intervals within the epoch for 1,000 repetitions. The resulting series of SHn values were ranked in ascending order, and the 50th number was taken as SHI (see Fig. 6).

To determine breath-to-breath respiratory period variability, we calculated the breath-to-breath consecutive difference in respiratory cycle duration (CDI-I) as a function of time and obtained an average measure as the mean of the absolute value of CDI-I (CDI-II) (10).

All data analysis was performed using custom-written software in LabView 7 (National Instruments) on a 1-GHz PowerBook G4 computer.

**Procedures**

**Baroreflex testing.** The Oxford method was used to test baroreflex sensitivity (BRS) (6, 37). This method involved reflex changes in HR in response to randomized 0.3-ml bolus injections of phenylephrine (1.5–3 μg/kg) or sodium nitroprusside (2–5 μg/kg) over 10 s. To derive BRS, a least squares linear regression was fit to R-R interval change in response to ramp changes in SBP induced by phenylephrine and sodium nitroprusside. The slope of the linear regression was taken as an estimate of BRS (ms/mmHg). BRS estimates were calculated separately for phenylephrine and sodium nitroprusside slopes.

**Cardiac pacing.** Cardiac pacing was achieved by delivery of 20-ms pulses at 1- to 3-mA current at rates slightly higher than the intrinsic HR and increasing in ~5 beat/min increments every 2 min, for a total of 10–20 min. The intrinsic HR and the individual animal’s capacity to maintain blood pressure determined the total number of pacing steps. Successful pacing was confirmed, in real time, using a storage oscilloscope (model 464, Tektronix). Postmortem examination of eight animals confirmed that the tips of the pacing wire were at or near the confluence of the superior vena cava and the right atrium.

**Sinoaortic denervation.** Sinusoidal denervation (SAD) was achieved in a two-step procedure (27): the sternoceildomastoid muscles were retracted, and the carotid sheath and the underlying carotid triangle were exposed. With the aid of a dissecting microscope (model SZ-STU1, Olympus), the aortic depressor nerve (ADN) was identified prior to its junction with the vagus nerve and cut. In some rats, baroreceptor afferent fibers also coursed through the sympathetic chain and/or the superior laryngeal nerve (SLN). Therefore, the sympathetic trunk was identified at its origin at the nodose ganglion and sectioned, and the SLN was cut at its junction with the vagus.
nerve. For isolation of the carotid baroreceptors, the carotid sinus nerve (CSN) was severed at its junction with the glossopharyngeal nerve, and the internal, external, and caudal sections of the common carotid artery were completely stripped. Then all carotid branches were painted with 10% ethanol and allowed to stabilize for 60 min. Consistent with previous studies and with the inherent variability in baseline HR and SBP associated with respiration taken into account, SAD was considered complete when baroreflex testing with phenylephrine and sodium nitroprusside produced <10 beat/min reflex changes in HR (38, 39).

Vagotomy. In rats, the vagus nerves travel along the common carotid artery within the carotid sheath. Vagus nerves were dissected free from the carotid sheath and surrounding tissue and sectioned bilaterally at the caudal and rostral ends.

Sham surgery. In eight rats, sham operations were performed to assess the effects of time and surgical stress on CVC. In these experiments, rats were subjected to the same experimental procedure, including tracheostomy, surgical dissection, isolation of baroreceptor afferent nerves, and insertion of the cardiac pacing electrode. The key difference, however, is that denervation was not performed in these animals.

Protocol
A schematic representation summarizing the study protocol is shown in Fig. 2. In all protocols, 15 min of baseline data collection was followed by baroreflex testing to establish the baseline BRS. Then cardiac, respiratory, and blood pressure activity were recorded simultaneously during cardiac pacing, which enabled the examination of the relations between CVC and respiratory period variability over a range of HR-to-fo ratios. At this point, the study was split into three separate protocols, with the effects of vagotomy (protocol I), SAD (protocol II), and SAD + vagotomy (protocol III) assessed in separate experiments. Animals in protocols II and III underwent further BRS testing to determine the completeness of SAD. In all protocols, variable-rate cardiac pacing was performed to ensure that the effects of denervation on CVC and respiratory period variability were assessed across a minimum of one HR-to-fo integer ratio. In addition, sham experiments were conducted separately in eight rats that underwent similar surgical preparation but were not subjected to cardiac pacing or denervation.

Statistical Analysis
Values are means (SD). Paired data were compared using Student’s paired t-test, and differences between CVC patterns were compared using Student’s unpaired t-test if factorial ANOVA was found to be significant. P < 0.05 was considered statistically significant.

RESULTS
Steady-state HR and SBP before SAD were 340 beats/min (SD 24) and 110 mmHg (SD 13), respectively. Immediately after SAD, we observed a rapid increase in SBP [+76 mmHg (SD 12)], which stabilized to baseline levels within 60 min [110 mmHg (SD 22) after SAD]. In contrast, we observed an immediate increase in HR after SAD that remained significantly elevated for the duration of the study [370 beats/min (SD 23) after SAD].

Baseline recordings were visually inspected for each animal before cardiac pacing. Although horizontal banding of RI plots were observed in 48% of recordings (14 of 29 rats), the apparent degree of CVC varied between, as well as within, the data epochs. This variation is illustrated in Fig. 3, which shows 300-s recordings from two experiments. The example in Fig. 3A shows no apparent horizontal structures on the RI plot, which is consistent with the absence of CVC. In contrast, the example in Fig. 3B shows periods of horizontal banding, consistent with the presence of CVC, as well as periods with no visible structure on the RI plot. Comparison of the two epochs shows breath-to-breath HR-to-fo ratio consistently >6:1 throughout the example in Fig. 3A and ~4:1 throughout the example in Fig. 3B. CDI-I and CDRI-I were smaller in Fig. 3B, consistent with the presence of CVC. SHfo was greater than SHI throughout the recording in Fig. 3A, consistent with no apparent structure in the RI plot and relatively large-magnitude variability in CDI-I, which did not vary throughout the recording. In contrast, SHfo fell below SHI in Fig. 3B during pattern I coupling, which was associated with a step-wise reduction in the magnitude of CDI-I.

Although CVC was not visually apparent in 15 of 29 baseline recordings, during cardiac pacing, 9 of these 15 “noncoupled” animals (60%) subsequently showed clear evidence of CVC, as illustrated in Fig. 4, which shows results obtained from one such animal before and during cardiac pacing. Before pacing (Fig. 4A), no horizontal structures were observed on the RI plot, consistent with the absence of apparent CVC. In contrast, the same animal during cardiac pacing (Fig. 4B) demonstrated banding structures on the RI plot that are consistent with pattern I and Vfo coupling. Comparison of the two sets of results shows HR-to-fo ratio consistently >4:1 throughout the recording in Fig. 4A, with relatively large-magnitude variability in the CDI-I time series that did not vary throughout the recording. In contrast, the recording in Fig. 4B shows periods of pattern I coupling with an HR-to-fo ratio

![Diagram](https://via.placeholder.com/150)
corresponding to 5:1, while the period of pattern IV hi coupling was associated with HR-to-fo ratio slightly >5:1. Although [CDI-I] during cardiac pacing was similar, there was significant variation in magnitude throughout the epoch, with significant step-wise reductions in CDI-I during pattern I coupling. [CDRI-I] is greater in Fig. 4A than in Fig. 4B. SHr is greater than SHt in Fig. 4A, which is consistent with no apparent structure in the RI plot or variation in the magnitude of CDI-I throughout the recording. In contrast, SHr fell below SHt in Fig. 4B during pattern I coupling, which was associated with a step-wise reduction in CDI-I.

The changes in CVC pattern and CDI-I with HR alterations in an animal with clear evidence of CVC are illustrated in greater detail in Fig. 5. HR was increased from ~319 to 360 beats/min over seven different ~120- to 180-s HR steps. The RI plot shows sequential transitions between different CVC patterns, as well as periods without CVC. From the beginning of the recording, at 6 s, a ~30-s period of pattern I coupling was observed. This was followed by a period of pattern IV lo coupling between 36 and 210 s. Between 210 and 360 s, three transitions from pattern I to pattern IV hi and back to pattern I were followed by a period of pattern I coupling between 385 and 420 s and pattern IV hi coupling between 420 and 600 s. The epoch appeared uncoupled from 600 s onward. A distinct relation between the pattern of CVC and the HR-to-fo ratio can be observed. Pattern I coupling was associated with an HR-to-fo ratio of 5, consistent with five heartbeats elapsing for every breath. Pattern IV lo coupling was associated with an

---

**Fig. 3.** HR, observed respiratory rate (fo), RI plot, HR-to-fo ratio, and consecutive respiratory period difference (CDI-I) time series from 2 representative experiments (A and B). No horizontal structures are observed in A, indicating the absence of apparent CVC throughout the 300-s recording. This is consistent with the HR-to-fo ratio occupying noninteger values >6 throughout the epoch. In B, 4 horizontal bands on the RI plot are consistent with 4:1 coupling. Absolute value of CDI-I ([CDI-I]) is 0.020 s (SD 0.040) in A and 0.0063 s (SD 0.0062) in B. Absolute value of consecutive breath-to-breath difference in RI interval ([CDRI-I]) is 0.043 s (SD 0.038) in A and 0.034 s (SD 0.045) in B. Proportional Shannon entropy (SHr) time series is consistently greater than threshold Shannon entropy (SHT) in A, indicating an apparent absence of CVC. In B, SHr > SHT in the presence of pattern I coupling is associated with a step-wise reduction in CDI-I.

---

**Fig. 4.** HR, fo, RI plot, HR-to-fo ratio, and CDI-I time series obtained from 1 animal in the absence (A) and presence (B) of cardiac pacing. In A, no horizontal structures were observed on the RI plot, consistent with no apparent CVC. HR-to-fo ratio was consistently >4:1 throughout the recording. In B, the RI plot is consistent with pattern I coupling, associated with a constant 5:1 HR-to-fo ratio and minimal CDI-I variation, as well as pattern IV lo coupling with an HR-to-fo ratio slightly >5:1 and greater CDI-I variation. [CDI-I] is 0.016 s (SD 0.012) in A and 0.013 s (SD 0.012) in B. [CDRI-I] is 0.075 s (SD 0.035) in A and 0.051 s (SD 0.043) in B. SHr time series is consistently greater than SHT in A, indicating an apparent absence of CVC. In B, SHr < SHT in the presence of pattern I CVC is associated with a step-wise reduction in CDI-I.
HR-to-$f_o$ ratio slightly below a whole number, whereas pattern IV$_{hi}$ coupling was associated with an HR-to-$f_o$ ratio slightly greater than a whole number. In contrast, the HR-to-$f_o$ ratios during uncoupled segments were noninteger, with values that were generally a little greater or smaller than the HR-to-$f_o$ ratios associated with pattern IV coupling. Figure 5 shows that transitions between different CVC patterns were accompanied by sharp step-wise changes in $f_o$ and CDI-I, again with pattern I coupling associated with SH$_{IV}$ less than SH$_T$ and step-wise reduction in CDI-I. These observations are consistent with consecutive breaths during pattern I coupling having similar cycle durations that abruptly shift to a pattern of greater breath-to-breath respiratory period variability with pattern IV coupling and without coupling.

In six animals, visual inspection of the RI plot showed no clear horizontal structures, even with cardiac pacing. In these experiments, incremental changes in HR did not appear to influence CDI-I, with the degree of variability at integer HR-to-$f_o$ ratios appearing similar to variability magnitude observed at noninteger HR-to-$f_o$ ratio. Moreover, the sharp step-wise changes in $f_o$ and CDI-I clearly apparent in Fig. 5 were not observed in these experiments, which is consistent with very weak or truly absent CVC.

The relations between CVC pattern, HR-to-$f_o$ ratio, and breath-to-breath CDI-I highlighted by Figs. 3–5 were consistently observed throughout the study and are statistically summarized in Table 2. Table 2 shows that pattern I was associated with whole number integer HR-to-$f_o$ ratios with smaller $|$CDRI$_{II}$| and CDI-I, indicating minimal RI$_1$ interval and respiratory period variability compared with pattern IV and uncoupling.

### Table 2. Differences in respiratory rate, breath-to-breath respiratory period variability, and heart rate-to-respiratory rate entrainment ratio between coupling patterns

<table>
<thead>
<tr>
<th>Coupling Pattern</th>
<th>I</th>
<th>IVlo</th>
<th>IVhi</th>
<th>Uncoupled</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$f_o$</td>
<td>69 (8.1)</td>
<td>68 (12)</td>
<td>62 (18)</td>
<td>62 (18)</td>
<td>0.21</td>
</tr>
<tr>
<td>CDI-I</td>
<td>0.0080 (0.0020)</td>
<td>0.017 (0.010)</td>
<td>0.023 (0.024)*</td>
<td>0.047</td>
<td></td>
</tr>
<tr>
<td>HR/$f_o$</td>
<td>5.1 (0.58)</td>
<td>5.5 (1.5)</td>
<td>6.9 (3.3)</td>
<td>6.9 (3.3)</td>
<td>0.11</td>
</tr>
<tr>
<td>SD(HR/$f_o$)</td>
<td>0.055 (0.014)</td>
<td>0.14 (0.12)</td>
<td>0.32 (0.32)*†</td>
<td>0.0067</td>
<td></td>
</tr>
<tr>
<td>RI$_1-1$</td>
<td>0.061 (0.019)</td>
<td>0.074 (0.010)*</td>
<td>0.081 (0.010)*†</td>
<td>0.0036</td>
<td></td>
</tr>
<tr>
<td>$</td>
<td>$CDRI$_{II}$</td>
<td>0.011 (0.0050)</td>
<td>0.040 (0.018)*</td>
<td>0.047 (0.025)* †</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Values are means (SD). $n$, total number of epochs from all experiments, which, in some experiments, included multiple epochs from 1 recording. Observed respiratory rate ($f_o$), mean absolute consecutive difference in breath-to-breath respiratory period ($|\text{CDI-I}|$), mean and standard deviation of heart rate (HR)-to-respiratory rate ($f_o$) ratio [SD(HR/$f_o$)], mean RI$_1$ coupling interval ($\text{RI}_1-1$), and mean absolute consecutive difference in coupling interval ($|\text{CDRI}_{II}|$) for pattern I, IV, and uncoupled data. $P$ values are for factorial ANOVA. *Significantly different from pattern I; †significantly different from pattern IV ($P < 0.05$).
uncoupled data. In the present study, *pattern III* coupling was present in only two experiments for ~30 s on each occasion (see Fig. 7). Even less prevalent was *pattern II* coupling, which was not observed in the nerve-intact state. Because of small numbers, *patterns II* and *III* were excluded from the statistical comparison in Table 2.

The effect of vagotomy on CVC was examined in 13 experiments. Vagotomy was associated with a significant decrease in mean *f*₀ [80 (SD 22) and 40 (SD 26) breaths/min before and after vagotomy, respectively, *P* < 0.05] but no significant changes in HR. In this cohort, visual inspection of RI plots clearly showed CVC in 10 animals before vagotomy with the heart beating spontaneously and in all animals during cardiac pacing. After vagotomy, RI plots from spontaneous (i.e., without cardiac pacing) epochs were again examined, and CVC was observed in nine animals. Although CVC was apparently absent in four animals after vagotomy, the absence of CVC may be due to noninteger HR-to-*f*₀ ratios, rather than a true absence of CVC. Therefore, cardiac pacing was repeated in these experiments, with three of the four animals subsequently demonstrating periods of CVC. The presence of CVC before vagotomy, after vagotomy, and during cardiac pacing was verified by at least one episode of statistically significant CVC where *SHₚ* was less than *SH₉₀*, as well as the relation between CVC pattern, HR-to-*f*₀ ratio, and CDI-I illustrated in Fig. 5. Examples of CVC and associated respiratory period variability after vagotomy are shown in Fig. 6.

The effect of SAD was examined in nine experiments. In this cohort, CVC was observed in all RI plots obtained before SAD with the heart beating spontaneously. This was verified by at least one episode of statistically significant CVC on the *SHₚ* time series and features consistent with CVC on the HR-to-*f*₀ ratio and CDI-I time series. After SAD, RI plots from spontaneous epochs were again examined, and brief periods of CVC were detected in two animals. An example from one animal is shown in Fig. 7. The post-SAD BRS was 8 beats/min

---

**Fig. 6.** Examples of CVC after vagotomy. **A:** *pattern I* coupling from 1 rat after vagotomy. Note 12–13 horizontal bands in the RI plot, indicating that 12 or 13 heartbeats elapse between each inspiration. **B:** *pattern II* coupling. Note horizontal banding in the RI plot, similar to *pattern I* coupling, except quantal changes in respiratory period vary between 2 values equivalent to 1 heart period. This is seen as quantal breath-to-breath fluctuations in the HR-to-*f*₀ ratio between 13 and 14. CDI-I is 0.053 s (SD 0.054) in A and 0.091 s (SD 0.071) in B. CDRI-I is 0.025 s (SD 0.039) in A and 0.039 s (SD 0.041) in B. *SHₚ* is 0.505 (*n* = 40, *SH₉₀* = 0.900) in A and 0.505 (*n* = 37, SH₉₀ = 0.892) in B.
classical "double banding" of the RI plot (horizontal bar), SH, HR-to-

with CVC, even with approximately whole number integer

Visual inspection of the RI plot for recordings obtained from
denervation, none of the seven animals demonstrated CVC.

After denervation, none of the seven animals demonstrated CVC.

Visual inspection of the RI plot for recordings obtained from
these seven animals during cardiac pacing verified the absence
of any RI plot structure consistent with CVC, even with
approximately whole number integer HR-to-fo ratio. SHa val-
ues for these animals were consistently greater than SHf,

A potential explanation for the persistence of CVC in two
rats after SAD may relate to the completeness of SAD. In rats,
the ADN generally runs separately from the vagus nerve but
can also run closely alongside the vagus nerve (27, 39). Even
though SAD was considered complete in all rats included in
protocoi II of the present study, this was based on an arbitrary
definition of a <10 beat/min change in HR after phenylephrine

DISCUSSION

The present study shows that CVC is a major determinant of
CDI-I. The study also demonstrates that the presence of CVC
on RI plots is dependent on the HR-to-fo ratio and, therefore,
that the absence of horizontal bands on the RI plot may not
accurately reflect the strength of the cardiac input to the
respiratory pattern generator. This was shown in the present
study, where up to 79% of recordings (23 of 29 rats) showed
clear evidence of CVC during cardiac pacing, even though, in
the same cohort of animals, CVC was graphically evident in
only 48% of animals (14 of 29 rats) with the heart beating
spontaneously. Our results indicate that, in addition to the RI
plot, the HR-to-fo ratio and CDI-I should also be taken into
account to ensure accurate determination of CVC. The present
study is therefore the first to systematically examine the role
of the vagus nerve and arterial baroreceptors in CVC having also
taken the HR-to-fo ratio into consideration.

The role of the vagus nerve in CVC has previously been
examined, but results have been inconclusive. Kenner et al.
(24) used an epinephrine infusion to achieve a whole number
HR-to-fo ratio and observed periods of coupling with transient
periods of "escape" that were associated with increased respira-

ory variability. The loss of these features after vagal dener-
vation led Kenner et al. to conclude that vagal afferents were
critical to CVC. However, Bucher et al. (2), using a variable
pneumothorax and cooling of the sinoatrial node to achieve
whole number HR-to-fo ratio, described CVC that persisted
after bilateral vagotomy. This discrepancy may be due to
failure in both studies to adequately consider the effects of
changes in the HR-to-fo ratio and CDI-I associated with vagal
denervation.

In this study, bilateral vagal denervation (with the CSN
and ADN intact) was associated with no significant changes in
CVC. Bilateral SAD (with the vagus nerves intact) was asso-
ciated with a marked reduction in CVC, although in a small
number of animals CVC persisted. In contrast, CVC was
completely abolished in all animals after bilateral SAD and
vagal denervation, even after we accounted for the HR-to-fo
ratio by cardiac pacing. These observations are consistent with
arterial baroreceptors playing a critical role in the CVC process
with negligible contribution from vagal afferents.

Fig. 7. CVC after SAD. RI plot shows pattern III coupling associated with
classical "double banding" of the RI plot (horizontal bar), SHa < SHf, and fo
and HR-to-fo ratios that alternate between 2 values. Quantal variations in
respiratory period were not equivalent to 1 heart period; thus RI - 1 interval
alignment is different for alternate breaths. Post-SAD baroreceptor sensitivity
for this animal is 8 beats/min with phenylephrine and 8 beats/min with sodium
nitroprusside. CDI-I is 0.017 s (SD 0.013), and CDRI-I is 0.043 s (SD
0.040).

The present study was therefore the first to systematically examine the role
of vagal afferents and arterial baroreceptors in CVC having also
taken the HR-to-fo ratio into consideration.
and sodium nitroprusside challenge (27, 38, 39). Thus it remains possible that not all fibers were eliminated and that some residual ADN fibers traveling along the vagus sheath remained intact to produce slightly /H1102110 beat/min reflex changes in HR.

Although all patterns of CVC were observed in this study, patterns II and III were relatively rare. In the nerve-intact state, pattern III was observed only briefly in two experiments, whereas pattern II was not observed. The rarity of these patterns may relate to the relatively small areas occupied by these two patterns on the HR-to-fi map described in our previous study (13). In addition, noisy dynamics inherent in the cardiorespiratory system may adversely affect these patterns and cause them to appear “uncoupled” where they may be present.

Two clear epochs of pattern II coupling were observed after vagotomy, which in this study was associated with a significant reduction in f0. An association between pattern II coupling and slow f0 in anesthetized humans has been described (12). One explanation for this association is that pattern II is a variant of pattern I coupling in which small variations in the fi of the respiratory oscillator cause variations in the HR-to-f0 ratio. According to our model, the impact of such small variations in fi is greatest at low frequencies and may explain why pattern II was observed only after vagal denervation.

**Mechanism of CVC**

In 1967, Hinderling (21) described the tendency of the HR-to-f0 ratio to assume integer values during cardiac pacing in human subjects. Hinderling proposed that the tendency for cardiac and respiratory rhythm to couple was the result of a cardiac influence on respiration but did not further elaborate on the possible nature of this influence. The present study extends Hinderling’s observations to the anesthetized rat and is the first to show that CVC during cardiac pacing is accompanied by sharp step-wise changes in f0 and CDI-I that are related to the HR-to-f0 ratio. For example, in the case of pattern I coupling, where we have proposed that each breath is triggered by the preceding heartbeat, the CDI-I is consistently smaller than in the other patterns of CVC or in the case of uncoupled epochs. Therefore, dynamic transitions from pattern IV or an uncoupled data segment to pattern I coupling would be accompanied by a step-wise reduction in CDI-I. This was clearly demonstrated in this study and is consistent with the hypothesis that CVC results from the entrainment, or resetting, of the respiratory rhythm by some cardiac cycle-related trigger.

This cardiac-trigger hypothesis adequately explains why CVC persists during atrial fibrillation (28), which is a pathological condition of the heart associated with irregular HR and diminished efferent vagal modulation of the sinoatrial node.
Taken together, these observations clearly preclude any significant role of respiratory modulation of the heart in the CVC process.

Recent experiments recording neuronal activity in the nucleus tractus solitarius and in the rostral and caudal ventral respiratory groups detected and quantified beat-to-beat cardiac modulation of neurons within central respiratory networks (4, 5). It was concluded that a cardiac cycle-related modulation is associated with the arterial pulse, which is evident in respiratory premotor, as well as motor, output. It was also suggested that the respiratory pulse modulation is likely to be peripheral and sensory, rather than of central origin (4, 5), which is consistent with our observation that CVC was abolished after SAD. Indeed, it is well established that neural stimulation of the CSN and the SLN alters respiratory timing (7–9, 33, 36, 40, 41, 43).

Although the respiratory influence on cardiac timing (respiratory sinus arrhythmia) is well known, the ability of the heart to variably influence respiratory timing is not well appreciated or understood (4, 5, 17, 19, 20). Such an interaction may have potential clinical relevance in conditions of impaired respiratory rhythm control and may help explain observations such as the onset of tachycardia with exercise, dyspnea associated with tachycardia, including patients in fast atrial fibrillation, hyper-ventilation associated with anxiety, and reduced incidence of apnea episodes during atrial overdrive pacing in patients with central and obstructive sleep apnea (15–17, 19, 20).

**Study Limitations**

These findings should be interpreted in the context of the following limitations, many of which are common to all locking studies. One limitation relates to the plasticity of biological systems, where preparations may be affected, in unaccountable ways, by the stimulation itself (18). In addition, complex interactions between the various parameters and restrictions on the ability to measure these variables in biological systems mean that experiments were conducted with less control than in the ideal situation, where all interacting variables are constant. In the present study, it was elected to modify HR, inasmuch as HR is readily amenable to physio-

logical manipulation. However, the $f_i$ of the respiratory oscillator, or the true strength of the cardiac stimulus, cannot be directly measured, and although these variables can be esti-

mated, these procedures carry their own set of limitations (13).

In the present study, it has been assumed that the $f_i$ and cardiac burst magnitude are relatively constant throughout a given experiment. Another limitation relates to the use of cardiac pacing as a means to alter HR. Because cardiac pacing cannot slow HR, the ability to explore regions of coupling associated with lower HR-to-$f_i$ ratios before vagotomy was limited. In addition, the present study was limited by how finely the HR and, therefore, the HR-to-$f_i$ ratio could be altered without excessive prolongation of the time needed to perform the study protocol. Finally, although we have demonstrated a dependency of respiratory period variability on cardiac activity, this study dealt entirely with peripheral pathways of CVC and does not provide further insights on central processes.

In summary, the present study shows that induced changes in HR resulted in changes in CVC pattern and CDI-I that are consistent with our model. This study has demonstrated the critical role of baroreceptor afferent nerves in CVC, and, therefore, it can be concluded that the putative cardiac afferent signals responsible for CVC are of baroreceptor origin. However, detail regarding the central processes of CVC requires further study.

**REFERENCES**


