In vivo assessment of temporal and transmural variability of cardiac repolarization in the dog, as a tool for evaluating pro-arrhythmic risk

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Is QT an accurate predictor for the risk of arrhythmia ?

- Prolongation of the QT interval of the ECG
  - Corresponds to a prolongation of the repolarization phase of the action potential
  - Produced by a number of drugs, in laboratory animals and humans
  - Generally considered as indicative of a risk of arrhythmia.
  - Evaluation is requested by ICH guidelines (S7B)
- However
  - Prolongation of QT interval as such is probably not an accurate indicator of the risk of arrhythmia
  - Other parameters of cardiac repolarization are more predictive

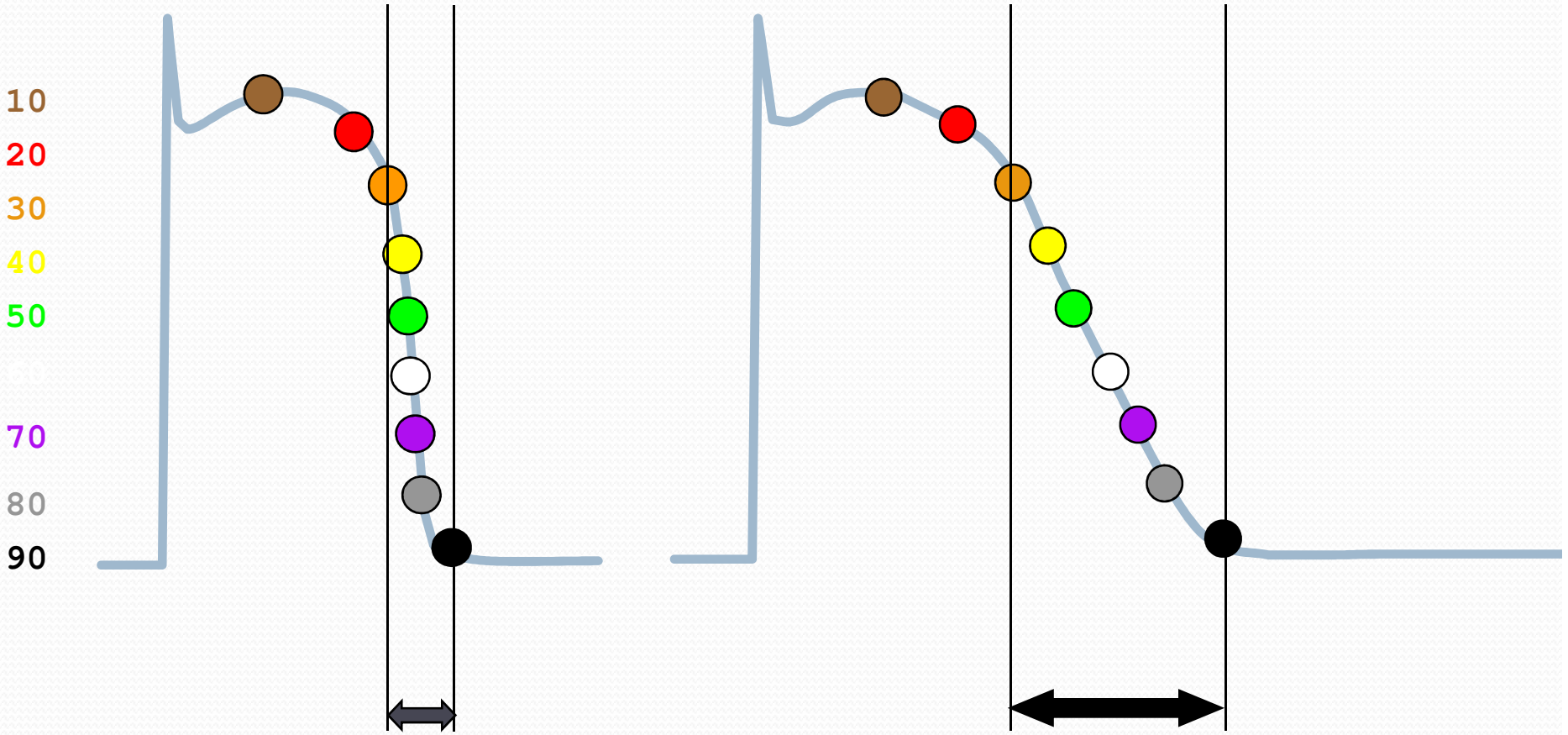
Are there more accurate predictors?

- Beat-to-beat variation of cardiac action potential duration (temporal variability)
  - In vitro models: SCREENIT (Pr Hondeghem)
  - In vivo: beat-to–beat QT variation
- Spatial variability of action potential duration
  - In vitro model: cardiac wedge
  - In vivo:
    - Change in T wave morphology

Beat-to-beat variability of repolarisation: in vitro evaluation The Screenit model

- Isolated paced rabbit heart
- In situ recording of action potentials (AP)
- Assessment of a proarrhythmic index: TRIad based on
  - Triangulation: change in AP morphology
  - Reverse use dependency: drug-induced AP prolongation more marked at low than at high heart rate.
  - Instability: increased beat-to-beat variability of AP duration

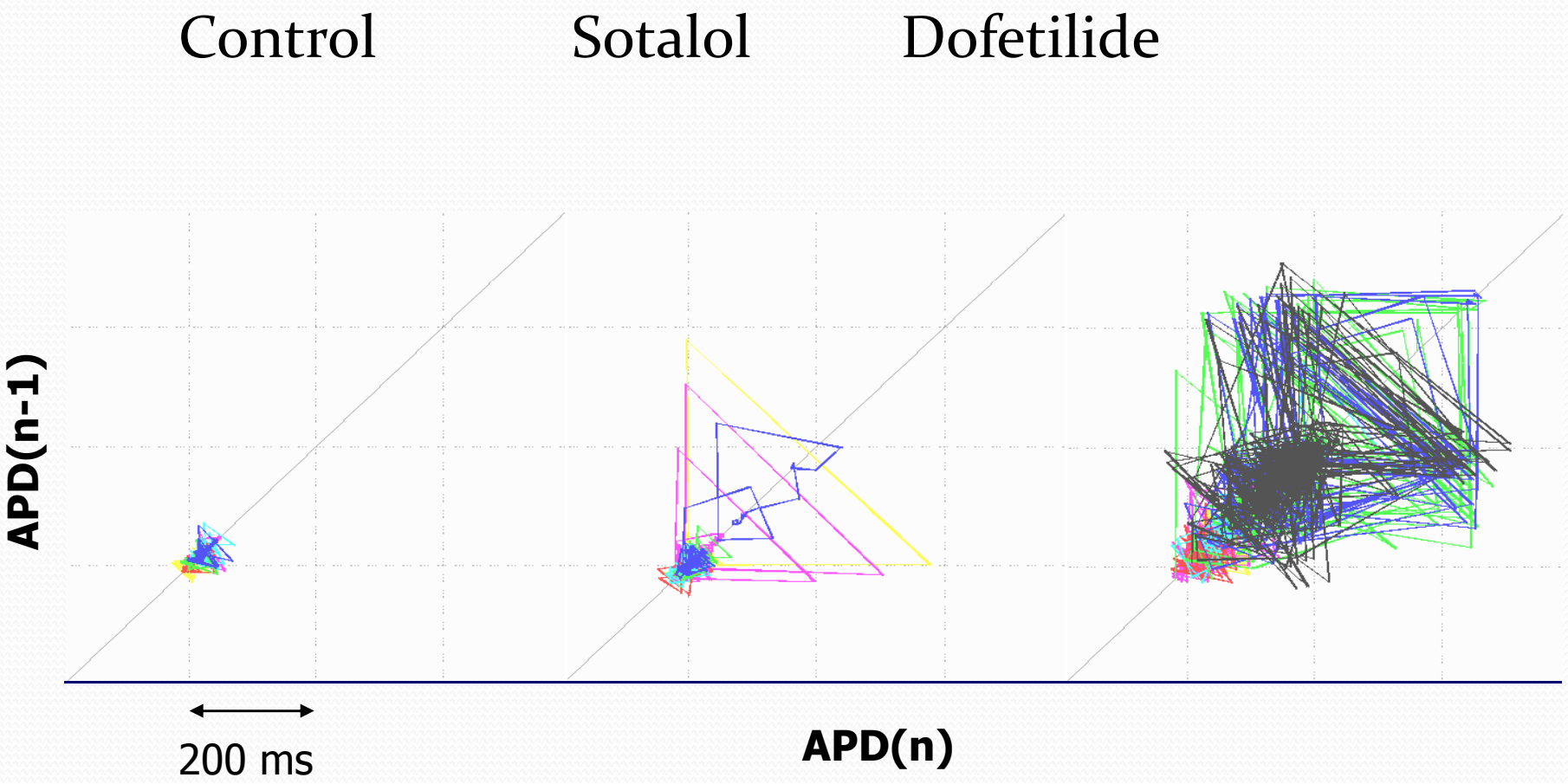
Action potential triangulation



From an Luc Hondeghem's presentation

Temporal variability (instability) of cardiac action potential

Poincaré plot



J Card Electrophys 2003;14:287

Beat-to-beat variability of repolarisation: in vivo evaluation from ECG

- The coefficient of variation of QT

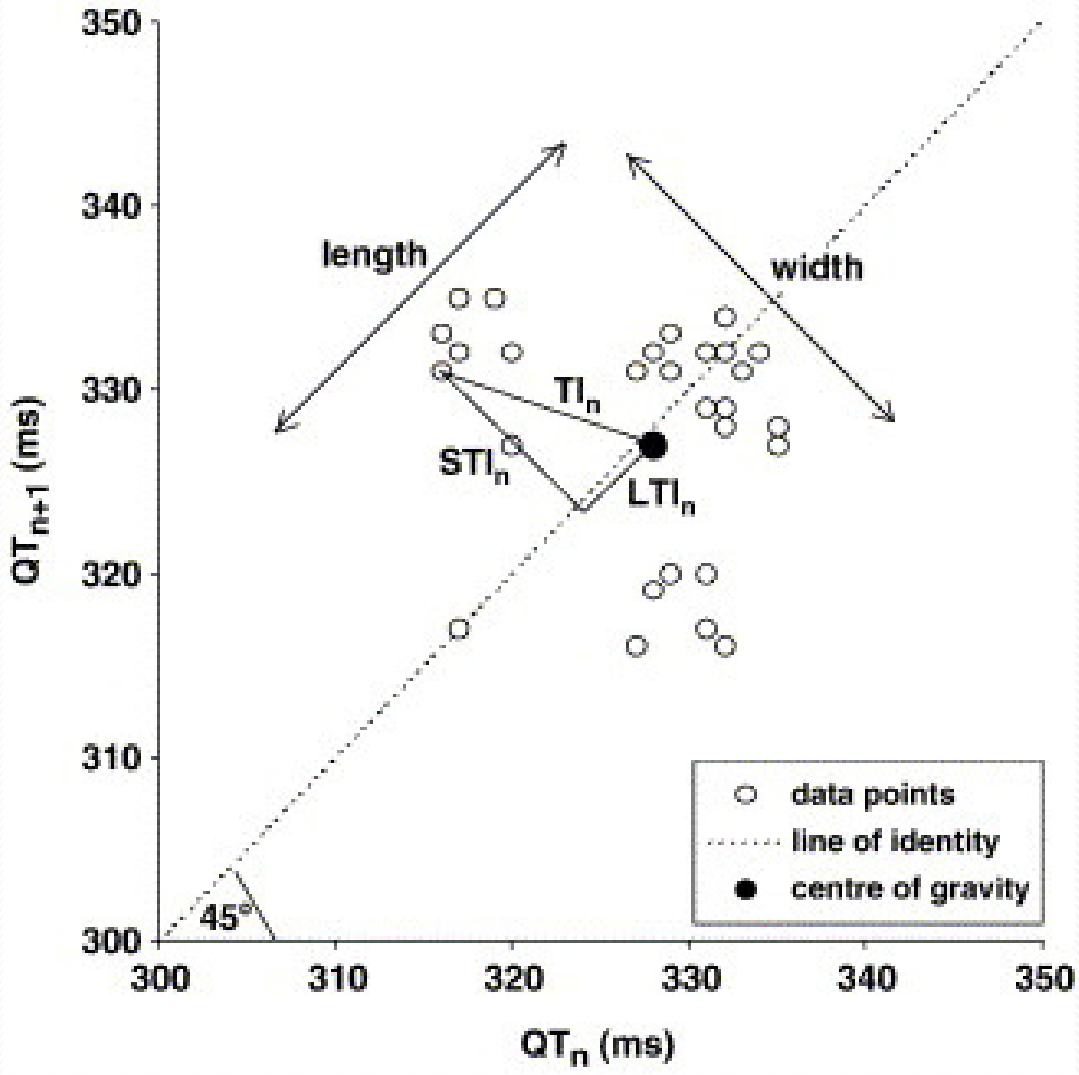
  $CV_{QT} = SD_{QT}/mean_{QT}$ (expressed in %)

- The formula of QT temporal dispersion described in the literature and used in clinical investigations:

  $QTdt = \log_{10} (CV_{QT}/CV_{RR})^2$

- The Poincare plot

Evaluation of QT temporal dispersion with a Poincaré plot

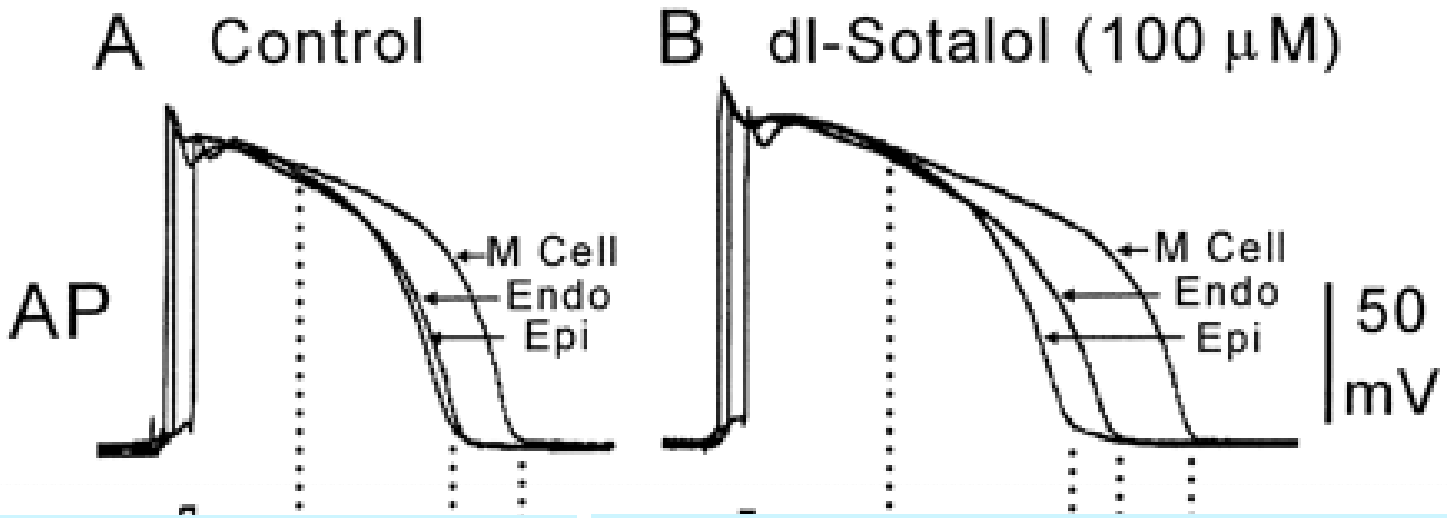


The width of the dispersion indicates short term instability and the length indicates long term instability

(Van der Linde et al, 2005)

Spatial heterogenity of cardiac repolarisation

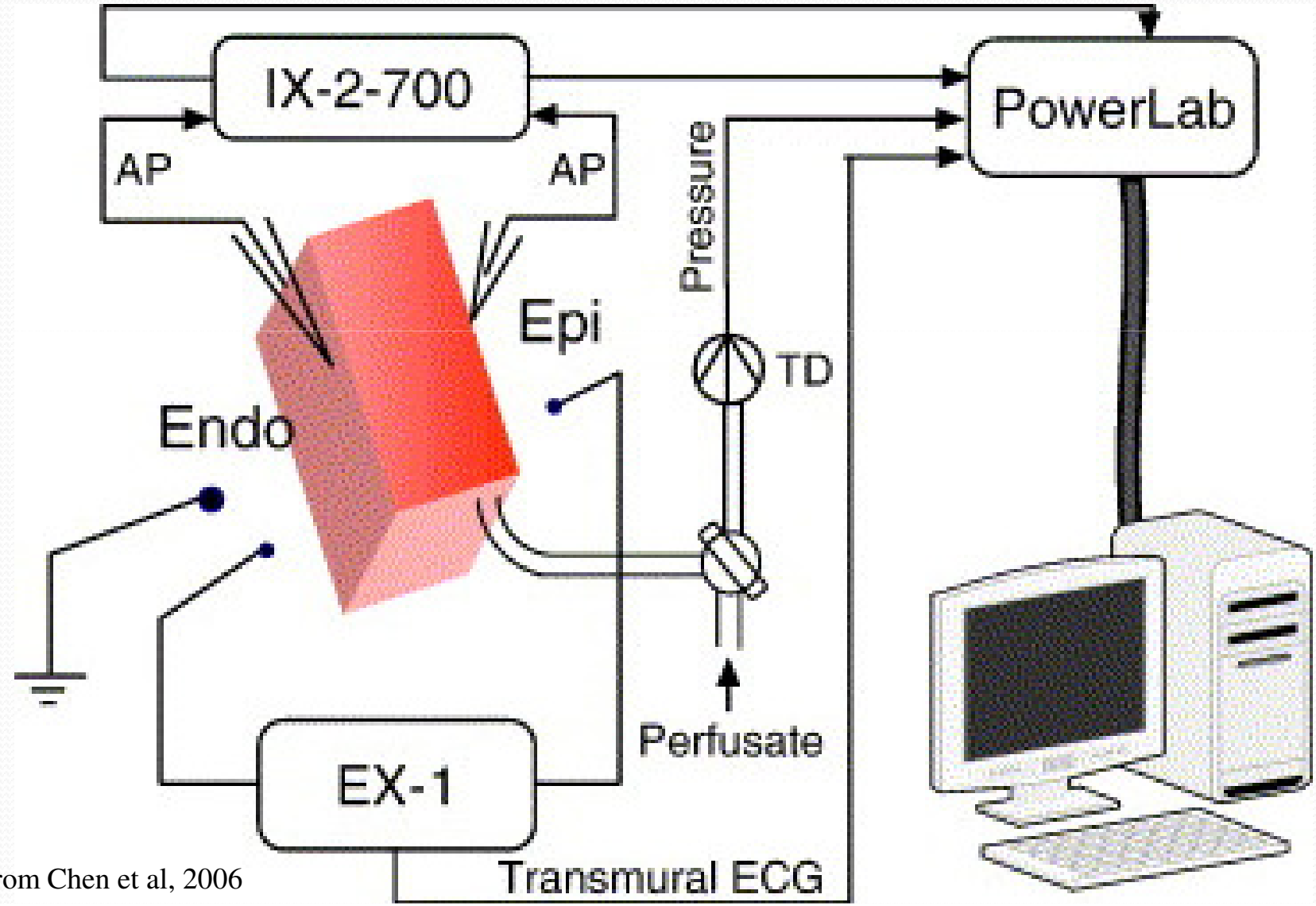
(fromYan and Antzelevitch, Circulation, 98, 1928-1936, 1998)



The different layers of cardiac myocytes repolarise at different rates

- Some compounds affect differently the different layers
- This may produce an increase in the differences and subsequently re-entry
- Considered as a major cause of the triggering of torsade-de-pointes in humans

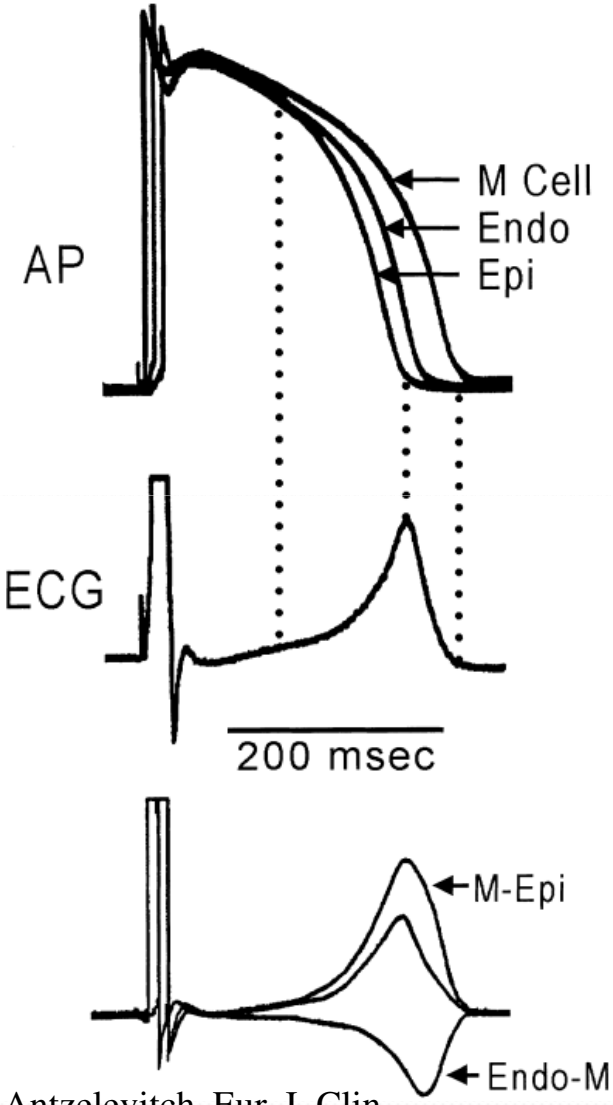
In vitro evaluation of spatial varaiability
Cardiac wedge preparation:



From Chen et al, 2006

In vivo evaluation of spatial variability
Change in the morphology of T wave

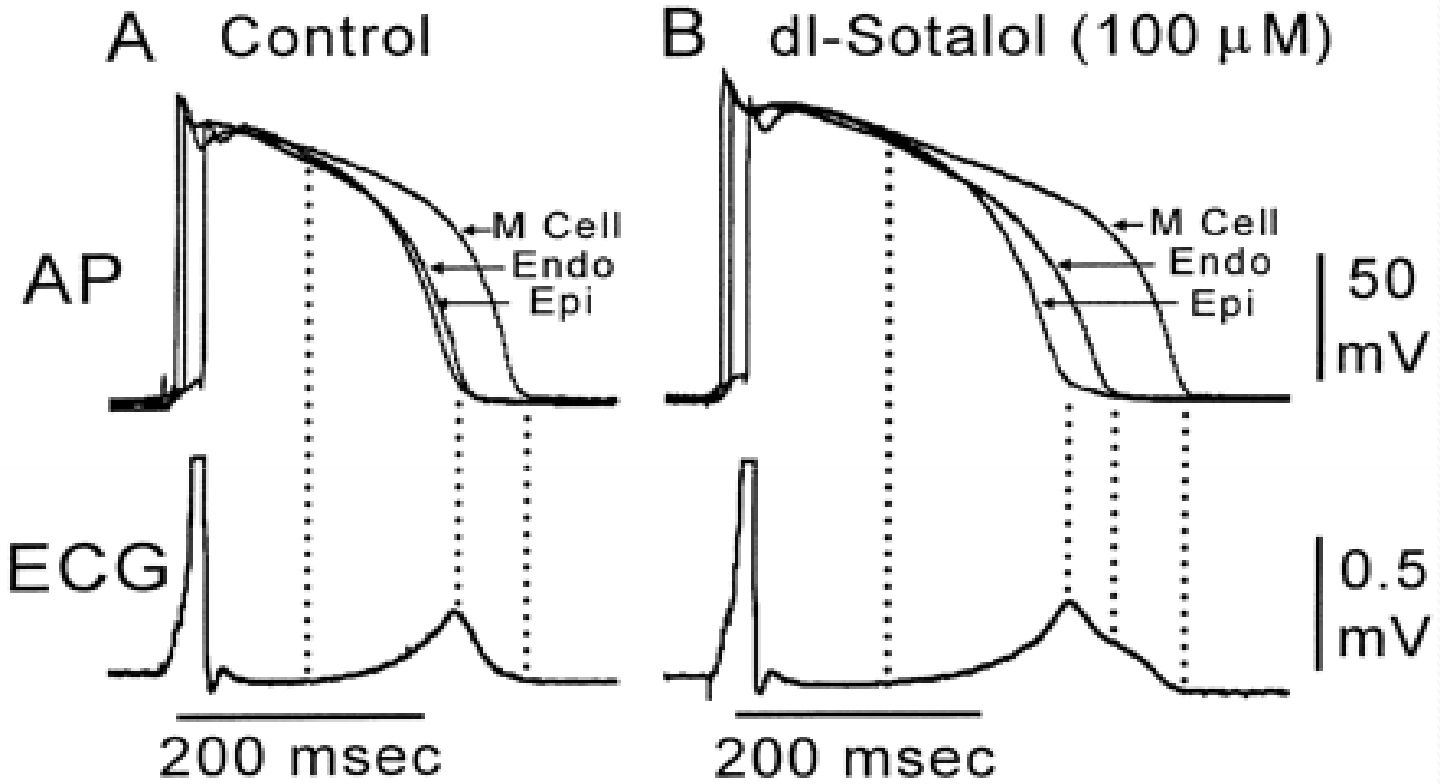
- T wave is the result of 2 opposing voltage gradients
  1. between mid-myocardium M cell and epicardium
  2. between M cell and endocardium
  - Full repolarization of epicardial cell correspond to peak of T wave
  - Full repolarization of M cells correspond to end of T wave



From: Antzelevitch, Eur. J. Clin Invest, 31, 555-557, 2001

Change in the morphology of T wave after I_{Kr} blocking

(fromYan and Antzelevitch, Circulation, 98, 1928-1936, 1998)



- Increase heterogeneity of repolarization of these different cell layers produced
  - Increase in Tpeak – Tend interval
  - Notching of the T wave

Experimental Plan: Aim

- Establish ***in vivo*** markers for the risk of arrhythmia
  - Assess the changes in dog ECG corresponding to spatial and temporal variability of cardiac repolarization
- Testing of astemizole, isoproterenol, and hypokalaemia, which are kown to be associated with a proarrhythmic risk
  - Confirm that ***in vivo*** indicators of repolarization variability are affected

Experimental Plan: study design

- **Effects of astemizole**
  - Cross over design: treatment of 9 dogs/group with a single intravenous injection of astemizole at doses of 0, 1 or 3 mg/kg
  - Another group of 3 dogs received single intravenous injections at increasing doses of 6, 9 and 15 mg/kg
- **Effect of Cisapride**
  - Cross over design: treatment of 9 dogs/group with a single intravenous injection of cisapride at doses of 0, 1.5 or 6 mg/kg
- **Effects of isoproterenol**
  - A group of 3 dogs received increasing doses (2.5, 5 and 10 µg/kg) of isoproterenol by the subcutaneous route
- **Effects of hypokalaemia**
  - Hypokalaemia was induced by treatment of 12 dogs with furosemide (5 to 60 mg/kg) over 12 days

ECG recording

- Evaluation of beat-to-beat variability of QT:
  - Coefficient of variation of QT

    $CV_{QT} = SD_{QT}/mean_{QT}$ (expressed in %)
- Evaluation of spatial variability of repolarisation
  - Morphology of T wave in precordial lead CV5RL
  - Scoring of notching

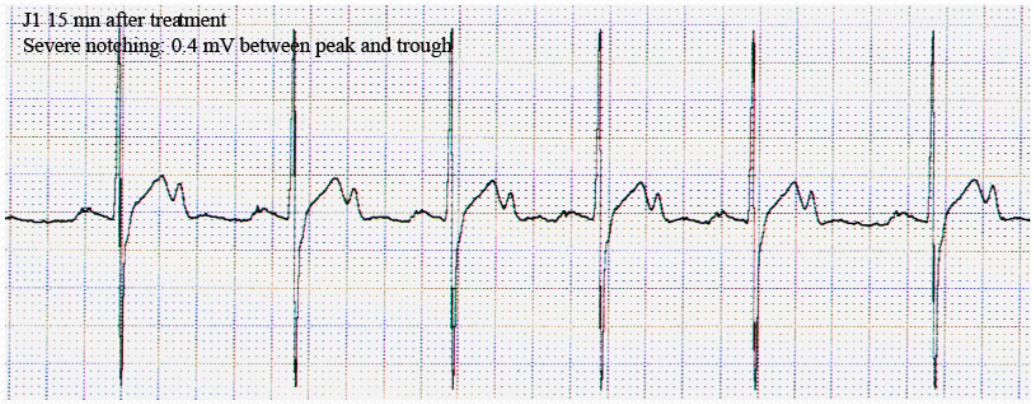
Evaluation of T wave morphology

- In the precordial lead CV5RL, the T wave is always monophasic and positive in healthy untreated dogs
- A grading system for the notching has been established

From 0 = No notching



To 5 = severe notching



Astemizole cross-over study

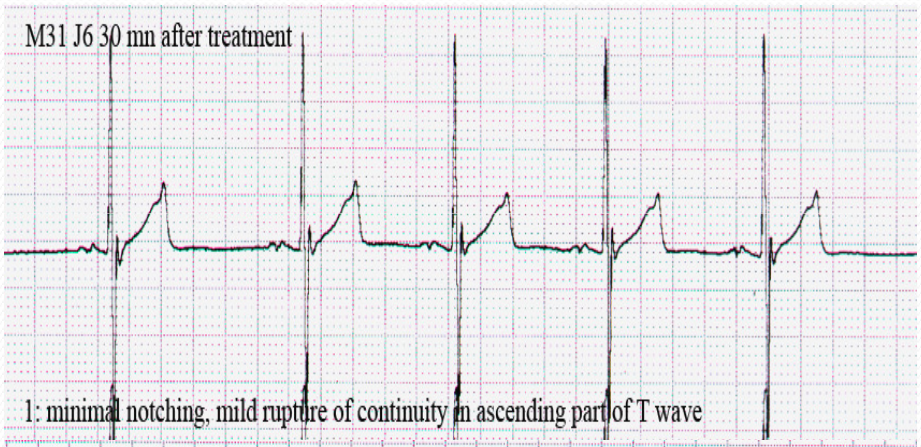
- Increase in mean QT
- Increase in the CV of QT

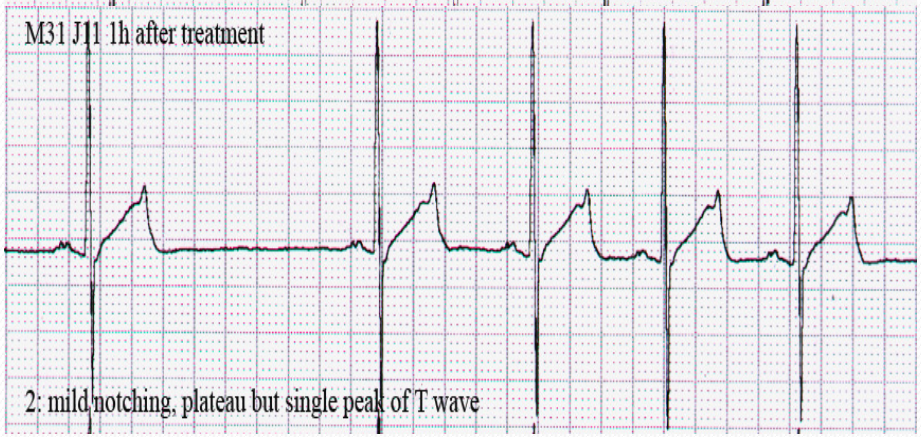
	CVQT mean values at 3 time points (%) (n=9) Lead CV5RL			**Difference compared to predose values**	
	Pre-dose	30 min	60 min	30 min	60 min
Control	1.51±0.33	1.47±0.51	1.62±0.37	**-0.04**	**0.11**
1 mg/kg	1.65±0.41	2.48±0.57	2.25±0.74	**0.84****	**0.61**
3 mg/kg	1.67±0.35	2.89±1.15	2.80±1.22	**1.22****	**1.13****
Change compared to control at 1 mg/kg	**0.13**	**1.01*****	**0.63***		
Change compared to control at 3 mg/kg	**0.16**	**1.42*****	**1.18****		

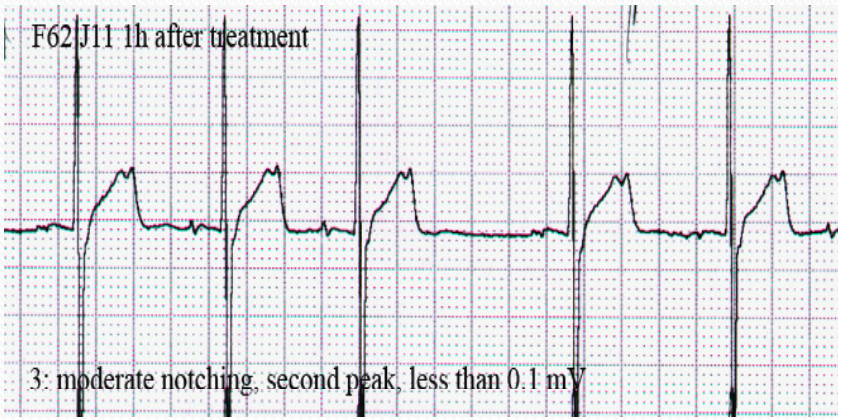
*: p<0.05, **: p<0.01, ***: p< 0.001

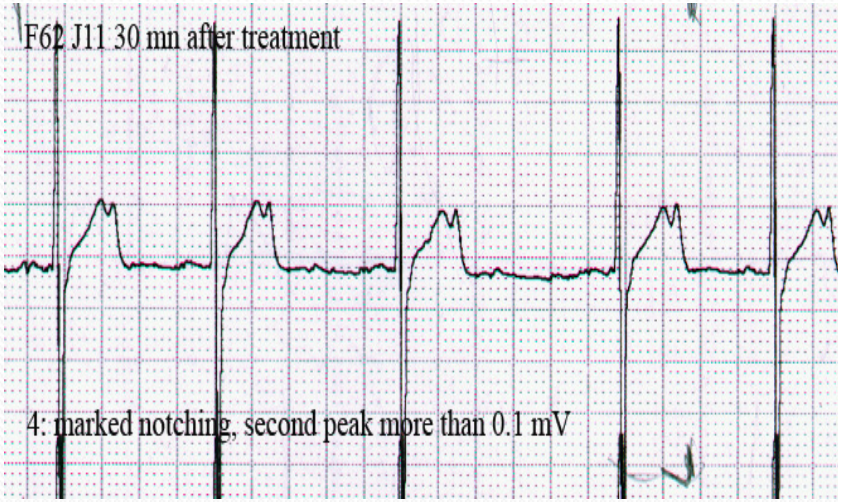
Astemizole cross-over study: Notching of T wave in CV5RL precordial lead







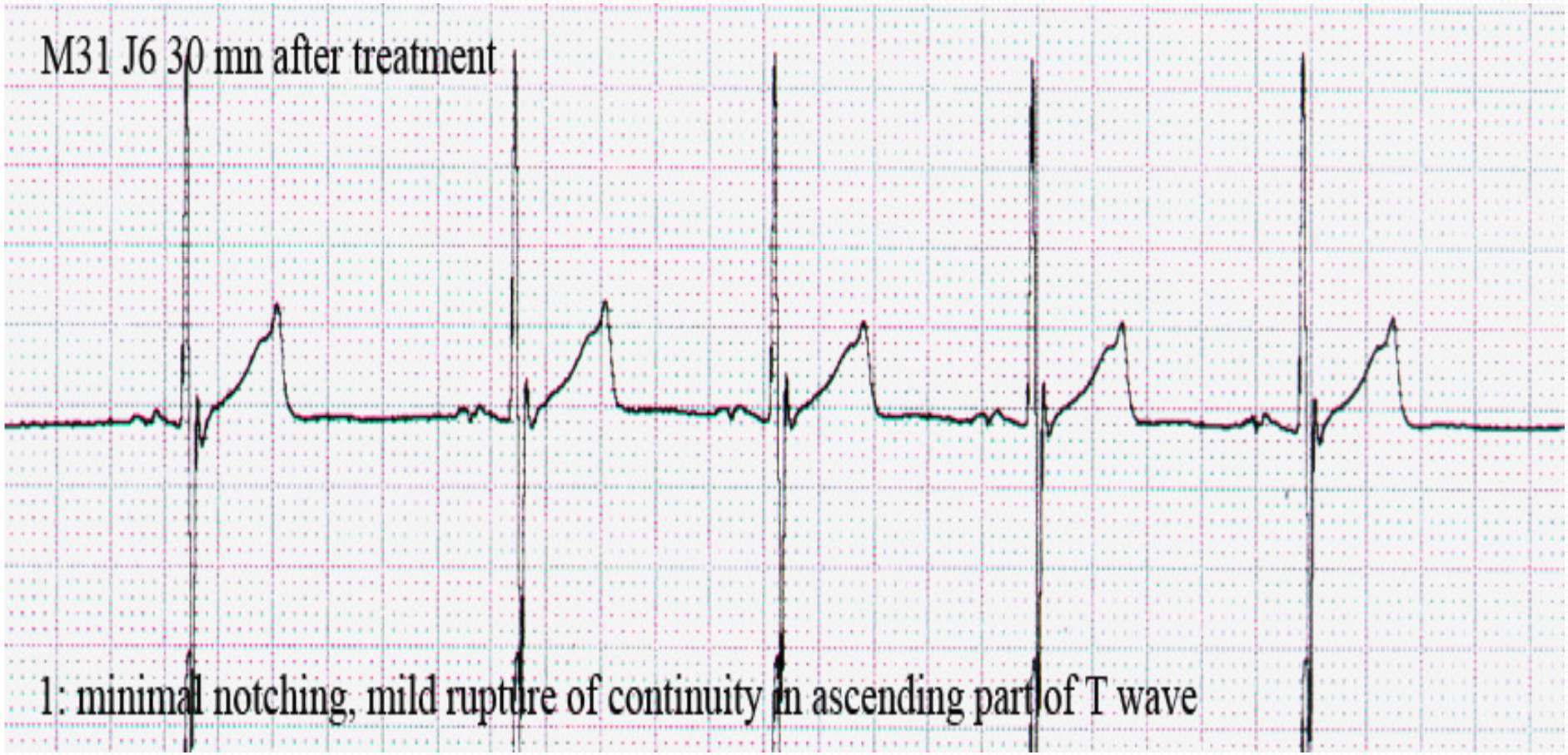




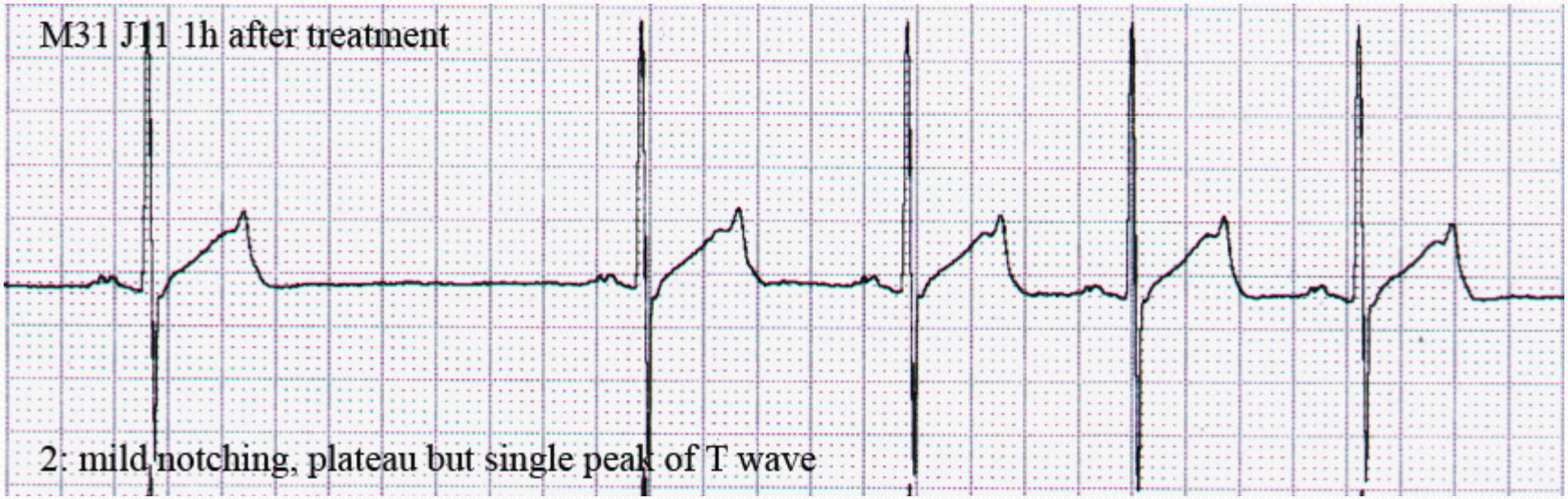
Normal T wave after vehicle treatment



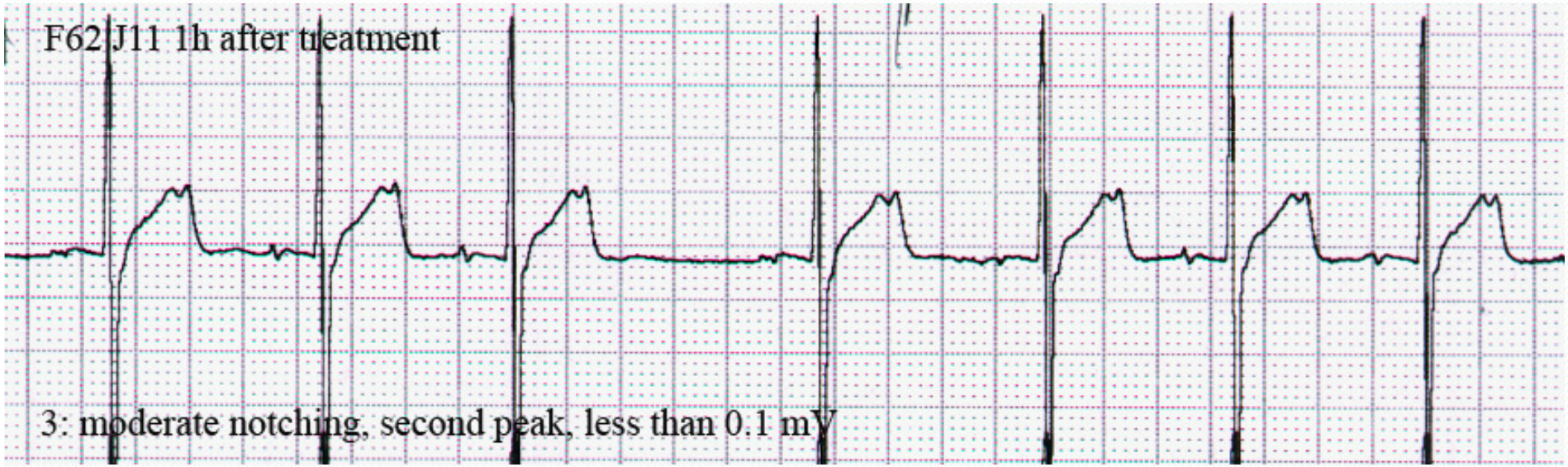
Notching after 1 mg/kg



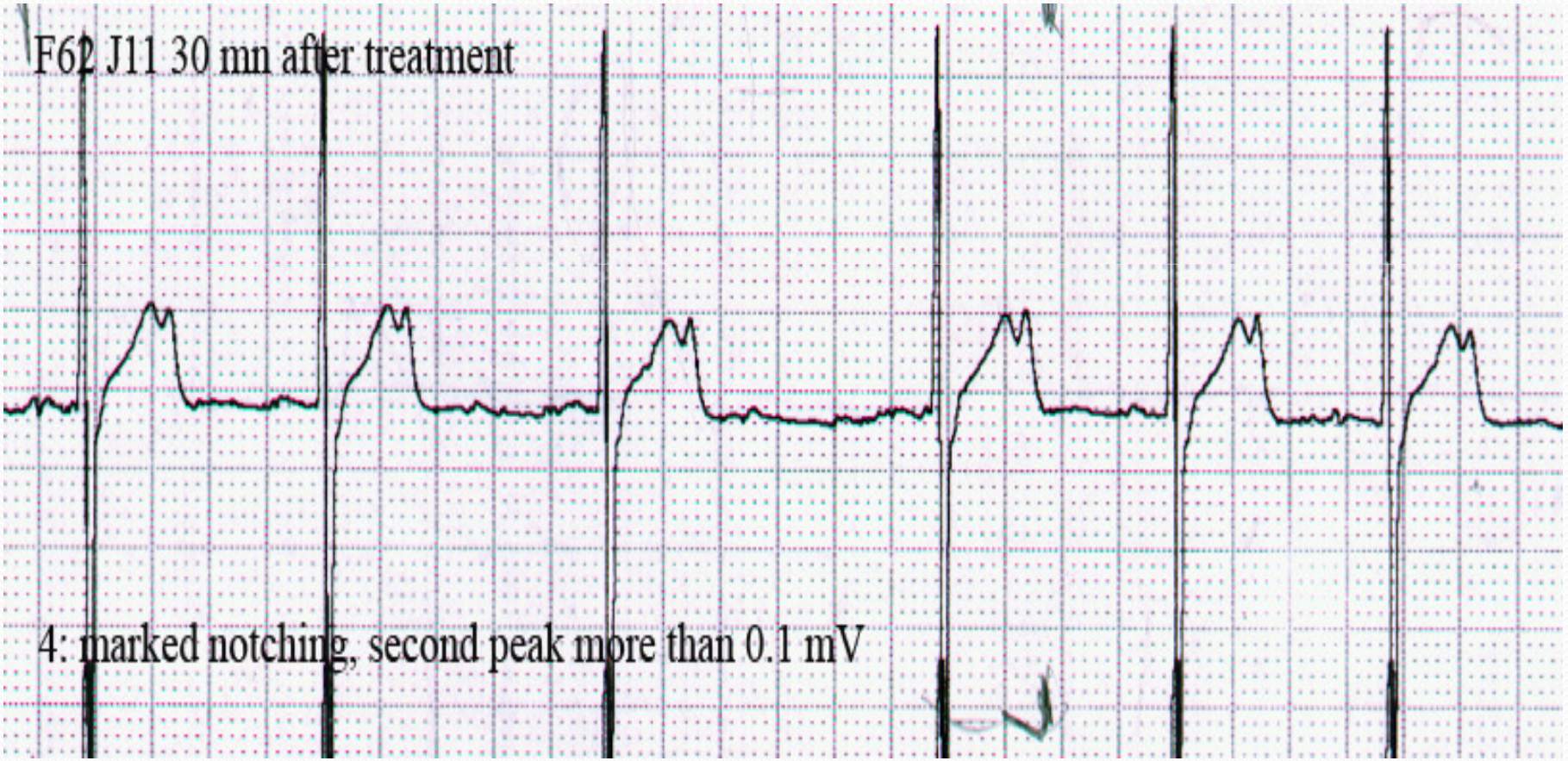
Notching after 1 mg/kg



Notching after 3 mg/kg



Notching after 3 mg/kg



Astemizole cross-over study

- Notching of the T wave in CV5RL

Mean score (n = 9) and *range mini max*			
	Before	0.5 hour	1 hour
Control	0.55 *0 - 2*	0.22 *0-1*	0.75 *0-2*
1 mg/kg	0.55 *0-2*	2.11 *0-3*	2.22 *0-4*
3 mg/kg	0.55 *0-1*	3.22 *1-4*	2.67 *0-4*

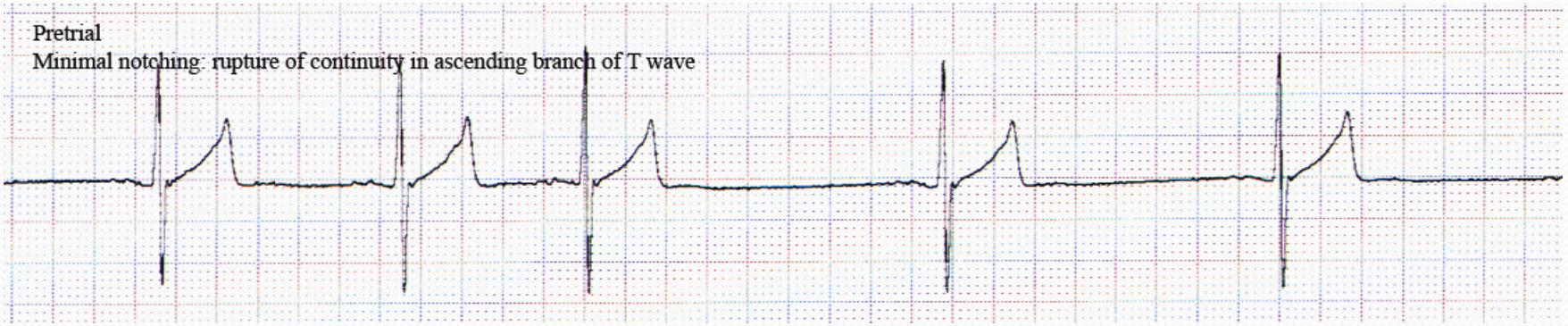
Astemizole increasing dose study

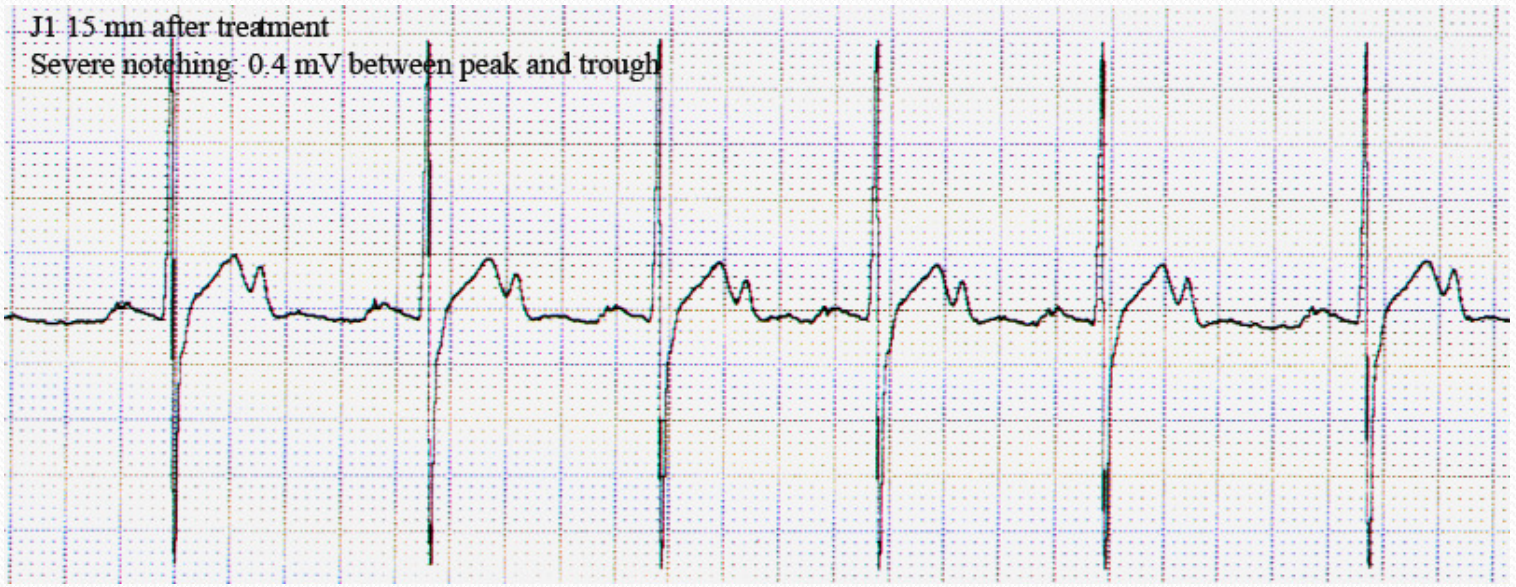
- Increase in mean QT interval, CV of QT

Changes (%) compared to baseline in coefficient of variation of QT after treatment with 6 mg/kg of astemizole (n = 3)	
Time after dose (min)	CV of QT
15	+53
60	+124

Astemizole increasing dose study

Notching of T wave



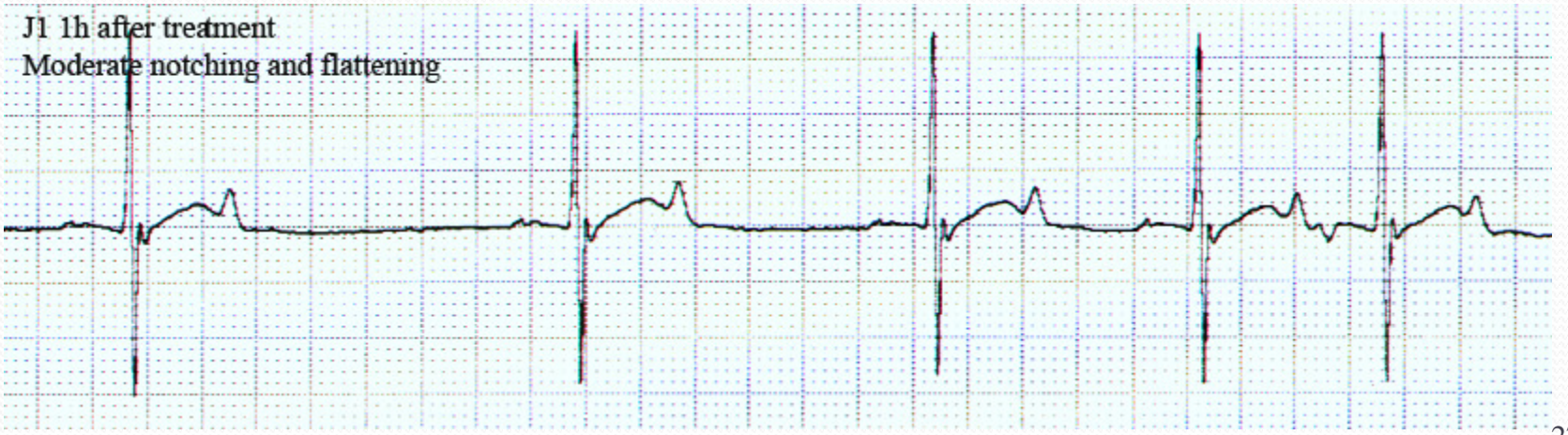


6 mg/kg

Astemizole increasing dose study: Notching of T wave

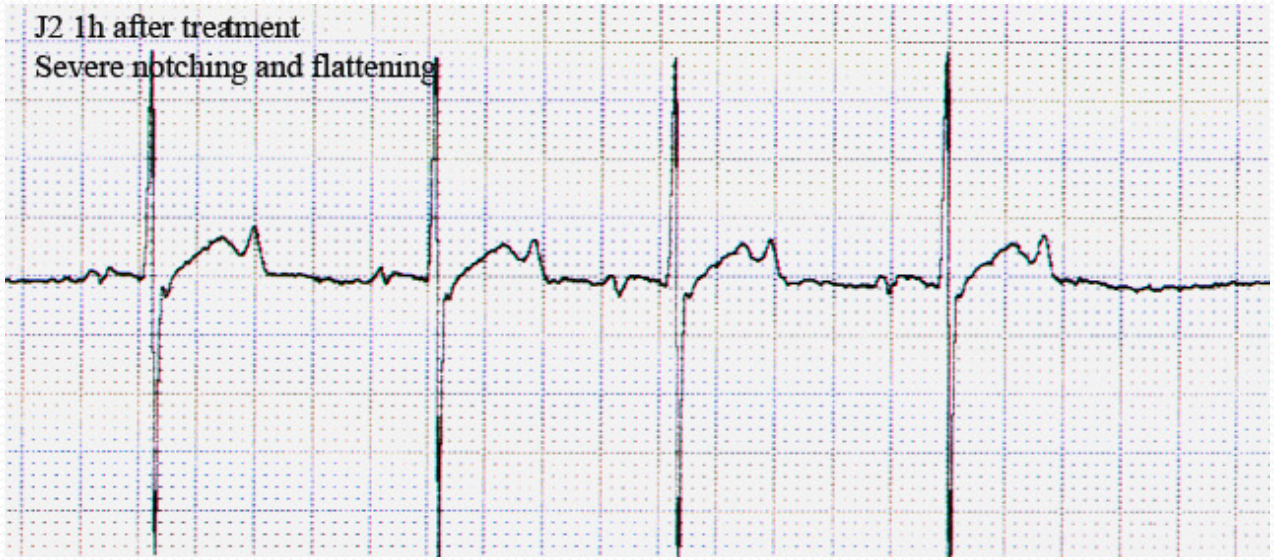


6 mg/kg



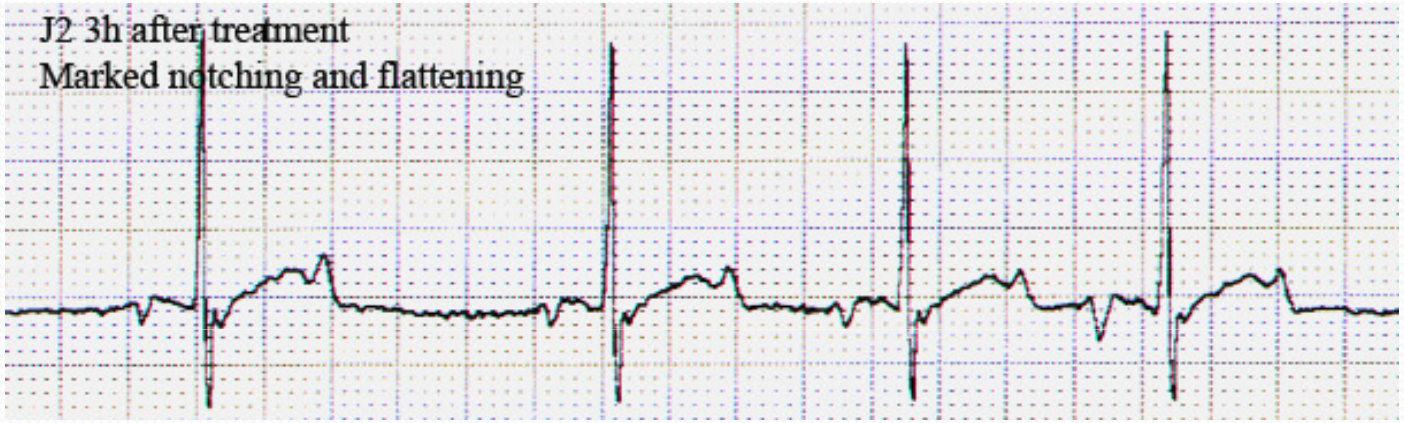
Astemizole increasing dose study: Notching of T wave



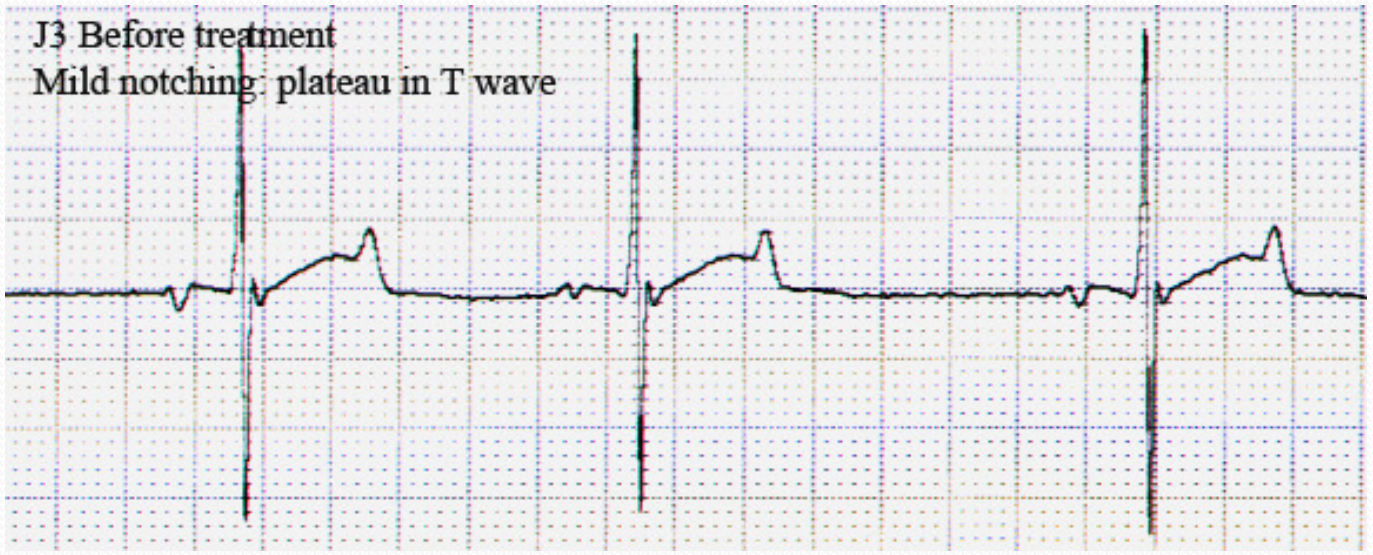


9 mg/kg

Astemizole increasing dose study: Notching of T wave



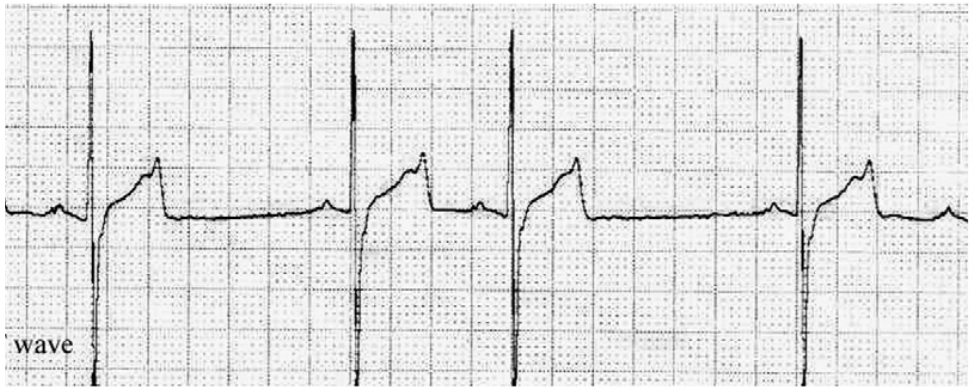
9 mg/kg



Effect of Astemizole: summary

- Increase in QT interval
- Increase in beat-to beat variability of QT
- Notching of T wave indicative of an increase in transmural heterogeneity of cardiac repolarization
  - Blocking of I_{Kr} occurs predominantly on the M cells, which are more sensitive to this blocking than epicardial or endocardial cells

Twave notching in the cross-over study with cisapride.







Mild notching 1 h after treatment with 1.5 mg/kg: (grade 2).

Marked notching with flattening of the T wave 1 h after treatment with 6 mg/kg (grade 4).

Severe notching of the T wave, 3 h after treatment with 6 mg/kg. (grade 5).

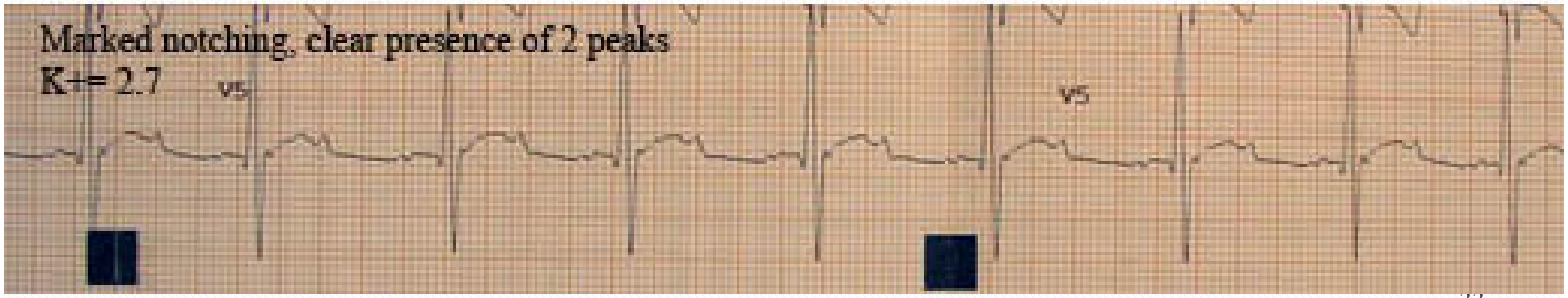
Hypokalaemia

- Increase in QT interval
- Change in morphology of T wave:
  - Notching, flattening, inversion, biphasic or triphasic aspect
  - Extracellular potassium prolongs the duration of cardiac action potential to a greater extent in the epicardium than in other myocardial layers, which is attributed to a predominant I_{to} current in the epicardium

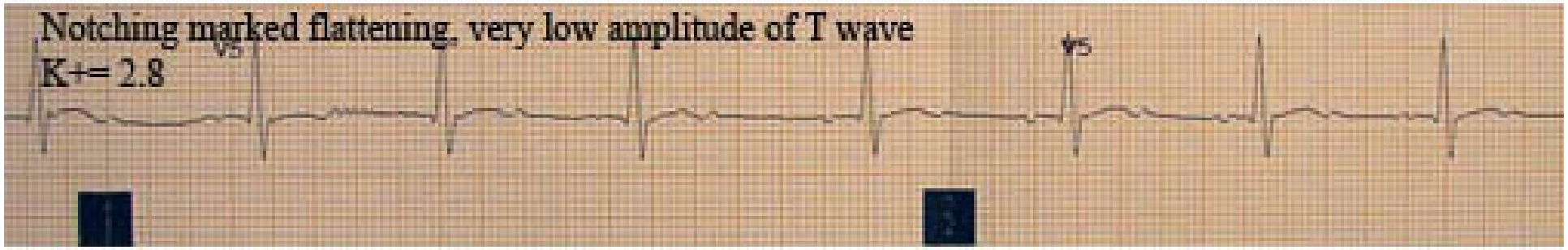
Effects of hypokalaemia on T wave morphology

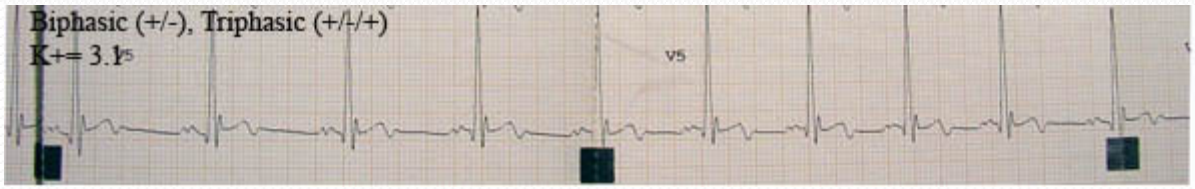


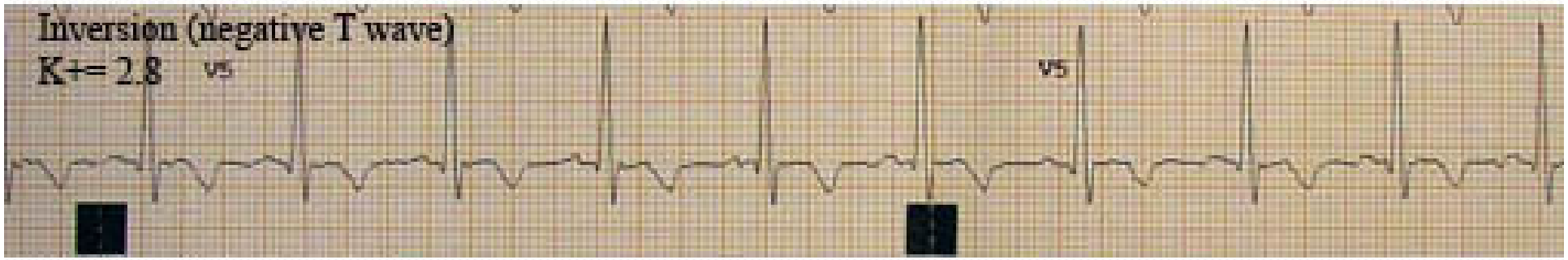




Effects of hypokalaemia on T wave morphology







Isoproterenol

- Decrease in QTc interval
- Notching of T wave
  - Isoproterenol produces a shortening of action potential in epicardial and in endocardial cardiomyocytes and has minimal effects on action potential of M cells
  - Results from intrinsic differences in I_{Ks} (on which isoproterenol act predominantly), among the 3 myocardial cell types

    Large augmentation in I_{Ks} current in epicardial and endocardial cells but not in Mcell in which I_{Ks} is weak

Isoproterenol: notching of the T wave







Conclusion:
In vivo evaluation of temporal and spatial variability of cardiac repolarization

- Coefficient of variation of QT found to be a good evaluator of beat-to-beat variability of QT
- Changes in morphology of T wave in CV5RL found to be a good evaluator of spatial variability
  - Easy: only one lead required
  - Allows detection of changes produced by drugs or conditions increasing transmural heterogeneity of repolarization by different mechanisms

Conclusion:
In vivo evaluation of transmural heterogeneity of cardiac repolarization

- Evaluation of QT spatial dispersion in dogs
  - Not always possible, time-consuming: needs recording of a number of leads
  - Not accurate

CONCLUSION

Action potential instability (beat-to-beat variability of cardiac repolarization) and transmural heterogeneity of repolarization are considered as key factors in triggering arrhythmias

Calculation of CV of QT can assess action potential instability

Evaluation of changes in morphology T wave recorded in CV5RL (notching) can evaluate transmural heterogeneity

Increase in CV of QT and T notching occurred in pro-arrhythmic conditions

Evaluation of CV of QT and T wave morphology in dogs may help predicting risk of arrhythmia of compounds under development

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- **A. Yvon, PhD**: scientific supervision
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- **F. Besse**: scanning the ECG tracings
- **A. Racaud and B. Geffray**: statistics

Thank you for your attention

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Back-up slides

ECG recording

- **Leads**

  Standard bipolar limb leads I, II, III, unipolar limb leads aVR, aVL, aVF and precordial leads CV6LL, CV5RL, CV6LU, V10.

- **Timing**
  - Astemizole
    - Cross over: before treatment and 0.5 and 1 hour after treatment (100 beats)
    - Increasing dose: before and 15 minutes, 30 minutes, 1 hour and 3 hours after treatment (40 beats)
  - Isoproterenol: before and 15 min, 30 min, 1 h, 3 h and 5 h after treatment (over 20 seconds)
  - Hypokalaemia: before furosemide dosing, then 1.5 and 3.5 hours after dosing (over 1 minute)

Evaluation of T wave morphology

Grading system for the notching

0. No notching
1. minimal notching, mild break of continuity in the ascending part of the T wave
2. mild notching, plateau but single peak of T wave
3. moderate notching, second peak, less than 0.1 mV between peak and trough, mild flattening
4. marked notching, second peak, 0.1 to 0.3 mV between peak and trough, moderate flattening
5. severe notching, second peak, more than 0.3 mV between trough and peak, marked flattening and/or trough at the isoelectric line or slightly below

Increase in QT interval associated with decrease in plasma potassium

