Beat Rate Variability of Pacemaker Cells

Heart rate/Beat rate variability (HRV/BRV); its sources and underlying mechanisms

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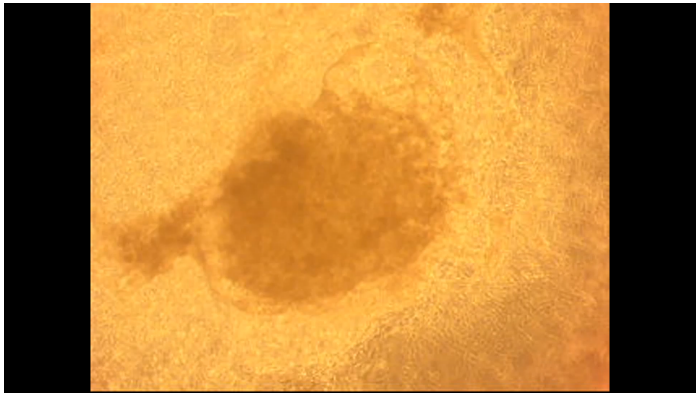

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הפקולטה לרפואה ע"ש רות וברוך רפפורט
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Haifa

5th Lugano Stem Cell Meeting
20th - 21st June 2016 - Università della Svizzera Italiana - Switzerland
STEM CELL MEETING

The study was initiated by the question….

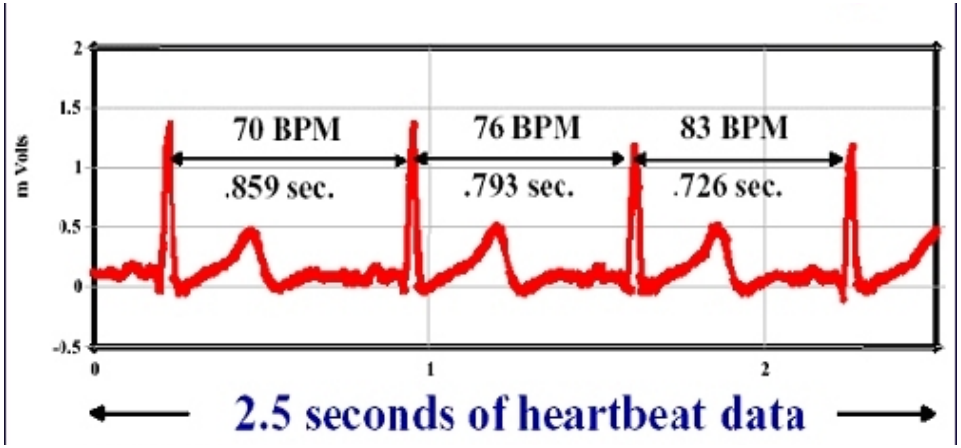
Can induced Pluripotent Stem Cell (iPSC) derived cardiomyocytes (iPSC-CM) serve as potential biological pacemakers?

Michael Rosen and Ira Cohen

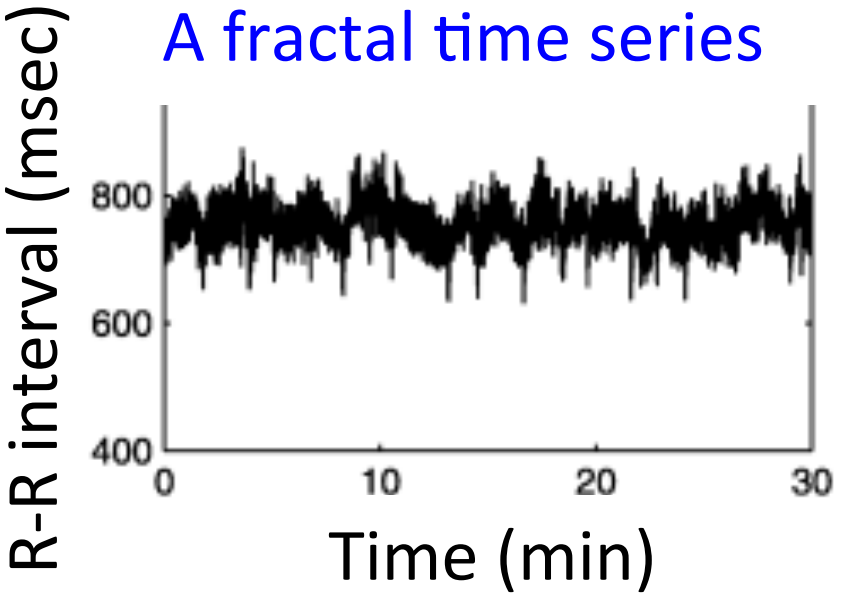
Why study Beat Rate Variability (BRV) in iPSC-CM?

- In addition to automaticity, a key feature of the human sinoatrial node (SAN) is **Heart Rate Variability** (HRV), which exhibits **self-similar fractal-like** oscillations
- Therefore, potential biological pacemakers, should not only fire spontaneously, but also exhibit BRV, similar to the SAN

Heart Rate Variability



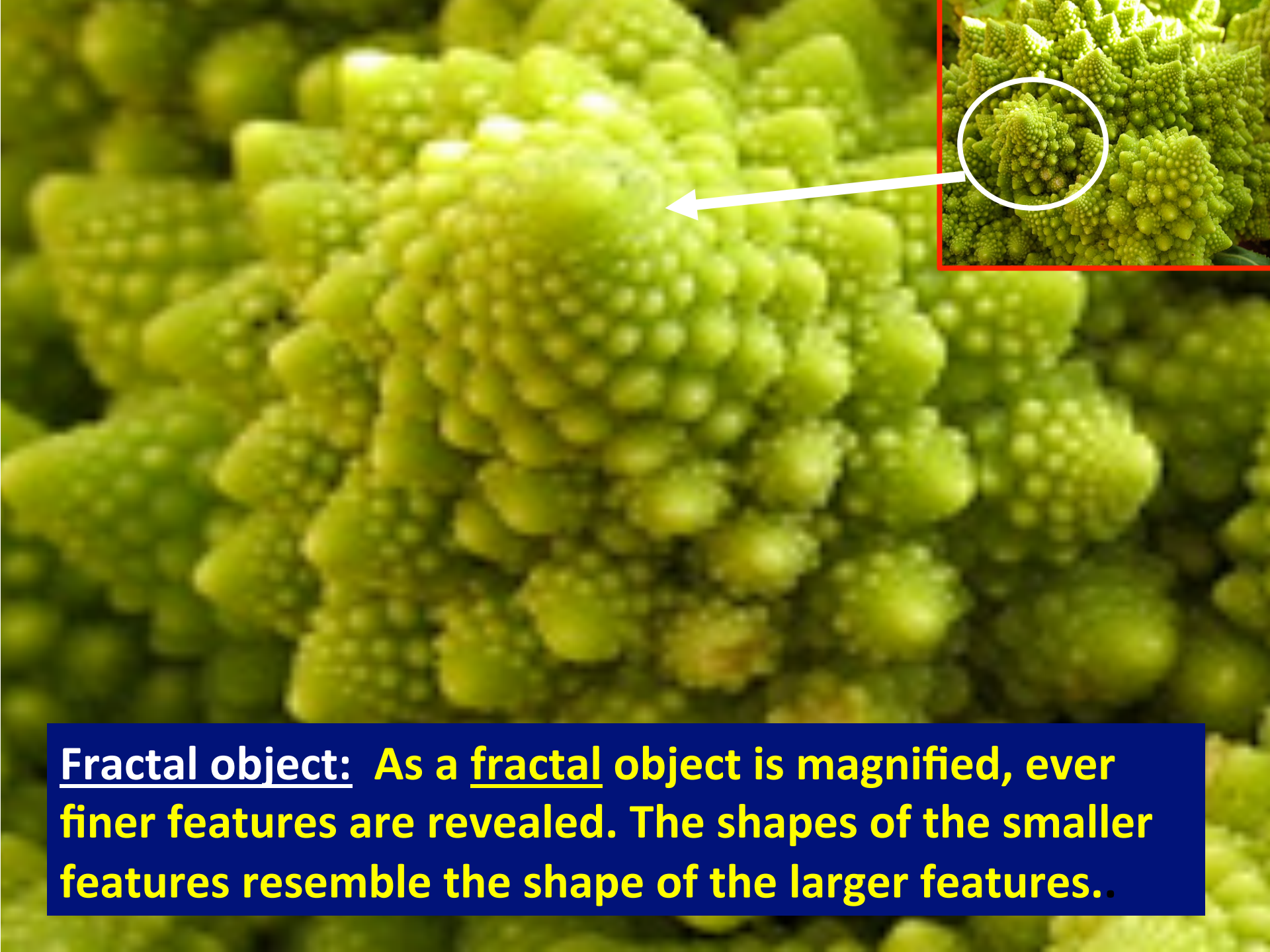
A fractal time series



- **Heart rate variability (HRV) refers to the beat-to-beat alterations in heart rate. Under resting conditions, the ECG of healthy individuals exhibits periodic variations in R-R intervals.**
- **This time series features self-similarity and fractal behavior.**

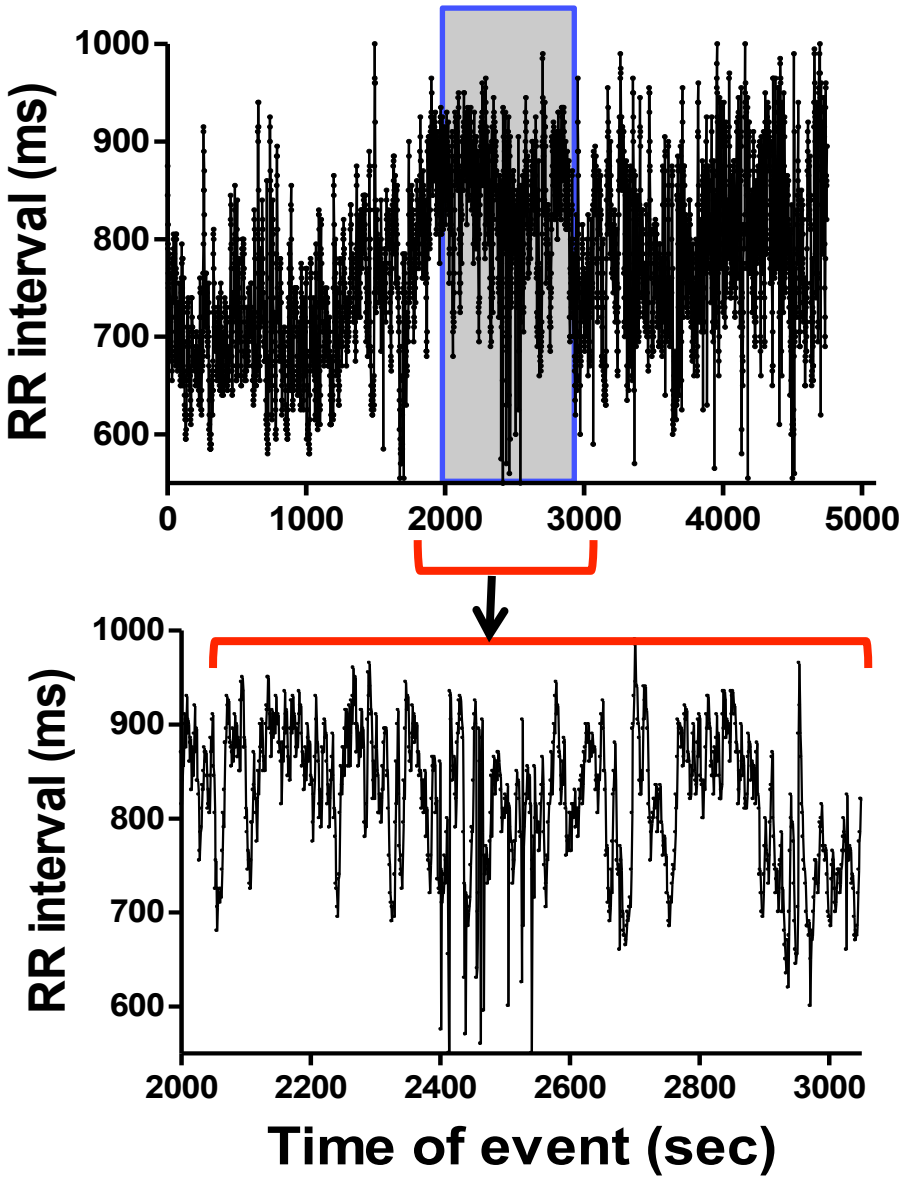
What are Fractality and Self-similarity?

- Fractal objects
- Fractal time series



Fractal object: As a fractal object is magnified, ever finer features are revealed. The shapes of the smaller features resemble the shape of the larger features..

A fractal time series: A plot of the R-R intervals versus time features self-similarity. ECG recorded from a 40-year old healthy volunteer

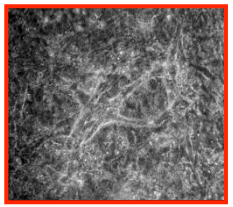


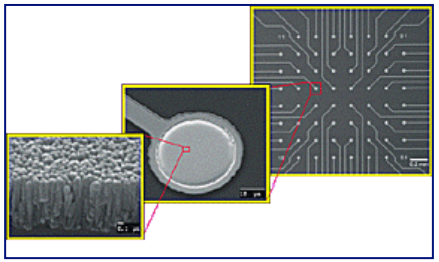
A time series can be considered self-similar (or fractal) if comparable fluctuations over multiple time scales can be identified. Namely, the time series looks "similar" on different times scales.

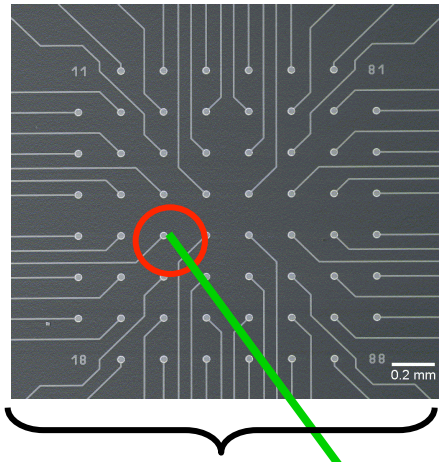
The key question

Does iPSC-CMs automaticity exhibit BRV and fractality?

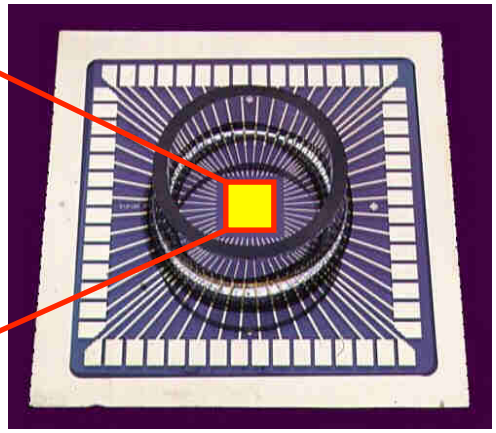
The Network level: The Micro-Electrode-Array (MEA)



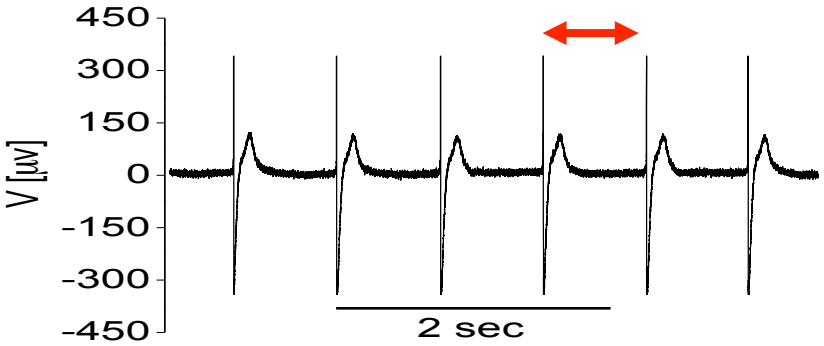


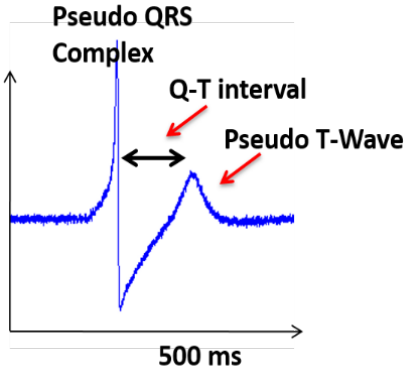


1.4 mm



Inter-beat interval (IBI)





Protocols

- Extracellular electrograms were recorded from hESC-CM and iPSC-CM cultures.
- The inter-beat intervals were analyzed for BRV properties.

Mandel et al, Circulation 2012

Circulation
JOURNAL OF THE AMERICAN HEART ASSOCIATION

Human Embryonic and Induced Pluripotent Stem Cell-Derived Cardiomyocytes Exhibit Beat Rate Variability and Power-Law Behavior

Yael Mandel, Amir Weissman, Revital Schick, Lili Barad, Atara Novak, Gideon Meiry, Stanislav Goldberg, Avraham Lorber, Michael R. Rosen, Joseph Itskovitz-Eldor and Ofer Binah

Conclusion:

BRV, self-similarity and fractality are inherent properties of the (isolated) cardiac "Network" and do not depend on extra-cardiac influences

But the common dogma says:

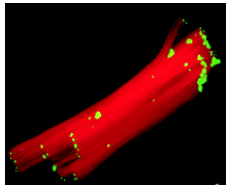
Heart Rate Variability is:

A measure of neurocardiac function that reflects heart-brain interactions and autonomic nervous system dynamics.

McCraty & Singer, 2002

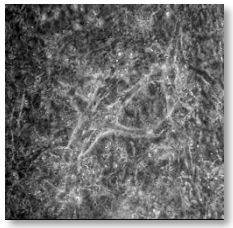
We think there are additional generators/ contributors to HRV/BRV

To investigate the sources of BRV/HRV, we generated the 3-level Hypothesis



First level: Cell

BRV originates from intracellular mechanisms (e.g., Ca^{2+} cycling)



Second level: Network

BRV is affected by the interaction between pacemakers, inter-cellular coupling

Third level: Heart

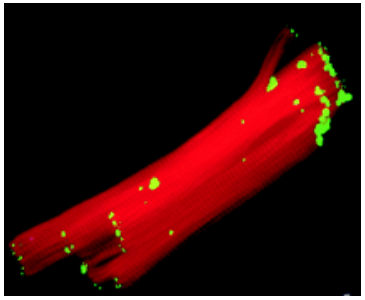
HRV is modulated by autonomic inputs, thermoregulation, hormones

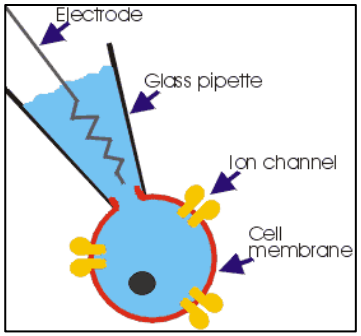
Integration

Binah et al. Integrating beat rate variability: From single cells to hearts. Heart Rhythm. 2013.

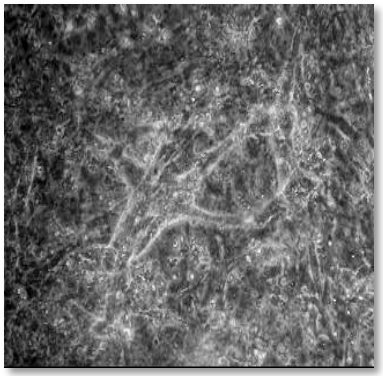
Testing the 3-level hypothesis in the same person: ECG, iPSC-derived network (contracting EB) and single cardiomyocytes

A single cardiomyocyte action potential (AP)

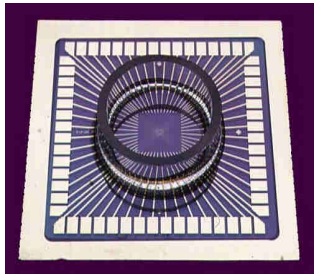




Electrograms Cluster/network (Embryoid Body, EB)



MEA



ECG

Electrophysiological recordings, Inter-Beat-Intervals (IBIs) versus time plots, and IBIs histogram at the three levels

Heart (ECG)

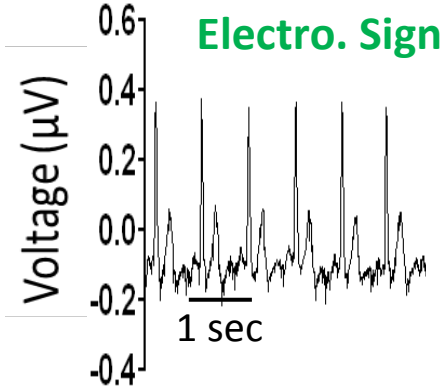
EB (MEA)

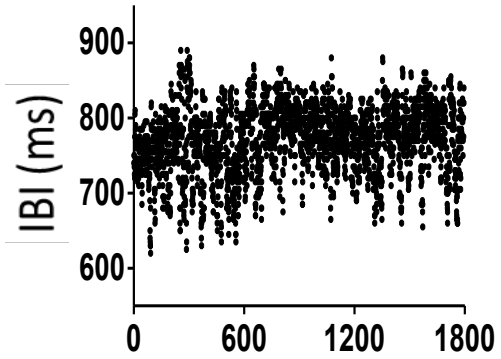
Single cell (AP)

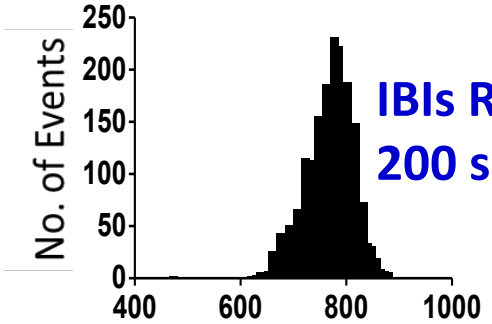
Electro. Signal

IBI versus Time

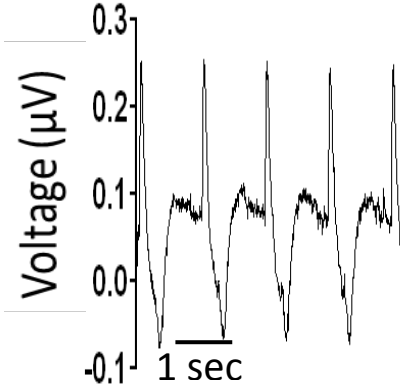
IBIs Histogram

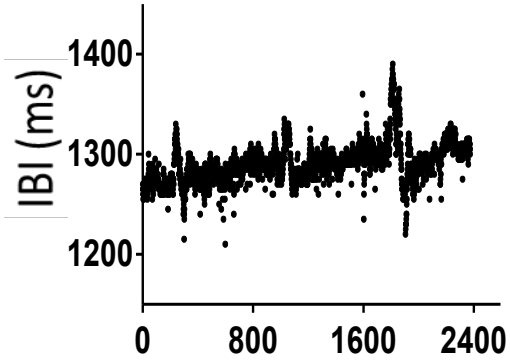


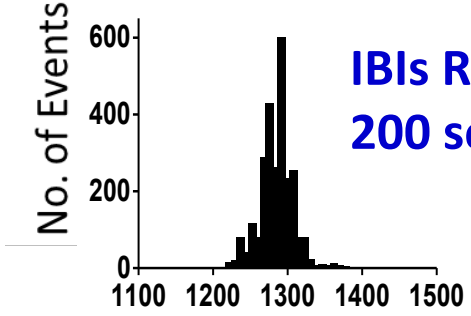




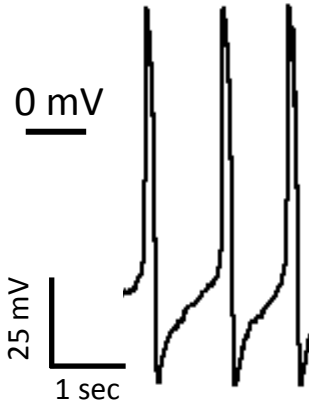
IBIs Range: 200 sec

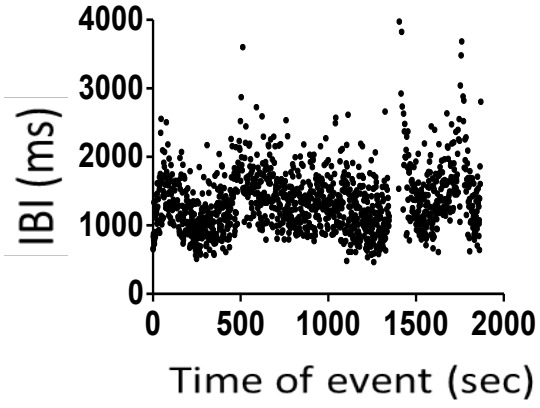


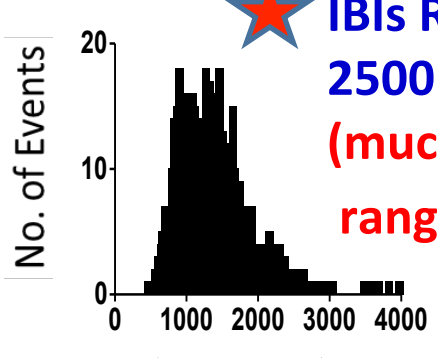




IBIs Range: 200 sec



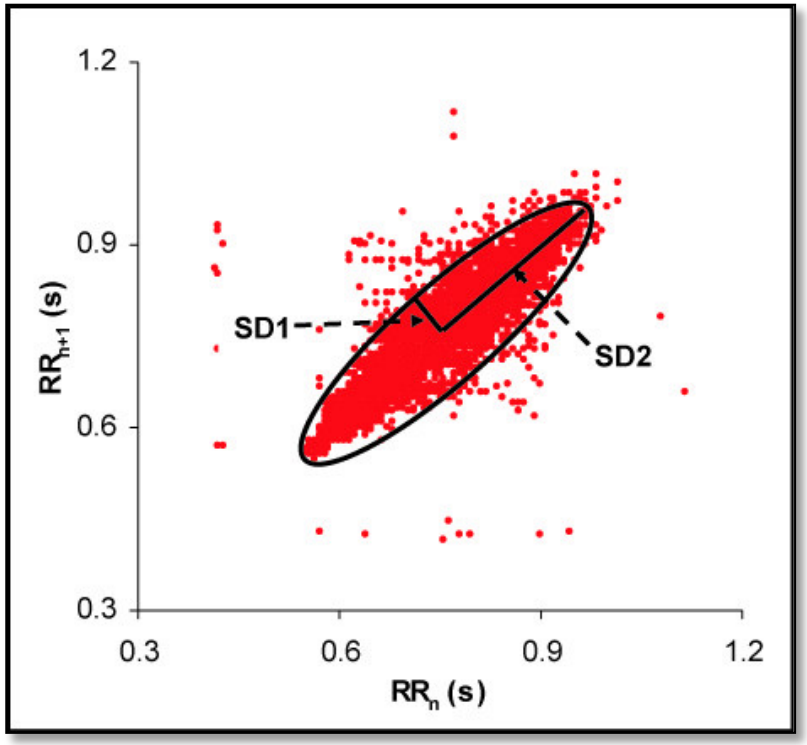




IBIs Range: 2500 sec (much broader range)

Poincaré plot: a quantitative measurement for the evolvement in time of the beat rate variability

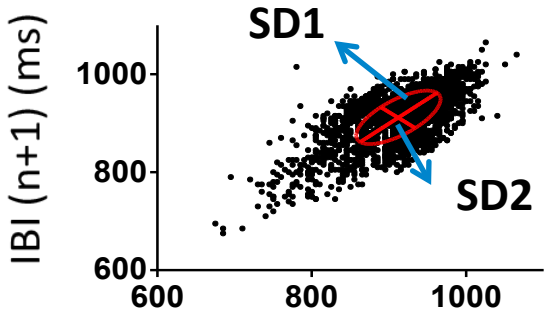
- A Poincaré plot is a common mean for quantifying self-similar fractal behavior, such as that of HRV.
- In a Poincaré plot, each RR interval is plotted against the previous interval, generating a scatter cloud in a 2-D array.
- The cloud is then fitted with an ellipse providing two quantitative indices: **SD1** - the standard deviation of the short-term IBI variability and **SD2** - the standard deviation of the long-term IBI variability.



Poincaré plots at the three levels.
Note difference in IBIs dispersion at the three levels

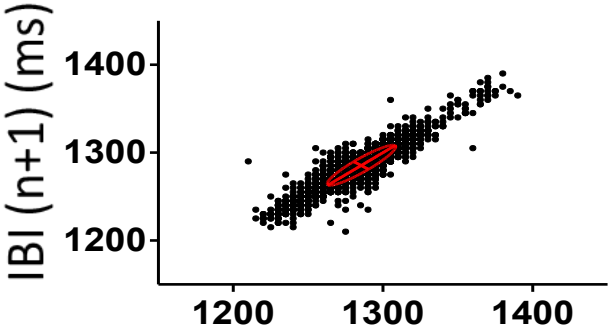
Heart

Range: 200 sec



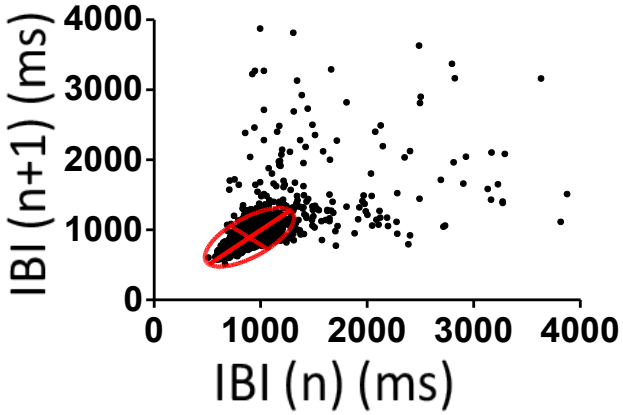
EB

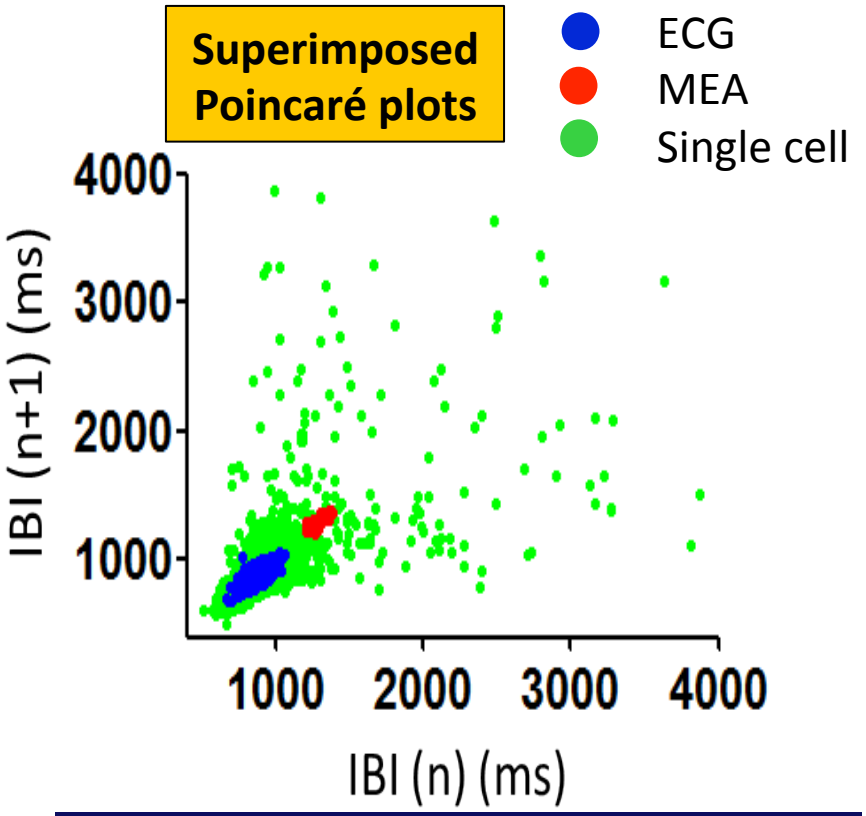
Range: 200 sec



Single cell

Range: 2500 sec

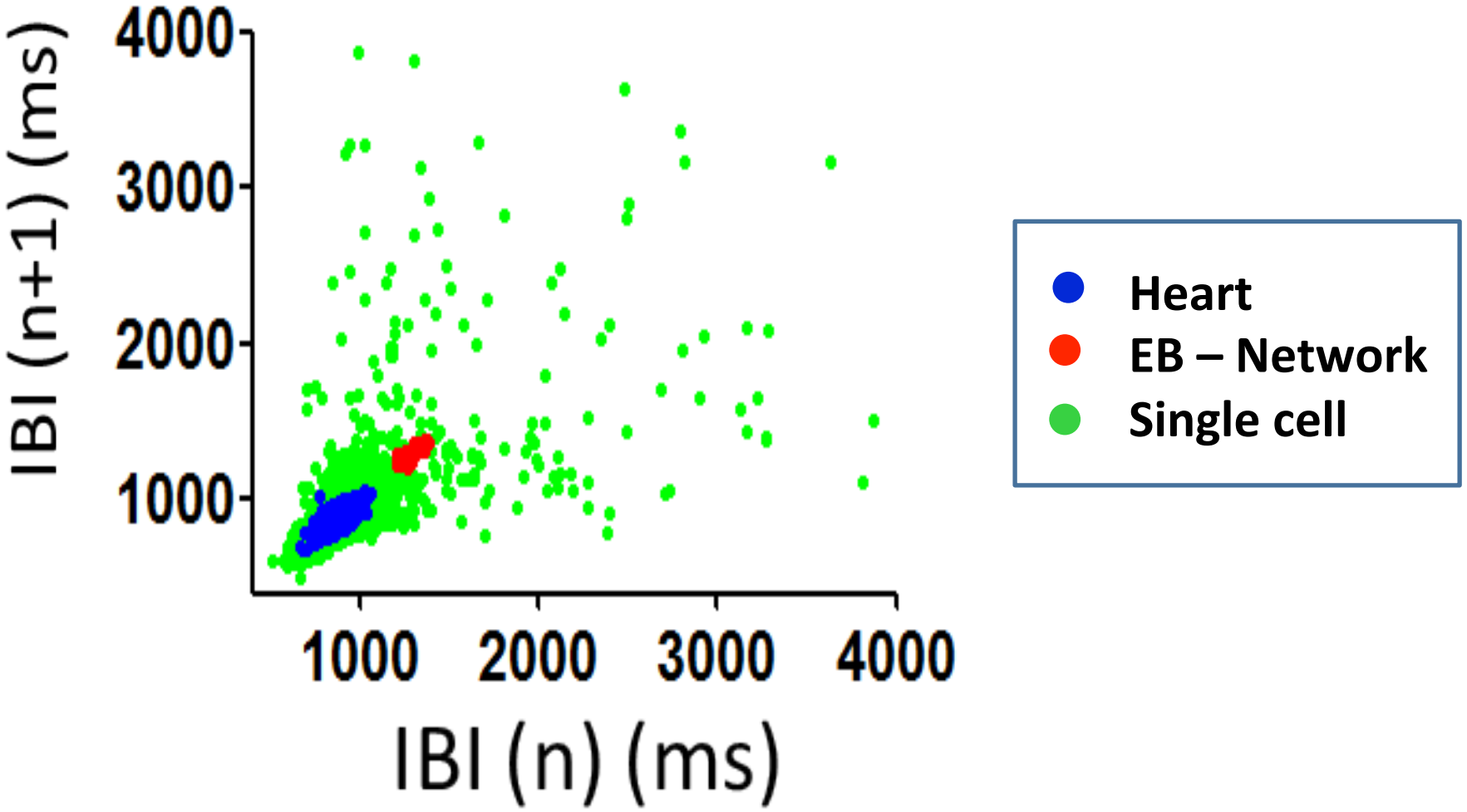




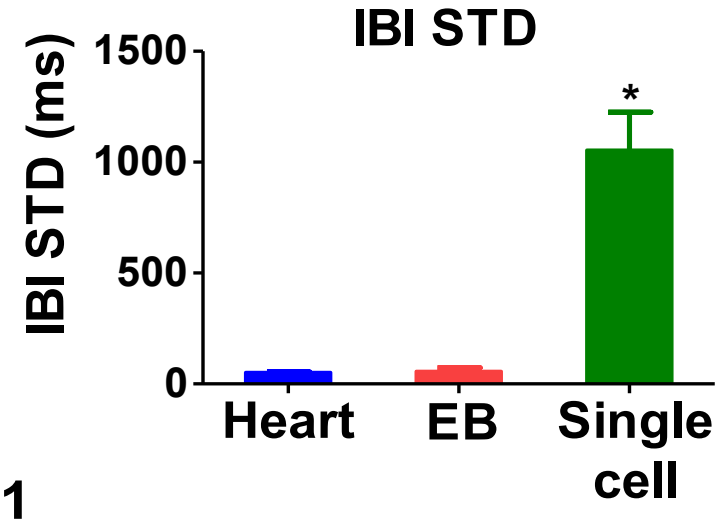
Single cell depicts the highest dispersion

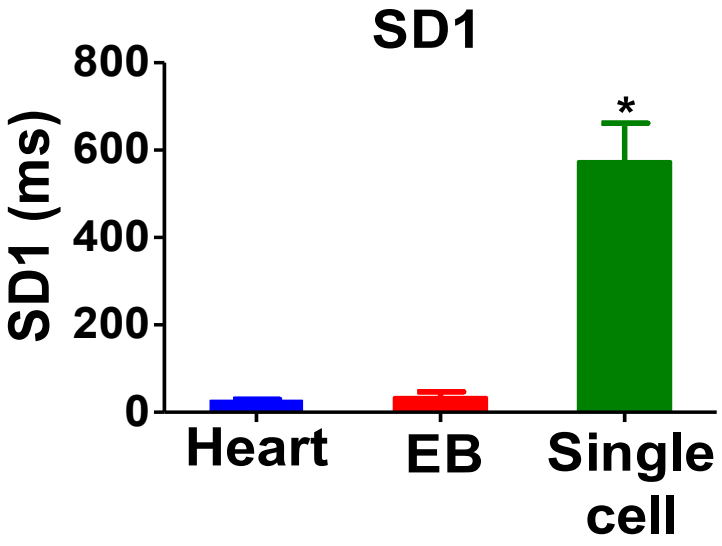
Superimposed Poincaré plots compare BRV from the three levels.

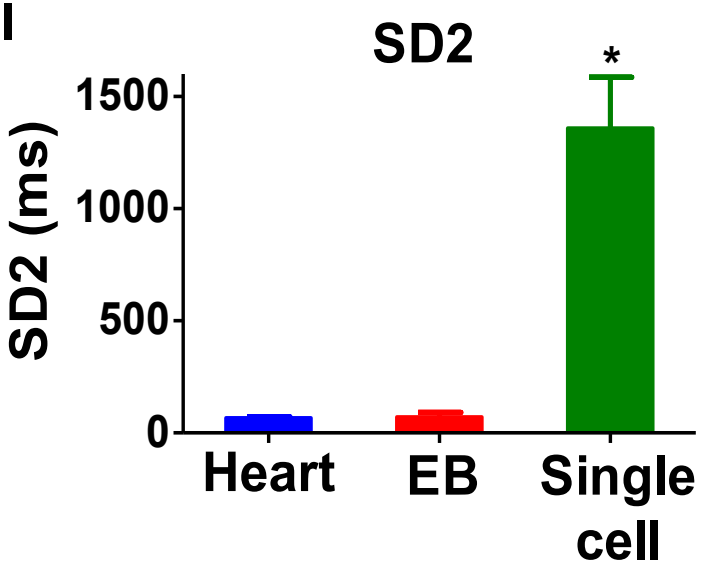
Single cell depicts the highest dispersion



IBIs Standard Deviation (IBI STD), SD1 and SD2 at the three levels

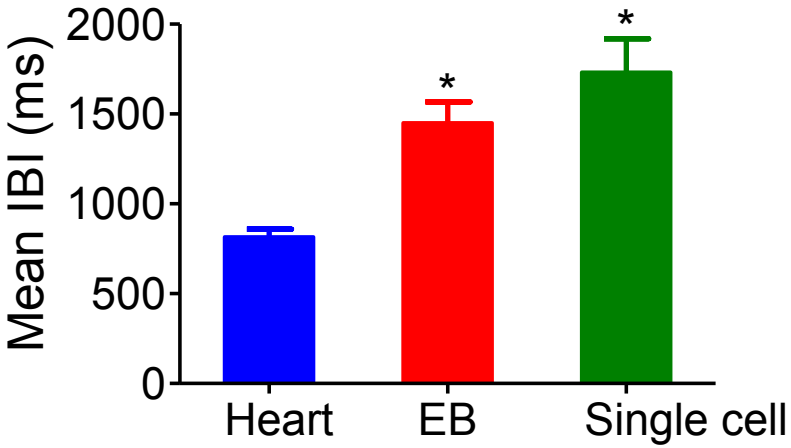


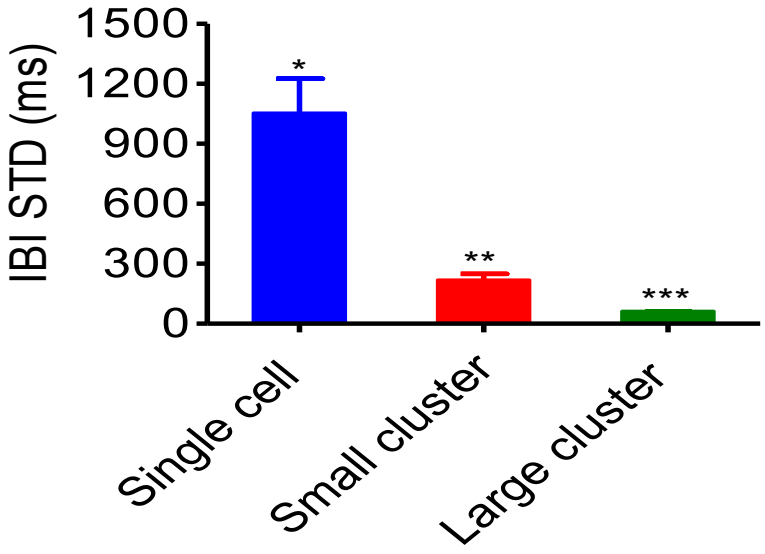


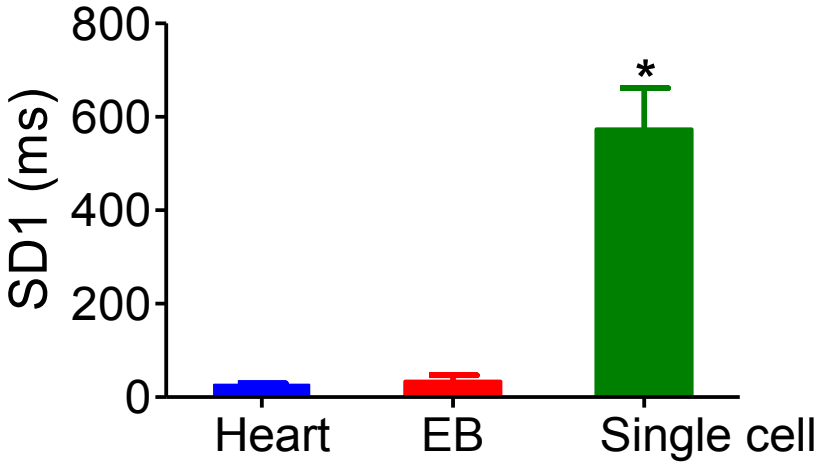


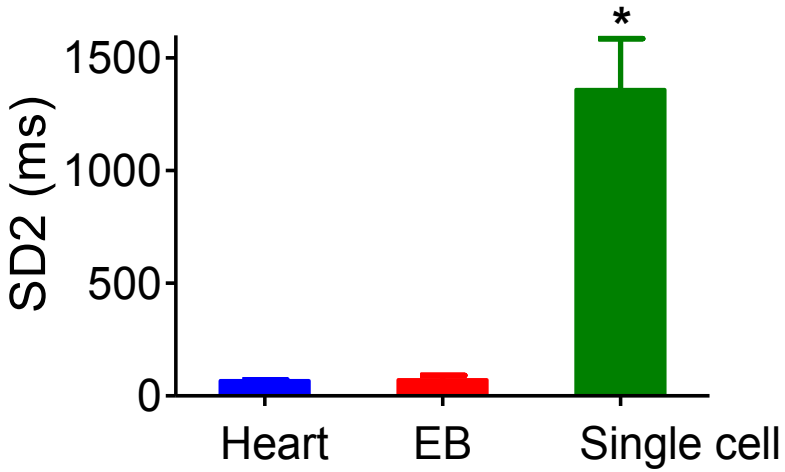
The larger dispersion of IBIs is at the single cell level

BRV measures at the3 levels: SD1 and SD2 (Poincare plot), and IBIs Standard Deviation (IBI STD)



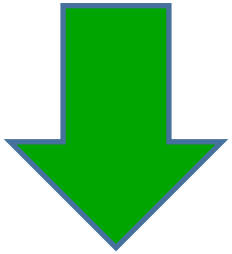






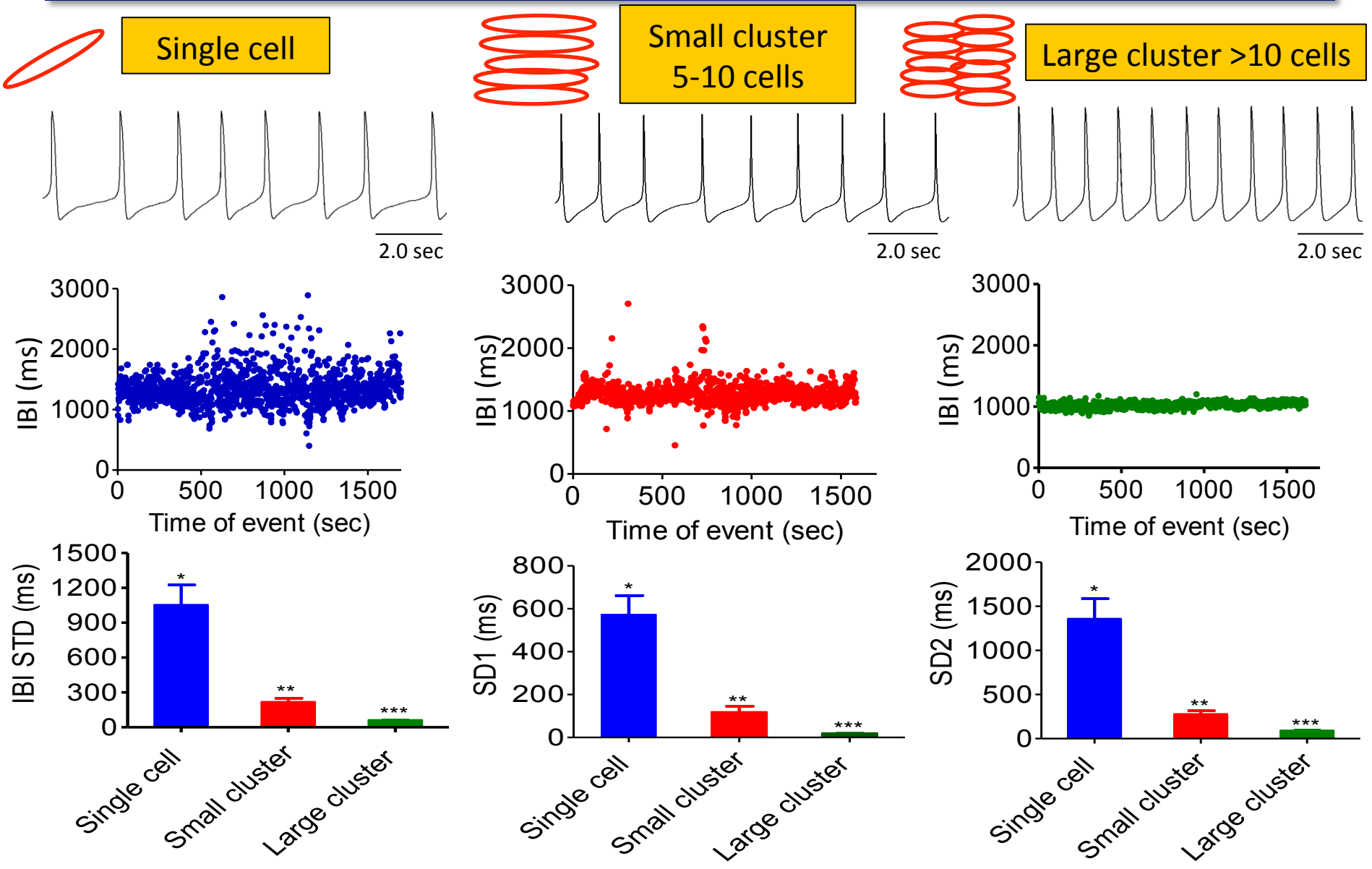
The larger dispersion of IBIs is at the single cell level

Digging deeper into the mechanisms of BRV



Does IBI variability depend on the number of cardiomyocytes in the ncluster?

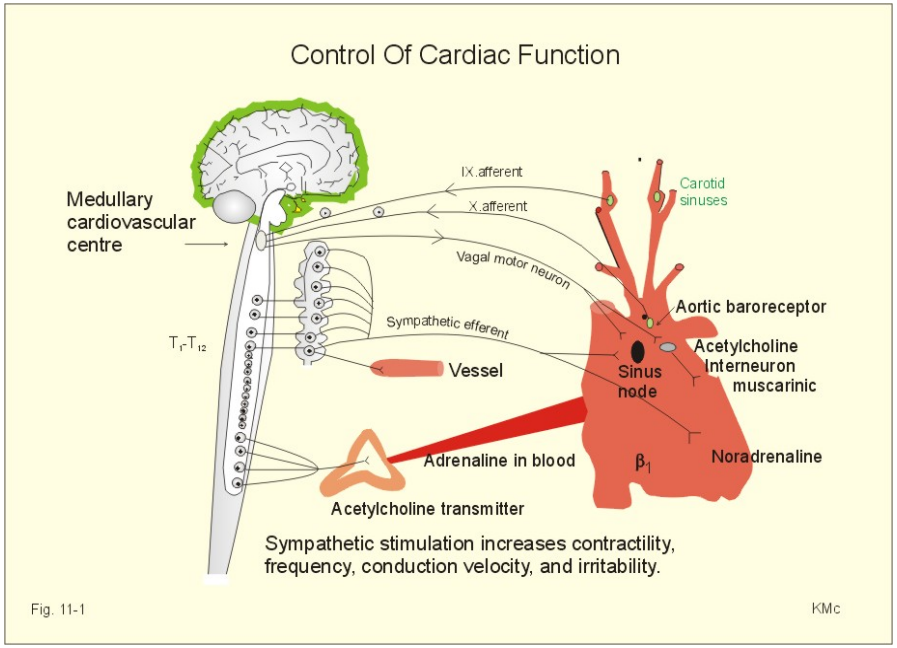
Inter-beat interval (IBI) variability decreases when the number of cells in the cluster increases

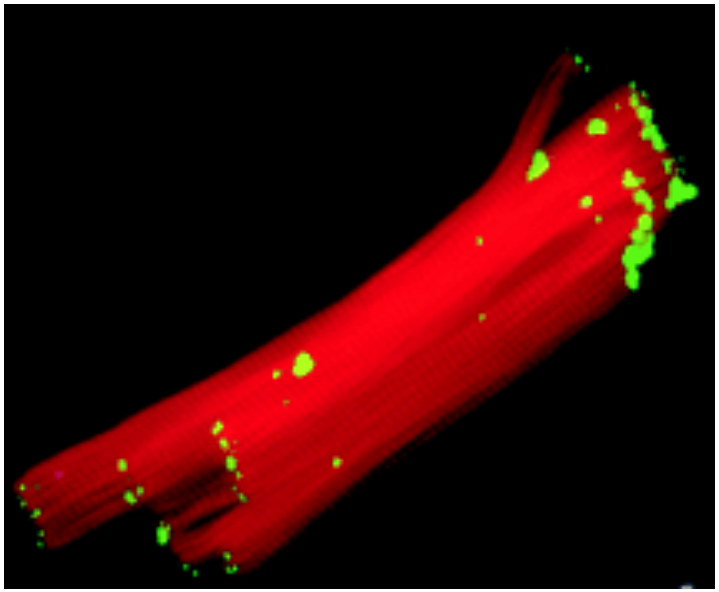


Why is HRV/BRV larger at the single cell level?

The *in situ* heart: Multiple (some are opposing) inputs which converge on the SA Node, dampen the oscillations

Single cell: Few (e.g., Calcium clock) (non-opposing?) inputs on the pacemaker machinery generate large variability



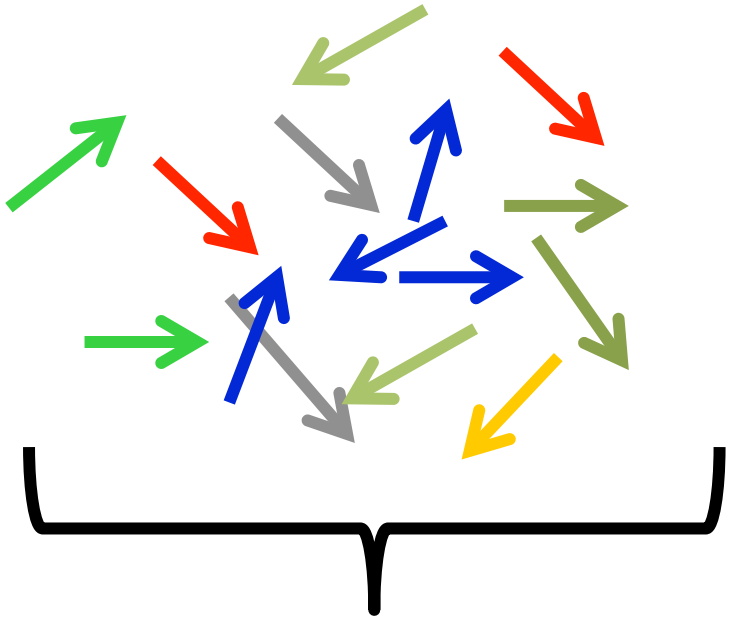


Low IBIs Variability

High IBIs Variability

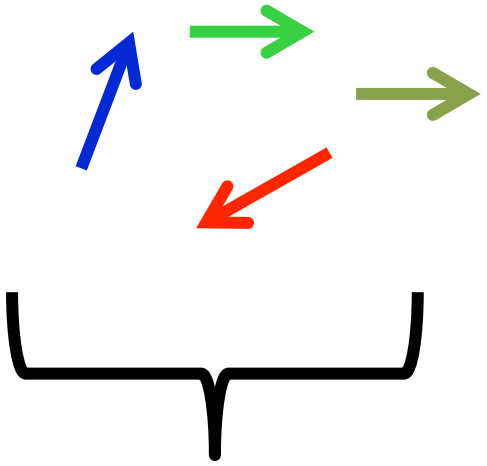
Why is HRV/BRV larger at the single cell level?

The Heart



Low Noise

A pacemaker cell



High Noise

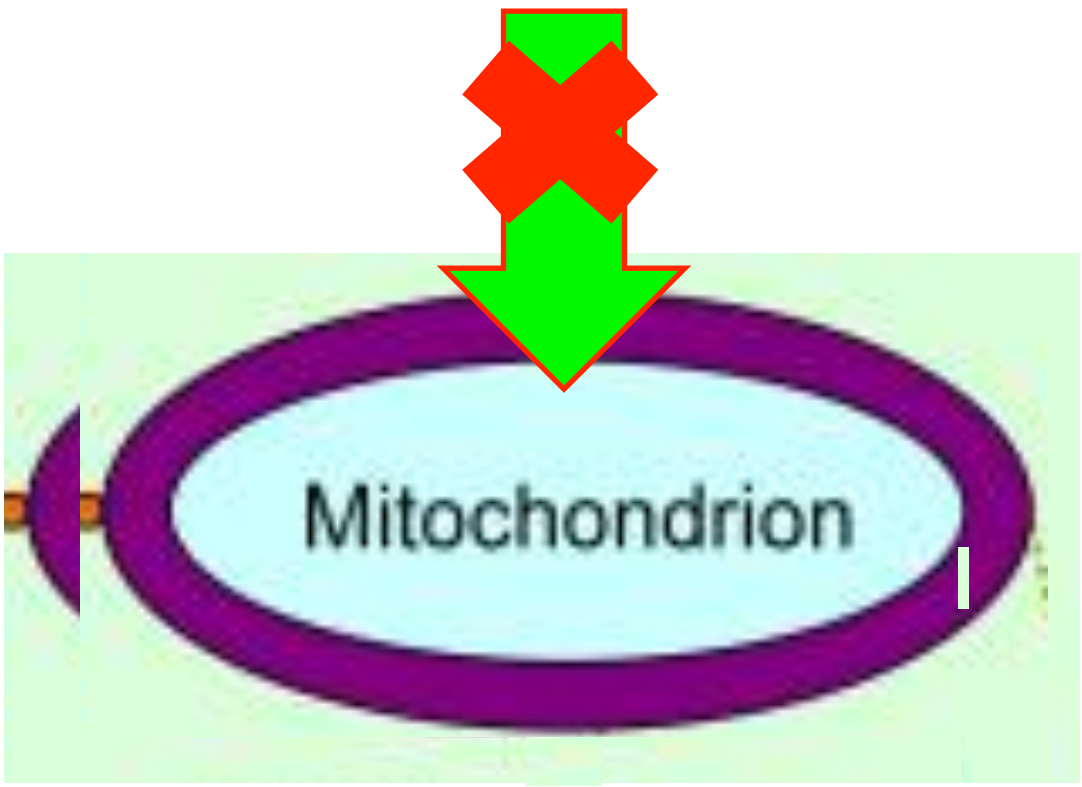
The higher the number of modulators/effectrs of automaticity - the smaller is the noise (IBIs variability)

What will happen to BRV if we decrease the number of interacting generators/effectors of automaticity in single a pacemaker cell?

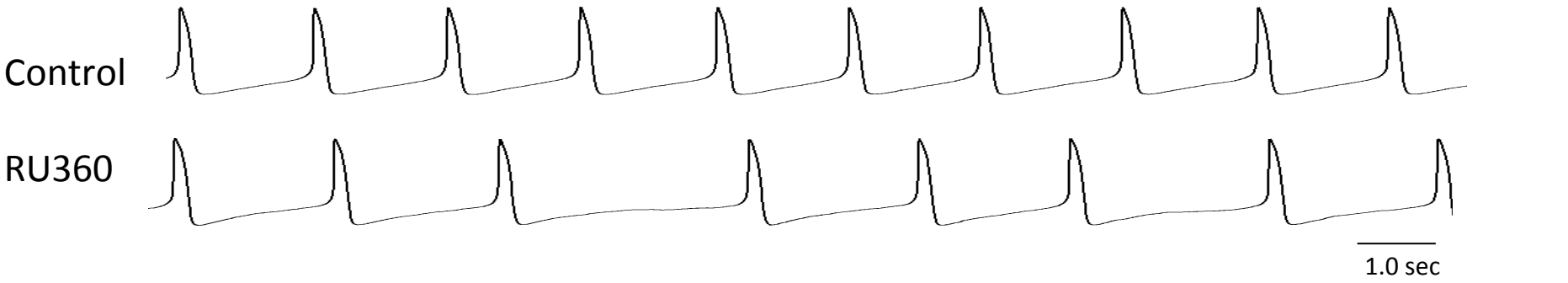
Any guess?

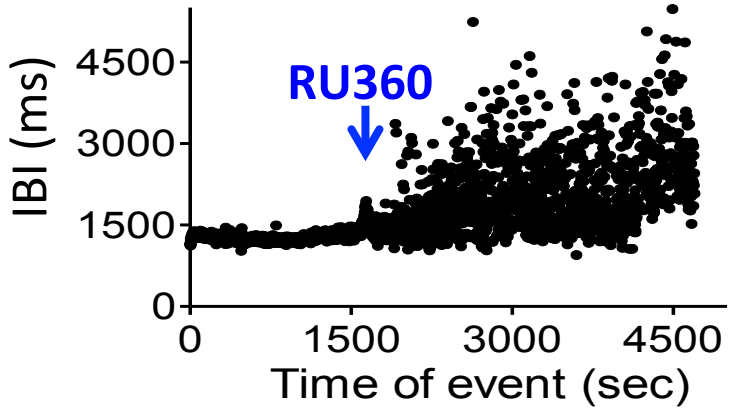
RU360 - a specific mitochondrial Ca^{2+} uptake blocker – interferes with intracellular Ca^{2+} handing, affecting automaticity

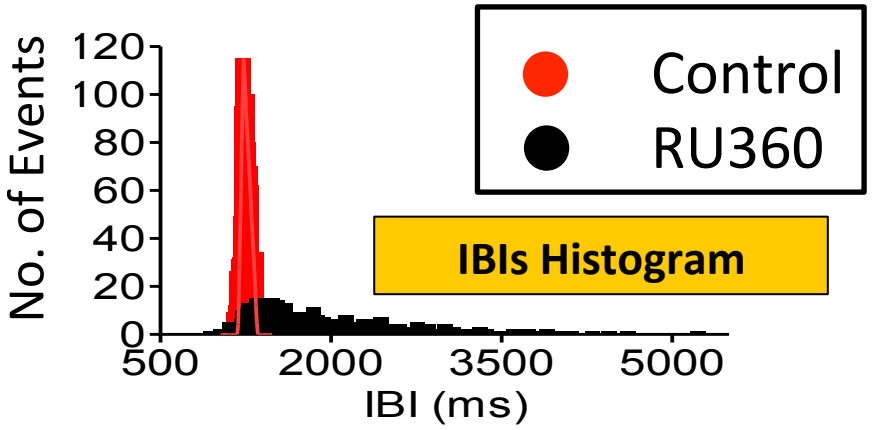
Ca^{2+}

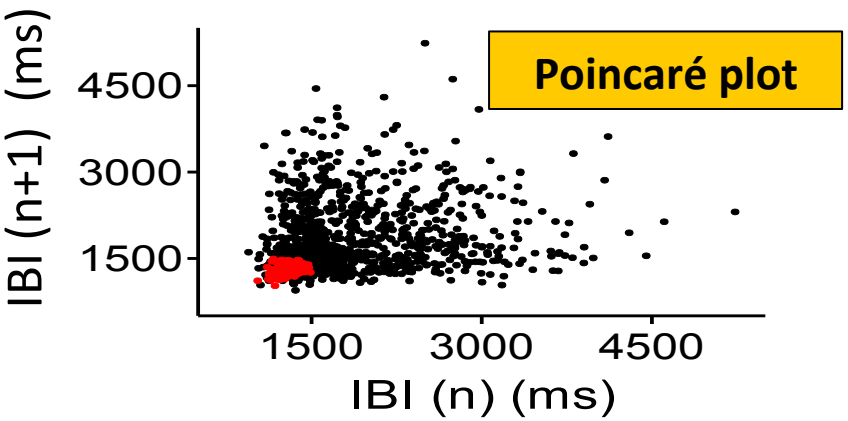


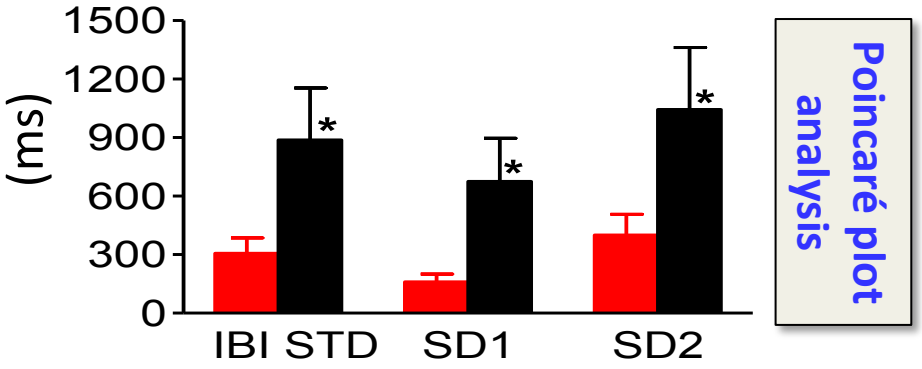
RU360 markedly increases IBI variability



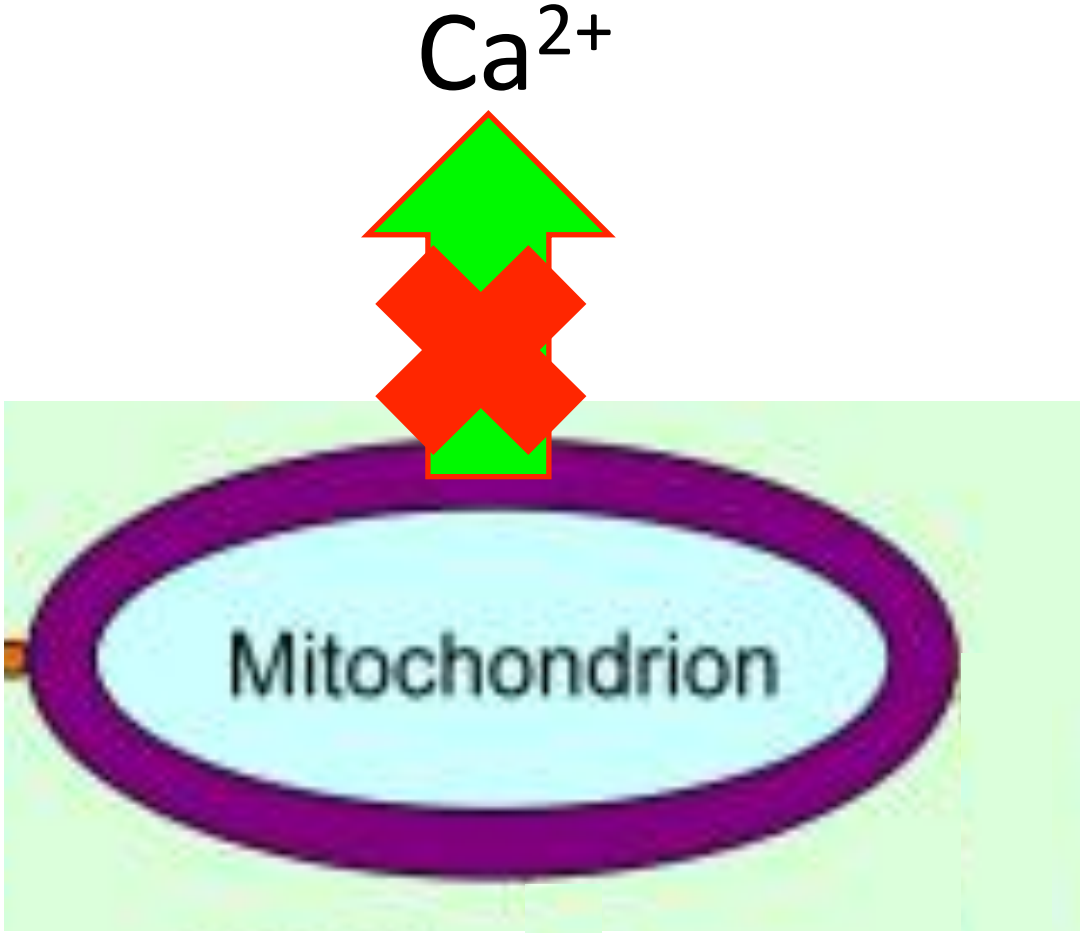




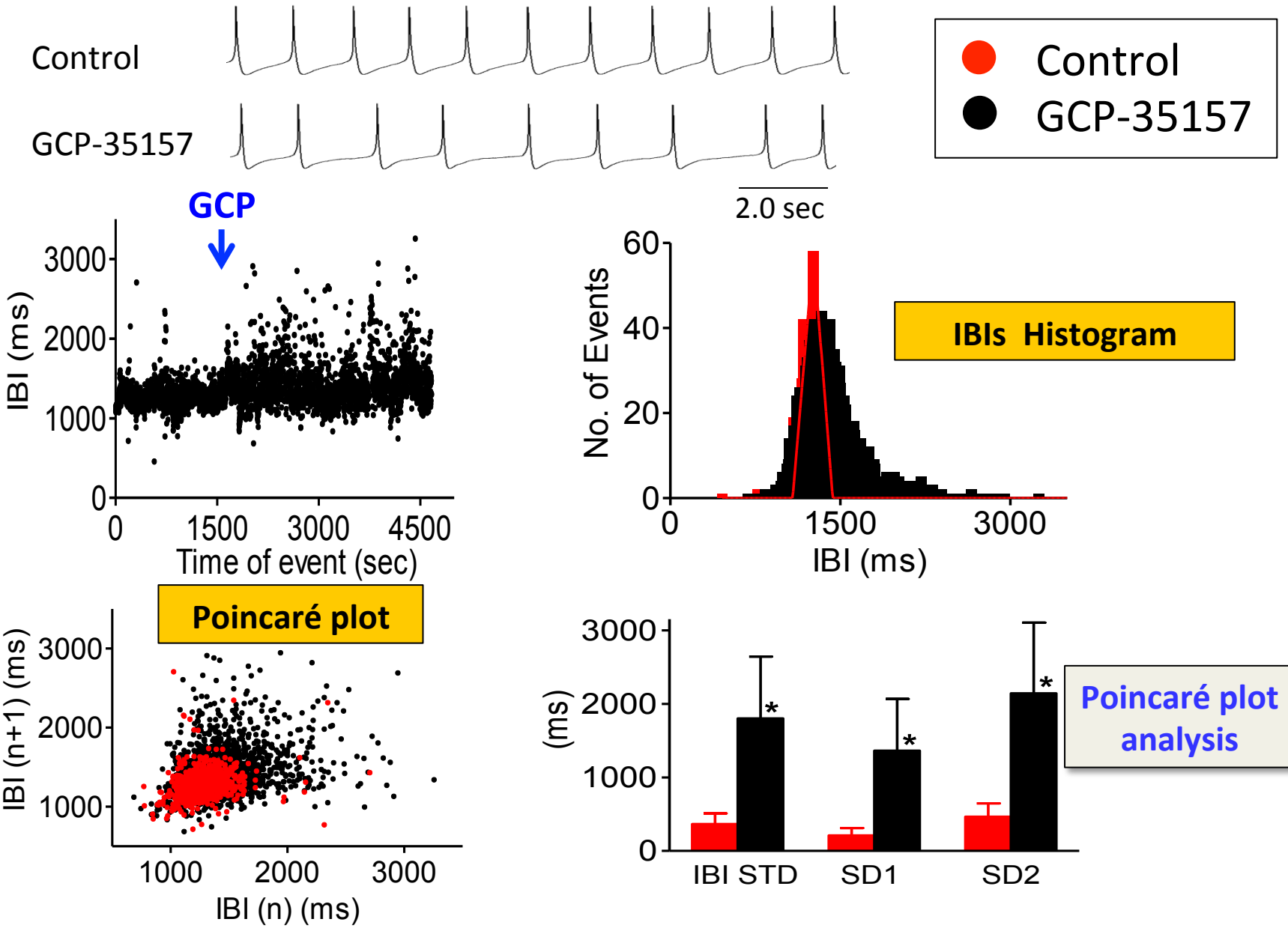




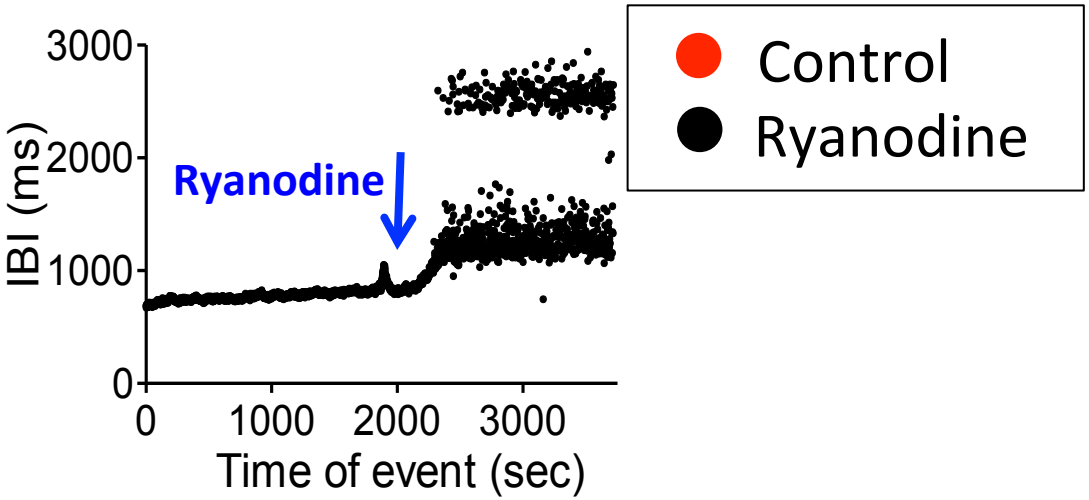
GCP-35157 – blocks mitochondrial Ca^{2+} efflux - interferes with intracellular Ca^{2+} handing, affecting automaticity

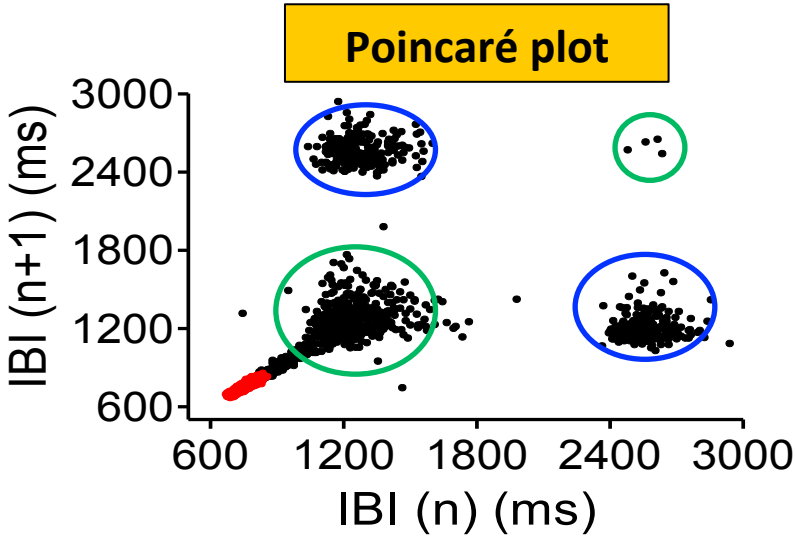


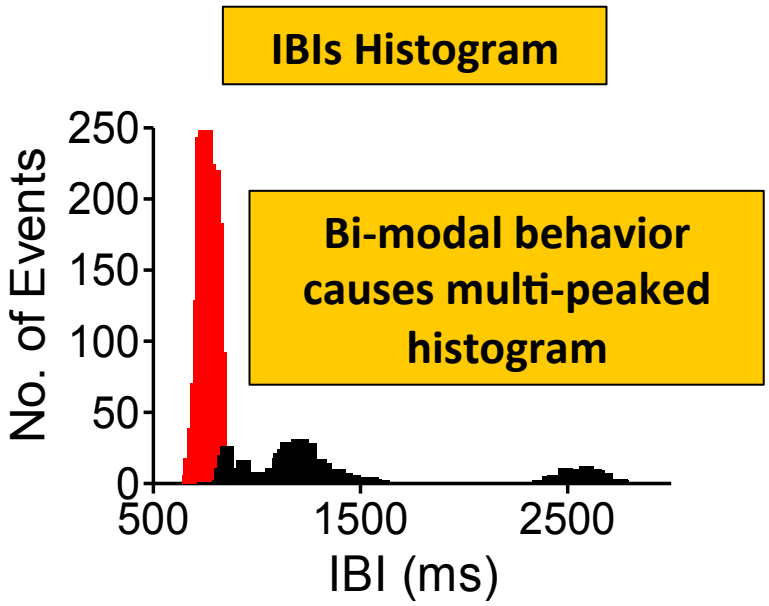
GCP-35157 markedly increases IBI variability



Ryanodine markedly increases IBI variability and causes bi-modal firing behavior







Why 4 populations in the Poincaré plot?

- Green-circled clouds represent groups firing at the same rate (e.g., 1200 or 2500 msec).
- In Poincaré plot IBI_{n+1} is plotted against IBI_n → blue-circled clouds represent points oscillating between the low and high firing rates.

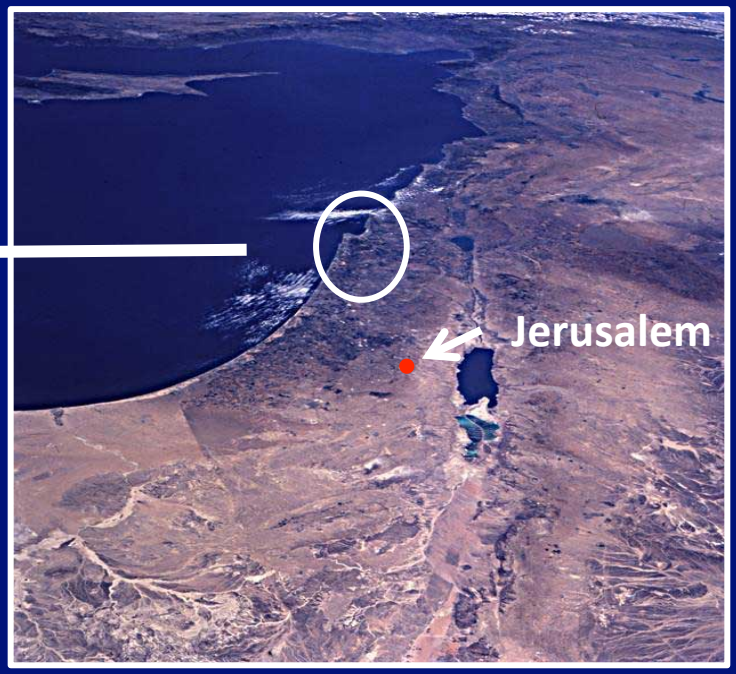
Summary

(1) BRV is an intrinsic feature of single human cardiac pacemaker cells derived from iPSC

(2) BRV is markedly enhanced at the single cell level compared to the cluster and the *in situ* heart. The major increase in IBIs variability occurs in the transition from interconnected network to a single cardiomyocyte

(3) Intracellular Ca^{2+} cycling contributes to IBIs variability and BRV/HRV

Thank you!

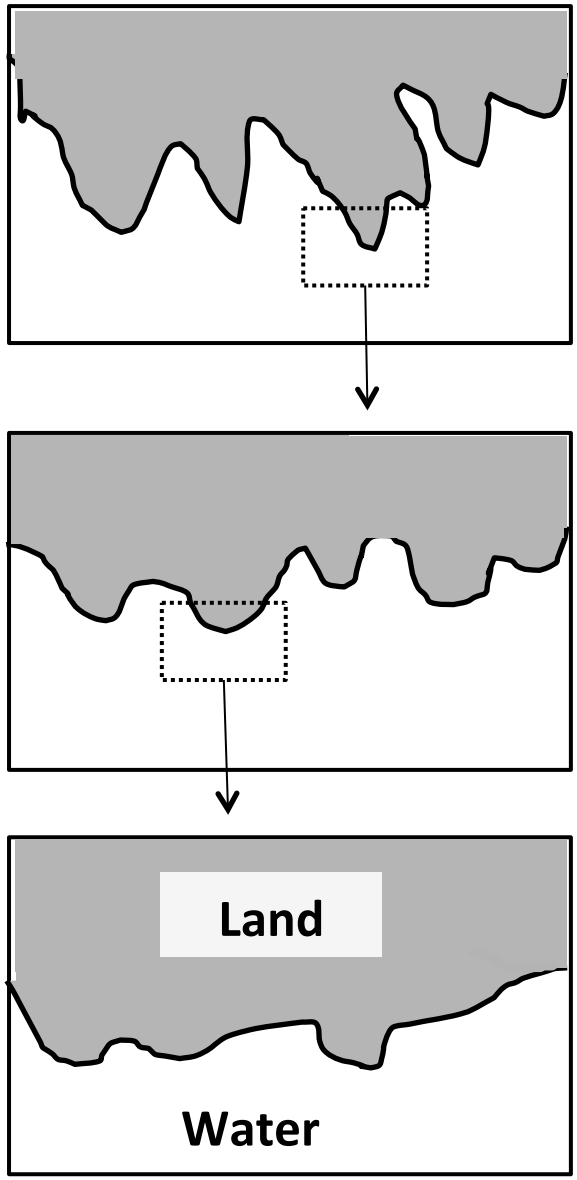


The coastline paradox – the higher the resolution of the measurement, finer detailed are revealed, and the longer is the coastline!

The "fine" coastline is missed

If the coastline of Great Britain is measured using fractal units 100 km long (left picture), then the length of the coastline is approximately 2,800 km. With 50 km units (right picture), the total length is approximately 3,400 km, approximately 600 km longer

A coastline features fractalilty and self-similarity

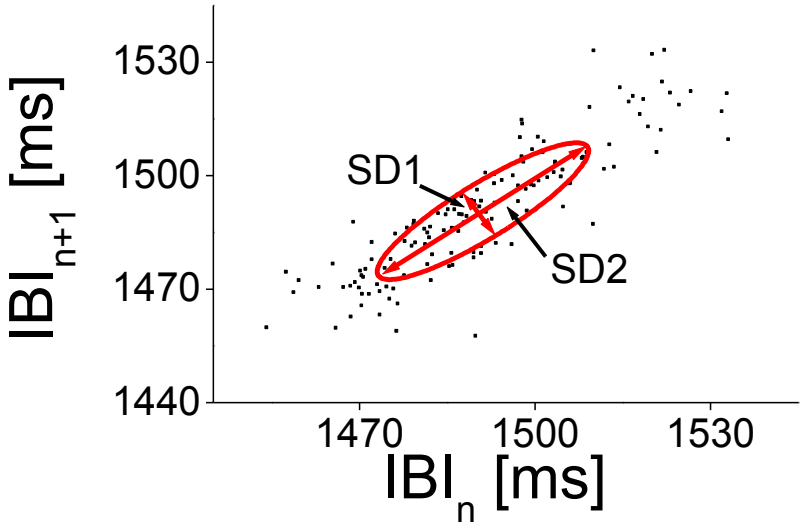


- The coastline has a fractal behavior

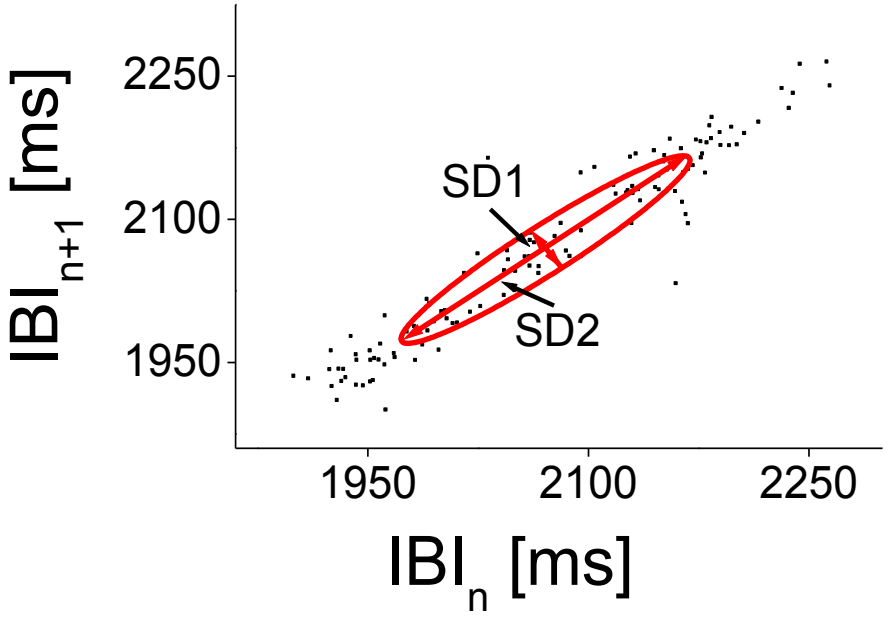
Fractals are objects or processes which when are magnified at different resolutions display similar or identical features of the whole unit. In other words, fractals have **self-similarity** at different magnifications.

Poincaré plot of the BRV in hESC-CM and iPSC-CM

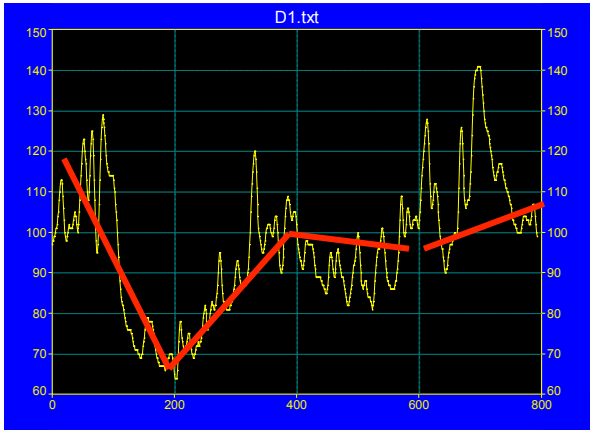
hESC-CM



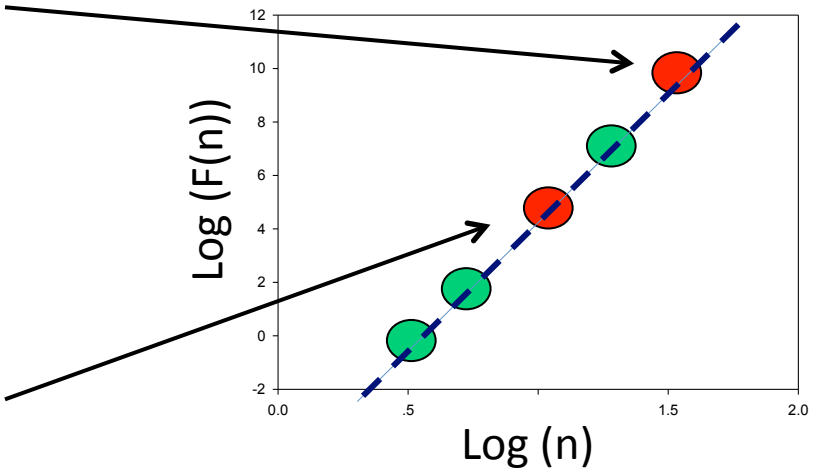
iPSC-CM



Conclusion: The IBIs of hESC-CM and iPSC-CM exhibit the 'human-like' ('cigar shape') dispersion, indicating fractal-like behavior of the BRV. The SD1 and SD2 values are similar to human ECG values.



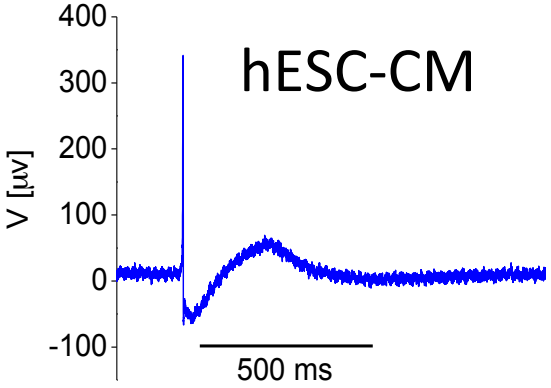
A measure of Self-Similarity: Detrended Fluctuation Analysis (DFA)

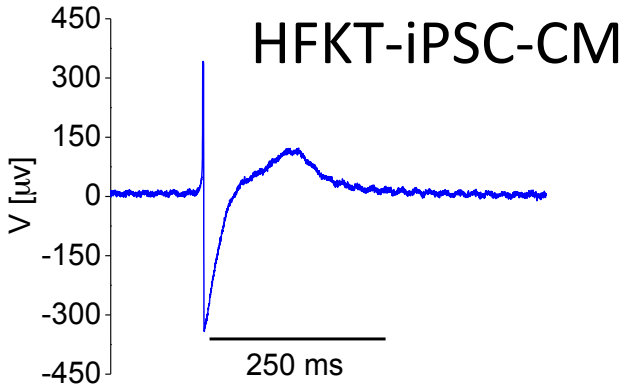


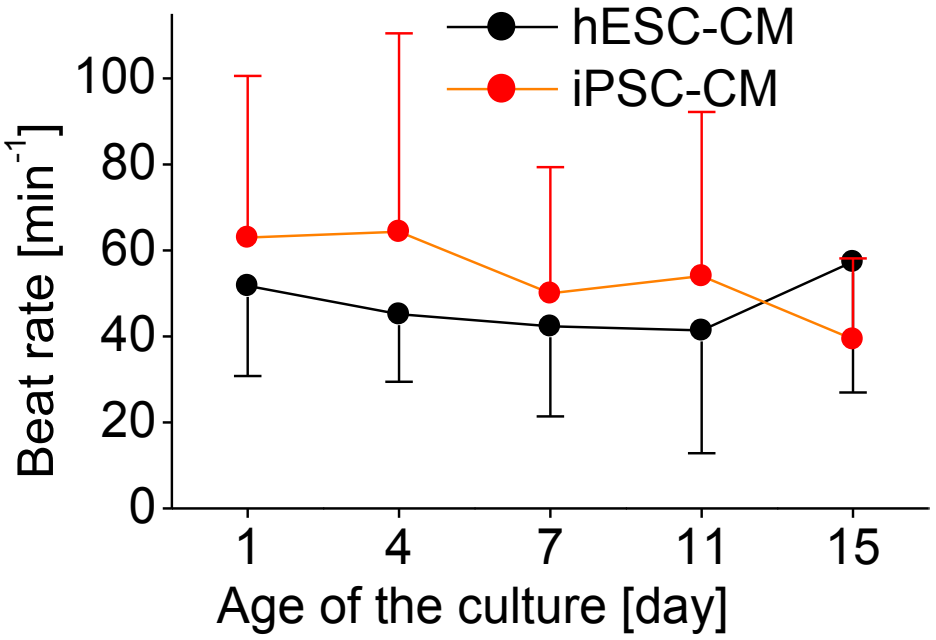
Calculating DFA

- The original time series of inter-beat-intervals (yellow trace) is integrated.
- The fluctuations *F(n)* of the integrated signal around the best linear fit in a window (red lines) of time of size *n*, is determined. The red circles denote two *F(n)* values calculated for two different time windows (100 msec, and 50 msec).
- The slope of the line relating *F(n)* to log *n* determines the scaling exponent (self-similarity parameter) of the time series.

hESC-CM and HFKT-iPSC-CM have similar spontaneous beat rate

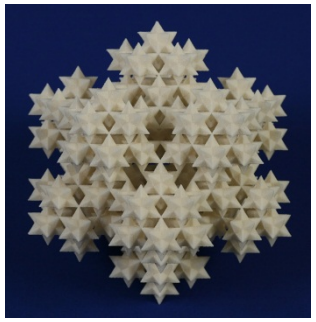


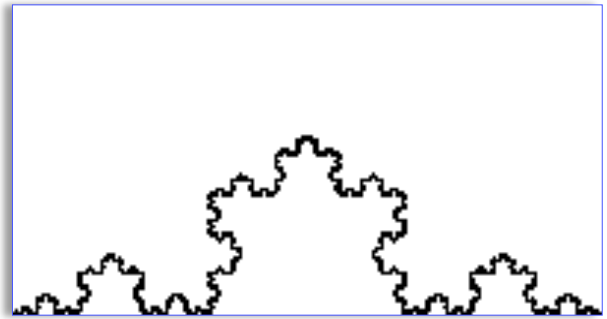


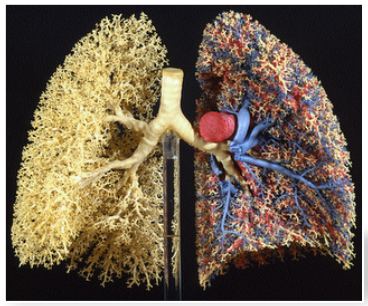


Self-similarity and fractal-like behavior

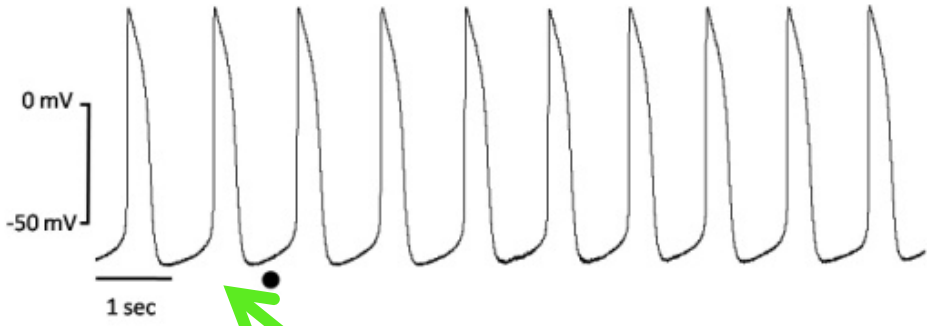
- A key feature of the sinoatrial node is its prominent beat-to-beat- variability, which shows self-similarity on different time-scales (fractal behavior).
- In healthy humans, the power spectrum of the inter-beat intervals follows a power-law behavior. Power laws describe fractal objects; i.e., objects that have fine structure at each scale, and appear similar at all scales of magnifications.

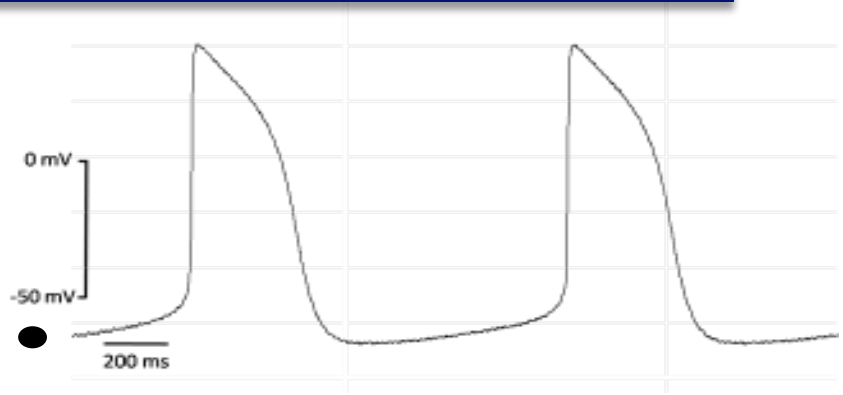


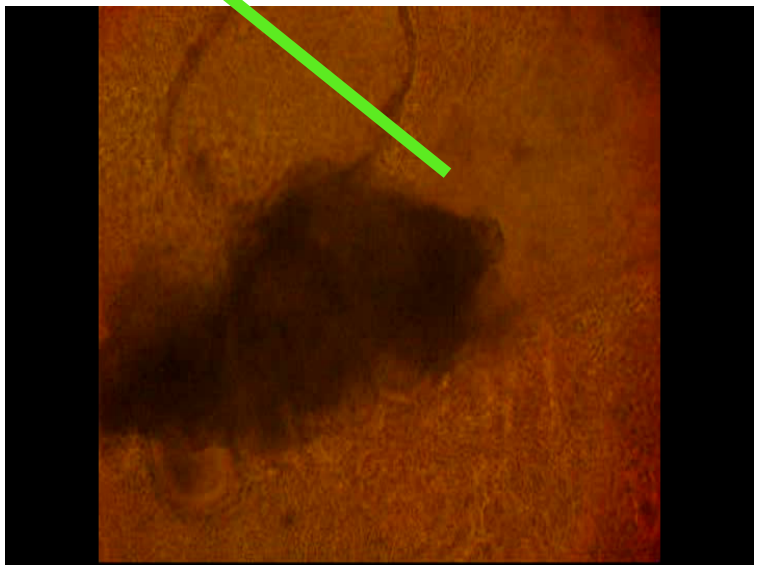


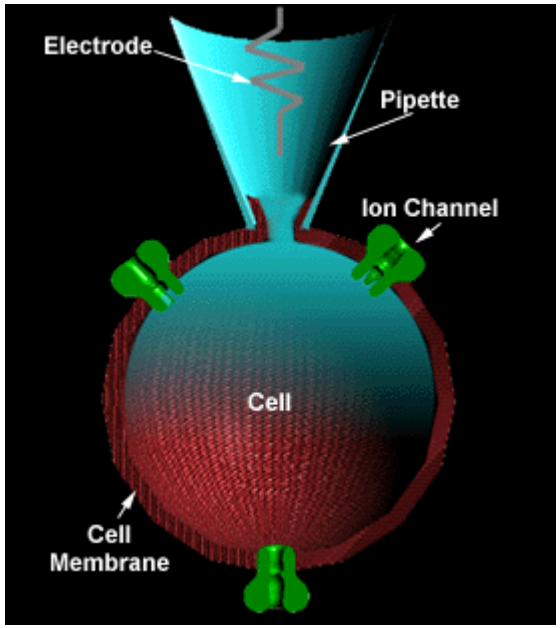


The single cell level: Spontaneous (pacemaker) electrical activity recorded from 33-day old HFKT-iPSC-Cardiomyocytes (using the patch clamp technique)



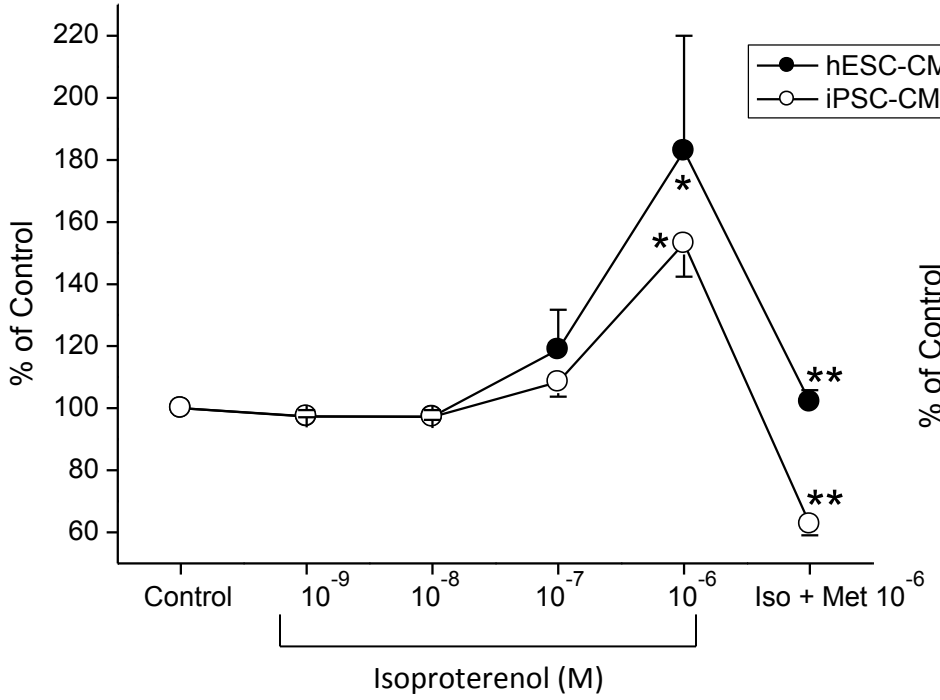




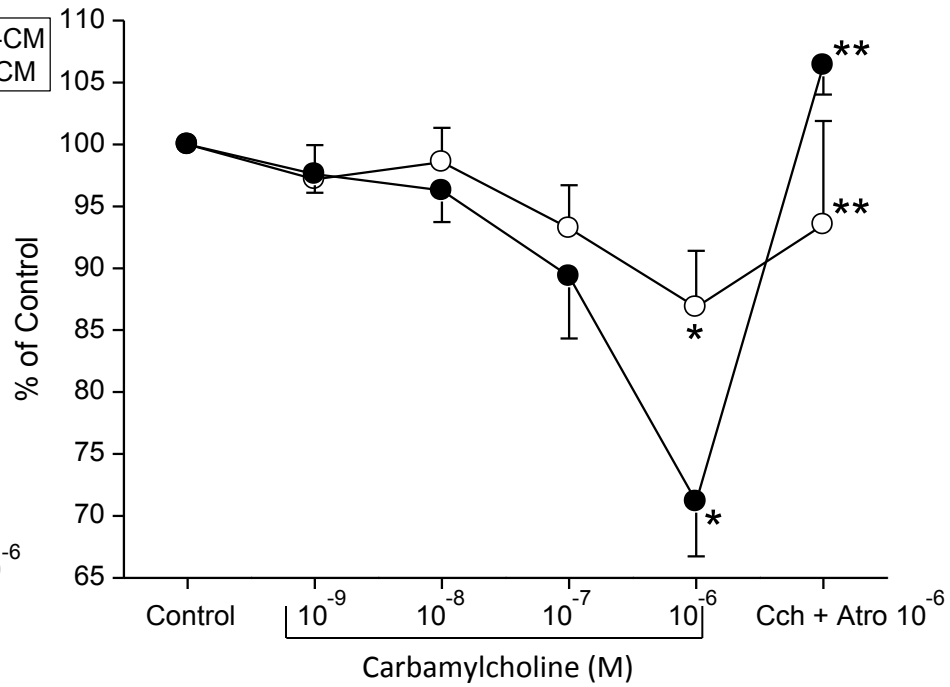


hESC-CM and iPSC-CM respond to autonomic modulation

Isoproterenol + Metoprolol



Carbamylcholine + Atropine

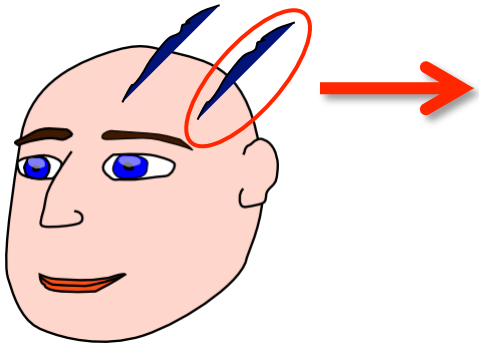


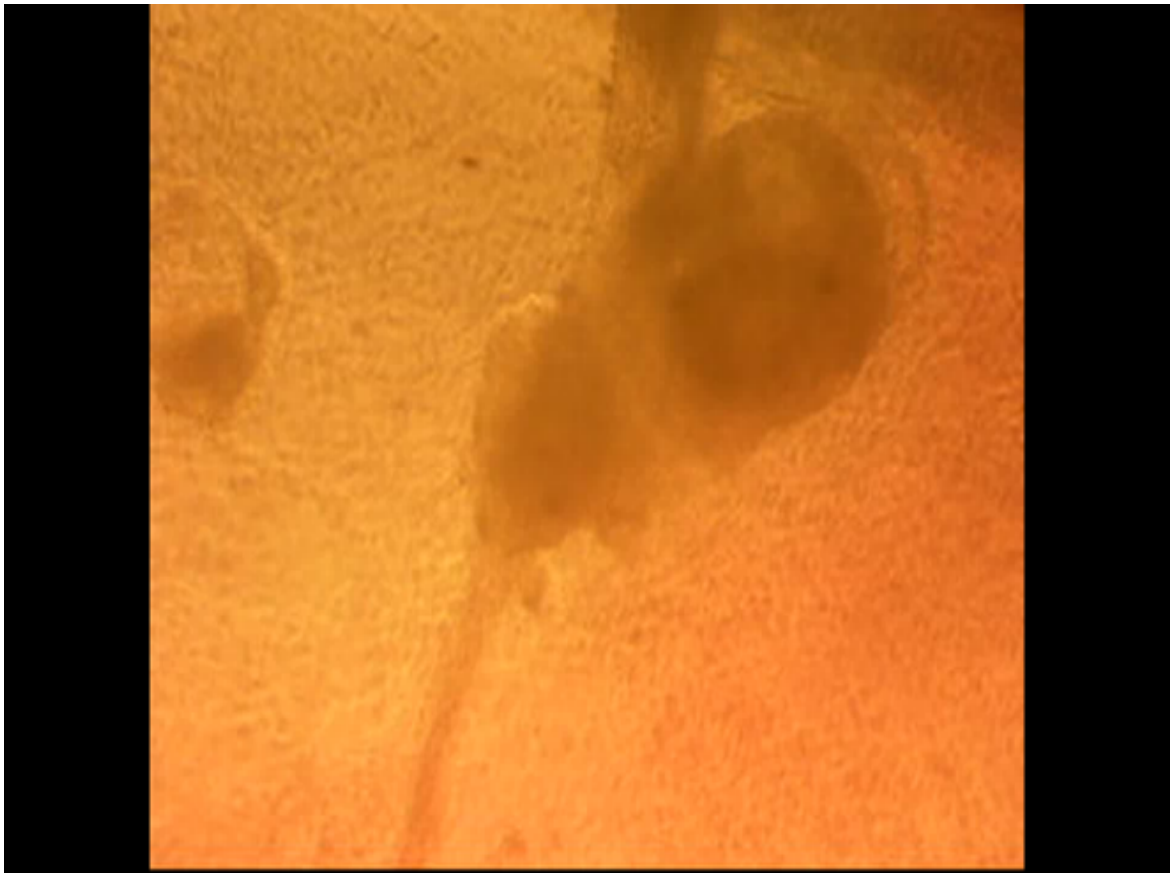
Iso – Isoproterenol, b-adrenergic agonist; Metoprolol - b-blocker;
Cch – Cholinergic agonist; Atropine – cholinergic blocker

The fundamental requirements from a cardiac pacemaker

- Cardiac-like Excitation-Contraction Coupling machinery
- Automaticity, pacemaker currents, responsiveness to Funny current (I_f) blockers, autonomic modulation
- Heart/Beat rate variability, like the sino-atrial node

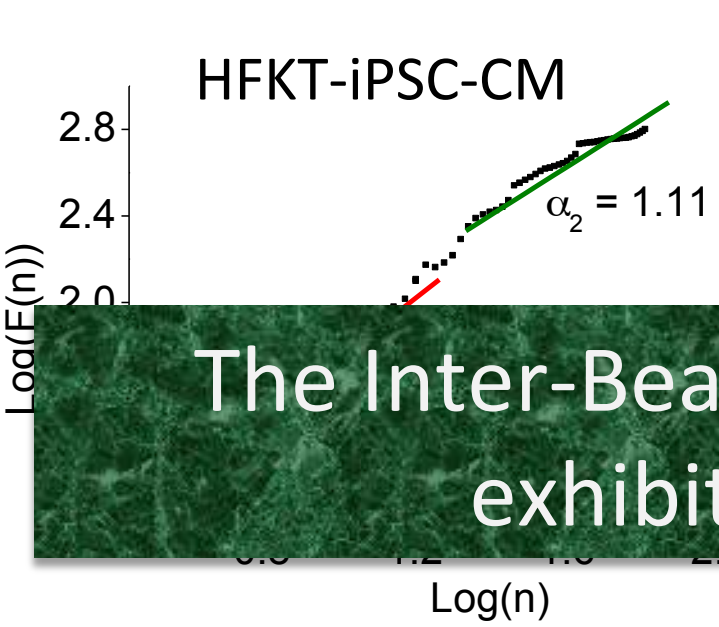
Functional properties of HFKT-iPSC-CM

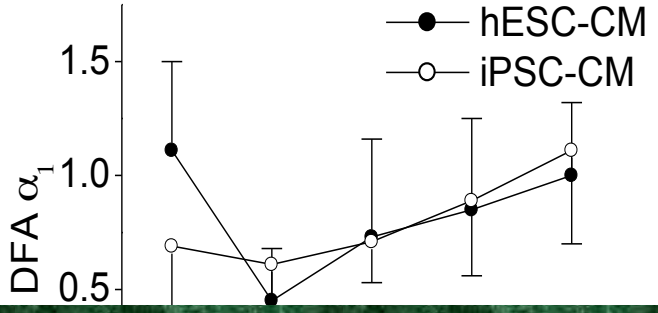




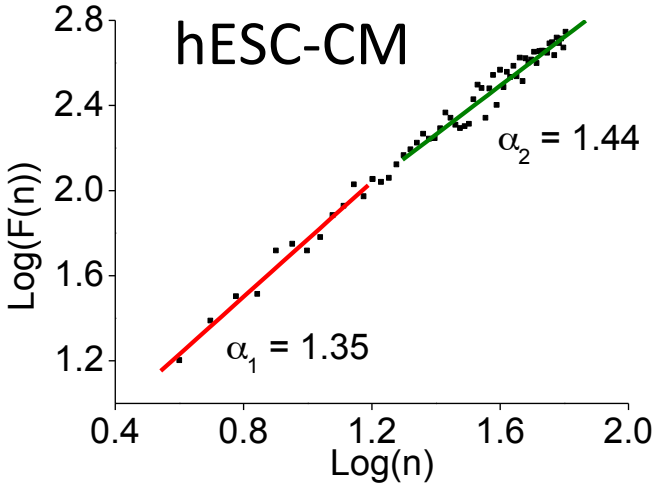
Yes, we can transform human hair into little hearts!

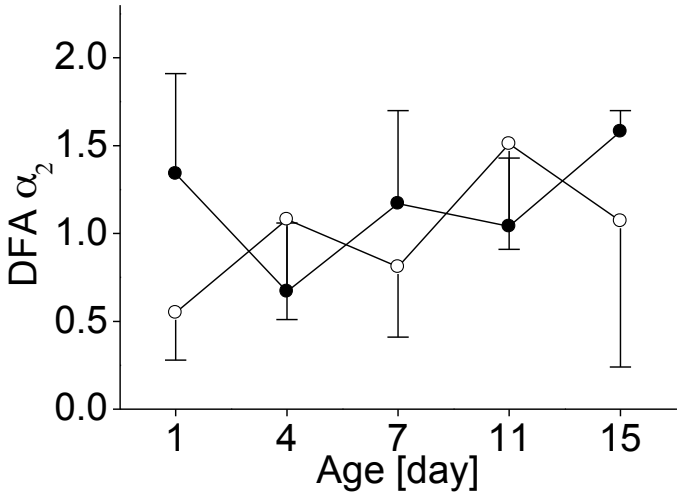
Detrended Fluctuation Analysis (DFA) of the Inter-Beat-Intervals of iPSC-CM and hESC-CM:
A slope around 1, indicates Self-Similarity





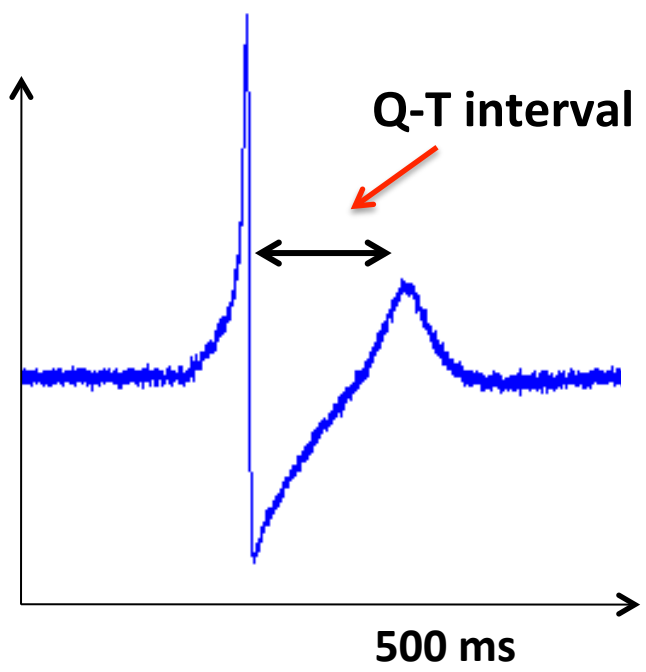
The Inter-Beat-Intervals time series exhibit Self-Similarity

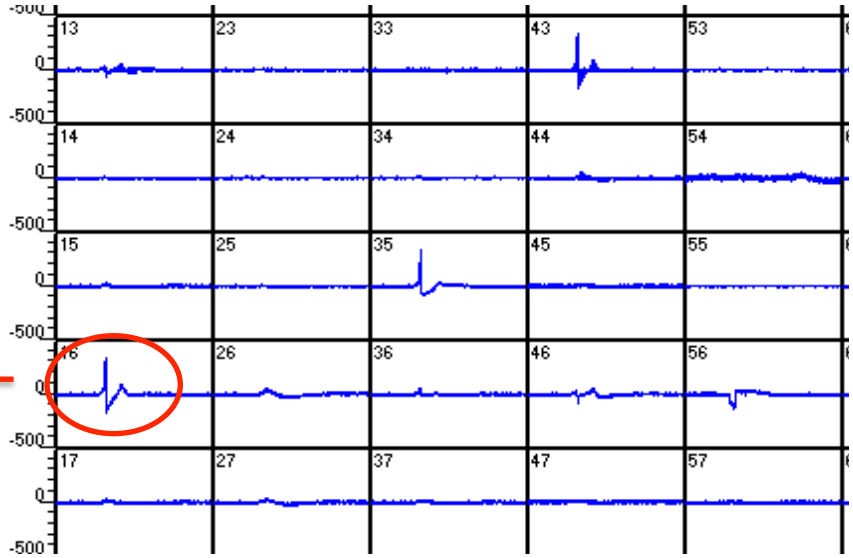




Functional HFKT-iPSC-CM

Extracellular electrograms are recorded by means of the Micro-Electrode Array (MEA) data acquisition system



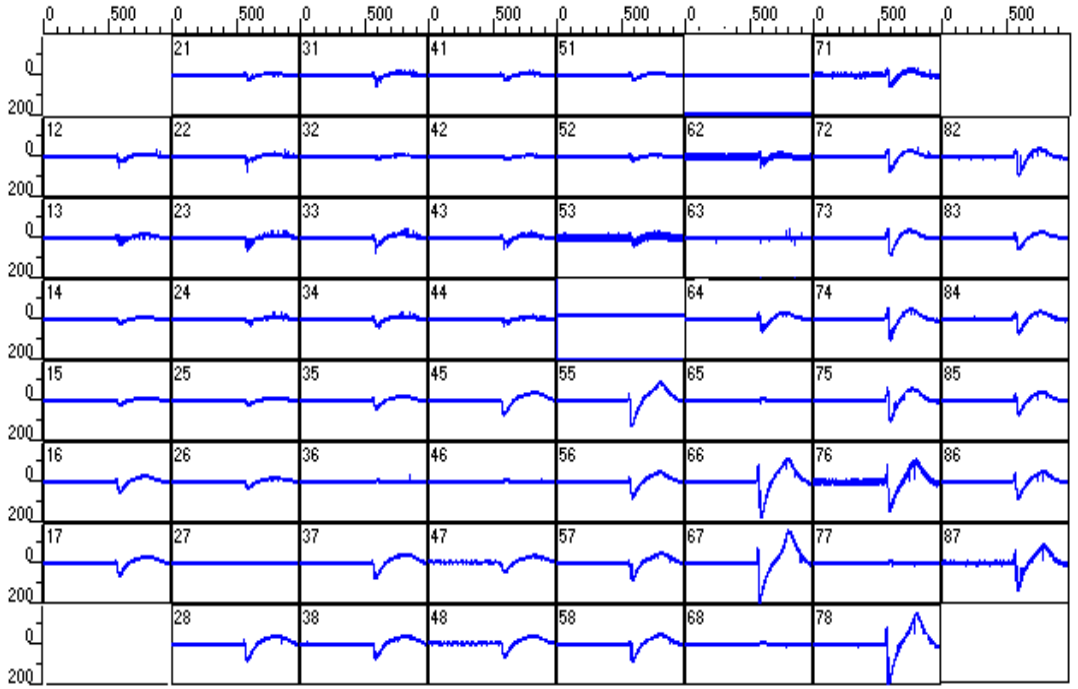


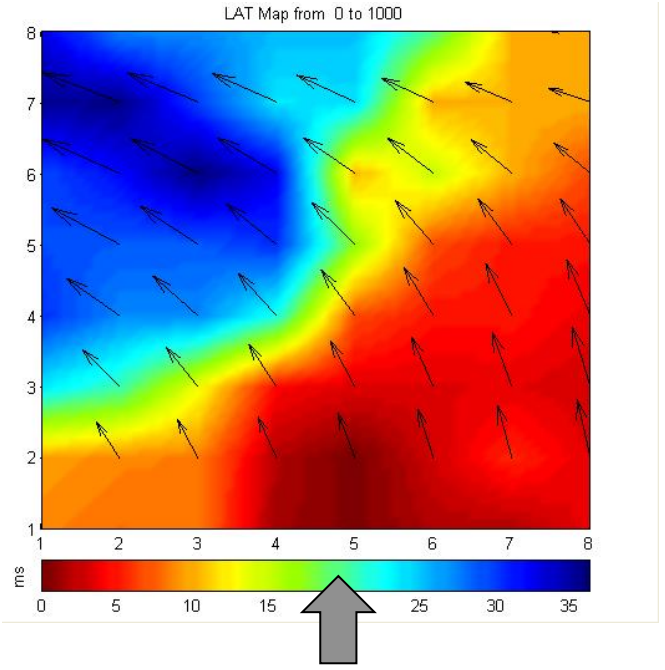
An excellent set-up for testing drugs for Q-T prolongation

Extracellular recordings from HFKT-iPSC-CM

Generating activation maps: conduction patterns and conduction velocity

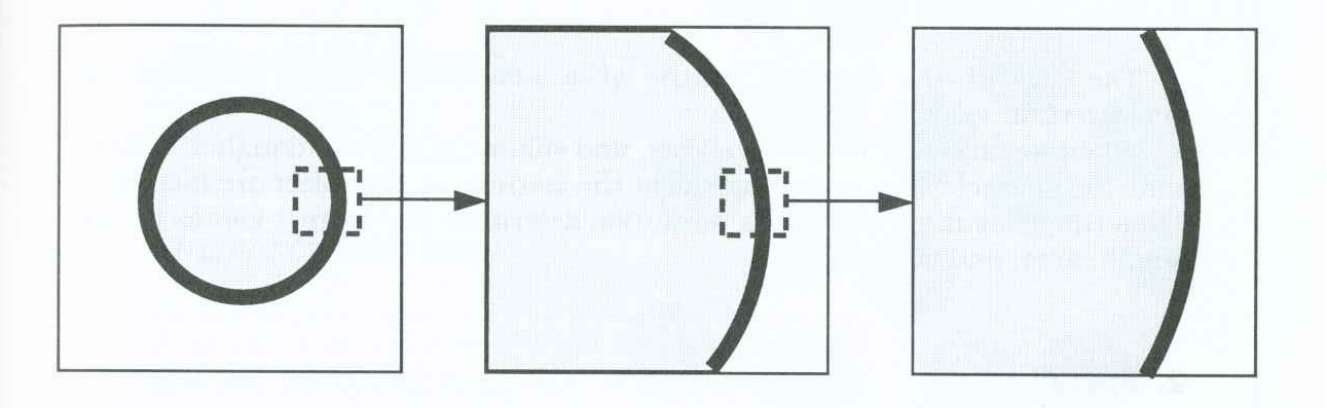
Extracellular recordings from the MEA



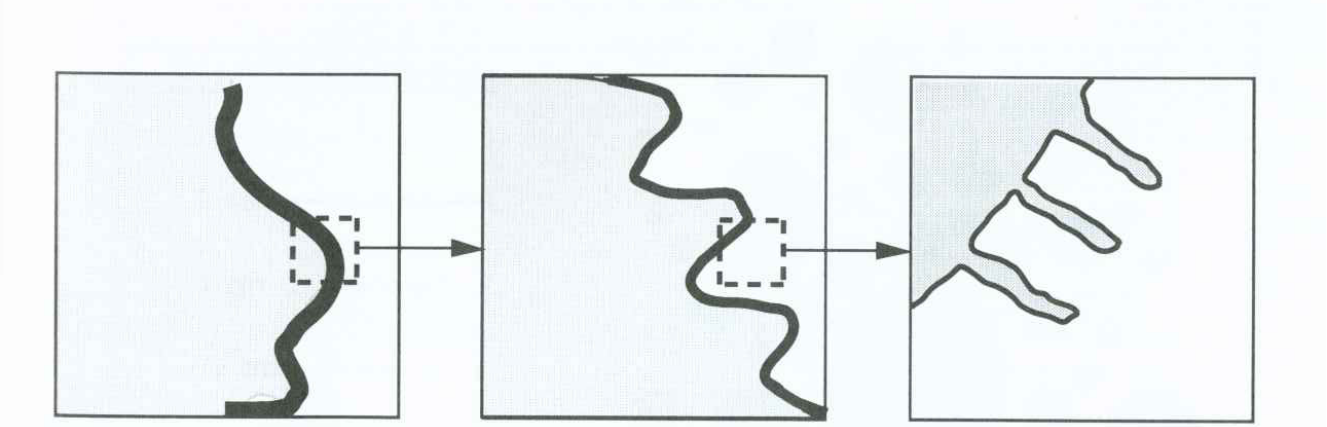


CV = 9.25 cm/sec

Non-fractal object: As a non-fractal object is magnified, NO new features are revealed.

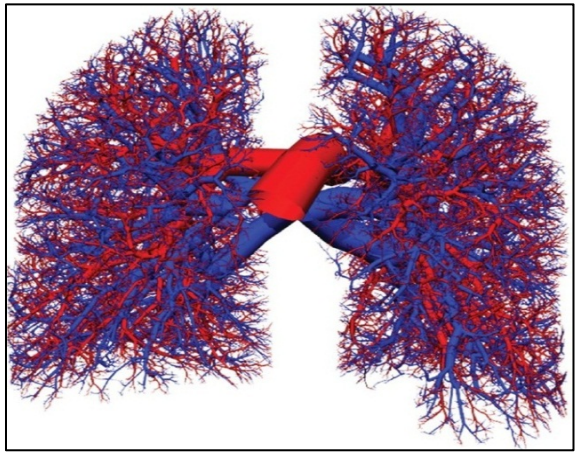


Fractal object: As a fractal object is magnified, ever finer features are revealed. The shapes of the smaller features resemble the shape of the larger features..



Fractal (showing self-similarity) objects in Nature

Ice frosts



Lung airways

Romanesco Brocoli