

# Stability of Ventricular Repolarization in Conscious Dogs with Chronic Atrioventricular Dissociation and His-Bundle Pacing

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**SANDERS, R., ET AL.: Stability of Ventricular Repolarization in Conscious Dogs with Chronic Atrioventricular Dissociation and His-Bundle Pacing.** *Following AVN ablation, eight dogs were surgically instrumented for chronic (continuous) HIS-bundle pacing. For data collection, implanted pacemakers were transiently programmed to pace in stepwise ascending ramps at rates from 50 to 200 beats/min in 30-beat/min steps. Each rate was held for 60 seconds. At each rate, ECG signals were collected from conscious dogs for measurements of QT intervals during the last 10 seconds of each paced rate to construct a QT-HR ramp. This QT-HR ramp was repeated twice on each day of study 10 minutes apart. Dogs were randomly assigned to two groups and studied weekly for a minimum of 6 weeks. Group 1 dogs had pacemakers programmed to a rate of 80 beats/min for the duration of the study. Group 2 dogs were paced at 80 beats/min for weeks 1–3, then increased to 140 beats/min for weeks 4–6. The difference between paired QT-HR ramps within 1 day was <3 ms. QT-HR ramps were statistically indistinguishable over the 6-week study for group 1 dogs. Group 2 dogs experienced a slight flattening in the slope of the QT-HR ramps from week 3 to week 6 due to a reduction in QT interval at low HRs (50 and 80 beats/min) only. This conscious HIS-pacing model in dogs is a sensitive, stable, and reproducible method to define ventricular repolarization characteristics over a range of programmable HRs and experimental conditions. (PACE 2004; 27:1475–1483)*

## **His-pacing, QT interval, repolarization**

### **Introduction**

Changes in cardiac repolarization are potential warning signs of proarrhythmic states, particularly those associated with drug exposure. The QT interval is a simple measurement that can serve as a convenient, though imperfect marker for altered repolarization times. It is routinely evaluated in preclinical tests and clinical trials as part of drug development. Despite its value, the QT interval is confounded by substantial heart rate (HR) dependencies that can mask or mimic drug related effects. The authors recently described an anesthetized, canine model of atrioventricular (AV) dissociation and His-bundle pacing to facilitate investigations of ventricular repolarization characteristics typified by the QT-HR relationship.<sup>1</sup> The inherent limitations of an acute anesthetized model prompted modifications to permit chronic evaluations in conscious dogs. This article includes a description of the adaptation to a conscious model, an evaluation of the stability of the

model over 6 weeks of study, a preliminary assessment of the effects of programmed daily pacing rate on the QT-HR relationship, and an echocardiographic evaluation of gross cardiac structural remodeling.

### **Methods**

#### **Animals**

Adult purpose-bred beagle dogs were used for this study (7 males, 1 female). Dogs were housed individually and cared for in accordance with United States Department of Agriculture (USDA) and National Institutes of Health (NIH) guidelines for animal welfare. Inclusion criteria included normal resting eight-lead electrocardiograms (ECGs) and echocardiograms.

#### **Surgical Instrumentation**

Following an 8-hour fast, dogs were sedated with an intramuscular injection of butorphanol (0.1 mg/kg) and acepromazine (0.02 mg/kg). General anesthesia was induced with an intravenous injection of propofol (6 mg/kg) and maintained by an inhaled mixture of isoflurane and oxygen. Introducer sheaths (7 Fr, Cordis, Warren, NJ, USA) were percutaneously inserted in the right jugular and left femoral veins. Using single-plane, lateral projection fluoroscopic guidance, a 5 Fr temporary transvenous pacing lead was advanced through the jugular sheath to the right ventricular apex. A 7 Fr ablation catheter (EP Technologies, Boston

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Supported in part by a grant from Pfizer Global Research and Development and the generous donation of pacemaker generators from Medtronic, Inc., Minneapolis, Minnesota.

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Received October 28, 2003; revised December 19, 2003; accepted July 8, 2004.

Scientific, Sunnyvale, CA, USA) was advanced through the left femoral vein to record the highest amplitude His potential, then withdrawn slightly caudal (posterior) to the presumed vicinity of the AV node (AVN). Radiofrequency energy was applied to a tip temperature of 65°C until complete AV dissociation was evident. Most dogs required only a single, short (30 s) application of heat to achieve permanent AV dissociation. Total radiation exposure time was consistently <5 minutes. Endocardial right ventricular pacing was initiated using a temporary generator (Model 5320, Medtronic, Inc., Minneapolis, MN, USA). Existence of remaining functional His-bundle tissue was confirmed by temporary endocardial His-bundle pacing, demonstrating an S-V interval within 5 ms of the preablation H-V interval, and a normal QRS configuration in surface lead ECGs.

Dogs were moved to an adjoining surgical suite and prepared for a right fifth intercostal thoracotomy. The chest and pericardium were opened to expose the lateral wall of the right atrium. A custom-made, active-fixation permanent pacing lead was inserted into the right atrium through a purse-string atriotomy. With a temporary pacing generator, stimuli were applied to the tip of this lead. The lead tip was manipulated until paced beats had a normal QRS configuration indicative of His capture. A capture threshold  $\leq 3$  mA, lead impedance  $< 500 \Omega$ , and a QRS duration  $\leq 50$  ms characterized optimal positioning. Once optimal placement was identified, the lead was fixed in position. The free end of the lead was tunneled subcutaneously to a site between the scapulae and connected to an implanted generator Kappa, Sigma, Thera series, single chamber generators (Medtronic, Inc.). Total surgery time improved during the study stabilizing to a minimum of approximately 2 hours.

His-pace generators were initially set to a fixed rate of 80 beats/min. Dogs were allowed at least 14 recovery days before beginning data collection. During recovery, dogs were trained to lie comfortably in a sling. Baseline ECGs were periodically checked to confirm His capture. Generators were interrogated for shifts in lead impedance and threshold voltages. Adjustments were made as needed to keep stimulus strengths approximately 2 V above threshold. Of 12 dogs instrumented for this study, 8 were in good His capture for 2–4 weeks prior to the beginning data collection. Four dogs were excluded because of premature lead fractures and failure to capture.

### Data Collection and Analysis

#### *Echocardiograms*

Dogs were sedated with an intramuscular injection of butorphanol (0.1 mg/kg) and acepro-

mazine (0.02 mg/kg) and paced at a fixed rate of 80 beats/min. Standard two-dimensional and m-mode images were obtained from the right parasternal window using a 5-MHZ probe (ATL Ultrasound, HDI 5000.). Measured indices of heart size and function included short-axis diastolic epicardial area defined by an ellipse bounded by the epicardium of the lateral left ventricular wall and the right endocardial surface of the septum, the short-axis end-diastolic and end-systolic left ventricular area, end-diastolic and end-systolic left ventricular internal diameters, and the short-axis diameter of the body of the left atrium. Left ventricular cross-sectional myocardial area was used as an index of hypertrophy and was calculated as the difference between epicardial area defined above, and the diastolic left ventricular area. Echocardiograms were performed presurgically, and again during weeks 1, 3, and 6 of the experimental protocol. Echocardiographic exams were performed at least 24 hours prior to scheduled ECG data collections to eliminate the effects of sedation on recorded ECGs.

#### **ECG Data**

Recorded ECGs consisted of eight lead surface ECGs including the six bipolar limb leads, and  $V_1$  and  $V_4$  unipolar leads. Lead  $V_1$  was centered half way between the costo-chondral junction and the sternum in the right fifth intercostals space. Lead  $V_4$  was positioned at the costochondral junction in the left sixth intercostals space. Both positions were marked with indelible ink or a skin tattoo to facilitate consistent placement over the 9 weeks of study. ECG data were collected from conscious unsedated dogs passively restrained in a sling. Analog ECG signals were digitized at 1 KHz and stored for later analysis using commercial data acquisition software Dataq Instruments, Inc. WINDAQ Pro acquisition software.

#### **QT Intervals**

QT intervals were measured manually for all dogs by a single interpreter (R.S.) to the nearest millisecond from the digitized ECG signal using on-screen cursors. The end of the T wave was defined as the zero-crossing of the first derivative of the original ECG signal. In dogs, precordial leads typically provide T waves with the most discrete transition to baseline. The study typically used lead  $V_1$  for QT measurements; in each dog the same lead was used throughout the study.

For the subset of dogs in group 1 (see below), RT intervals, defined as the time between peak of the R wave and end of the T wave, were measured for the same single lead used in the manual measurements using an automated data analysis system (EMKA Technologies Inc, ECGAUTO data

analysis software.) For this system, a dog-specific library of beats with user-defined R peak and T end was used to automatically measure the RT intervals. The end of the T wave was again defined as the zero-crossing of the first derivative of the ECG signal. The EMKA analysis system is based on a proprietary shape-based algorithm that is immune to the effects of a pacing artifact and occasional P wave encroachments on the body of the T wave. New waveform shape libraries were constructed for each dog and day of data collection. An average of 3 (range 1–5) waveforms were used for each data set. Each automated measure was over-read by a single observer (N.B.O.) and excluded if the automated measure was obviously in error. The average RT interval for 5–10 beats during the last 15 seconds at each paced rate was used only to compute estimates of statistical power over the 6-week course of the study.

QT-HR relationships were constructed to examine the effects of changing HR on the QT interval. Pacing generators were transiently programmed to deliver stimuli at preestablished ramped steps in rate beginning at 50 beats/min, and increasing in 30-beats/min steps to a maximum of 200 beats/min. Each paced rate was held for 1 minute. At each paced rate, QT intervals were measured for a minimum of 3 beats during the last 10 seconds of the 60-second ramp-step. Beats were excluded if the dissociated P-wave coincided with the end of the t wave.

### Experimental Protocols

ECG data were collected once weekly for 6 consecutive weeks. To evaluate the within-day reproducibility of the model, the study compared paired QT-HR ramps generated on the same day, separated by 10 minutes. Comparing weekly RT-HR relationships for group 1 animals over the 6-week experimental protocol assessed long-term reproducibility of the model. An averaged RT-HR relationship constructed from the paired daily ramps was used for this assessment.

To evaluate the effect of background daily pacing rate, dogs were randomly assigned to one of two 4-dog groups. The experimental data collection protocols described above were identical for both groups. Groups differed only in their background (24 hour) programmed pacing rate. Group 1 dogs were kept at daily pacing rate of 80 beats/min for the entire 6-week protocol. Group 2 dogs were paced at 80 beats/min until just after the third week of data collection when their daily rates were increased to 140 beats/min for weeks 4–6. In a subset of two group 2 dogs, the pacing rate was restored to 80 beats/min for 3 additional weeks following the sixth week of data collection. QT-HR relationships

were constructed as described above in the ninth week for these two group 2 dogs.

### Statistical Analysis

Echocardiographic indices were compared individually across weeks using a repeated measures ANOVA. Paired ramps of QT-HR data generated within a day were compared using a repeated measures ANOVA with paced rate representing the single, five-level repeated factor. Weekly QT-HR data were compared using a grouped, repeated measures ANOVA. For this analysis, two repeated measures were included, paced rate for the ramp, and ramps for each of the 6 weeks of the protocol.

For long-term stability comparisons, only the second repeated factor (weeks) was evaluated. Statistically significant F-ratios for time, group, or the group time interactive effect were investigated in more detail using orthogonal linear contrasts.

Estimates of statistical power as a function of sample size were calculated using commercial software designed for this purpose. These calculations were based on a normalized effect size calculated from the ratio of the desired detectable difference in QT interval (ms), to the population standard deviation estimated by the  $\sqrt{MS_{error}}$  from the applicable ANOVA.<sup>2</sup> For within-a-day effects, statistical power estimates were based on hand-measured QT intervals. For estimates of statistical power to detect a change over 6 weeks, the automated measures of RT interval for the subset of group 1 dogs were used.

All statistical calculations were performed using commercial software (Statistica version 6.1, StatSoft Inc., Tulsa, OK, USA) with a nominal type I error rate of 5%.

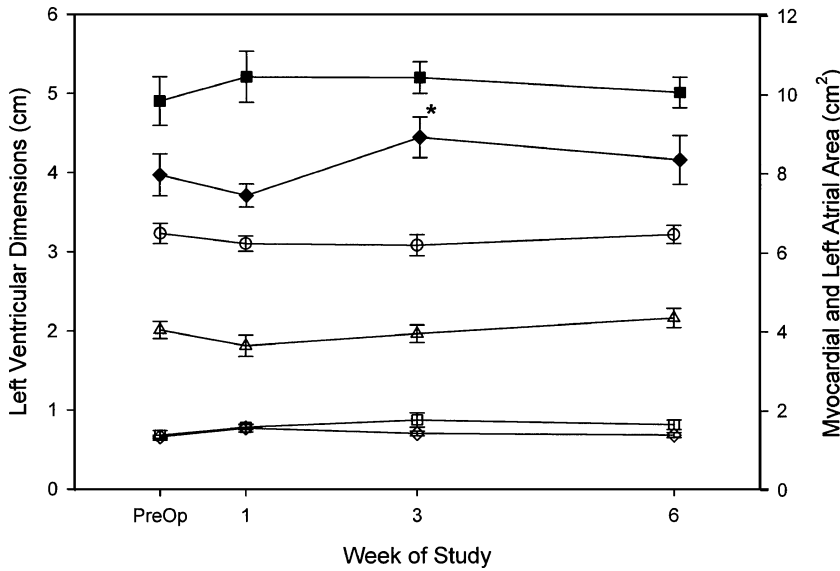
## Results

### Technical Aspects of Model

Following a transient increase just after implantation, lead impedance and threshold voltages were stable for the remainder of the study. All animals that entered the study completed the 6-week main study with stable (normal) QRS duration and morphology.

### Structural Remodeling

Figure 1 summarizes selected echocardiographic indices of left ventricular and left atrial size. There was no significant change in left ventricular end-diastolic or end-systolic size. There was no significant change in myocardial mass estimated from the short-axis myocardial area. His-bundle pacing had no significant effect on left ventricular systolic performance estimated by left ventricular fractional shortening or end-systolic



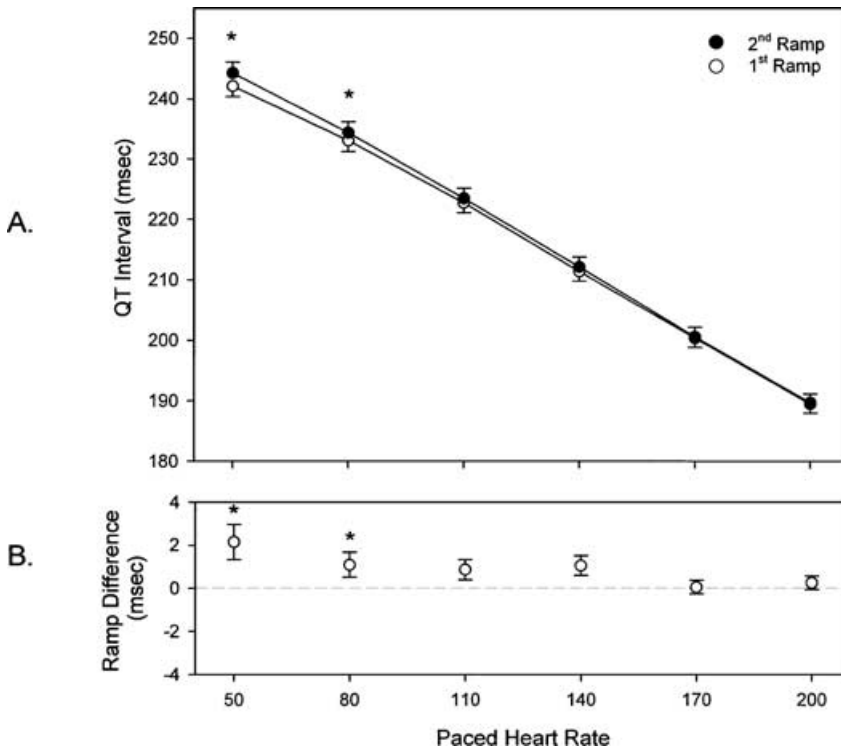
**Figure 1.** Selected echocardiographic findings are depicted as a function of time of study for all dogs of the study. Open symbols represent left ventricular dimensions: open circles being left ventricular end-diastolic dimension, open triangles being left ventricular end-systolic diameter, open squares being diastolic septal thickness, and open diamonds being diastolic left ventricular wall thickness. Filled symbols represent myocardial cross sectional area (filled squares), and left atrial internal area (filled diamonds). Only the left atrial area changed significantly during the study, and then only transiently increasing for week 3 of the study. (\* $P < 0.05$ )

diameter. The only recognized change was an increase in left atrial size (average increase 11.9%) that became statistically significant only for the 3-week study interval ( $P = 0.005$ ).

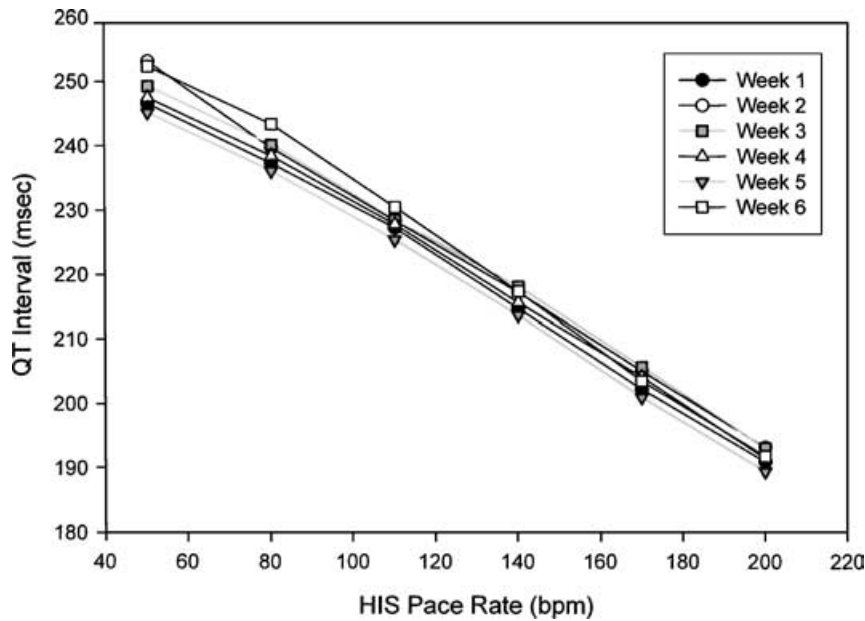
**QT-HR Relationships**

As with the anesthetized His-pace model,<sup>1</sup> HIS-pacing QT-HR data in conscious dogs assumes a linear relationship. Paired ramps collected on the

same day were highly reproducible as depicted by the nearly super imposable plots in Figure 2. The average difference in QT intervals between the two ramps was  $<3$  ms over the entire range of paced rates; the second ramp having (longer) QT intervals than the first. Even this small difference was consistent enough to be statistically significant at paced rates of 50 and 80 beats/min ( $P \leq 0.01$ ).



**Figure 2.** Short-term reproducibility of the QT-heart rate (HR) relationship is shown for all dogs of the study as (A) nearly superimposable lines for the first and second ramps generated 10 minutes apart on the same day, and (B) the average magnitude of the difference in QT intervals over the range of paced HRs between the first and second ramps. Paired daily ramps were statistically different at rates of 50 and 80 beats/min, but by a difference of  $\leq 3$  ms (\* $P < 0.05$ ).



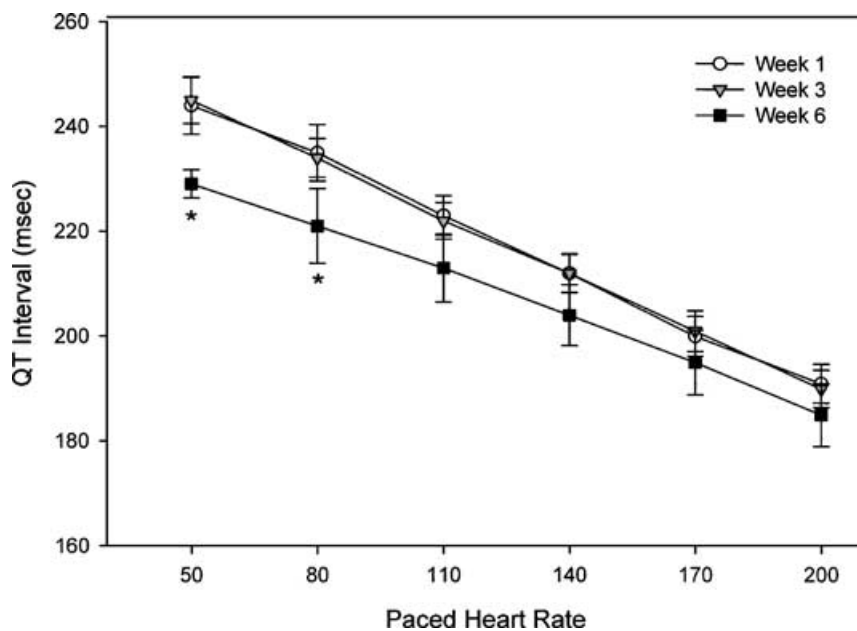
**Figure 3.** Chronic reproducibility of the QT-heart rate (HR) relationship is shown as the similarity between the group-averaged ramps for 6 sequential weeks of the study. No differences were detected from one week to the next in this study.

The QT-HR relationship remained stable over the 6-week course for group 1 dogs paced at a constant rate of 80 beats/min (Fig. 3). Similar stability was observed for group 2 dogs from weeks 1–3. When the group 2 daily pacing rate was then increased to 140 beats/min, a reduction in the maximal QT interval observed at low pacing rates slowly became evident. A statistically significant reduction in the QT interval at 50 beats/min (average, 14.9 ms,  $P = 0.003$ ) was detected for group 2 dogs by week 6 of the study Figure 4. This tachycardia induced change had resolved by the ninth

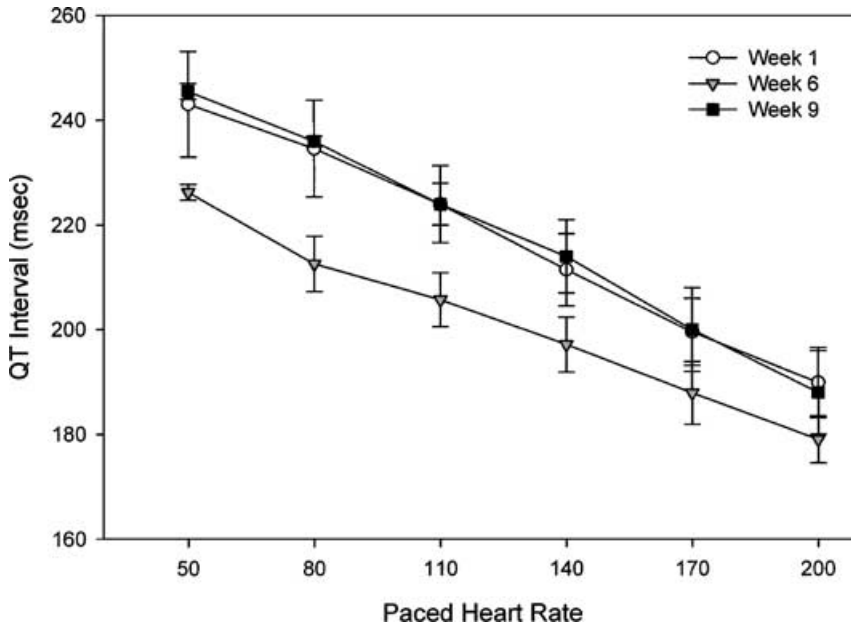
week in the two group 2 dogs paced at 80 beats/min from week 6 to week 9 (Fig. 5).

Figures 6 show estimates of statistical power to detect the effects of an acute intervention on QT for at least one paced rate. For acute interventions conducted on the same day, the  $MS_{error}$  for paired QT-HR ramps is  $12.9 \text{ ms}^2$ . Related estimates of statistical power show this model to be capable of detecting 6-ms changes with samples sizes as small as seven animals with a statistical power  $>0.8$ .

For the subset of animals analyzed, automated measures of RT interval were more precise than



**Figure 4.** The effect of increasing the daily background pacing rate on the QT-heart rate (HR) relationship is shown for group 2 dogs. Ramps for weeks 1 and 3 are nearly superimposable. Following 3 weeks of an increase in daily pacing rate from 80 to 140 beats/min, the group-averaged ramp for week 6 is flattened with shorter QT intervals at lower pacing rates. Significant differences between week 6 and weeks 1 and 3 are evident for paced rates of 50 and 80 beats/min ( $*P < 0.05$ ).

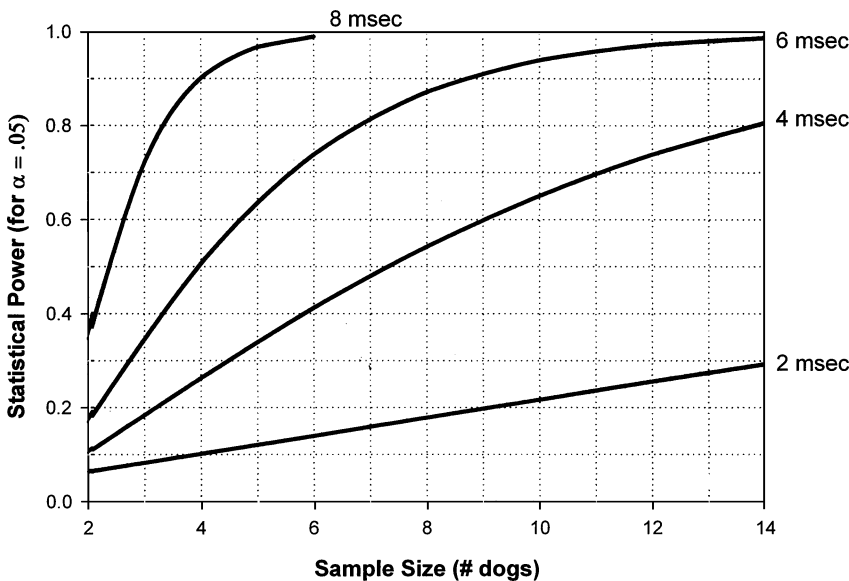


**Figure 5.** The effect of increasing daily pacing rate on the QT-heart rate (HR) relationship is shown to be reversible by 3 weeks of restored pacing at 80 beats/min in two of the group 2 dogs. The QT-HR relationship at week 6 is shifted down and flattened compared to week 1, but is restored to week 1 levels, 3 weeks after restoring the daily pacing rate back to 80 beats/min.

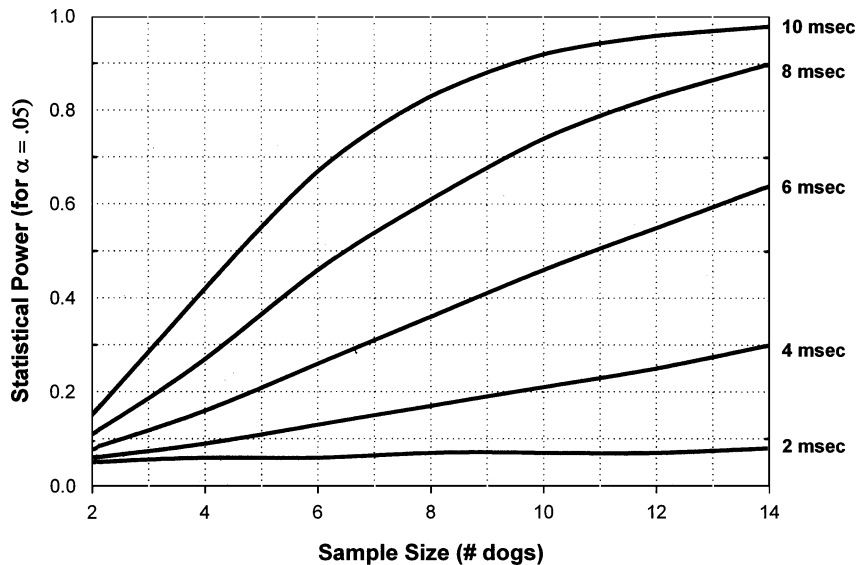
hand measures of QT intervals of the same data. The effect is seen as a substantially lower  $MS_{error}$  term for the repeated measures ANOVA (42 vs 82  $ms^2$ ). The difference likely reflects difficulties in identifying the onset of the QRS in animals with mild pacing induced preexcitation, and human inconsistencies in identifying the end of the T wave, particularly as their morphology changes slightly from day to day. Figure 7 shows the estimates of statistical power for detecting a difference in RT interval for at least one paced rate over the course of 6 weeks.

The aforementioned statistical power estimates ignore the linear relationship between QT (RT) and paced HR and are suitable for finding a difference at any single paced rate irrespective of the others. If the linear slope of the relationship is instead used as the index of an interventional effect, the statistical power improves. By way of comparison, Figure 8 shows the statistical power for detecting a change in RT interval for group 1 dogs based on a change in slope.

As currently employed, the model's statistical power for chronic interventions is lower. For



**Figure 6.** Short-term (within a day) statistical power to detect a difference in QT interval for at least one paced heart rate, calculated from the results of paired QT-heart rate (HR) ramps, is plotted as a function of sample size and effect size. Each curve depicts the power-sample size relationship for a given effect size ranging from 2 to 8 ms.



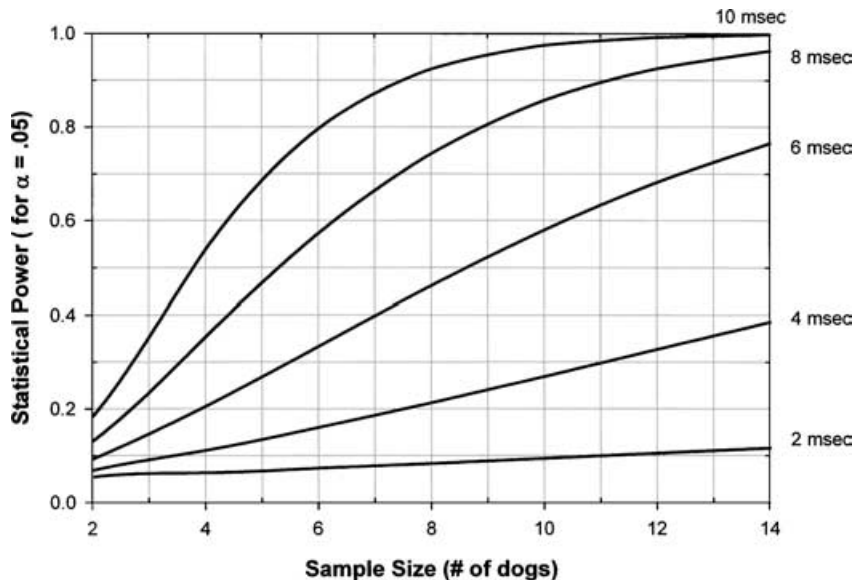
**Figure 7.** Long-term (weeks) statistical power to detect a difference in RT interval for at least one paced heart rate, calculated from the results of sequential weekly RT-heart rate (HR) ramps, is plotted as a function of sample size and effect size. Each curve depicts the power-sample size relationship for a given effect size ranging from 2 to 10 ms.

chronic interventions, sample size predictions for detecting single digit changes in QT interval (milli seconds) with statistical power of at least 0.8 would be 14 animals.

**Discussion**

The QT interval, and its response to interventions, is a valuable but indirect and imperfect marker of repolarization-related arrhythmias. The mechanism(s) by which prolonged QT intervals relate to increased proarrhythmic risk is complex and probably includes the potential for reentry and triggered activity.<sup>3-5</sup> Though incompletely understood mechanistically, the association between QT prolongation and the propensity for arrhyth-

mias has been well established.<sup>6</sup> One of the major problems using QT interval as a biomarker for proarrhythmic risk is the substantial HR dependence of the measurement. The authors recently described an acute anesthetized canine model of AVN ablation with His-bundle pacing as a tool for evaluating QT intervals independent of changing HR.<sup>1</sup> His-bundle pacing maintains the normal sequence of ventricular activation and preserves the natural myocardial sequence of depolarization and repolarization. The acute anesthetized model in this study is sensitive and can be used to evaluate QT-HR relationships at targeted times of interest, like the maximal effect of an experimental intervention. However, it is also potentially



**Figure 8.** Long-term (weeks) statistical power to detect a difference in the slope of the RT-heart rate (HR) relationship, is plotted as a function of sample size and effect size. Each curve depicts the power-sample size relationship for a given effect size ranging from 2- to 10-ms equivalents. Millisecond equivalents were computed as the change in RT interval at 50 or 200 beats/min paced HR for a given change in slope.

confounded by the effects of anesthetic drugs, hypothermia, and was inherently limited to acute interventions. This conscious model is an improvement that avoids these problems and enjoys additional advantages. Interventions can be applied acutely or chronically and by a variety of means including oral administration of test compounds. Data can be collected at any time of interest, repetitively over short or long periods of interest. A conscious animal model also leaves intact, neural and endocrine modifiers of myocardial electrophysiology.

The study did not detect gross morphological evidence for ventricular remodeling in this pacing model. Ventricular size and systolic performance were unchanged throughout the study. The only detectable change was a mild increase in left atrial size, an expected finding with AV dissociation and asynchrony. This supports the presumption that His-paced ventricles are mechanically and electrically normal, at least over the course of several weeks. In contrast, models of complete AV block are associated with left ventricular hypertrophy and electrical remodeling characterized by action potential prolongation, increased dispersion of repolarization, and increased susceptibility to drug induced arrhythmias.<sup>7,8</sup>

As indicated by the power calculations only small numbers of animals are required to detect small changes in the QT-HR relationship following an acute intervention. The sensitivity of the model was slightly lower for chronic interventions because of greater variance in the QT-HR relationship when measured over many days or weeks. This study was not designed to identify specific causes for this difference in sensitivity, though it probably reflects a mixture of physiological and technical factors. Autonomic tone is known to alter ventricular repolarization and the QT interval.<sup>9-11</sup> Since autonomic tone (and other unspecified natural modifiers) were not controlled, greater variance was expected in the data when measured repetitively over longer periods of time. Interestingly, a clear pattern for changing QT-HR relationships was not identified over the 6-week study in group 1; it did not follow a pattern of steadily increasing (or decreasing) QT intervals that might be ex-

pected from a systematic change in autonomic tone as dogs became more familiar with the data collection process.

Technical issues related to defining QT intervals are also likely to be a factor in the greater variance noted over the length of the study. The morphology of the normal T wave is fairly stable over short periods in dogs (i.e., within 1 day), but is more labile over longer periods. Human bias in defining the beginning of the QRS and the end of the T wave are more likely to develop with slight variations in waveform morphology typically seen over the course of weeks. When automated analysis was used instead of manual measurements, measurement precision nearly doubled as evidenced by the near 50% reduction in  $MS_{\text{error}}$  for the effect of time (weeks). Given these physiological and technical issues, it was not surprising to find reduced detection sensitivity over longer periods of time. Regardless of cause, this finding may be important for effective experimental study designs to identify chronic interventional effects on repolarization. Inclusion of a control, untreated group would allow an estimation (and subtraction) of the effect of time alone, for comparisons to a treatment group.

The daily programmed pacing rate had an effect on the QT-HR relationship in as little as 3 weeks. In the authors' original article describing the acute anesthetized His-pacing model, they reported a small hysteresis or memory effect of recently different HRs on the instantaneous QT interval.<sup>1</sup> The reduction in the maximal QT interval observed for group 2 dogs may reflect a similar, but larger and longer memory effect induced by the higher pacing rates imposed for the last 3 weeks of the study. Their results underscore the importance of standardizing experimental pacing protocols to maximize the sensitivity of the model to detect true interventional effects.

## Conclusions

The modified AV-ablated His-paced model for conscious dogs provides reproducible measures of ventricular repolarization over a period of many weeks. It offers a flexible experimental platform for investigating interventional effects on repolarization over a wide programmable range of HRs.

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