# Evolution and Resolution of Long-term Cardiac Memory

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***Background***—Cardiac memory (CM) refers to T-wave changes induced by ventricular pacing or arrhythmia that accumulate in magnitude and duration with repeated episodes of abnormal activation. We report herein the kinetics of long-term CM and its association with the ventricular action potential.

***Methods and Results***—Dogs were paced from the ventricles at rates of 110 to 120 bpm for ≈3 weeks. CM characterized by gradual sinus rhythm T vector rotation toward the paced QRS vector evolved in all dogs regardless of pacing site (left ventricular [LV] anterior apex or base, posterior LV, or right ventricular free wall). Cardiac hemodynamics and myocardial flow (microsphere studies) were unaltered by the pacing. Recovery time for the memory T wave to return to control increased with duration of the previous pacing. The protein synthesis inhibitor cycloheximide markedly ($P<.05$) and reproducibly attenuated evolution of CM. When pacing was performed from the atrium, CM did not occur. Standard microelectrode techniques were used to study action potential from the LV free wall of control and CM dogs. CM was associated with increased action potential duration in epicardial and endocardial but not midmyocardial cells, significantly altering the transmyocardial gradient for repolarization.

***Conclusions***—CM is a dynamic process for which the final T vector is predicted by the paced QRS vector and which is associated with significant changes in epicardial and endocardial but not midmyocardial cell action potential duration, such that the transmural gradient of repolarization is altered. It is unaccompanied by evidence of altered hemodynamics or flow, requires a change in pathway of activation, and appears to require new protein synthesis. **(*Circulation*. 1998;97:1810-1817.)**

**Key Words:** action potentials ■ electrocardiography ■ electrophysiology ■ T-waves ■ pacing



The term "cardiac memory," used with respect to the ECG, was described and named by Rosenbaum et al[1] and had been reported independently by Chatterjee et al.[2] Cardiac memory is a characteristic of the T wave during sinus rhythm that, after periods of ventricular pacing or ventricular arrhythmia, assumes a vector approaching that of the paced or arrhythmic QRS complex.[1,3] A key aspect of the T-wave change described by Rosenbaum et al[1] is "accumulation," ie, repeated periods of exposure to the inciting event augment and sustain the T-wave change during subsequent sinus rhythm. Equally important is that the T-wave change occurs in hearts that have no demonstrable hemodynamic or structural abnormalities or ischemia.

Our purpose in the present study was to determine the kinetics and stability of cardiac memory and its association with changes in the cardiac action potential and to test whether it shares properties in common with neuronal long-term potentiation. This is not necessarily a far-fetched idea, given the frequent lessons in nature relating to conservation of proteins and of signaling processes. To this end, we used a model of pacing-induced long-term cardiac memory in the intact dog to ask the following questions: (1) Can T-wave memory be verified to occur in the absence of any manifestation of cardiac failure or ischemia? (2) Are there means for its accurate quantification? (3) Is it a purely HR-dependent process or is an altered activation pathway essential to its genesis? (4) Does it manifest the property of accumulation and if so, what are the kinetics of its evolution and resolution? (5) Is its course modified by protein synthesis inhibitors? and (6) Is it associated with action potential changes that might alter the voltage gradients contributing to the T wave?

## Methods

### Pacemaker Implantation

Control ECGs were recorded from 20 mongrel dogs of either sex weighing 22 to 27 kg. Anesthesia was induced with propofol 6 mg/kg IV, followed by inhalation of isoflurane (2%). Under sterile conditions, the chest was opened, the heart suspended in a pericardial cradle, and a Medtronic permanent pacing lead (model 6917) attached to the epicardium of the left atrium or the right or left ventricle. The lead was connected to a Medtronic MINIX 8340 pulse generator that was placed in a subcutaneous pocket. The incisions were closed, and the animals were allowed to recover for 2 to 3 weeks, during which time they were laboratory trained and the ECG stabilized.

Ventricular or atrial pacing was instituted (mode VVO; rate, 110 to 120 bpm; amplitude, 3.3 to 5 V; pulse width, 0.35 to 0.5 ms) at a rate 10% to 15% faster than that of the animal's sinus rhythm. All

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**Selected Abbreviations and Acronyms**

APD = action potential duration
HR = heart rate
LV = left ventricle, left ventricular
MAP = mean arterial pressure

ECGs were recorded with animals standing quietly in a sling. Every 2 to 3 days, the pacemaker was shut off for 1 hour and the ECG was recorded at the end of that period. For all ECGs, six limb leads and lead $V_{10}$ were acquired at a 500- to 1000-Hz sampling rate on a computer using PO-NE-MAH data acquisition software (Gould Instrument Systems, Inc) and the PC-EKG program (ISI, Inc). Frontal vector images were reconstructed using the pseudo-orthogonal lead system I-aVF-$V_{10}$. The distance from the origin of a vector loop to its most remote point was considered to be the amplitude of the QRS or T-wave vector, and the vector angle was calculated as the angle between this line and the 0 axis.

Twenty-four-hour monitoring was performed on four animals on random days to ensure reliable pacemaker capture. At no time was the extent of the capture <75%. Once cardiac memory developed and stabilized, pacing was discontinued and some animals were allowed to recover for 3 to 5 weeks. The others were anesthetized with sodium pentobarbital 30 mg/kg IV, and their hearts were removed and prepared for cellular electrophysiological study.

For studies of protein synthesis inhibition, cycloheximide (Aldrich Chemical Co) was given to three dogs. A cumulative dose totaling 2400 mg was administered over a period of 2 days (two subcutaneous injections) before pacing. We arrived at this dose on the basis of the work of MacLean and Ungar[4] and our own (unpublished) work on dose ranging in dogs and rats.

For studies of hemodynamics and of myocardial flow, four additional adult mongrel dogs weighing 24 to 27 kg were anesthetized with isoflurane (2%) and mechanically ventilated. A thoracotomy was performed in the left fifth intercostal space under sterile conditions. Tygon catheters (ID, 0.04 to 0.05 in; OD, 0.07 to 0.09 in; Cardiovascular Instrument Corp) were placed in the descending thoracic aorta, the apex of the LV, and the left atrium. Instrumentation for cardiac pacing was as described above, and pacing was performed from the anterior LV site.

After recovery from surgery, hemodynamic studies were performed as a baseline before and immediately after pacing was instituted. Recordings were made again at 3 weeks of continuous ventricular pacing and after pacing was discontinued. All hemodynamic measurements were made with the dog lying on its right side in a conscious state. The previously implanted fluid-filled Tygon catheters were connected to transducers (Statham Instruments, Inc) to measure aortic pressure (MAP), LV pressure, and left atrial pressure. The LV pressure signal was electronically differentiated (Differentiator Signal Conditioner, Gould Electronics) to measure LV dP/dt. The data were recorded on an eight-channel recorder (Gould model 3800). Mean values were derived for aortic pressure and left atrial pressure. To test $\beta$-adrenergic responsiveness, isoproterenol (Elkins-Sinn) was given as intravenous bolus injections at doses of 0.1 and 0.5 $\mu$g/kg, and the maximum changes of LV dP/dt, HR, and MAP were measured.

Regional myocardial blood flow was determined by injecting 2 mL of colored microspheres within 15 seconds into the previously implanted left atrial catheter. At the same time, a 15-mL sample of aortic blood was obtained by a steady withdrawal (Harvard Apparatus) at a rate of 7.5 mL/min for 2 minutes for determination of blood microsphere concentration. Cardiac pacing was then initiated. When hemodynamic parameters achieved a steady state, hemodynamic measurements and isoproterenol and colored microsphere (of a different color) injections were repeated. The pacer was then kept on for 3 weeks, after which the same experimental protocol was repeated with the heart paced and then at the spontaneous sinus rhythm (45 minutes after the pacemaker was turned off). Two additional colors of microspheres were injected for this protocol.

At the end of the entire experiment, the heart was removed, weighed, and cut into small (≈1 g) samples from three regions of myocardium: LV anterior wall, LV posterior wall, and septum. LV anterior and posterior wall samples were further cut into epicardial, midmyocardial, and endocardial layers. Tissue samples were digested and the microspheres were retrieved and then trapped in a polyester membrane filter (10 $\mu$m in pore size, 25 mm in diameter; Poretics Corporation). The color dye was then digested from the microspheres with the use of 100 $\mu$L of dimethylformamide. The photometric absorption of each 100-$\mu$L sample was then measured by a diode array ultraviolet/visible spectrophotometer (model 8452A; Hewlett-Packard Co). The composite spectrum of each dye solution was resolved at peak frequencies into the contributions from the individual colored spheres by use of a matrix inversion technique.[5] We calculated regional myocardial blood flow from the coronary circulation by determining microsphere concentrations from aortic blood samples in a similar manner for each colored sphere using the standard equation[5]

$$CBF_{reg} = (ABS_{reg} \times F_{ao}) / (\Sigma ABS_{ref} / m_{reg})$$

where $CBF_{reg}$ is the regional coronary blood flow, $ABS_{reg}$ is the absorbance of the specific color, $F_{ao}$ is the aortic withdrawal rate, $ABS_{ref}$ is the absorbance of the aortic blood sample, and $m_{reg}$ is the mass of the tissue sample. The analysis was performed with the use of a commercial software program (Triton Technology Inc) on an IBM computer.

## Microelectrode Methods

Dogs were anesthetized with sodium pentobarbital (30 mg/kg IV). Hearts were removed through a left lateral thoracotomy and immersed in cold Tyrode's solution equilibrated with 95% $O_2$–5% $CO_2$ and containing (in mmol/L) NaCl 131, $NaHCO_3$ 18, KCl 4, $CaCl_2$ 2.7, $MgCl_2$ 0.5, $NaH_2PO_4$ 1.8, and dextrose 5.5. Endocardial, epicardial, and transmural strips (≈1.5×1.0×0.1 cm) from the anterolateral LV, one third of the distance from base to apex, were filleted with surgical blades either parallel or perpendicular (transmural) to the surface.[6,7] Endocardial and epicardial preparations were obtained from positions directly opposite one another. The same region was studied in each animal, and it was ≥3 cm from the pacemaker site. The preparations were placed in a tissue bath, superfused with Tyrode's solution warmed to 37°C (pH 7.35±0.05), and allowed to equilibrate at a cycle length of 2000 ms. Solutions were pumped through the bath at a rate of 12 mL/min, with chamber content changed three times per minute. The bath was connected to ground with a 3 mol/L KCl/Ag/AgCl junction. Experiments were not started until preparations had fully recovered and displayed stable electrophysiological characteristics, which required 2 to 3 hours for endocardial and transmural strips and 4 to 6 hours for epicardial strips.

Transmembrane potentials were recorded with the use of 3 mol/L KCl-filled glass capillary microelectrodes (tip resistances of 10 to 20 MΩ) coupled by Ag/AgCl junction to an amplifier with high-input impedance and capacity neutralization (model KS-700, World Precision Instruments). Action potentials and $V_{max}$ were displayed on a digital storage oscilloscope (model 4074, Gould) and stored in digitized form in a personal computer for consequent analysis. $V_{max}$ was obtained by electronic differentiation with an operational amplifier, and the system was calibrated as previously described.[8] For stimulation of preparations, standard techniques were used to deliver 1- to 2-ms square-wave pulses 2.0× threshold through bipolar polytetrafluoroethylene-coated silver electrodes.[8]

## Statistical Analysis

Data are expressed as mean±SE. Significance of differences compared with control were determined by use of a one-way ANOVA followed by the Duncan multiple range test or Bonferroni's test when the F value permitted.[9] Statistical significance was determined at a value of $P<.05$.

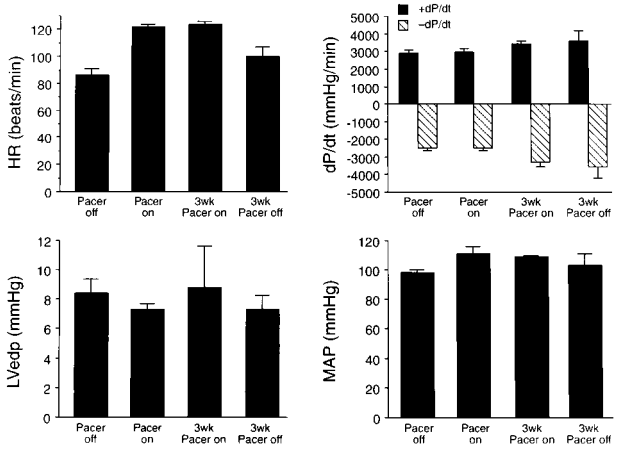


**Figure 1.** Hemodynamic studies of four dogs during a control period (Pacer off), immediately after the onset of ventricular pacing (Pacer on), on the last day of pacing (3 wk Pacer on), and 45 to 60 minutes after the pacemaker off was turned off (3 wk Pacer off). Data are presented for HR, LV dP/dt, LV end-diastolic pressure (LVedp), and MAP. No significant change was seen in any variable ($P>.05$).

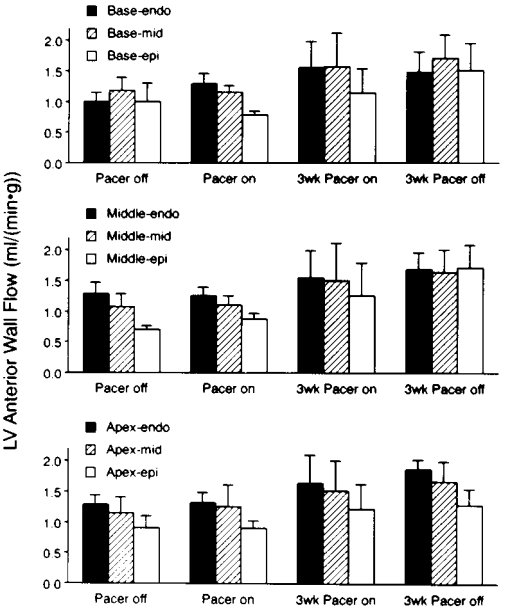


**Figure 2.** LV anterior wall flow measured in four dogs by use of microsphere techniques. Data are presented for endocardium (endo), midmyocardium (mid), and epicardium (epi) from the basal, mid, and apical LV anterior wall. Studies were done during control (Pacer off), immediately after the onset of pacing (Pacer on), on the last day of pacing (3 wk Pacer on), and 45 to 60 minutes after the pacemaker was turned off (3 wk Pacer off). No significant changes were seen ($P>.05$).

## Results

### Studies of Myocardial Pressures and Flows

Initially, we tested whether the pacing protocol induced cardiac failure or myocardial ischemia. Hemodynamic recordings made from dogs at baseline and after 3 weeks of cardiac pacing are summarized in Fig 1. In the baseline setting, resting HR averaged 86±5.1 bpm. Pacing was initiated at 122±1.7 bpm, which resulted in no statistically significant alteration in LV end-diastolic pressure, positive or negative $dP/dt_{max}$, or MAP. After 3 weeks of ventricular pacing, resting HR on return to sinus rhythm was 101±6.7 bpm ($P>.05$ versus rate before pacing). Importantly, there were no hemodynamic signs of decompensation of ventricular function as evidenced by the absence of changes in any of the hemodynamic values with the pacemaker turned on or off.

To further explore whether 3 weeks of ventricular pacing led to any detectable signs of heart failure, hemodynamic responses to bolus infusions of isoproterenol (0.5 μg/kg) were determined before the onset of pacing and at the end of 3 weeks of pacing with the pacemaker turned off. There were no significant differences in percent changes in $dP/dt_{max}$ (125±21% versus 131±21%) or in MAP (−40±3% versus −36±0.2%). Peak HRs observed after the bolus infusion were also insignificantly different between the two conditions (237±10.2 versus 215±18.4 bpm).

As detailed in "Methods," regional myocardial blood flow was assessed with the use of colored microspheres. Results obtained from the basal, middle, and apical regions of the anterior LV wall, which were further subdivided into endocardial, midmyocardial, and epicardial zones, are summarized in Fig 2. As shown, flow was little affected 45 minutes after the pacemaker was turned on. After 3 weeks of pacing, there was a nonsignificant trend for flow to increase in all regions. Finally, flow was not further affected 45 minutes after the pacemaker was turned off. Similar data were obtained from the posterior LV wall and from the septa of these same hearts (data not shown). Thus, these data indicate that myocardial ischemia did not develop during the pacing period and therefore did not contribute to the electrophysiological changes observed in these hearts.

### ECG Studies

The time course of typical ECG evolution during cardiac memory induction is shown in Fig 3. Direction, magnitude, and evolution of the T-wave change differ in any given lead, limiting the value of any form of quantification of a single lead. In Fig 4, data from the same dog as in Fig 3 are presented using a frontal plane vectorcardiogram. Here, a distinctive pattern of counterclockwise T-wave vector rotation is readily seen. The record in Fig 4 is representative of

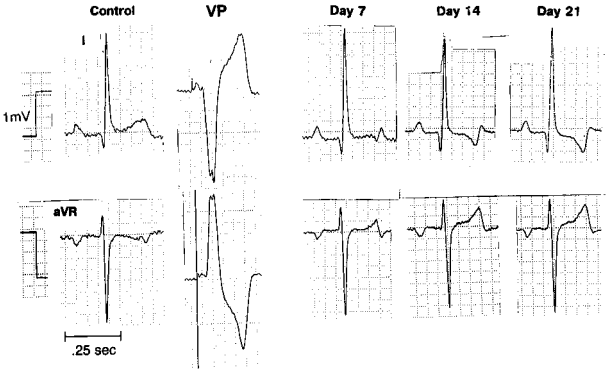


**Figure 3.** Cardiac memory evolution over 21 days of ventricular pacing (VP). Depicted are leads I and aVR from one dog during control, during VP, and at the end of 1 hour after pacing was discontinued on days 7, 14, and 21. In both leads, evolution of the T wave is such that it tracks the vector of the paced QRS complex.

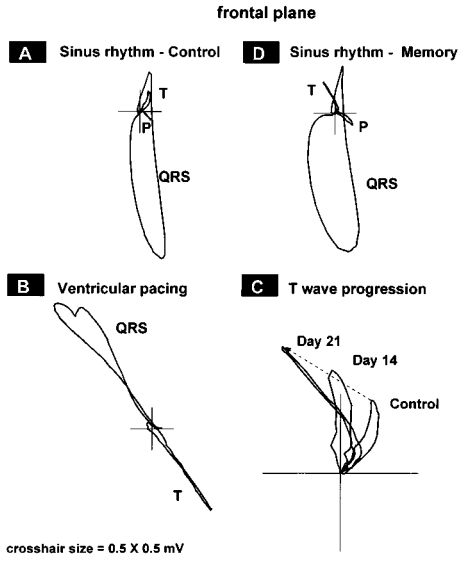


**Figure 4.** Frontal plane vectorcardiographic depiction of cardiac memory from the same dog presented in Fig 3. A shows P, QRS, and T-wave vectors in control, before the onset of pacing. B, The QRS and T vectors during ventricular pacing. C, The T-wave vector alone (note enlarged scale as well as dotted line indicating the change in vector) during sinus rhythm in control (identical to A) and on days 14 and 21. Note the increase in amplitude of the vector as well as the shift in vector angle from that in control to one that approximates the QRS vector during pacing (B). D, A record made within 1 hour of returning to sinus rhythm on day 21.

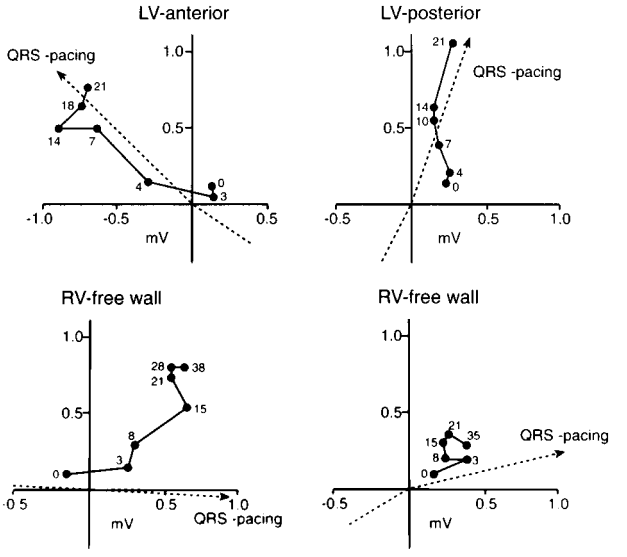


**Figure 5.** Relationship between pacing site and direction of the sinus rhythm T-wave vector change in the frontal plane vectorcardiograph of four dogs, paced respectively from the anterior and posterior LV and the right ventricular free wall. The broken line and arrow depict the paced QRS vector angle in each experiment. The solid circles represent the vector angle and amplitude in control (indicated as 0) and on individual days during which pacing was discontinued for 1 hour and T-wave vector angle and amplitude were recorded. Hence, in the first animal, (LV anterior) recordings from days 0, 4, 7, 14, 18, and 21 are presented. Note that in the animals paced from the LV sites, the T-wave vector increases in amplitude through day 21, and its angle approximates that of the paced QRS complex by day 4 to 7. With right ventricular pacing, vector amplitude again increases and the vector angle approaches that of the paced QRS complex but not with the same precision as seen during LV pacing.

the changes seen in 13 dogs, all of which were paced from the anterior wall of the LV. The distinctive pacing-induced ECG pattern had a wide QRS complex directed upward and rightward (mean frontal angle, −140°) and a wide QRS-T angle (approaching 172°); see Table 1 and Figs 3 and 4B.

As the duration of ventricular pacing increased in all animals, a gradual alignment of the sinus rhythm T-wave vector with the paced QRS vector occurred. This resulted in rotation of the sinus T-wave vector toward the paced QRS vector (ie, opposite to the paced T-wave vector (Fig 4 and Table 1). In addition to the T-wave vector angle, the magnitude of the vector increased as memory evolved (Table 1 and Fig 4). The correlation between the magnitude of the paced QRS vector and the magnitude of the T-wave vector was 0.48 ($P<.05$). Finally, whereas the T-wave vector was altered considerably, no consistent changes in the QT or $QT_c$ intervals occurred (Table 1).

To further test the association between the paced QRS-T axis and the final T-wave vector, we performed additional

**TABLE 1. ECG Sequelae of 21 Days of Ventricular Pacing (13 Dogs Paced From the Anterior Wall of the LV)**

| | Control Sinus Rhythm | Day 21+ Ventricular Pacing | Day 21 Sinus Rhythm |
|---|---|---|---|
| RR, ms | 611±17 | 519±8* | 586±22 |
| QT, ms | 224±4 | 221±5 | 220±4 |
| $QT_c$ | 288±4 | 313±4* | 289±4 |
| QRS vector magnitude, mV | 2.1±0.15 | 3.0±0.20* | 2.1±0.17 |
| QRS vector angle, degrees | 34.1±6.4 | −139.8±2.7* | 34.8±8.3 |
| T-vector magnitude, mV | 0.39±0.05 | 2.02±0.15* | 0.81±0.09* |
| T-vector angle, degrees | −37.0±18.3 | 35.2±3.8* | −144.0±3.5* |
| QRS-T angle, degrees | 94.6±9.8 | 172.3±1.3* | 160.9±4.0* |
| Paced QRS–sinus rhythm T-wave angle, degrees | 82.5±7.4 | NA | 9.1±2.4* |

*$P<.05$ compared with control. Values are mean±SE.

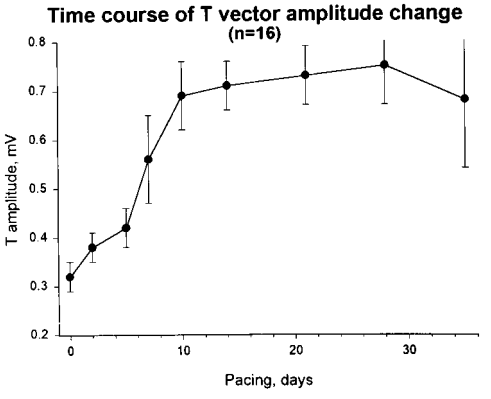


**Figure 6.** Time course of sinus rhythm T-wave vector amplitude expressed as the absolute T-wave vector amplitude in 16 dogs. Note the attainment of a steady state in ≈10 days.

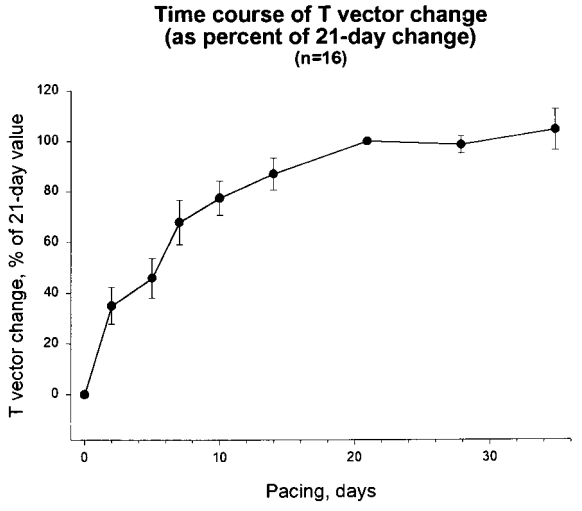


**Figure 7.** Time course of sinus rhythm T-wave vector change expressed as percent of the value at 21 days in the same 16 dogs as in Fig 6. The T-wave vector on day 21 was expressed as 100% and that before pacing as 0%. The value on any day between control and the end of the experiment was expressed as percent of the day 21 to 0 distance value. Using Fig 4C as a reference point, the day 21 value is 100%, the control value is 0%, and the day 14 value would be expressed as a percent of the distance. Note that when this method, which incorporates amplitude and vector angle in the measurement, is used, a steady state is reached by ≈3 weeks.

experiments using pacing sites on the posterior wall of the LV or the right ventricular free wall. As expected, changes in pacing site resulted in different paced QRS-T axis directions (Fig 5). Nonetheless, in all animals, the T-wave vector in sinus rhythm was still influenced by the vector of the paced QRS complex.

The time course of T-wave vector change is plotted in Figs 6 and 7. Fig 6 demonstrates the absolute changes in T-wave vector amplitude. Note that a steady state is reached in ≈10 days. In Fig 7, the overall change in T-wave vector (the difference between the control T-wave vector and that in sinus rhythm after a period of ventricular pacing) is plotted. This variable incorporates both vector angle and vector amplitude and requires ≈21 days to attain a steady state.

Fig 8 shows the time course of memory dissipation in two groups of dogs. The first group was paced for 21 to 25 days. A steady state of memory was maintained for 2 days, and then

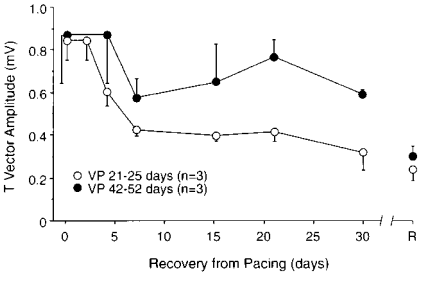


**Figure 8.** Time course of memory dissipation presented as the change in T-wave vector amplitude from the day of cessation of pacing (0) through 30 days of recovery. Results from two groups of animals are presented. The reference (R) T-wave value on the right is that recorded before the onset of pacing. See text for discussion. VP indicates ventricular pacing.

**TABLE 2. ECG Sequelae of 21 Days of Atrial Pacing (4 Dogs Paced From Right Atrial Appendage)**

| | Control Sinus Rhythm | Day 21 Atrial Pacing | Day 21 Sinus Rhythm |
|---|---|---|---|
| RR, ms | 688±83 | 500±0* | 720±142 |
| QT, ms | 234±9 | 219±10* | 238±7 |
| $QT_c$ | 284±8 | 310±14 | 287±19 |
| QRS vector magnitude, mV | 2.0±0.16 | 2.1±0.25 | 2.1±0.18 |
| QRS vector angle, degrees | 57±10.5 | 57±11.7 | 62±11.6 |
| T-vector magnitude, mV | 0.39±0.08 | 0.39±.04 | 0.36±0.06 |
| T-vector angle, degrees | −94±68 | −155±9* | −62±79 |
| QRS-T angle, degrees | 118±40.1 | 148±13.8 | 136±26.9 |

*$P<.05$ compared with control. Values are mean±SE.

recovery to levels near control was achieved by 7 days. The second group was paced for 42 to 52 days. Here, a steady-state level of memory persisted for 4 to 5 days. Memory remained for the entire period of observation such that at 30 days, the T vector still differed from control. This stresses the important role of accumulation in the persistence of the memory process. It also demonstrates that whereas the magnitude of the peak pacing-induced T-wave changes was similar in both groups, these changes persisted longer in the group that was paced longer.

As a control for the effects of increasing HR alone, we performed atrial pacing in another group of four dogs (Table 2). Atrial pacing at the same rates and durations comparable to those in the ventricular pacing protocol induced no significant change in vector angle and amplitude and no memory. Hence, atrial pacing, inducing a change in HR without a change in activation pathway, did not induce cardiac memory.

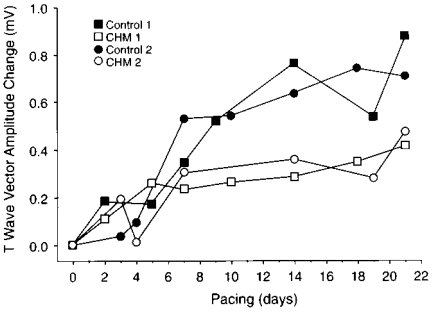


**Figure 9.** Effects of administration of cycloheximide (CHM) on evolution of cardiac memory in one dog. T-wave vector amplitude change is used as the form of measurement here. Four consecutive experiments were done. The sequence was control 1–CHM 1–control 2–CHM 2. For control 1, a standard protocol was used to induce memory. Note the evolution of a steady state in T-wave amplitude by about day 14. Complete resolution of the memory was then permitted, after which the animal was administered CHM and pacing was reinstituted. The result was only partial induction of memory, as seen in a curve that was displaced downward and to the right of control. Resolution of the memory process was permitted, after which a second control experiment was done. The results were nearly identical to the initial control. After resolution of the memory, a second course of CHM was given, and the pacing protocol again was repeated. In this case, the result was essentially identical to that of the initial CHM experiment.

**TABLE 3. Epicardial, Endocardial, and Midmyocardial Cell Recordings From Control Dogs and Dogs LV Paced for 21 Days and Manifesting T-Wave Memory**

| | n | MDP, −mV | Os, mV | Phase 1 Notch, mV | $APD_{50}$, ms | $APD_{90}$, ms |
|---|---|---|---|---|---|---|
| Control | | | | | | |
| Epi | 16 | 88±1† | 12±2† | −3±2 | 190±10† | 241±9† |
| M | 24 | 90±1 | 20±2 | 0±3 | 285±9 | 346±12 |
| Endo | 18 | 88±1 | 24±2 | ‡ | 201±6† | 265±5† |
| Memory | | | | | | |
| Epi | 30 | 84±1*† | 11±2† | 1±1* | 231±5*† | 273±5*† |
| M | 20 | 87±1* | 19±1 | 2±2 | 299±13 | 336±12 |
| Endo | 22 | 85±1* | 28±2† | ‡ | 229±5*† | 296±12* |

MDP indicates maximum diastolic potential; Os, phase 0 overshoot; Epi, epicardial; M, midmyocardial; and Endo, endocardial.

Preparations were equilibrated for 6 hours in Tyrode's solution with $[K^+]_o$=4 mmol/L; basic cycle length=2000 ms.

*$P$<.05 compared with control; †$P$<.05 compared with M within each group.

‡The endocardium had no notch.



The next set of experiments made use of cycloheximide to test the role of new protein synthesis in the genesis of cardiac memory. In one of the three animals studied, four sequences of ventricular pacing were performed. Between each run, the animal remained in sinus rhythm for a time sufficient for the T wave to return to control levels. Before the second and fourth runs, cycloheximide was administered as described in "Methods." Note in Fig 9 the evolution of a reduced magnitude of T-wave vector change with cycloheximide treatment compared with control. Note as well that the two cycloheximide runs were entirely reproducible, as were the two control runs. The other cycloheximide-treated dogs underwent a single drug trial preceded and followed by control trials. Results were similar to the above: at day 21 of pacing after cycloheximide administration, the magnitude of the T-wave vector change for the three animals was 63±8% of that in the control run ($P$<.05).

## Cellular Electrophysiological Studies

Cellular electrophysiological data for epicardial, endocardial, and midmyocardial cell preparations from control and long-term–memory animals are presented in Table 3. Cardiac memory resulted in prolongation of the epicardial and endocardial APDs in the absence of any change in the midmyocardial cells. These changes would significantly alter the transmural gradient for repolarization and would be expected to contribute to the T-wave changes that characterize memory on the ECG. Also noted were small but significant reductions in maximum diastolic potential (MDP) in all three cell types.

The relationships between the changes in APD in epicardium and endocardium are further explored in Fig 10. Multiple recordings from an LV epicardial and endocardial slab of a control animal appear in Fig 10A; Fig 10B shows records from slabs comparably located from an animal with cardiac memory. In both panels, the effects of changing pacing cycle length on $APD_{50}$ and $APD_{90}$ are demonstrated. In the setting of memory, the prolongation of APD was such that the endocardial and epicardial durations become more similar to one another than was the case in the control condition. In Fig 10, this change in gradient is indicated by the cross-hatched area inscribed between the epicardial and endocardial $APD_{50}$ and $APD_{90}$ curves. In the control animal, the epicardial area only minimally overlapped that from the endocardium. In the memory setting, the two areas were largely overlapping, reflecting a lesser gradient than in control.

## Discussion

The first question posed in this study related to the possibility that myocardial ischemia or failure might contribute to the occurrence of memory. It has been important to discriminate between memory and ischemia clinically because the ST-T–wave changes of ischemia can be mimicked by those of memory.[1,2] It is equally important to determine whether

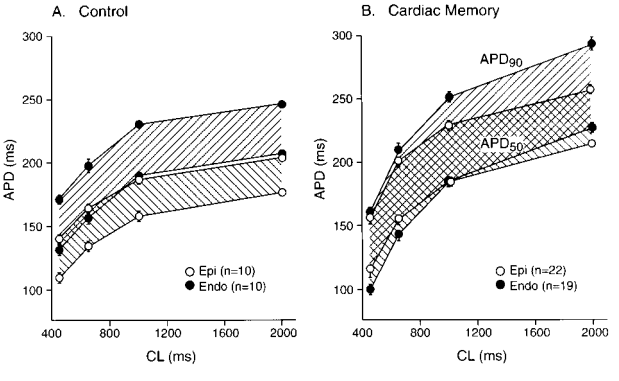


**Figure 10.** APD to 50% ($APD_{50}$) and 90% ($APD_{90}$) of repolarization measured from epicardium (Epi) and endocardium (Endo) of LV in a control dog (A) and a dog with cardiac memory (B). Preparations were paced over a range of cycle lengths (CL; horizontal axis) from 2000 to 400 ms. Three to five minutes was required for equilibration at each cycle length. Recordings were made at 10 minutes. Three sets of preparations were used per heart, and multiple impalements were made per preparation. The shaded areas are those inscribed between $APD_{50}$ and $APD_{90}$ of the epicardial and endocardial preparations. In A at almost all cycle lengths, almost all values for endocardium differ from those at corresponding cycle lengths in epicardium ($P$<.05 for all). In B, statistical significance ($P$<.05) was seen only for $APD_{90}$ at the two longest cycle lengths. See text for discussion.

failure or ischemia occurs in this canine model, because prior studies[10] have shown that chronic pacing of the canine heart at critical rates does in fact induce failure and repolarization changes. Had this been the case in the present study, the conclusions concerning cardiac memory would have been invalidated. Our microsphere studies demonstrate that myocardial flow was not diminished to any area of the ventricles. Moreover, there was no change in hemodynamics, indicating that congestive heart failure was not of concern. In another study,[11] we measured the capacitance of ventricular myocytes from dogs with cardiac memory induced by use of the same protocol as in the present study (H. Yu, PhD, and I. Cohen, MD, PhD, preliminary data, 1997). Capacitance was 107±17 pF in the control group and 105±18 pF in the memory group, indicating that myocyte size had not changed. Hence, it is reasonable to conclude that the pacing protocol induced a change in electrical function unassociated with deterioration of flow or contractile performance or with myocyte hypertrophy. These findings emphasize that the T-wave changes of cardiac memory are clearly distinguishable in origin from those in a variety of pathological conditions that induce both structural and electrophysiological changes. However, they do not rule out the possibility that cardiac memory is an early marker of a pathological process that is not yet expressed at a macroscopic level, that is reversible, and whose identification may suggest the need for early intervention and prevention.

Figs 6 through 8 demonstrate that both the accumulation and resolution of cardiac memory can be quantified accurately. Earlier studies, including our own,[12] have quantified alterations in T-wave amplitude or area as the prime descriptors of memory. However, it is clear from data such as those shown in Fig 3 that the use of single (or even several) ECG leads can give varying results concerning the magnitude and extent of the memory process. In contrast, vectorcardiography provides an opportunity to consider the T-wave vector angle and amplitude concomitantly in a way that is readily and consistently measurable and that is concordant with the original descriptions of cardiac memory in human subjects[1]; that is, the vector of the "memory T wave" during sinus rhythm tracks the vector of the paced QRS complex. The latter either completely or partially predicts the former, with the magnitude and direction of change apparently reflecting the site of origin of ventricular activation. In all instances, the increase in vector amplitude is a consistent phenomenon, reaching a steady state in ≈3 weeks. The resolution of the process clearly is slower than its accumulation, as shown in Fig 8.

There are a number of determinants of the electrical gradient that underlie the Q-T interval and T wave, including the apico-basal[13,14] and epicardial-endocardial[15] differences in ventricular myocardial action potentials, whose timing relative to one another is also influenced by the activation sequence of the ventricle. In the present study, we have deliberately focused on the epicardial-endocardial gradient in one region (the LV anterobasal free wall) as well as on the role of activation. With respect to the epicardial-endocardial gradient, the major effect of the ventricular pacing protocol is to change the voltage-time course of repolarization in endocardium and epicardium but not midmyocardium, such that the differences in repolarization time among the three are diminished. We must emphasize that these action potential data can be interpreted in a limited context only. Although we studied epicardium, endocardium, and midmyocardium, this was at one site only in the LV. As such, although it is clear that long-term memory is associated with action potential changes, the extent to which gradients for repolarization have been altered across the entire mass of the left and right ventricles is not considered. Such information awaits detailed mapping studies of ventricular repolarization in the setting of memory.

Our earlier work[11,12] and reports in the literature[16] suggest that the induction of memory is based on fundamental alterations in a subset of ion channels that determine the voltage-time course of repolarization. One of the determinants of the action potential that is altered is the phase 1 notch.[17] In preliminary studies, we have shown that the transient outward current, $I_{to1}$, which is a major determinant of the notch, has altered activation kinetics[11] and that its density and the mRNA for its channel protein Kv4.3 are significantly reduced in long-term cardiac memory (H. Yu, PhD, et al, preliminary data, 1997). Fig 8 suggests that these alterations, once in place, persist for long periods. It is important to stress, however, that we do not believe Kv4.3 is the one channel uniquely involved in the memory process. The fact that repolarization is prolonged in epicardium (which has a large $I_{to1}$[18–20]) and endocardium (which has a small $I_{to1}$[20]) and is unchanged in midmyocardium (which has a prominent $I_{to1}$) together suggests that additional channels play an important role. Candidates include $I_K$, $I_{Ca,L}$, $I_{K1}$, and $I_{Na}$. A reduction in $I_{K1}$ could also explain the reduction in membrane potential demonstrated in the memory setting (Table 3). Another possible explanation for the reduction in membrane potential is the finding by our associates (J. Gao, PhD, and I. Cohen, MD, PhD, preliminary results, 1997) that Na/K pump current is reduced in the setting of memory.

The other factor contributing importantly to the gradient for repolarization is the ventricular activation pathway. For this reason, it was important to determine the extent to which cardiac memory is influenced by changes in HR alone as opposed to the combination of altered rate and activation pathway. It is apparent from Table 2 that a change in sinus rate alone is insufficient to significantly alter the T-wave vector. When Tables 1 and 2 are compared, it is clear that the changes induced by an altered pacing rate plus an altered activation pathway significantly exceed those resulting from a rate change alone. Therefore, we conclude that the activation pathway is critical to the expression of long-term memory.

It is of particular interest that the time course of onset of cardiac memory is modified by cycloheximide. Our reason for using cycloheximide was in part the potential analogy that exists between memory in heart and memory in central nervous system. In the central nervous system, memory is a process of long-term potentiation induced by repetitive exposure to a signal that results in new protein synthesis.[21,22] The process is common to complex mammalian life forms as well as to far simpler organisms such as aplysia. Neuroscientists have relied on cyclo-

heximide as an inhibitor of new protein synthesis,[23,24] using it to differentiate the mechanisms responsible for long-term memory (requiring new protein synthesis) and those responsible for short-term memory (dependent on channel phosphorylation).[22] The dose of cycloheximide we used was derived from experience by others in the dog[4] and from our own preliminary dose-ranging experiments in rat and dog (data not reported). Although we did not measure any independent descriptor of new protein synthesis, the literature clearly provides evidence for the expectation of its inhibition.[23,24] When cycloheximide was administered, the evolution of memory was significantly delayed. Moreover, as shown in Fig 9, both the effect of cycloheximide and the recovery from its effect were reproducibly demonstrable in the same animal. Therefore, it would appear that new protein synthesis does play a role in the evolution of long-term memory. The caveat here is the nonselective nature of the activity of cycloheximide: it inhibits synthesis of a broad spectrum of proteins.

Given the absence of any structural remodeling or deterioration of coronary flow or mechanical function, we assume that the expression of new protein synthesis in memory is manifested uniquely at the subcellular level. This is of particular importance in evaluating memory-associated changes in action potential in light of our preliminary description of changes in Kv4.3 mRNA and functional properties of $I_{to1}$ that accompany the memory process (Reference 11 and H. Yu, PhD, et al, preliminary data, 1997). These observations support the speculation that fundamental alterations in channel density as well as protein structure may occupy the induction of long-term cardiac memory.

In conclusion, long-term cardiac memory is a clearly defined alteration in the T wave induced in settings in which ventricular activation is changed in the absence of myocyte hypertrophy, cardiac ischemia, or hemodynamic deterioration. Its expression can be delayed by inhibition of new protein synthesis, and it is associated with changes in the action potential that appear to reflect alteration in specific ion channels. Definition of the role of the memory process in the modulation of the effective refractory period and of cardiac arrhythmias and further definition of its transduction pathways and molecular determinants are needed. Finally, we stress that results of the present study relate entirely to the process of long-term cardiac memory, a process requiring days to weeks for its induction and weeks to months for resolution. They are not to be confused with the changes seen in short-term memory, which requires minutes to induce and minutes to hours for resolution.[12,25]

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# Evolution and Resolution of Long-term Cardiac Memory

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