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Nonlinear analysis of a *Drosophila* ECG time series.

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The objective is to introduce a new computational approach to the genetic analysis of *Drosophila* cardiac function, using nonlinear dynamics together with gene knockout.

Chaos theory and a unique software, the Point Correlation Dimension, PD2i, were applied for the first time to the study of the genetic nature of *Drosophila* cardiac dynamics. The PD2i software (Skinner *et al*., 1994), calculates the degrees of freedom in small sub-epochs of a data series, such as the digitized electrocardiogram (ECG). The PD2i algorithm has an advantage over other chaos quantifiers in that it can analyse a non-stationary data series. Originally the PD2i software was designed for the detection of human heartbeat pathologies, and it was found to have a 100% sensitivity in predicting ventricular fibrillation among high-risk patients (Skinner *et al*., 1993). In the present study the PD2i was applied for the first time to an abnormality in *Drosophila* cardiac function resulting from gene knockout of potassium channels. The result was that the *Drosophila* mutants *eag* (which encode subunits of K+ channels) show periodic low-dimensional excursions in the PD2i range of 2.2 to 3.7 degrees of freedom that neither the normal control nor the randomized-phase surrogate data show ($p < 0.01$). Statistical measurements (*e.g*., running SD's, Power spectrum, 1/f noise) did not detect any differences in the same data set.

The *Drosophila eag* mutant heartbeat data are similar to that of the ischemic myocardium of a mammalian heart, although in the latter the excursions are to 1.2 dimensions. Low dimensional chaos turns out to be the most serious indicator of genetically induced cardiac disorders. Only in cardiac mutations are observed the characteristic low dimensional excursions registered by the PD2i.

Drosophila heartbeat: Optical recording and digitization

All measurements were made on *Drosophila melanogaster*. *eag*-gene mutants (ether-a-go-go), concerning K^+ channels which are related with heart pathologies, and wild type controls were provided by the Bloomington *Drosophila* Stock Center, U.S.A. Optical ECG records were taken at a stage P1 (white puparium) of *Drosophila* development (Bainbridge and Bownes, 1981) when it is both immobile and transparent and the dorsal vessel (Rizki, 1978) is easily viewed. The object was placed on a glass slide in a drop of distilled water under a microscope (magnification 350×). Fluctuation in light intensity due to movement of the dorsal vessel tissue is captured by photocells fitted to the one eyepiece of the microscope. Experts from the Onassis Cardiac Center, Athens, Greece, designed this analogue signal acquisition equipment.

The captured analogue signal was then digitized at 1 kHz sampling rate by data acquisition card and LabVIEW data capturing software supplied by "National Instruments" and stored in a

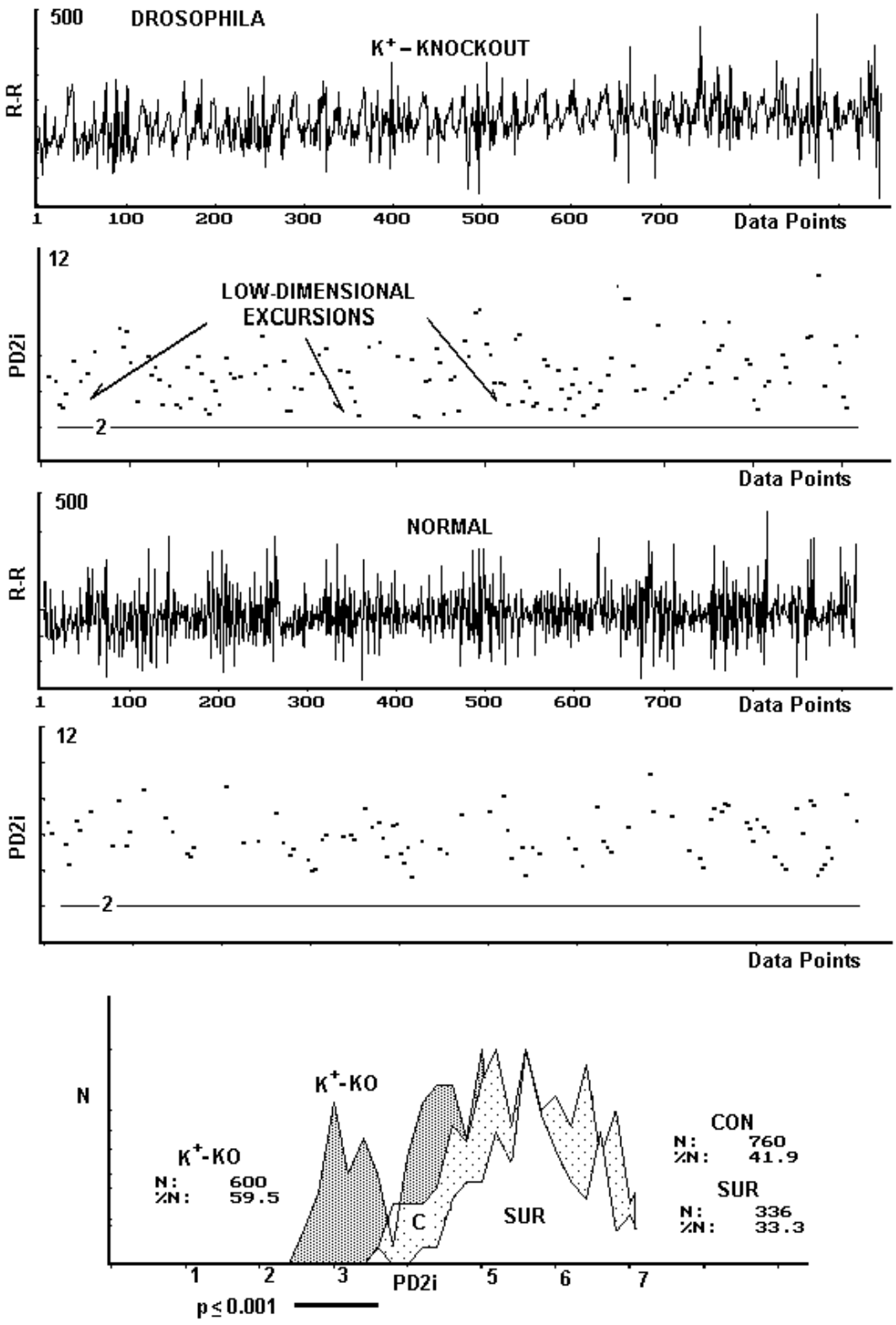

Figure 1. A low dimensional excursion in the *Drosophila* eag mutant ECG time series detected by the PD2i software.

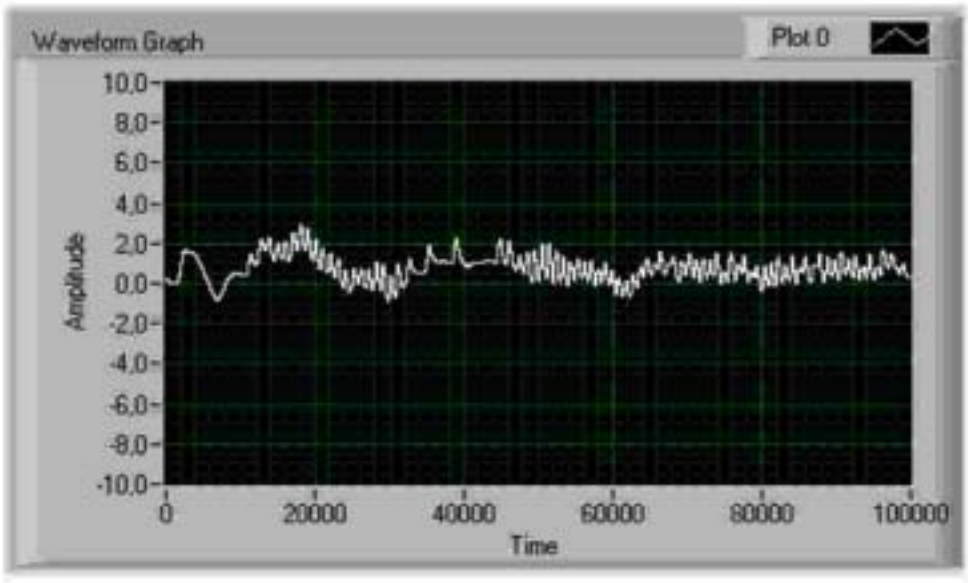

Figure 2. A *Drosophila* ECG displayed by the LabVIEW data capturing software.

Pentium III computer. 600000 data points (more than 1000 heartbeats) were taken for each sample. Optimal data gain was empirically estab-lished to be equal to 5.

The PD2i Software

The correlation dimension (D2) of a time-series is defined as C(r,n) ~ r expD2 where C(r,n) is the cumulative num-ber of all rank-ordered vector -difference lengths within a range (r) and n is the number of vector-difference lengths (the "~" sign means, "scales as"). Grassberger and Procaccia (1983) developed a simple algorithm (D2) for calculating this reconstructed dimension.

Mathematical stationarity is presumed during the collection of data in the above D2 application, a presumption which is not tenable for biological systems. The "pointwise" scaling dimension was suggested by Farmer, Ott and Yorke (1983) to be an estimate of D2 that was perhaps less sensitive to nonstationarities, because the reference vector is fixed. Since the reference vector is chosen sequentially for each digitized point in the time-series, dimension is estimated as a function of time.

The "point-D2" estimate of the correlation dimension (PD2i) was developed by Skinner and associates (1991). Like the D2i, each PD2i reference i-vector remains fixed, while each of the j-vectors run through the whole data series. But for the PD2i, the j-vectors that will contribute to the small log-r values must arise from a subepoch that manifests scaling characteristics similar to those surrounding the i-vector. Basically the PD2i reference vector seeks its own sunspecies of stationary data with which to make the vector-difference lengths; this occurs by a process that involves, 1) the plot length (PL) of the small log-r values in the scaling region (*i.e.*, as observed in the log-log plot of the cumulative histogram of the rank-ordered vector difference lengths vs the range), 2) the linearity criterion (LC) for this scaling region, and 3) the convergence criterion (CC) of the slope of this scaling region vs the embedding dimension. Part of the success of the PD2i software revolves around the rejection of values that do not result in linear scaling and clear convergence; these rejections also eliminate PD2i estimates that could result from contamination of the small log-r values (*i.e.*, by other nonstationary subepochs, noise or artifacts in the data; contamination by cardiac arrhythmias is also eliminated).

The Chaos software is unique in its class, since it is a deterministic data processor and can analyze non-stationary time series. Its basic advantages are:

- Reliability: it is a deterministic (not a stochastic) measure whose results are valid for individual subjects and representative at any point of the data series. Thus, it displays unique precision and

predictability compared to other, traditional quantifiers of chaos as Lyapunov exponents, Generalized dimensions, Entropies, etc.;

- Sensitivity: deterministic dimensional measures are inherently more sensitive to the output of the system than classical stochastic measures as the mean, standard deviation, power spectrum, etc.;

- Efficiency: it does not require data stationarity and can track rapid dimensional changes within small points of time;

It was not until recently considered that biological data are random and spurious. Chaos theory and the PD2i software for the first time allow interpreting them as deterministic. Standard tools for the analysis of biological data as: Power spectrum, Fourier transform, Mean and Standard deviation, Entropy, and so forth treat these data "averagely" (statistically), while the PD2i software is capable of identifying individual qualitative specificities within an unfolding data series. This capability of PD2i allowed us to apply it for distinguishing the individual dynamical properties of the various *Drosophila* mutants heart dynamics.

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