Chapter 2

Electrocardiographic T wave and its relation with ventricular repolarization along major anatomical axes

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ABSTRACT

Background: The genesis of the electrocardiographic T wave is incompletely understood and subject to controversy. We have correlated the ventricular repolarization sequence with simultaneously recorded T waves.

Methods and Results: Nine pig hearts were Langendorff perfused (atrial pacing, cycle length 650 ms). Local activation and repolarization times were derived from unipolar electrograms sampling the ventricular myocardium. Dispersion of repolarization time was determined along 4 anatomic axes: left ventricle (LV)–right ventricle (RV), LV:apico-basal, LV:anterior-posterior, and LV:transmural. The heart was immersed in a fluid-filled bucket containing 61 electrodes to determine \( T_p \) (Tpeak in lead of maximum integral), \( T_{Te} \) (Tp to Tend) and \( T_{pTe}_\text{total} \) (first Tpeak in any lead to last Tend in any lead). Repolarization was nonlinearly distributed in time. \( RT_{25} \) (time at which 25% of sites were repolarized, 288±26 ms) concurred with \( T_p \). \( T_{pTe} \) was 38±8 ms and \( T_{pTe}_\text{total} \) was 75±9 ms. \( T_{pTe}_\text{total} \) correlated with dispersion of repolarization time in the entire heart (73±18 ms), but not with dispersions of repolarization times along individual axes (LV-RV, 66±17 ms; LV:apico-basal, 51±18 ms; LV:anterior-posterior, 51±27 ms; mean LV:transmural, 14±7 ms, all n=9).

Conclusions: We provide a correlation between local repolarization and T wave in a pseudo-ECG. Repolarization differences along all anatomic axes contribute to the T wave. \( T_{pTe}_\text{total} \) represents total dispersion of repolarization. At \( T_{p} \approx 25\% \) of ventricular sites have been repolarized.
INTRODUCTION

The T wave on the ECG (T-ECG) represents repolarization of the ventricular myocardium. Its morphology and duration are commonly used to diagnose pathology and assess risk of life-threatening ventricular arrhythmias. However, the physiological background of the T wave is incompletely understood thereby hampering reliable interpretation of the T wave.

Interpretation of the T wave morphology on body surface ECGs (T-ECG) is different from that on local electrograms (T-local). In a far-field recording (e.g. a 12-lead ECG), the T wave represents repolarization in the entire heart. In a near-field recording (e.g. a local electrogram), the T wave can be used to accurately determine local repolarization moments. Unlike our understanding of T-local, knowledge of the T-ECG is still incomplete. Previous studies have focused mainly on a single dominant repolarization gradient to explain the T-ECG morphology. It has become generally accepted that concordance of the T-ECG with the QRS complex is explained by opposing sequences of transmural activation and repolarization. Whereas activation progresses from subendocardial to subepicardial myocardium, repolarization was assumed to progress in the opposite direction. Additionally, T-ECG-peak and T-ECG-end were explained by the end of repolarization of, respectively, the subepicardial and M cell layers. There is, however, controversy regarding the presence of a functional M cell layer in human hearts. M cells have been observed only in human wedge preparations, but were absent in vivo. In dogs, total rather than transmural dispersion of ventricular repolarization is more determinative for the peak-to-end interval of the T-ECG (TpTe). Other studies have suggested that an apico-basal gradient of repolarization is responsible for the T-ECG morphology. These observations have resulted in a persisting debate concerning the dominant repolarization differences giving rise to the T-ECG.

We aimed to clarify the genesis of the T-ECG by correlating the ventricular repolarization sequence with simultaneously recorded T-ECGs in isolated perfused pig hearts. Pig hearts most likely lack an M cell layer, like human hearts.

METHODS

See the Data Supplement for detailed methods.

Experimental setup

The experimental protocol was approved by the local ethical committee on animal experimentation. Male pigs (n=9; body weight, 34±12 kg) were anesthetized and a 12-lead ECG was recorded in supine position (in vivo ECG). The heart was excised and perfused...
according to Langendorff with a 1:1 blood-Tyrode’s mixture. The apex was fixed to avoid swinging of the heart, but to allow rotation and movement in the vertical plane.

**Electrophysiological study and data acquisition**

After removal of the sinus node area, the atrium was paced at a cycle length of 650 ms. Transmural multielectrode (0.5 mm diameter) needles were inserted in a predefined pattern to obtain transmural local unipolar electrograms throughout the heart. Exact locations of terminals were reconstructed after termination of the experiment. The heart was surrounded by a perfusion-fluid-filled bucket containing 61 regularly distributed electrodes (1 electrode at the bottom of the bucket) to obtain a pseudo body surface ECG (BS-ECG). The reference signal was the assembled average of all 61 electrodes.

In the BS-ECG and in vivo ECG, QRS duration, QRS vector, QT interval, QT interval corrected for heart rate via Fridericia\(^{18}\) (QTc\(_F\)), T-ECG duration (T-duration), T-ECG vector (T-vector), and T\(_p\)T\(_e\) interval were determined (see Figure 1). QRS\(_{onset}\) and QRS\(_{end}\) were defined as respectively the first deflection and last J-point of the QRS complex in any ECG lead. Integrals of the QRS complex and T-ECG (defined as area under the QRS and T curve) were calculated between manually set markers. These were used to identify main (in vivo) and detailed (BS-ECG) QRS and T vectors in 3 planes: frontal, transversal and sagittal (in vivo: lead I, V6 and V2 equal 0 degrees, and rotation to lead aVF, V2, and aVF denotes a positive angle; in BS-ECG, similar definitions were used).

In the ECG lead of maximum (positive or negative) T-integral, the onset (T\(_o\)), peak (T\(_p\)), and end (T\(_e\)) of the T-ECG were manually determined relative to QRS\(_{onset}\) using the tangent method (Figure 1).\(^{19}\) QT interval and T\(_p\)T\(_e\) were defined as interval between QRS\(_{onset}\) and T\(_e\), and between T\(_p\) and T\(_e\) in a single lead. We additionally determined T\(_p\)T\(_e\)\(_{total}\) in the BS-ECG as interval between earliest T\(_peak\) in any lead and last T\(_end\) in any lead.

At each local recording site, local activation times and local repolarization times (RTs) were automatically determined as the interval between QRS\(_{onset}\) to time of the minimum derivative of QRS complex and maximum derivative of T-local, respectively (Figure 1). All signals and time markers were visually validated. Recordings with ST-elevation or a flat T-local were excluded from analysis of RTs.

RT\(_{min}\) and RT\(_{max}\) were start and end of repolarization, respectively. The volume distribution of repolarization was described with time points at which, respectively, n=5%, 25%, 50% (median RT), 75% and 95% of the recording sites were repolarized (RT\(_n\)). Dispersion of repolarization (dRT) was defined as range of RTs along a specific anatomic axis: left ventricle (LV)–right ventricle (RV), LV:apico-basal, LV:anterior-posterior, and LV:transmural. Maximum and mean LV:transmural dRT (dRT\(_{max}\)LV:transmural, dRT\(_{mean}\)LV:transmural) were, respectively, maximum and mean of all dRTs per LV needle. The dRT\(_{total}\) was the maximum range of RTs across the entire heart (Figure 1). Signal analysis was performed offline using a custom-made data analysis program written in Matlab 2006b.\(^{20}\)
Statistics

Continuous variables were given as mean±SD if normally distributed and in median (25th-75th percentile) if not normally distributed. Evaluation of normality was based on...
on Q-Q plots and correspondence of mean and median. Differences in characteristics between the in vivo ECG and BS-ECG, between $RT_n$ and T-ECG parameters, and between dRT values and $T_{pTe}$ or $T_{pTe_{total}}$ were tested using a paired $t$ test. We determined the amount of similarity (combination of distance and correlation) between $RT_n$ and T-ECG parameters and between dRT values and $T_{pTe}$ or $T_{pTe_{total}}$ by calculating intraclass correlation coefficients (ICCs) using a 2-way mixed model of absolute agreement.\textsuperscript{21} Diff-

![Diagram A](image1)

**Figure 2:** **A:** Electrode placement of the 12-lead ECG of a pig (left) and a typical example of a 12-lead in vivo ECG recording (right). In the in vivo ECG, the T-ECG is concordant in all extremity leads and discordant in precordial leads V1 to V4. **B:** Typical example of an ex vivo BS-ECG recording of the same heart as in Figure 2A. It is a schematic view of an unfolded bucket with the electrograms arranged in the configuration of the electrodes. *Bottom* refers to the recording derived at the bottom of the bucket. The QRS vector in this BS-ECG is directed to the left and posterior (arrow QRS), and the T-vector is directed to the left and anterior (arrow T). Most leads show a discordant T-ECG with the QRS complex, similar to the in vivo precordial leads.
ferences in $RT_{50}$ between different regions along an anatomic axis were tested with a Friedman analysis. Differences and ICCs with a $P$ value of $\leq 0.05$ were considered statistically significant.

RESULTS

In vivo standard 12-lead ECG and ex vivo BS-ECG

Figure 2 shows a typical example of a porcine in vivo 12-lead ECG in supine position (Figure 2A) and the BS-ECG of the same heart in Langendorff perfusion (Figure 2B). In general, in the in vivo ECG the T-ECG was positive in precordial and inferior leads and flat, biphasic, or slightly negative in leads I, aVR, and aVL.

Overall, the T-ECG in BS-ECG was discordant with the QRS complex in most leads and positive in anterior leads. The Table features the main characteristics of the ex vivo BS-ECG and in vivo 12-lead ECG. In comparison with the in vivo ECG of the same animal, the QRS vector in the BS-ECG is rotated in each plane (frontal: $47\pm 74^\circ$; $P=n.s.$; transversal: $56\pm 60^\circ$; $P=0.024$; sagittal: $49\pm 39^\circ$; $P=0.005$). There was no significant change in T-vector between the in vivo ECG and BS-ECG (frontal: $-3\pm 90^\circ$, transversal: $-74\pm 105^\circ$, sagittal: $56\pm 93^\circ$; all $P=n.s.$) but the change in T-vector showed a larger SD than the change in

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$P$ values for a paired $t$ test between the in vivo ECG and BS-ECG (n=9). BS-ECG indicates body surface ECG; $n$, number of experiments; QTc_F, corrected QT interval via Fridericia; RR, interval between two successive QRS complexes; $T_pT_e$, $T_p$ to $T_e$ interval in the lead with maximum T-ECG integral.
QRS vector. QRS duration, QT interval, QTc_F, T-duration and TpTe in the BS-ECG were not statistically different from the in vivo ECG (Data Supplement).

**Activation and Repolarization**

Figure 3A and 3B shows typical examples of, respectively, activation and repolarization maps of 1 heart. In all hearts, activation times were obtained from 87±27 recording sites throughout the heart. Overall, activation occurred earliest at the left septum and latest at the RV basal free wall (as in Figure 3A). Transmural activation along needles was almost simultaneous. Activation was complete within 49±9 ms (see Data Supplement).

In all hearts, repolarization times were obtained from 61±21 recording sites per heart. Overall, repolarization started at the anterior or posterior RV. Repolarization in the LV progressed from basal to central (between apex and base) regions. Repolarization of

![Figure 3: Typical activation (A) and repolarization (B) map in transversal slices at basal (top), central (middle) and apical (bottom) level. Red indicates early areas and blue indicates late areas. A: Activation starts at the septal basal and central region and progresses to the posterior central region and the anterior apex. The free walls of the ventricles activate latest (left ventricle [LV] preceding right ventricle [RV]). Activation of subendocardial (endo) and opposite subepicardial (epi) regions occurs almost simultaneously. The maximum difference in this animal was only 18 ms (asterisk at the central anterior LV wall, with epi earlier than endo). B: Repolarization starts at the anterior base of RV and septum and at the posterior basal region. Latest repolarization occurs in the LV endo basal and central regions. Transmural repolarization was generally simultaneous although a region with a large transmural difference of 48 ms (asterisk at the basal posterior LV wall, endo repolarizing later than epi) was observed.](image)
apical regions varied remarkably between hearts. Final repolarization was at the central free walls (RV before LV; see also Figure 3B). Transmural repolarization was simultaneous, although there were isolated sites with larger transmural differences (asterisk in Figure 3B, crowding of isochrones).

On average, RT$_{min}$ was at 251±22 ms and RT$_{max}$ at 324±33 ms (n=9 hearts). RT$_{25}$ was at 288±26 ms (at midrepolarization), RT$_{50}$ at 303±28 ms and RT$_{75}$ at 309±30 ms. From the onset of repolarization, it took 52±13 ms to fully repolarize the first 50% of the recording sites. From that moment it took only 21±7 ms to complete repolarization.

**T-ECG and the overall repolarization sequence**

T$_o$ occurred always earlier than RT$_{min}$ (−27±15 ms; $P<0.05$; ICC=0.56; $P<0.005$). T$_o$ was not significantly different from RT$_{max}$ (−3±10 ms; $P=n.s.$) and had a large ICC (0.95; $P<0.001$).

We compared time of T$_p$ on the BS-ECG (T$_p$= 283±28 ms) with the distribution of repolarization (RT$_{min}$, RT$_{25}$, RT$_{50}$, RT$_{75}$, and RT$_{max}$). Figure 4 shows that RT$_{25}$ had the smallest difference with the time of T$_p$ (6±12 ms; $P=n.s.$) and largest ICC (0.89; $P<0.001$). More precisely, at T$_p$ the percentage of repolarized sites was 19.5±12.6%.

**Figure 4:** T$_p$ versus repolarization time (RT) values. Bars represent the difference between the RT value (RT$_{min}$, RT$_{25}$, RT$_{50}$, RT$_{75}$, RT$_{max}$) and T$_p$ in ms. Error bars represent SDs. Intraclass correlation coefficients (ICCs) are given above the bars and were all significant ($P<0.05$). Note the small difference between RT$_{25}$ and T$_p$ and the large ICC.

**T-ECG and the distribution of RT along 4 anatomic axes**

RT distribution along the 4 anatomic axes was studied in relation to T-ECG morphology. Figure 5 shows an example of the time relation per predefined axis between the T-ECG in a BS-ECG lead (with maximum T-ECG integral, opposite the RV), and RT$_{5r}$, RT$_{25r}$, RT$_{50r}$, RT$_{75r}$, and RT$_{95r}$. The figure (top panel) demonstrates that half of RV (RT$_{50}$) had already repolarized...
Figure 5: Left: Example of the T wave (of the BS-ECG lead with maximum T wave integral) and repolarization time (RT) distribution (RT₅ to RT₉₅ with whiskers for RTₛₒ, RT₅₀, and RT₇₅) within a region per anatomic axis: LV-RV-septum (upper), LV:apico-basal (second), LV:antero-posterior (third), LV:transmural (fourth), entire heart (lower). At the background of the lower section a histogram of the number of repolarized recording sites is depicted (grey bars; grey line indicates cumulative percentage). n is the number of sites per region.

Right: Comparison of RT₉₅ (median [25th-75th percentile]) values between the regions along an anatomic axis in 9 hearts. Differences in RT₉₅ between regions along each axis were tested with a Friedman analysis (P value per axis is shown). n is number of sites per region (mean±SD).
before RT$_5$ of LV, and that at RT$_{55}$ in the RV, 50% of LV (RT$_{50}$) still had to repolarize. We restricted analysis of RT distribution along other axes only to the LV, because it has the largest contributing mass and harbored more electrodes. In the figure, the dispersion of RT$_{50}$ values within the LV:antero-posterior, LV:apico-basal or LV:transmural axes was not large enough to explain the T-duration. In this example, LV repolarization starts at the basal posterior midendocardial region and ends in the basal anterior subendocardial region, illustrating that repolarization along all axes plays a role in the time relation between the T-ECG and the repolarization process. The bottom panel of the figure shows the RT distribution of the entire heart along the T-ECG. The grey histogram of repolarized recording sites re-emphasizes that the majority of sites repolarized after the moment of T$_p$.

Figure 5 summarizes RT$_{50}$ values per anatomic axis of all animals. RT$_{50}$ values were significantly different between regions within the LV:apico-basal axis (central region was 17±9 ms longer than apical or basal region; n=7), and LV:transmural axis (the subendocardial or midendocardial region was 17±13 ms longer than the subepicardial or midepicardial region; n=9).

**T$_p$T$_e$ and dispersion in repolarization**

T$_p$T$_e$ has been associated with heterogeneity in RT, either in wedge preparations$^6$ or in the whole heart.$^{12,22}$ We, therefore, studied dispersion of repolarization moments (dRT) along each of the 4 axes and over the entire heart in relation to T$_p$T$_e$ on the BS-ECG (Figure 6). Locations of maximum and minimum RT across each single axis differed between animals, so the direction of the vector connecting the pair of values constituting the dRT$_\text{total}$ was not similar among animals. T$_p$T$_e$$_\text{total}$ on the BS-ECG was least different from dRT$_\text{total}$ (Figure 6: mean difference of 1±13 ms; paired t test $P=0.758$), with an ICC of 0.626 ($P=0.03$). T$_p$T$_e$ was least different from dRT$_\text{maxLVtransmural}$ (Figure 6: mean difference of 4±20 ms; paired t test $P=0.552$). However, the ICC of 0.306 was low and statistically not significant. Other dRT values that were not statistically significant from T$_p$T$_e$$_\text{total}$ or T$_p$T$_e$ (Figure 6) had larger differences and lower ICC values with T$_p$T$_e$$_\text{total}$ or T$_p$T$_e$.

**DISCUSSION**

The main findings of the present study are that (1) differences in repolarization moments along all axes contribute to the T-ECG morphology; (2) T$_p$T$_e$$_\text{total}$ measured on the BS-ECG reflects total dispersion of repolarization within the heart; (3) at the moment of T-ECG peak, ≈25% of the recording sites are repolarized; and (4) onset of T-ECG precedes onset of repolarization. We provide the first complete correlation between local repolarization and the simultaneously recorded T-ECG on the surface ECG.
In vivo ECG versus ex vivo ECG

The in vivo and pseudo-ECG closely resemble each other, although transversal and sagittal QRS vectors differ. It likely results from a different position of the heart in the thorax versus the bucket (Table and Data Supplement). Therefore, we assume that activation in the hearts was unchanged between in vivo and ex vivo. This is supported by the unchanged QRS duration. In addition, the in situ ventricular activation sequence of a dog heart recorded by Durrer et al.\textsuperscript{23} did not change after isolation.

Repolarization and T-ECG

We showed significant differences in RT\textsubscript{50} along LV:apico-basal and LV:transmural axes. However, differences (≈17 ms) were too small to explain an average T-ECG duration of 97 ms. It suggests that total dispersion of RT is reflected in the entire T-ECG. Indeed, we demonstrated that the interval between first peak to last end of all T-ECGs did correspond with total dispersion of RT, corroborating results from Xia et al.\textsuperscript{22} Although maximum transmural dRT was least different from T\textsubscript{p}T\textsubscript{e}, its ICC was low and not statistically significant. The lack of correlation may be attributed to the fact that the maximum

Figure 6: Dispersion of repolarization time (dRT) values along various anatomic axes (mean±SD; n=9) with corresponding T\textsubscript{p}T\textsubscript{e} and T\textsubscript{p}T\textsubscript{e}_total (solid line=mean; dashed line=SD; n=9). To exclude influences of other axes contributing to the left ventricle (LV):transmural axis, we also obtained dRT\_maxLVtransmural and dRT\_meanLVtransmural. Note that mean T\textsubscript{p}T\textsubscript{e}_total is least different from mean dRT\_total (intraclass correlation coefficient [ICC]; P<0.05) and mean T\textsubscript{p}T\textsubscript{e} is least different from mean dRT\_maxLVtransmural (ICC=ns). Paired t test: * P<0.05 dRT compared with T\textsubscript{p}T\textsubscript{e}, † P<0.05 dRT compared with T\textsubscript{p}T\textsubscript{e}_total.
difference represents a single outlier value, which may not affect T wave morphology. The mean transmural dRT more reliably represents transmural dispersion but did not correlate with $T_pT_e$ as demonstrated before.\textsuperscript{12}

$T_e$ precedes the first measured RT. This is unlikely the result of a sampling error, because it occurred in all hearts. We suggest that regional differences in action potential (AP) morphology (phase 1 and 2) rather than differences in RTs contribute to genesis of the first part of the T-ECG. $T_e$ coincides with the last measured RT, consistent with the fact that RT in a unipolar electrogram relates to the steepest part of AP downstroke,\textsuperscript{23} which approximates $APD_{90}$ (AP duration at 90\% of repolarization).\textsuperscript{24}

Fuller et al\textsuperscript{25} found a near equality of time of T wave peak and mean RT, which contrasts with our results that 75\% of recording sites repolarizes after $T_p$. Methodological differences may explain this discrepancy. We used a single surface ECG lead instead of a root mean square of epicardial recordings. Not only is the former clinically more relevant, the Data Supplement indicates that the root mean square of BS-ECGs shows similar results and does not explain the discrepancy. Furthermore, Fuller used only epicardial electrograms, which may shift or transform RT distribution and underestimate RT dispersion. Differences in data presentation (medians and quartiles versus means) and species (pigs versus dogs) may cause additional discrepancy. We underscore that the finding that $T_p$ reflects RT\textsubscript{25} cannot indiscriminately be applied to a randomly chosen lead because each has a different view on the dominant vector. Nonetheless, we suppose it is applicable to the lead with maximum T wave area.

Our data show that the time during which the first 50\% of sites repolarize is about twice as long as the time required for repolarization of the last 50\% of sites. A possible physiological explanation for the nonlinear repolarization process is that repolarization accelerates exponentially as a result of radial propagation of the repolarization wave. This matches the repolarization course illustrated in Figure 5 (bottom panel). The highly skewed apparent velocity of the repolarization process has thus far not been described, although its reflection in an asymmetrical T wave is common knowledge ($T_o$ to $T_p$ interval of $59\pm7$ versus $T_pT_o$ interval of $38\pm8$ ms).

We speculate that the first part of a normal T-ECG primarily depends on regional differences in repolarization time course (i.e. changes in AP morphology) preceding the moment of full repolarization (i.e. steepest part of AP downstroke). When full repolarization has started, continuation may accelerate depending on the degree of electrotonic interaction, which may determine the last part of the T wave.

**Pig versus human: In vivo ECG and repolarization sequence**

The QRS configuration of the in vivo porcine ECG in our study is in agreement with another study in pigs.\textsuperscript{26} The in vivo porcine T-ECG in our study resembles a normal T-ECG in human\textsuperscript{27} as the polarity is positive in most leads and T-vectors have similar angles (if
corrected for differences in position of the heart in the thorax).²⁸ QRS duration in pig, however, is shorter than in man.²³ This is because of a more extensive transmural Purkinje network²⁹ and the smaller size of porcine hearts compared to human. Nevertheless, the general activation sequence observed in our study resembles the activation sequence described in humans²³ and pigs.³⁰,³¹

The porcine repolarization sequence in our study resembles that in earlier reports,²²,³¹ although repolarization of the septal region occurred later in our study. Methodological differences (endocardial monophasic action potentials recorded sequentially at 50–70 sites instead of local transmural electrograms recorded simultaneously at 61±21 sites [our study]) may have caused the minor differences in repolarization sequences, although correlation between RT and monophasic APD₉₀ is high.²⁴

**CONCLUSIONS**

We provide the first complete correlation between local repolarization and the corresponding electrocardiographic T wave. A main conclusion of this study is that total dispersion of repolarization along all anatomic axes is determinative for the T wave on the pseudo-ECG of the isolated perfused pig heart. \( T_pT_e_{\text{total}} \) (first peak to last end of all T-ECGs) across ECG leads represents total dispersion of repolarization. Moreover, at the T wave peak, only \( \approx 25\% \) of ventricular sites are repolarized. Therefore, the start of the T wave is likely the result of regional differences in early repolarization (phase 1 and 2 of the AP), and the nonlinear repolarization process may be reflected in the final part of the T wave.

**ACKNOWLEDGEMENTS**

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REFERENCES


SUPPLEMENTAL METHODS

Experimental setup
Pigs were premedicated with ketamine (10-15 mg/kg, Nimetek, Eurovet Animal Health BV) and midazolam (1-2 mg/kg, Actavis, IJsland) intramuscularly and anesthetized with 15 mg/kg pentobarbital (Nembutal, Ceva Santé Animale) intravenously. The animals were intubated and ventilated with room air and oxygen plus isoflurane (1:1 + 1.5%). After a midsternal thoracotomy, heparin 5000 international units (Leo Pharma) was injected intravenously. Tyrode’s solution was infused, after which 4 to 5 L diluted blood was collected.

Electrophysiological study and data acquisition
Transmural multi-electrode (0.5 mm diameter) needles were inserted in a predefined pattern. In the left ventricle 16 to 26 needles (harboring 4 electrodes per needle) were inserted, and in the right ventricle 12 to 19 needles (harboring 2-4 electrodes per needle). Twenty-four to 35 of the terminals were located in the septum. A 256-channel amplifier (BioSemi, 24 bit dynamic range, 122.07 nV LSB, total noise 0.5 µV) was used. Signals were recorded with a sampling frequency of 2048 Hz (bandwidth (−3dB) DC −400 Hz). The active common mode electrode was positioned in the aortic root. Stimulation pulse amplitude was 1.5 times the stimulation threshold and pulse width was 1 ms.

Vector determination in ECG
In the in vivo ECG we were able to determine only the main vector due to a low number of leads. Integrals were automatically calculated by an algorithm in Matlab between manually set markers. The main vector (in vivo) was determined by calculating the angles using the integrals of all leads. In the BS-ECG exact vectors were determined. First, the time of the peak of the QRS-complex or T-ECG was determined in the BS-ECG lead with the absolute maximum integral (positive or negative) of the QRS-complex or T-ECG, respectively. We suppose that at this time the vector has the maximum amplitude and therefore we determined the vector angles at this moment. At this time the angle was calculated between the lead with the maximum and minimum voltage potential (see Figure S1). In the BS-ECG the three vector planes (frontal, transversal and sagittal) were assigned based on the position of the heart in the bucket and with similar directions as in the in vivo ECG.

Dispersion of repolarization
Measured RT in a unipolar electrogram relates to the steepest part of action potential (AP) downstroke, which is close to APD90 (AP duration at 90% of repolarization).
Dispersion of repolarization (dRT) was defined as the range of RTs along a specific anatomical axis: LV–RV, LV:apico-basal, LV:anterior-posterior and LV:transmural. Along each axis the recording sites were divided according to regions (all sites in dRT LV–RV and only LV sites in other axes), after which the maximum range between these regions was determined. E.g. along the LV:apico-basal axis all LV recording sites were subdivided into the apical, central or basal region after which the maximum difference in RT values between these regions was determined. These dispersion measures were not completely independent of dispersion along other axes. Especially dRT LV:transmural may be influenced by dispersion along other axes, because each transmural region includes basal as well as apical recording sites. Therefore, we also obtained the maximum and mean LV:transmural dRT (dRT_maxLVtransmural, dRT_meanLVtransmural). Per LV needle we determined the dispersion of repolarization (defined as the range between any of at least 3 terminals on a needle). Subsequently, we determined the maximum and mean of all these dRTs.

**Figure S1:** Example of determination of the exact QRS and T vector in the BS-ECG. The lower recording is the lead with the largest QRS integral. In this lead the peak of QRS is determined. At this time the upper recording shows the lead with the minimum voltage potential at the time of QRS peak (A1). Between these two leads the vector angles were calculated. In this example the T vector was calculated between the upper lead (largest T integral at time of T wave peak (B1)) and lower lead (minimum voltage).
Root Mean Square (RMS)
Because a single BS-ECG lead may be limited by the view from the dominant axis of the cardiac vector, the RMS (as global ECG measure) may provide more reliable information on the underlying repolarization process. However, a single BS-ECG lead is clinically more relevant than the RMS and we suppose that the BS-ECG lead with the largest T-integral is a close approximation of this RMS. Nevertheless, to validate the use of a single BS-ECG lead over the RMS we compared them. The RMS was composed by superimposing all BS-ECGs.

\[
RMS = \sqrt{\frac{\sum_{i=1}^{61} BSECG_i^2(t)}{61}}
\]

where BSECG(t) denotes the BS-ECG signal at time t, of lead i. Subsequently, we determined the peak of the T wave in the RMS (T_{p,RMS}) and compared this with T_p (of the maximum T-ECG integral) and the RT values (RT_{min}, RT_{25}, RT_{50}, RT_{75}, RT_{max}).

Supplemental Results

In vivo standard 12-lead ECG and ex vivo BS-ECG
In general, in the in vivo ECG the amplitude of the QRS-complex was smallest in lead aVR (6/9 animals) and largest in lead V1 or V2 (8/9 animals). In most cases, the QRS-complexes of the extremity leads were biphasic and had no dominant polarity, causing a large variation in the angle of their main QRS-vector in the frontal plane between animals. However, the QRS-vectors in the transversal and sagittal plane were rather constant (both had a smaller SD in QRS-vector compared to that in the frontal plane, (see Table 1: in vivo).

Comparison of the QRS-vectors and T-vectors between the in vivo ECG and BS-ECG showed a rotation of the QRS-vector in all planes and no change in T-vector, although the latter showed a larger standard deviation than the change in QRS-vector. After correction for the change in QRS-vectors, the change in T-vectors remained not significant and the standard deviations were still large (frontal: −50±84 degrees, transversal: −49±127 degrees, sagittal: −33±82 degrees, all P= n.s.).

The differences in QRS-vectors (in transversal and sagittal plane) between the BS-ECG and the in vivo ECG may be expected as a result of a different position of the heart in the thorax versus the bucket. In the thorax the LV was situated towards posterior and the apex pointed towards anterior, whereas in the bucket the LV was rotated more to the front and the apex was directed to the bottom. This positional change corresponds with less negative transversal and sagittal QRS-vectors in the BS-ECG versus the in vivo ECG (see Table 1).
Single BS-ECG lead versus the RMS

In Figure S2 an example of a comparison between the BS-ECG from the lead with the maximum T-integral (blue) and the RMS (red) is shown. In particular, comparison demonstrated that the time of the peak of the T wave in the RMS ($T_p_{RMS}$) is statistically not different from $T_p$ (of max T-integral). The mean difference is $-5 \pm 10$ ms ($T_p - T_p_{RMS}$, paired $t$ test, $P= n.s., n=9$). Comparison of $T_p_{RMS}$ with the RT values demonstrated that $T_p_{RMS}$ was not significantly different from $RT_{25}$ ($T_p_{RMS} - RT_{25}$, mean of $-1 \pm 8$ ms, paired $t$ test, $P= n.s., n=9$), and this had also the largest ICC value (0.96). This result is similar to the comparison between $T_p$ of the maximum T-integral and the RT values. As a consequence, about $24 \pm 12\%$ of the sites is repolarized at the moment of $T_p_{RMS}$, which is not significantly different from the percentage repolarized at the moment of $T_p$ derived from the lead with maximum T-integral.

Figure S2: Copy of the bottom panel of Figure 5 with addition of the T wave of the root mean square (RMS) of all BS-ECG leads (red) for the comparison with the T-ECG from the lead with the maximum T-integral (blue). The onset ($T_o$), peak ($T_p$) and end ($T_e$) of the T-ECG are marked with black dashed lines.

Activation and Repolarization

Earliest moment of activation was always a few milliseconds later than QRS$_{onset}$ on the BS-ECG (difference of $4\pm 2$ ms, one-sample $t$ test $P<0.001$) and latest activation was always earlier than QRS$_{end}$ on the BS-ECG (difference of $-11\pm 4$ ms, one-sample $t$ test $P<0.001$).

Transmural activation and repolarization

Transmural activation along needles was virtually simultaneous. The mean difference between sub-endocardium (endo) and sub-epicardium (epi) along a needle was $0\pm 2$ ms ($P= n.s.$ different from $0$, Wilcoxon signed-rank test per animal with Bonferroni correction, $n=8$, one animal has only one endo-epi needle and was excluded).
Transmural repolarization was also simultaneous. The averaged difference in RT between epicardial and endocardial sites along a needle was 12±7 ms ($P=\text{n.s.}$ different from 0, Wilcoxon signed-rank test per animal with Bonferroni correction, n=8).

**Supplemental References**

