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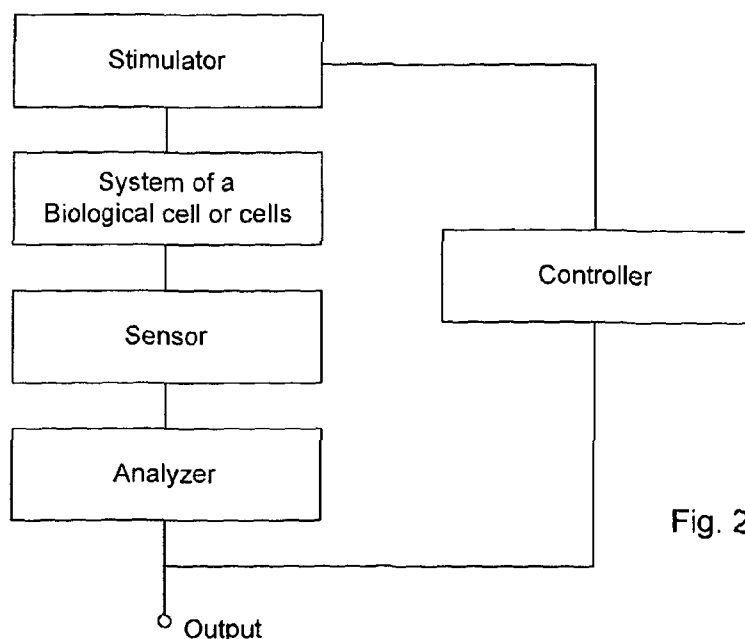


Fig. 2(a)

(57) Abstract: An apparatus is disclosed comprising: a stimulator for applying periodic stimulation to a biological cell system under investigation; a sensor for sensing the response of the cell system to the stimulation and producing an output; a controller adapted to: consistently change the period of the stimulation applied by the stimulator such that the period is either decreasing or increasing such that the state of the cell system passes from or to a region of monostability of response with respect to monostability; and then reverse the direction of the change in period such that the period is respectively consistently increasing or decreasing; and an analyzer arranged to analyze the sensor output to assess the location of a transition between the cell system having regular rhythm response and potentially irregular rhythm response with respect to stimulation.

BIOLOGICAL CELL SYSTEM INSTABILITY INVESTIGATION APPARATUS AND METHOD

FIELD OF THE INVENTION

This invention relates to an apparatus and method for the investigation of biological cell system instability, for example, but not exclusively, cardiac cells, such as in the examination and assessment of the proarrhythmic effects of drugs and heart arrhythmogenicity for pharmaceutical drug screening as well as drug discovery, and in the examination and assessment of heart arrhythmogenicity (i.e. the degree of the diseased state of the system).

BACKGROUND OF THE INVENTION

Between 0.5 million and 1 million Europeans and North Americans die each year of sudden cardiac death (SCD), which causes 10–20% of all deaths among adults in the Western world. While the implantable cardioverter defibrillator improves survival in high-risk SCD patients, at the present time standard antiarrhythmic drug therapy has failed to reduce, and in some instances has increased the incidence of SCD. Many reports of clinical trials for experimental therapies showed that antiarrhythmic drugs are only partially effective and may have many adverse effects; specifically, the most important of these is the potential to generate new life-threatening arrhythmias (proarrhythmia) such as ectopy, monomorphic ventricular tachycardia (VT), Torsade de Pointes, ventricular fibrillations (VF), conduction disturbances or bradycardia. In fact, cardiac pacing for irregular heartbeat using either an external device or an implantable cardioverter-defibrillator (ICD) is more effective than antiarrhythmic drugs at terminating life-threatening arrhythmias if they occur. However these devices do not prevent arrhythmia. Therefore, antiarrhythmic drug therapy still remains the mainstay of long-term arrhythmia treatment. Consequently, discovering safe and effective antiarrhythmic drugs up till now continues to remain the most pressing problem.

Pharmaceutical companies invest multi-billions of dollars per year in discovering and developing new medicines. The drug-development process normally proceeds through various phases over many years. Pre-clinical studies involve in vitro (test tube) and in vivo (animal) experiments using wide-ranging doses of the drug under

study to obtain preliminary efficacy, toxicity and pharmacokinetic information. Such tests assist pharmaceutical companies to decide whether a drug candidate has scientific merit for further development as a potential new drug. Overall, about 1,000 potential drugs are tested before just one reaches the point of being tested in a clinical trial. However, many programs are aborted after decades of costly yet fruitless efforts to limit side effects or toxicity of candidate drugs and many antiarrhythmic drugs are recalled from the market on stage of clinical trials due to proarrhythmic effects that led to increased mortality. Accordingly, tools that can abbreviate the research by revealing proarrhythmic effects on early pre-clinical studies are desirable.

DESCRIPTION OF THE RELATED ART

Theoretically, the discovery, development and use of antiarrhythmic drugs should be based upon a complete understanding of factors responsible for the susceptibility to, and initiation of cardiac arrhythmias. Although many factors have been identified to affect heart rhythm, the underlying mechanisms of initiation and development of cardiac arrhythmias have been only partially understood. As a result, antiarrhythmic drugs are only partially effective and have many adverse effects.

Nonlinear dynamical phenomena have great impact on initiation of electrical instabilities – one of the immediate causes of cardiac arrhythmias. Therefore we use nonlinear physics approach to elucidate cause-effect relationships of these phenomena that is necessary condition for solution of problems with control over nonlinear processes in cardiac dynamics. Here we apply our findings to reveal hidden proarrhythmic effects of drugs and to assess efficacy of antiarrhythmic drugs on different levels of cardiac dynamics from intracellular, cellular to the whole heart.

Over the past four decades, the restitution hypothesis, first proposed in 1968 by Nolasco & Dahlen, have become one of the key tools used to predict the onset of arrhythmias both in experimental/clinical and mathematical modeling settings. The restitution hypothesis is based on the phenomenon of period doubling bifurcations in relationship between action potential duration (APD) and the preceding diastolic interval (DI). The hypothesis states that when the restitution curve slope is equal or

greater than one (≥ 1), oscillations in APD, called alternans (i.e. long and short beat-to-beat variations in APD), will result. Despite great attention the restitution hypothesis remains still controversial: "A number of studies have shown cases where either a restitution curve slope is greater than one but alternans or fibrillation do not occur or a restitution curve slope is less than one but alternans or fibrillation nevertheless occur"

Developing the idea of Nolasco and Dahlen, several in vitro and/or cell culture-based methods/restitution protocols have been identified for predicting onset of electrical instability (alternans) and/or the deterioration of tachycardia into fibrillation. It has now become clear that none of the protocols tested provide a reliable prediction of the onset of alternans.

Examples of devices, methods, systems, and computer programs for detecting propensity for ventricular fibrillation using action potential curves, as well as for evaluating a risk of the occurrence of cardiac arrhythmias in a heart based on restitution hypothesis, can be found in various patents e.g. EP 0 922 256 (B1), US Patent 6094593, US Patent 7047067. However, the fact that restitution hypothesis is still controversial results in limitations of applicability of these ideas.

Recent studies reveal that the traditional methods/protocols based on restitution hypothesis have two essential limitations. The first limitation lies in the fact that traditional protocols predict the onset of instability with a considerable delay. Second limitation is that traditional protocols allow capturing of only a particular case of cardiac dynamics. Both limitations are caused by the nonlinear effects induced by application of protocols *per se*. Unexpected nonlinear effects appear because the nonlinear properties of electrical processes of the heart have been only partially understood and, consequently, have not been properly taken into account when developing the protocols, that has resulted in non-reliable predictions of cardiac instabilities by these protocols. In particular, the multistability property has not been taken into consideration, although it has a considerable impact on cardiac dynamics.

From a nonlinear dynamics perspective, multistability means the coexistence of different stable regimes for a fixed set of stimulation parameters at which well-timed stimulus may convert one regime into another. Experimental and modeling observations of multistability have been described in electrophysiology over many

years in many species. The variety of particular cases shows unpredictability and impossibility to control the phenomenon.

Nevertheless, in one modeling study, it is shown that the main cause of variety of particular cases (even when obtained at the same conditions) is the dependence of cell responses to the initial conditions of stimulation. Different initial conditions may result in different responses within multistability region where several stable states may coexist. It is significant that each of stable states is uniquely determined by certain initial conditions. Moreover, in another study it is shown that location of multistability region on the PCL-axis is depended on ionic properties of a cell. Our findings provide following explanations of variety cases observed in experimental studies: (i) due to impossibility to implement equal initial conditions for every cell (or other object under investigation) in experimental conditions, different results would inevitably be observed in experiment because they are produced from different initial conditions; (ii) ionic properties are always varied from cell to cell that also leads to different experimental results.

Moreover, in the latter study, the existence of multistability property in human cardiac dynamics by example of human ventricular cell model is shown. Figure 1 illustrates the existence of multistability property in human cardiac dynamics. The presented time series of transmembrane voltage change of a human ventricular single cell are obtained under external stimulation by electrical impulses with magnitude $I_{stim}=38 \mu A/cm^2$ and period of stimulation $PCL=235$ ms. Simulation performed using human ventricular cell model (Ten Tusscher & Panfilov, 2006, *Alterans and spiral breakup in a human ventricular tissue model*, Am J Physiol Heart Circ Physiol. 291:H1088-H1100) with parameters setting of the model corresponds to slope 0.7 (Table 1). Two different rhythms, the 2:1 rhythm and 1:1 rhythm, are shown to coexist at the same period of stimulation. A premature impulse delivered at a given time suddenly converts the 2:1 rhythm (corresponding to a conduction block) into the stable 1:1 rhythm (corresponding to tachycardia) or vice versa. It shows that multistability property may be considered as underlying mechanism of sudden triggering of life-threatening ventricular tachyarrhythmia.

The present invention seeks to provide a method and a system for examination and assessment of the proarrhythmic effects of drugs and heart arrhythmogenicity. The

invention also seeks to provide the effective and rapid screening of drugs which allows one to reveal hidden proarrhythmic effects of drugs.

SUMMARY OF THE INVENTION

5 The invention provides an apparatus comprising:

a stimulator for applying periodic stimulation to a biological cell system under investigation;

a sensor for sensing the response of the cell system to the stimulation and producing an output;

10 a controller adapted to: consistently change the period of the stimulation applied by the stimulator such that the period is either decreasing or increasing such that the state of the cell system passes from or to a region of monostability of response with respect to stimulation; and then reverse the direction of the change in period such that the period is respectively consistently increasing or decreasing; and

15 an analyzer arranged to analyze the sensor output to assess the location of a transition between the cell system having regular rhythm response and potentially irregular rhythm response with respect to stimulation.

The invention also provides a method comprising:

20 applying periodic stimulation to a biological cell system under investigation; sensing the response of the cell system to the stimulation and producing an output;

consistently changing the period of the stimulation applied such that the period is either decreasing or increasing such that the state of the cell system passes
25 from or to a region of monostability of response with respect to stimulation; and then reversing the direction of the change in period such that the period is respectively consistently increasing or decreasing; and

analyzing the sensor output to assess the location of a transition between the cell system having regular rhythm response and potentially irregular rhythm response
30 with respect to stimulation.

The stimulation protocol used by the apparatus and method according to the invention is also referred to herein as the U-turn protocol. The cell system having a regular rhythm response with respect to stimulation may be referred to as being in a stable state (or monostable state) with respect to stimulation. The cell system having
5 a potentially irregular rhythm response with respect to stimulation may be referred to as being in an unstable state with respect to stimulation (i.e. existing in one of a plurality of states; multistability).

Described herein embodiments of the invention, in effect, improve the process of antiarrhythmic drug discovery by revealing proarrhythmic effects on early pre-
10 clinical study, i.e. before clinical trials. The method, named as U-turn protocol, entails specific sequence of stimuli applied using a given set of rules and induces effects that reveal hidden potentially dangerous arrhythmogenic regimes which are not detectable via currently used traditional protocols but may occur in natural conditions, e.g. at clinical trials. Embodiments of the present invention contain
15 means of application of the U-turn stimulation protocol to an object under investigation to assess the effects of a drug, to discover new drug compounds, to assess a toxicity or drug overdose and to assess heart arrhythmogenicity. The system for assessing the proarrhythmic effects of drugs and heart arrhythmogenicity comprises the method of nonlinear analysis of data received from a single object, the
20 mean of categorization of the objects, and the mean of comparison by categories. The method and the system can be applied to an object of investigation such as isolated or cultured cardiac single cell, number of cells or a whole heart *in vitro* or *in vivo*. The method and the apparatus are suitable for use in laboratory conditions for screening an individual object as well as in pharmaceutical industry for screening large number
25 (more than thousand) of individual patch-clamped cells using planar array chip-based technology.

Some tangible benefits of the invention can include:

- The invention can abbreviate the research of new drugs by revealing hidden proarrhythmic effects on early pre-clinical studies that reduces
30 pharmaceutical company expenses required in the discovery and development of new drugs.

- The invention provides new opportunities for discovering safe and effective antiarrhythmic drug by using new methods and system for drug screening that provides (1) reliable prediction of potentially dangerous arrhythmogenic regimes, (2) safe assessment - earlier prediction of appearance of cardiac instabilities, (3) high sensitivity which allows the detection of dynamical consequences of intracellular changes.
- The invention improves the state of affairs with already marketed drugs by revaluation of their effect on cardiac dynamics and by correcting the dose of medications.
- The invention aids the decision-making efforts in the selection and development of new drugs as well as helps to assess the risk-benefit ratio of a given drug.

The key aspects of embodiments of the invention can be summarized in five major points:

1. Nonlinear dynamical phenomena greatly impact on initiation of electrical instabilities which is one of the immediate causes of cardiac arrhythmias.
2. Currently used traditional protocols elaborated for predicting onset of electrical instability (alternans) have two essential limitations: (1) prediction of the onset of instability with a considerable delay, (2) capturing only a particular case (as oppose to a more complete picture) of cardiac dynamics.
3. The multistability property has been not taken into account in a prognosis of onset of electrical instability.
4. The new method, named the U-turn protocol, can reveal hidden potentially dangerous arrhythmogenic regimes which are not detectable via currently used traditional protocols but may occur in natural conditions during clinical trials. The U-turn protocol entails specific sequence of stimuli applied using given set of rules in order to induce pre-programmed nonlinear effects which would uncover any kind of instability including simultaneously coexisted regimes (multistability) which signify potential arrhythmogenicity.

- 5 5. The newly proposed the U-turn protocol provides following advantages in comparison with currently used traditional protocols: (1) reliable prediction of potentially dangerous arrhythmogenic regimes, (2) safe control - earlier prediction of appearance of instabilities, (3) high sensitivity - allowing
10 detection of dynamical consequences of intracellular changes, (4) rapid screening .

The present invention relates to a method and a system for examination and assessing the hidden proarrhythmic effects of drugs and heart arrhythmogenicity.

10 The invented new method, named as the U-turn protocol, is elaborated for examination of the proarrhythmic effects of drugs and heart arrhythmogenicity in vitro and in vivo experiments, e.g. at pre-clinical studies. The main goal of the U-turn protocol is to reveal dangerous arrhythmogenic regimes which are not detectable via currently used traditional protocols but may occur in natural conditions, e.g. at clinical trials. The U-turn protocol entails sequence of stimuli applied using a give
15 set of rules. The invented U-turns protocol involves changing the period of stimulation (PCL) or frequency on each beat and recording the corresponding values of characteristics of the output response at each beat. U-turn means the change in the direction from decreasing in period of stimulation to increasing, or vice versa, but only after the moment when the system reliably has reached instability mode. Such
20 kind of forcing yields qualitatively different effects on the output response when system is driven in forward and backward directions that allows to assess dynamical properties of the system. Application of the U-turn protocol induces pre-programmed effects which uncover any kind of instability including simultaneously coexisted regimes (multistability) signified potential arrhythmogenicity.

25 The present invention contains means of application of the U-turn stimulation protocol to an object under investigation to assess the effects of a drug, to develop new drug compounds, to assess a toxicity or drug overdose and to assess heart arrhythmogenicity.

30 The new system for assessing the proarrhythmic effects of drugs and heart arrhythmogenicity comprises a method of nonlinear analysis of data received from a single object, means of categorization of the objects, and means of comparison of

assessments. There are four main purposes of data analysis: (a) identifying the instability onset and multistability phenomena, (b) discerning cause-effect relationship of arrhythmogenicity, (c) assessing arrhythmogenicity, and (d) decision-making how to reduce arrhythmogenicity and to improve the state of the object under investigation.

The U-turn protocol can be applied in any species to an object of investigation such as isolated or cultured cardiac single cell, number of cells (isolated or cultured tissue) or to a whole heart *in vitro* (e.g. Langendorff perfused whole heart) or *in vivo* (e.g. during an open heart bypass surgery).

The method and the system are suitable for using in laboratory or any other research/industrial/clinical environment for screening an individual object as well as in pharmaceutical industry for screening large number (more than thousand) of individual patch-clamped cells using planar array chip-based technology.

BRIEF DESCRIPTION OF THE DRAWINGS

Embodiments of the invention will now be described, by way of example only, with reference to the accompanying drawings in which:

Fig. 1 shows a time series of transmembrane voltage of a human ventricular single cell obtained under external stimulation by electrical impulses;

Fig. 2(a) shows a schematic illustration of an apparatus according to an embodiment of the invention, and Fig. 2(b) is a flow chart of an elementary method according to an embodiment of the invention;

Fig. 3 shows a time series of transmembrane voltage, and a plot of action potential duration (APD) against pacing cycle length (PCL) for a method embodying the invention;

Fig. 4 shows four plots of action potential duration (APD) against pacing cycle length (PCL) giving a comparison of a method embodying the invention (Figs. 4 A & B) and a conventional method (Figs. 4 C & D);

Fig. 5 shows plots of action potential duration (APD) against pacing cycle length (PCL) giving each giving a comparison of theory and results according to the invention;

Fig. 6 shows results obtained during a process for assessing the proarrhythmic affect
5 of a drug;

Fig. 7 shows results of the effects of a hypothetical drug that alters calcium inactivation;

Fig. 8 shows the results of a modeling study for potential drug discovery; and

Fig. 9 shows the results of a modeling study on a hypothetical drug that alters
10 potassium conductance.

Table 1: The model parameter settings. This Table is a copy of contents from Table 2 from Ten Tusscher & Panfilov, 2006. "Parameters that were varied were maximum conductance of the I_{Kr} , I_{Ks} , I_{pCa} , and I_{pK} currents (all in nS/pF). An additional parameter that was used was the time constant (τ_f) for the f gate. For the slope 1.1
15 setting, the time constant was as given in the paper [Ten Tusscher 2006]; for the other settings the time constant was multiplied by a factor (given in right column) for the voltage range $V > 0$ mV, thus rescaling inactivation but not recovery kinetics (τ_{finact})."

Parameter Settings	Restitution slope	G_{Kr}	G_{Ks}	G_{pCa}	G_{pK}	τ_{finact}
Setting 1	Slope 0.7 (flat)	0.134	0.270	0.0619	0.0730	x0.6
Setting 2	Slope 1 (normal)	0.153	0.392	0.1238	0.0146	normal
Setting 3	Slope 1.8 (steep)	0.172	0.441	0.8666	0.00219	x2

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

Fig. 2(a) shows schematically an apparatus embodying the invention. A controller controls a stimulator to produce a periodic stimulus for application to the biological cell system under investigation. The controller can be embodied as a conventional personal computer or similar device running appropriate software, or could be embodied in dedicated hardware. In one example the stimulator is a signal generator configured to output an electrical voltage as the periodic stimulation. However, other examples of the stimulation include electrical signal, mechanical force, change of pH, addition of reagents, an interference causing a change of ionic properties, or other types of internal or external stress on the system under investigation, or a combination of any of these.

The biological cell system under investigation can be an isolated or cultured single excitable cell (neuron, stomach cell, etc.), a plurality of excitable cells (isolated or cultured tissue) or a whole organ such as heart, nerve system, stomach, etc. (*in vitro* or *in vivo*) or a whole organism, of any species. The cell system can be entirely *ex vivo*. Isolated cells may be obtained by biopsy.

A sensor is arranged to sense the response of the cell system, and the specific sensor used depends on the nature of the system under investigation and on the stimulation. Examples include sensors to detect voltage, ion concentrations, ionic currents, force generated or other physical and/or chemical response characteristics. In more detail, the sensor could measure: electrical signals such as voltage or ECG from the surface of the body or from inside the body via catheters; ion transients (Ca^{2+} , Na^{+} , K^{+} , H^{+} etc), ion currents (I_{CaI} , I_{K} , I_{Na} , etc); forces, such as mechanical force; or pressure; or any combination thereof.

An analyzer is provided that receives the output of the sensor and determines characteristics, such as the period of the response, and whether there has been an abrupt change in the ratio of the period of the stimulation to the response. The analyzer can perform further analysis, as described below, such as to determine the boundary of monostability of the cell system, the effects of factors such as drug dosage on the system, or categorizing the cell system. The analyzer produces an output, which is also fed-back to the controller to effect how the controller changes

the stimulator, as explained further in relation to the method. The analyzer could be integral with the controller, for example if the analyzer is embodied as further software running on the same computer as the controller software.

An exemplary method according to the invention is outlined by the flowchart of Fig. 2(b), which is self-explanatory. Detecting that a transition has occurred from or to a region of monostability can be achieved, for example, by determining whether there has been an abrupt change in the ratio of the period of the stimulation to the response. Of course this detection typically does not coincide with the precise location of the boundary of the monostable region. The system may exhibit a single apparently stable output under certain conditions even when in a region of potential instability (including multistability). Embodiments of the invention attempt to assess the location of the boundary transition between a regular rhythm response and a potentially irregular rhythm response to stimulation by reversing the direction of the change in period (the U-turn). The 'direction' of the change in period refers for example to whether it is increasing or decreasing. Consistently changing the period means always changing it in the same direction, even if the step size of the change is varied. The question of whether sufficient data has been obtained, after the U-turn has been performed, can be based on various factors, for example by detecting that the system has returned to the original state of monostability or non-monostability, but this is not essential. In a further modification, multiple U-turns can be performed. Measuring changes in the location of the transition between stable and potentially unstable regions, under different condition, can be used to perform drug screening, for example to assess whether a drug or combination of drugs, at a specific dosage, is proarrhythmogenic, anti-arrhythmogenic, or neither. The location itself can also provide information for categorization of an object system under investigation, such as diseased, or the degree of the diseased state of the system.

As well as changing the period of the stimulation, the controller can also adjust other parameters of the stimulation, such as the step size of the change of the period, the stimulus magnitude, the stimulus shape, and the stimulus duration. An example of decreasing step size might be an experimental run in which the period of the stimulation starts as follows: 1000ms, 750ms, 500ms, 400ms, 380ms, 360ms and so on. The further parameters of the stimulation can be changed during an experimental

run, or some or all of them can be kept constant during an experimental run, but changed from experiment to experiment.

The present invention provides a method that allows one to reveal and to control nonlinear dynamical properties in experiments. Instead of trying to reach in experimental setting unrealizable conditions such as the equal initial condition for identical cells, initial conditions are controlled during experiment by assigning such kind of stimulation protocols in which initial conditions are changing by a given rule. It was surprisingly found that the new protocol that involves controlling initial conditions and changing the period of stimulation (PCL) on each beat and recording the corresponding action potential duration (APD) at each beat in forward and backward direction (Fig. 3 A-D) provides several tangible benefits for pharmaceutical drug screening as well as drug discovery.

Figure 3 demonstrates the simplest version of the U-turn protocol in action obtained using human ventricular cell model (Ten Tusscher & Panfilov, 2006) with parameters setting corresponded to slope 0.7 (Table 1). The presented simulation data are exemplified applying the proposed protocol to a cardiac ventricular cell in experimental conditions. Fig. 3A: The time series of transmembrane voltage is obtained as a result of U-turn protocol application with step change of pacing cycle length (PCL) from stimuli to stimuli of 20 ms. The sequence of action potentials (APs) indicated on the left half of the plot shows pacing with decreasing (PCL) whereas the APs-sequence indicated on the right half shows pacing back with increasing PCL. The down-pointing arrow indicates the point of U-turn from decreasing to increasing PCL. Current values of action potential duration (APD) are measured for each impulse with recording the resulting dependence of APD vs PCL. Fig. 3B: The recorded result of dependence of APD vs PCL. Dark left-pointing triangles and light right-pointing triangles are corresponded to current values of the APD and the PCL obtained from APs-sequences with decreasing and increasing PCL, respectively. When the PCL gradually decreases, the APD values 'hold on' near the 1:1 stable state while the stable branch becomes unstable, and after that the U-turns is applied; because of at the moment the system is in an unstable state, subsequent gradually decreasing in PCL leads the system to the nearest steady state but, at the moment, there is another steady state corresponding to the 2:1 rhythm. The system 'holds on' to the 2:1 rhythm (upper branch) as long as it remains stable otherwise

returns back to the main branch corresponding to the 1:1 rhythm. As a result the protocol drives the system through both coexisting stable states depicting one state in forward direction and another one in backward directions.

The main idea of this new protocol is to make U-turns during the stimulation process.

5 A U-turn means the change in the direction (e.g. from decreasing in period of stimulation (i.e. decreasing pacing cycle length) to increasing it), but preferably only after the moment when the system has reached instability region (Fig. 3 A). Also, the direction of U-turns could be opposite, i.e. from increasing to decreasing the pacing cycle length. Such protocol embraces a wide range of initial conditions and, if a
10 multistability property is inherent to the system under study, and if the applied protocol passes a multistability zone, then in one direction the dynamical trajectory reaches one steady state but in the opposite direction it goes close to another steady state, and this coexistence of several trajectory indicates multistability (Fig. 3B). Accordingly, coexistence of two or more regimes simultaneously signifies potential
15 arrhythmogenicity. Consequently, the U-turn protocol provides possibility to reveal hidden potential dangerous arrhythmogenic regimes which are not detectable via currently used traditional protocols but may occur in natural conditions, e.g. during clinical trials.

Moreover, the proposed experimental protocol is highly sensitive, that means, it
20 allows to detect small changes in the ionic properties of a cell. This is because dynamical consequences of induced small changes in the chemical composition of a cell which become visible due to protocol application over wide range of initial conditions and pacing frequencies (see Experiment 1).

Figure 4 shows the comparison of the U-turn protocol embodying the invention (Fig.
25 4 A-B) to the dynamic restitution protocol traditionally used to detect the onset of arrhythmia (Fig. 4 C-D). Both protocols are applied to Ten Tusscher & Panfilov 2006 model for two parameters settings leaded to flat and steep restitution curves (Table 1) and results for each setting are presented by columns. A-B: Map of the APD vs PCL shows results of the U-turn protocol application that reveals
30 multistability in both settings of the model. However, for the case of steep restitution curve coexisted regimes occur at considerable higher values of PCL than at the case of flat restitution curve. C-D: The values of APDs, obtained using the dynamic

restitution protocol, are plotted against the PCLs (the result reproduced from paper Ten Tusscher and Panfilov 2006). While in the case of steep restitution curve the dynamic restitution protocol detects instability onset, for the case of flat restitution curve there is no instability observed by the dynamic restitution protocol until the PCL is smaller than 200 ms that contrasts with the U-turn protocol prediction. A dashed line is used to indicate the instability onset predicted by the dynamic restitution protocol and a delay in the prognosis comparing with the U-turn protocol. While the dynamic restitution protocol shows monostability, the U-turn protocol reveals multistability in the human ventricular cell model that agrees with the experimental observations on mammalian cells.

Comparison of applying the U-turn protocol (Fig 4A B) and dynamic restitution protocol (Fig 4C, D) is shown by simulating human ventricular cell model. At high values of PCL both protocols demonstrate similar dynamics. However, in region of low values of PCL, which is considered as the most dangerous, the U-turn protocol reveals bi/multistability - coexistence of two different rhythms at the same values of PCL- in both cases (Fig. 4 A-B), while the dynamic restitution protocol predicts monostability (Fig. 4 C-D) in both cases and alternans only in one case (Fig. 4 D). Consequently, the dynamic restitution protocol fails to capture multistability. Meanwhile the case observed by the U-turn protocol contains the case of dynamic restitution protocol that reveals one of two coexisted regimes. Accordingly, dynamic restitution protocol shows particular case of more general picture of complex dynamics embraced by the U-turn protocol.

These results reveal why in pre-clinical studies traditional protocols for drugs screening show positive effect of drugs, and yet at the stage of clinical trials the same drugs may demonstrate more complex dynamics, e.g. having pro-arrhythmic effects. The obtained difference is caused by underestimation of dynamical consequences of drug influence in pre-clinical studies due to using unreliable protocols.

Figure 4 highlights two dangers associated with using dynamic restitution protocols for investigation of the onset of arrhythmias. First danger is unreliable prediction, where the dynamic restitution protocol predicts no alternans above PCL value of 200 ms (Fig. 4C) as compared to those at 300 ms, revealed by the U-turn protocol (Fig. 4A). Second danger is a delay in prediction of alternans. For example, the dynamic

restitution protocol predicts alternans at PCL value of 320 ms, while the U-turn protocol shows that side effects may occur at PCL value of 420 ms. Consequently, the dynamic restitution protocol in one case fails to detect instabilities and in other case predicts alternans but with a considerable delay, altogether showing dangers of using such kind protocols for drug screening. In contrast, the newly proposed U-turn protocol predicts the appearance of instabilities in both cases and, which is the most important, at higher PCL as compared to the theoretically predicted bifurcation point obtained by S1-CI-S2 protocol that is clearly seen in Figure 5. Prediction of instability appearance at higher PCL is a necessary condition for drugs screening and that may move forward developing safe anti-arrhythmic drugs.

Figure 5 shows the relationship between theory and the U-turn protocol results. Bifurcation diagrams of the Ten Tusscher & Panfilov (2006) model are shown superimposed with the U-turn protocol results. Two model parameters settings representing flat and steep restitution curves (Table 1) are used for simulations. The bifurcation diagrams show the steady state values of the action potential duration obtained by stimulation of cell model with constant value of PCL (black dots) using the S1-CI-S2 protocol described in Surovyatkina *et al.* 2007 [Surovyatkina E., Egorchenkov R., Ivanov G., *Multistability as intrinsic property of a single cardiac cell: a simulation study*, Conf Proc IEEE Eng Med Biol Soc. 1-4244-0788-5/07, 927-930]. While bifurcation diagram can be performed using model simulations only and needs long time for calculations, the U-turn protocol is developed for both simulation and experimental studies. The U-turn protocol rapidly reveals contours of bifurcation diagram of a system under investigation. The U-turn protocol results shown in dark and light triangles depict all stable branches of bifurcation diagram, highlighting dangerous regions with instabilities or multistability. It is important to note that the U-turn protocol signifies the deterioration in stability at higher PCL values than theoretical predicted onset of instability (branches indicated by right-pointing triangles in Figs. 5A and 5B diverge at higher values of PCL than black branches do) that is significant for making reliable prognosis of the onset of arrhythmogenicity.

Importantly, the time of an experiment with the U-turn protocol can be much faster in comparison with that of the dynamic restitution protocol. One embodiment of the U-turn protocol according to the invention involves changing the period of

stimulation (PCL) on each beat, whereas in the dynamic restitution protocol, a series of 50 stimuli is applied at a specified BCL/PCL, after which cycle length (PCL) is decreased. The U-turn protocol does not contain preparatory 50 stimuli but includes the change in the direction from decreasing to increasing (or vice versa) period of stimulation while in the dynamic restitution protocol, a period of stimulation is decreasing only. Therefore, the run time of U-turn protocol is about in 25 times shorter than the run time of the dynamic restitution protocol.

Embodiments of the invention provide a method and system for examination and assessing the proarrhythmic effects of drugs and heart arrhythmogenicity in vitro and in vivo experiments. The invention contains (a) a U-turn stimulation protocol for examination of the proarrhythmic effects of drugs and heart arrhythmogenicity, (b) application of the U-turn stimulation protocol to an object under investigation under different conditions, and (c) a system of data analysis of the received information from the experiments.

A **first** embodiment of the invention provides a method for examination of the proarrhythmic effects of drugs and heart arrhythmogenicity. The main goal of the method, named as U-turn protocol, is to reveal dangerous arrhythmogenic regimes which are undetectable via already existing methods but may occur in natural conditions, e.g. during clinical trials. The protocol comprises means of applying a specific sequence of stimuli, according to a given set of rules, to the object under study.

The applied stimulus can be an electrical, mechanical or other type of signal of internal or external stress on the system, and may comprise for instance, in the case of an isolated cell, either the externally applied transmembrane current or stretching and compressing cell cultures to simulate in vivo mechanically active environments, and in the case of a whole heart, an electric current just as used at cardiac pacing procedures to control heart rhythm or mechanical force simulating cardiac massage.

The stimulation process entails sequence of stimuli applied using a given set of rules. The U-turns protocol according to this embodiment involves changing the period of stimulation (PCL) or frequency on each beat and recording the corresponding values of characteristics of the output response at each beat.

The step-size of PCL changing from beat to beat could be of any size and may vary during the protocol run but should be physiologically reasonable. For example 20ms linear or sine or sawtooth.

5 The amplitude and duration of each stimulus is specified according to the object under investigation. For instance, in the case of an isolated cell the applied magnitude of the stimulus must be large enough to trigger an action potential in the cell, and in the case of a whole heart, stimulus should not provide electric shock that reset the heart rhythm but must be large enough to control heart rate.

10 Amplitude of the applying stimulus could be constant or varied in time. For example, the Amplitude within sequence of stimuli can change by sinus wave during the application time.

The invented method and system are for any species, including human. The signal can be applied to an object of investigation such as isolated or cultured cardiac single cell, number of cells (isolated or cultured tissue) or to a whole heart *in vitro* (e.g. 15 Langendorff perfused whole heart) or *in vivo* (animals). In human application to a whole heart, the invention can be applied *in vivo* such as during an open heart surgery and *in vitro* to a heart that was rejected for transplantation.

The U-turn protocol can be also applied to a large number (more than thousand) of individual patch-clamped cells using planar array chip-based technology 20 (production companies such as Axon Instruments, Nanion, Sophion, Cytocentrics, and Molecular Devices).

The invented U-turn protocol is suitable for application wherein the object under investigation is an isolated or cultured single excitable cell (neuron, stomach cell, etc.), plurality of excitable cells (isolated, e.g. from biopsy or cultured tissue) or a 25 whole organ such as nerve system, stomach, etc. (*in vitro* or *in vivo*) of any species.

The output of U-turn protocol application can be, for instance, in the case of an isolated cell - voltage, ion concentration(s), ionic current(s), and in the case of a whole heart – non-invasive as well as the invasively recorded ECG signal.

A **second** embodiment of the invention provides means of application of the U-turn 30 stimulation protocol to an object under investigation under different conditions.

To assess the *effects of a drug* an experiment could include one condition in which the drug is administered and a control condition in which there is no drug, therefore the U-turn stimulation protocol should be applied at least two times to the same object of investigation.

- 5 To discover *new drug compounds*, the U-turn protocol can be performed at first with single component for the purpose of determining the proarrhythmic effect of given component, and then the U-turn protocol could be applied in repeated experiments in which new components that are sequentially added. So, the solution results in discovery of desirable compound (See the description of Experiment I).
- 10 To assess *toxicity* or drug overdose, the U-turn the protocol can be applied also in repeated experiments, in which drug dose or exposure time is varied (See the description of Experiment II).

To assess heart arrhythmogenicity, the U-turn stimulation protocol can be applied to a whole heart using invasive and non-invasive techniques.

- 15 A **third** embodiment of the invention provides a system for assessing the proarrhythmic effects of drugs and heart arrhythmogenicity. The system comprises the method of nonlinear analysis of data received from a single object, the means of categorization of the objects, and the means of comparison of assessments.

Analysis of data received from a single object

- 20 There are four main purposes of analysis of data received from a single object: (a) identifying the instability onset and multistability phenomena, (b) discerning cause-effect relationship of arrhythmogenicity, (c) assessing arrhythmogenicity, and (d) decision-making of how to reduce arrhythmogenicity and to improve the state of the object under investigation.

- 25 *Categorization of the objects*

The reaction of objects to the stimulation protocol application would be different from cell to cell, from heart to heart. Therefore, in order to obtain comparable sets, the objects should be categorized into groups by initial state of the objects. Depending on homogeneity of objects the number of groups may be varied from two

(arrhythmogenic and stable) to any reasonable numbers of groups organized according to the degree of arrhythmogenicity that determines on the base set of attributes originated by results of analysis of primary data. Other categories or types to be considered in the objects under investigation include: male/female; various
 5 species (e.g. rat, guinea pig, rabbit, human); different diseases and different progression of the disease state (e.g. light or severe ischemia); and various types of cells, tissue, whole organs (e.g. ventricular, atria, SA node).

Comparison of assessments

1. Comparison of results (the onset of instabilities, the dispersion (height) of
 10 multistability region, etc.) of a single object from trial to trial to assess efficacy and toxicity of drugs application.
2. Comparison of results from each object inside of each category to assess reproducibility and reliability of the effect of the drugs.
3. Comparison by categories to assess the benefit-risk balance.

15

Figure 6 shows the U-turn Protocol Chart. The same U-turn protocol is repeatedly applied to two ventricular cells and results for each cell are represented by columns (Fig. 6A and 6D). The primary result is flat restitution curve (RC) for both cells (lower plots by X' in Figs. 6B and 6E). The main goal is to increase steepness of RC
 20 but to exclude pro-arrhythmic effects. A theoretical drug that increases calcium inactivation time constant ($\tau_{f\ inact}$) by multiplier N is applied in six experiments for N of 0.6, 1, 1.5, 1.8, 1.9 and 2. Results of the last experiment for N of 2 are shown by upper plots in Figs. 6B and 6E. Overall result of set of experiments for each cell is shown in C and F. Pro-arrhythmic effect of drugs is indicated by down-pointing arrows in Fig. 6C. The rectangles in Figs. 6C and 6F highlight the area of
 25 acceptable values of N led to the desirable effect of drug application.

The process of assessing the proarrhythmic effects of drugs and heart arrhythmogenicity involves several steps, explained in more detail below with reference to Figure 6:

- 30 i. **Obtaining Input Data** – Conducting time series (Fig. 6 A, D) analysis to characterize the magnitude and phase of each beat (a system's response to a stimulus), for instance, in the case of an isolated cell - max magnitude of

signal (e.g. voltage, ions concentrations, ionic currents *etc*), the action potential duration (APD), and in the case of a whole heart - RR-interval, PQ-interval, QT- interval.

- ii. **Mapping the results** – The map is plotted for characteristics of each beat against the stimulation period/frequency (Fig. 6 B, E).
- iii. **Test the results for multistability** – Conducting split data test (i.e., detecting more than one branch along the x-coordinate that is period or frequency of stimulation). Characterizing multistability region by finding beginnings and endings of splitting branches, so determining specific boundaries that mark multistability region.
- iv. **Selecting attributes of object** – Originating set of attributes on the base of test results (iii) for the categorization of the objects.
- v. **Categorization of the objects** – Categorizing objects into two (or more) groups by initial state of objects: arrhythmogenic and stable.
- vi. **Selecting an alteration condition attribute** – Setting an attribute reflecting the alteration condition
- vii. **Test for the response-alteration condition** – Repeating steps (i) to (iii) with data of experiments under alteration condition.
- viii. **Plotting diagram of overall result experiments of each object**– The diagram is plotted for characteristics of each beat against the condition attribute (e.g., Fig. 6 C, F).
- ix. **Test the results for arrhythmogenicity** – Considerable gap in a diagram corresponding to a single value of factor N (e.g., Fig. 6 for N=1.9 and 2) indicates a pro-arrhythmic effect of applied dose of a drug.
- x. **Comparison inside of a category of the objects** – Comparison of overall maps inside of each category and evaluation of the effect of alteration condition as ‘positive’, ‘negative’ or ‘no effect’.
- xi. Repeating steps (vii) to (ix) until reaching desirable result that consists reducing arrhythmogenicity in category of arrhythmogenic objects and excluding pro-arrhythmic effects in category of stable objects (Fig. 6 C, F).

This analysis provides information on the anti-arrhythmic and pro-arrhythmic effects of drug components that affect intracellular, cellular, and whole heart processes. Results of analysis allow one to decide what substance should be incorporated into the drug, or which channel should be modified. As a result, the specificity of a compound is gradually established. Consequently, the present invention will improve the discovery of new drugs by revealing proarrhythmic effects on early study of components selection.

The invented means of examination of experimental data via U-turn protocol application allows one to determine the most effective and safe components in the formulation of the drug and to find the optimum dosages and combinations of those components in order to manufacture the most effective drug.

The following description of experiments further illustrate aspects of the present invention.

Experiment I

Figure 7 shows results of the Experiment I that exemplify the invention in action for assessment of the safety and effectiveness and toxicity of a different dose of a theoretical drug that affects calcium inactivation. Initial data obtained using human ventricular cell model (Ten Tusscher & Panfilov, 2006) with parameters setting corresponded to flat restitution curve (Table 1) that is considered as a control case (Fig. 7A). Increasing in calcium inactivation time constant ($\tau_{f\text{ inact}}$) by multiplier N leads to increasing the steepness of stable branch (Fig. 7B) and occurrence of side effects at high values of PCL (Fig. 7C-7D) that signifies toxicity with drug which has the pro-arrhythmic effect in overdosage. The diagram APDs vs factor N shows comparison by overall results of set of experiments to assess effectiveness and toxicity of a different dose (Fig. 7E). Down-pointing arrows in Fig. 7E highlight values of N that lead to overdosage whereas values of N in the middle of diagram show effectiveness of drug application.

For example, in the first experiment, the U-turn protocol applies to a single cell without addition of a drug. The result of first experiment (Fig. 7A) is used as a control case for further consideration. Examination of these experimental results shows that (i) a stability region of PCL where a single stimulus produces an action potential AP is long enough and spread from PCL of 2000 ms to PCL of 300 ms; (ii) stable branch is flat. To improve steepness of stable branch we increase a voltage-dependent calcium inactivation time constant in the cell model by multiplication by a factor N (see Table 1) that exemplifies increase in drug dose application. A range of N factor values are taken from paper [Ten Tusscher & Panfilov, 2006] where suggested values of N were verified with experimental data. However, in opposite to the approach described in [Ten Tusscher & Panfilov, 2006], in the present experiment the only one parameter of the model, factor N , changes from experiment to experiment, while another parameters of the model do not change. In the control case [Ten Tusscher & Panfilov], factor N is equal to 0.6. The increase in calcium inactivation time constant leads to increasing the steepness of stable branch but stability domain of PCL decreases comparing to the control case of $N=0.6$. However, further increase in factor N leads to unexpected consequences. While in the case of $N=1.8$ branches generated by forward and backward pacing have slightly parted in the domain of long PCLs (Fig. 7B); in the case of $N=1.9$ in the same domain of low PCL additional branch occurs (Fig. 7C). Eventually in the case of $N=2$ the domain of instability spreads on considerable region of long PCLs (Fig. 7D) that exemplifies a toxicity or drug overdose, especially overdose with drug which has the pro-arrhythmic effect in overdosage.

In the case of large series of similar experiments, automated express analysis of obtained results is conducted in a diagram manner by plotting all recorded APD-values of given experiments against of values of factor N (Fig. 7E). Considerable gap in a diagram (indicated by down-pointing arrows in Fig. 7E) signifies pro-arrhythmic effect of applied dose of drug. The values of N in the middle of diagram correspond to safe and effective doses of drugs.

Experiment II

A modeling study aims to show opportunities for embodiments of the invention for new drug discovery. Figure. 8 shows the results of the Experiment II that exemplifies

the effective application of theoretical anti-arrhythmic drugs that reduces pro-arrhythmic effect induced by overdose of drug in previous experiment using another component of drug that affects to potassium conductance. Fig. 8A: - result of previous experiment with pro-arrhythmic effect induced by overdose of drug. Fig. 8B: - increasing in maximal plateau K^+ currents conductance G_{pK} from 0.073 to 0.16nS/pF leads to reducing dynamical instability in cell response to applied stimulation. Importantly, other parameters of the model are the same as those used in the previous experiment.

Experiment III

Figure 9 shows the result of Experiment III that exemplified negative influence of the theoretical drug that affects slow and rapid potassium currents, in particular, maximum conductance of the I_{Ks} and I_{Kr} currents and time constant for the x_s -gate. Fig. 9A: - result corresponded to 'normal' case for model parameters settings in (Table 1) suggested by Ten Tusscher and Panfilov, (Ten Tusscher & Panfilov, 2006) that is considered as a control case in the experiment. Fig. 9B: - result of altered K^+ properties, specifically, increasing in time constant for the x_s -gate and maximum conductance of the slow potassium currents and decreasing in maximum conductance of the rapid potassium currents lead to a more pronounced and significant shift arrhythmogenic zone to the domain of longer PCL that signifies the side effect of theoretical drug led to shortening region of normal heart functioning and increasing risk of arrhythmia developing at relatively small elevation of heart rate.

It will be understood that the present invention has been described with reference to particular embodiments, which provide an illustration of the principles of the invention. It will be further understood that numerous modifications may be made by those skilled in the art without departing from the true spirit and scope of this invention.

CLAIMS

1. An apparatus comprising:
 - a stimulator for applying periodic stimulation to a biological cell system
 - 5 under investigation;
 - a sensor for sensing the response of the cell system to the stimulation and producing an output;
 - a controller adapted to: consistently change the period of the stimulation applied by the stimulator such that the period is either decreasing or increasing such
 - 10 that the state of the cell system passes from or to a region of monostability of response with respect to stimulation; and then reverse the direction of the change in period such that the period is respectively consistently increasing or decreasing; and
 - an analyzer arranged to analyze the sensor output to assess the location of a transition between the cell system having regular rhythm response and potentially
 - 15 irregular rhythm response with respect to stimulation.
2. An apparatus according to claim 1, wherein the controller is adapted to reverse the direction of change in period after the analyzer has detected, from the sensor output, that the state of the cell system has passed from or to a region of
- 20 monostability of response with respect to stimulation.
3. An apparatus according to claim 1 or 2, wherein the controller is adapted to reverse the direction of the change in period more than once.
- 25 4. An apparatus according to claim 3, wherein the changing of the period as a function of time is one of a saw-tooth, sine-wave, staircase function or combination thereof.
5. An apparatus according to any preceding claim, wherein the stimulation
- 30 applied is any of an electrical signal, mechanical force, change of pH, addition of reagents, an interference resulting in a change of ionic properties, or any combination of thereof.

6. An apparatus according to any preceding claim, wherein the cell system under investigation is any of: an isolated or cultured single cell, a plurality of cells in an isolated or cultured tissue, or a whole organ.
- 5 7. An apparatus according to claim 6, wherein the cell or cells are cardiac cells, or the organ is a heart.
8. An apparatus according to any preceding claim, wherein the analyzer is
10 adapted to derive the borders of an arrhythmogenic region.
9. An apparatus according to any preceding claim, wherein the stimulator is adapted to change one or more parameters of stimulation, as well as or instead of period, the parameter being selected from: step size of the change of the period;
15 stimulus magnitude; stimulus shape; stimulus duration.
10. A method comprising:
applying periodic stimulation to a biological cell system under investigation;
sensing the response of the cell system to the stimulation and producing an
20 output;
consistently changing the period of the stimulation applied such that the period is either decreasing or increasing such that the state of the cell system passes from or to a region of monostability of response with respect to stimulation; and then reversing the direction of the change in period such that the period is respectively
25 consistently increasing or decreasing; and
analyzing the sensor output to assess the location of a transition between the cell system having regular rhythm response and potentially irregular rhythm response with respect to stimulation.
- 30 11. A method according to claim 10, wherein the step of reversing the direction of change in period is performed after detecting, from the sensor output, that the state

of the cell system has passed from or to a region of monostability of response with respect to stimulation.

12. A method according to claim 10 or 11, wherein the steps of changing the period and reversing the direction of the change in period are repeated more than
5 once.

13. A method according to claim 12, wherein the changing of the period as a function of time is one of a saw-tooth, sine-wave, staircase function or combination
10 thereof.

14. A method according to any one of claims 10 to 13, wherein the analyzing step comprises identifying the instability onset and multistability phenomena by assessing the sensor output data points to determine at least one of: the degree of branching,
15 the maximum width between different branches, the position of the onsets of branching, the distribution of data points between the braches.

15. A method according to any one of claims 10 to 14, wherein the stimulation applied is any of an electrical signal, mechanical force, change of pH, addition of reagents, an interference resulting in a change of ionic properties, or any combination
20 of thereof.

16. A method according to any one of claims 10 to 15, wherein the cell system under investigation is any of: an isolated or cultured single cell, a plurality of cells in an isolated or cultured tissue, or a whole organ.
25

17. Method according to claim 16, wherein the cell or cells are cardiac cells, or the organ is a heart.

18. A method according to any one of claims 10 to 17, wherein the analyzing step comprises deriving the borders of an arrhythmogenic region.
30

19. A method of assessing the effect of a drug or combination of drugs on a biological cell system, comprising performing the method according to any one of claims 10 to 18 on the cell system both with and without the addition of the drug or combination of drugs, and comparing the location of the transition between the cell system having regular rhythm response and potentially irregular rhythm response with respect to stimulation

20. A method according to claim 19, comprising performing the method of any one of claims 10 to 18 with one or more different dosages of the drug or combination of drugs.

21. A method according to claim 19 or 20, wherein the analyzing step further comprises assessing whether the drug or combination of drugs, at a specific dosage, is proarrythmogenic, anti-arrythmogenic, or neither.

22. A method according to any one of claims 10 to 21, comprising consistently changing one or more parameters of stimulation, as well as or instead of period, said parameter being selected from: step size of the change of the period; stimulus magnitude; stimulus shape; stimulus duration.

23. A method according to claim 22, wherein the analyzing step further comprises assessing whether the change of said parameter of stimulation is proarrythmogenic, anti-arrythmogenic, or neither

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Fig. 1

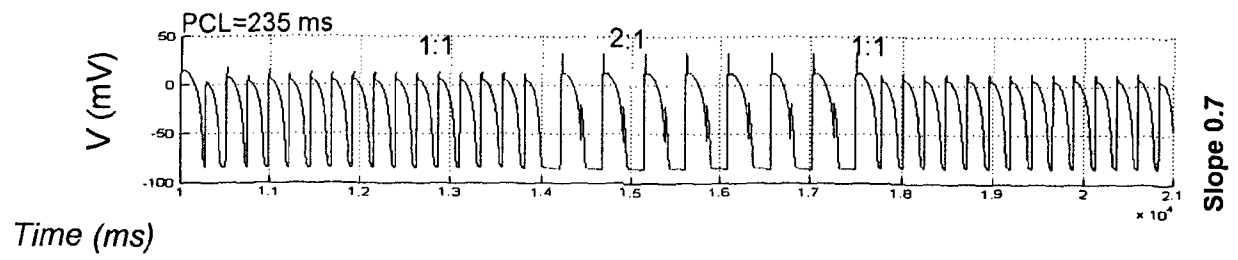
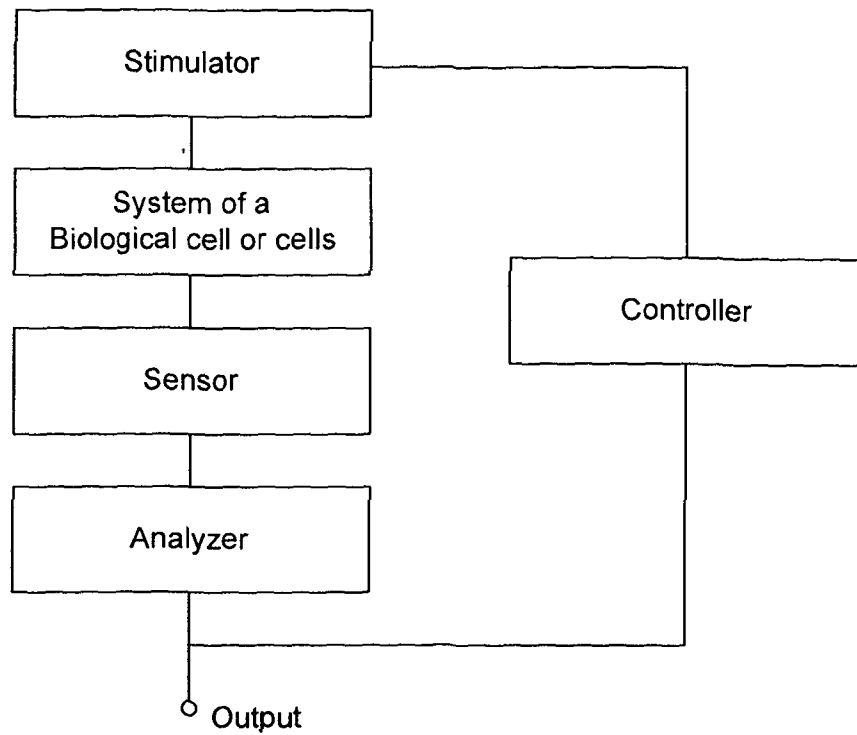
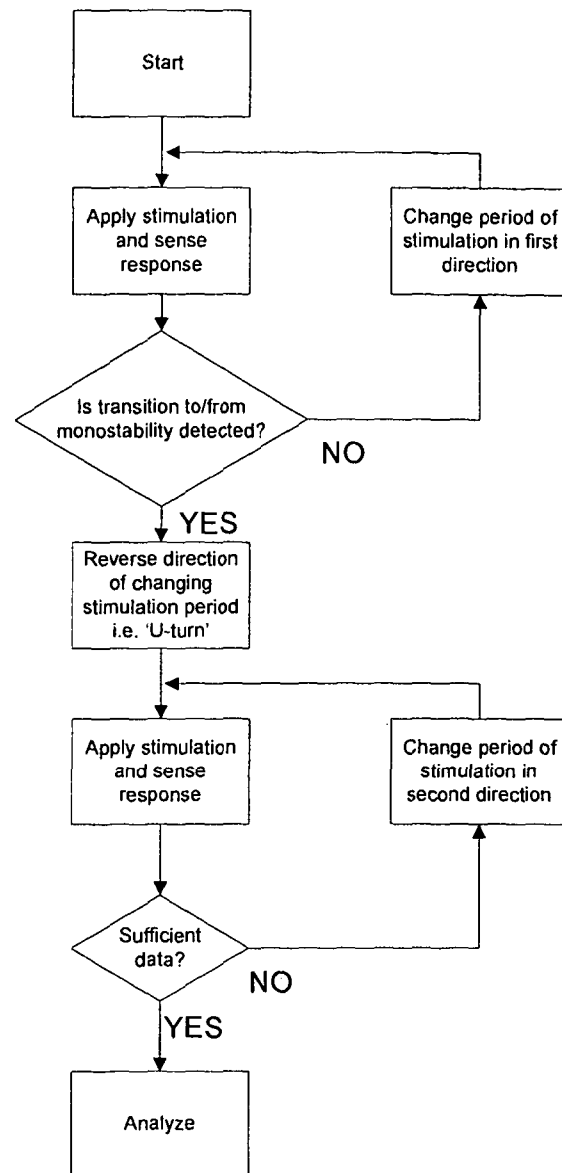


Fig. 2(a)



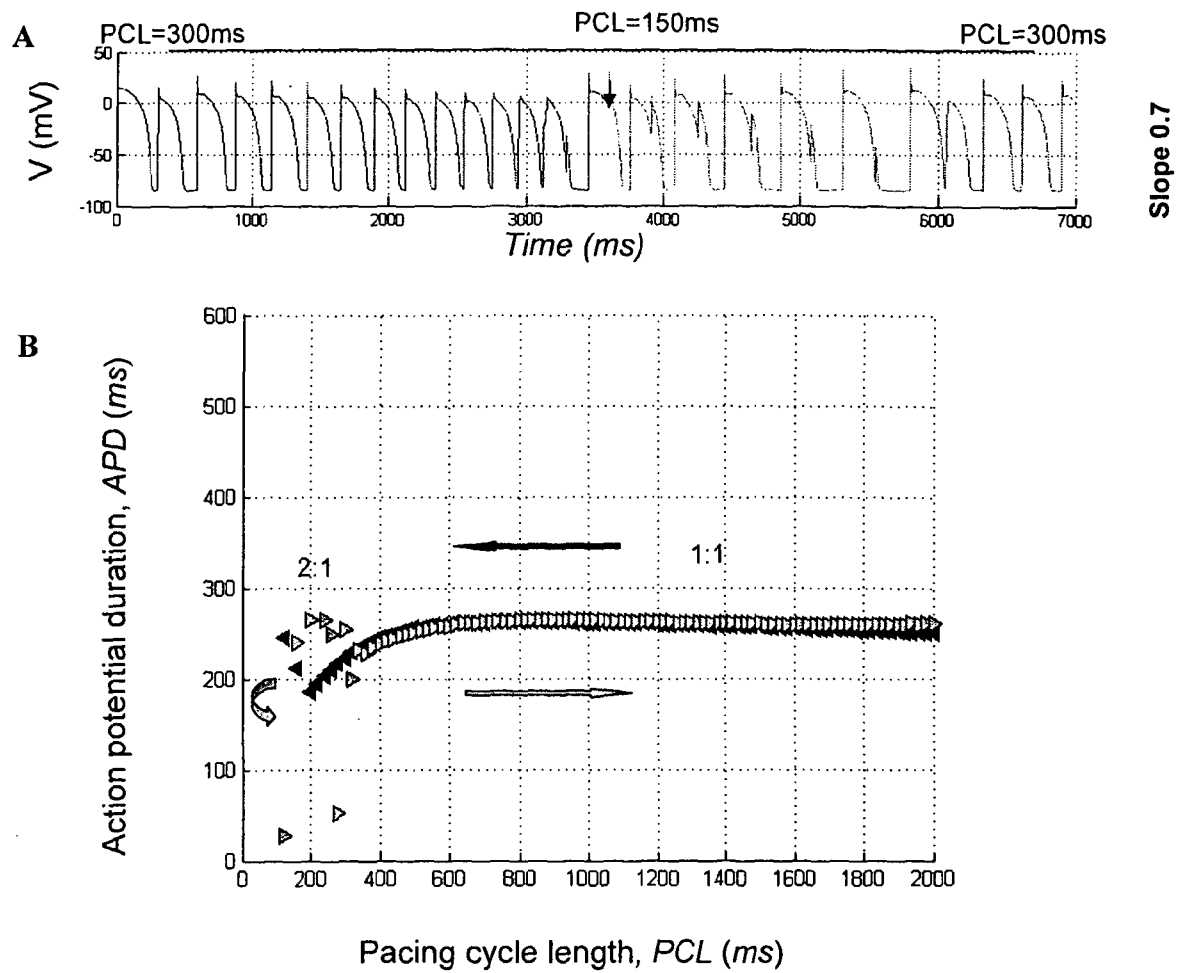
2/9

Fig. 2(b)



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Fig. 3



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Fig. 4

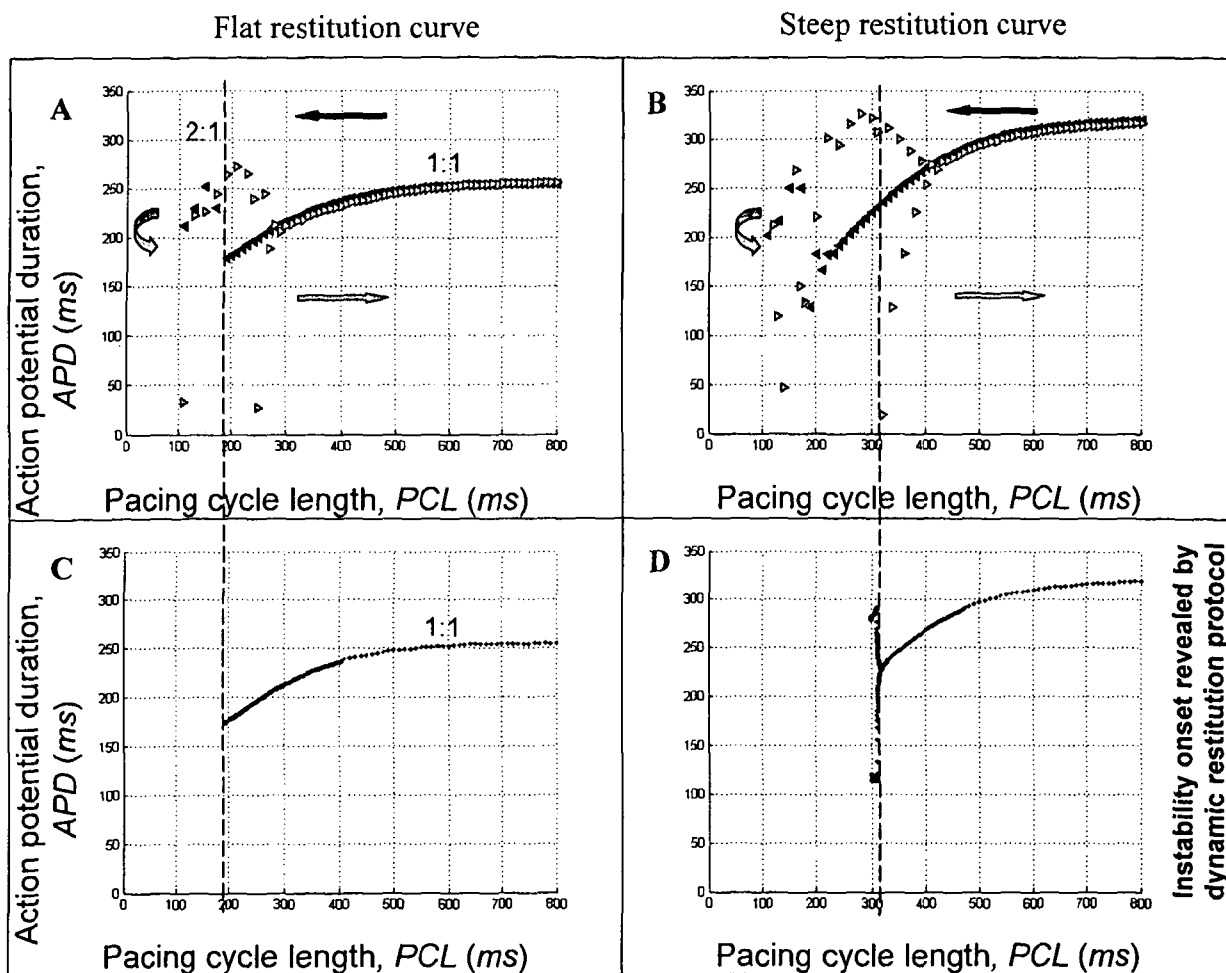
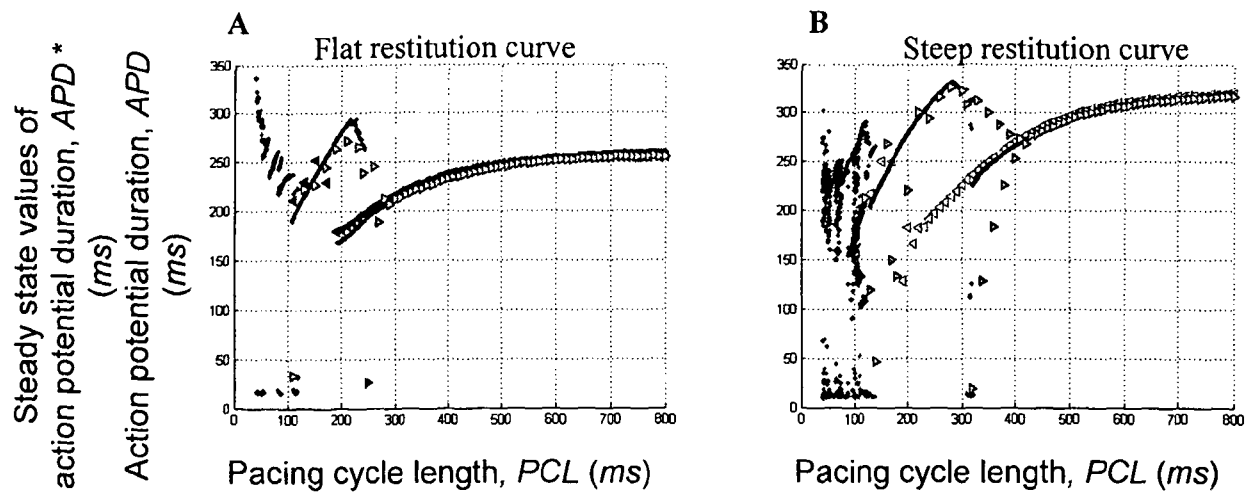


Fig. 5



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Fig. 6

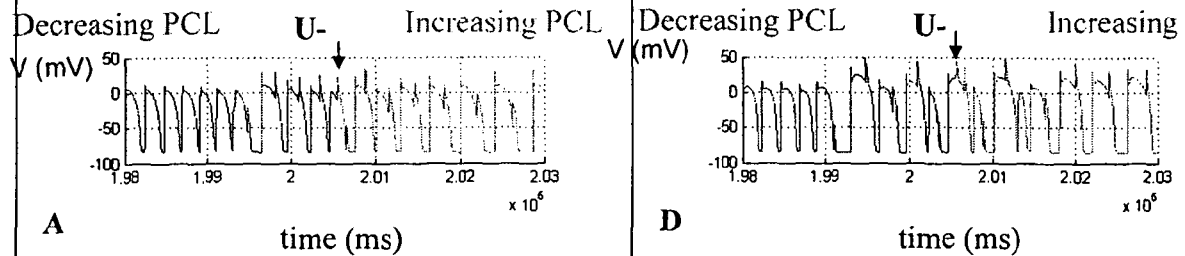
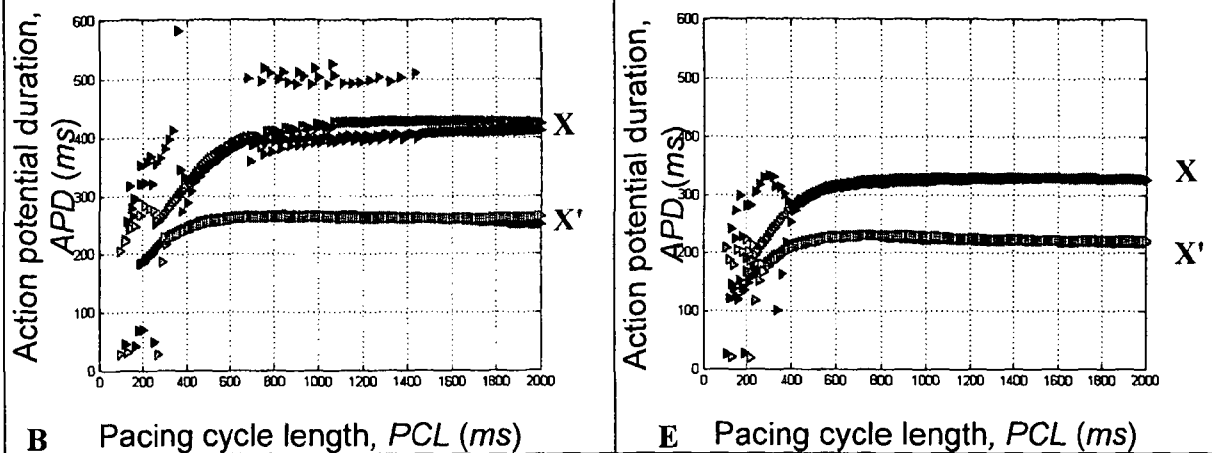
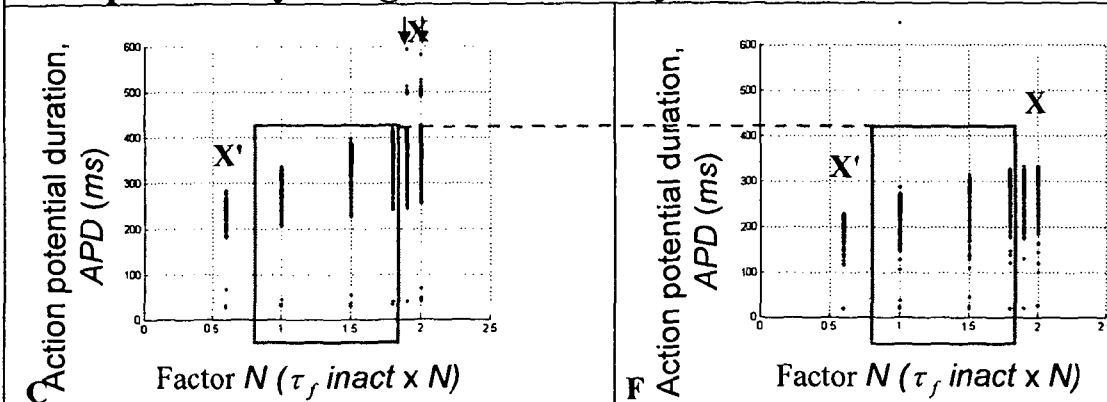
Run the U-turn protocol-**- Mapping result - Test the results for multistability-Adding drug-****Plotting diagram of the overall result experiments of each object - Comparison by categories of the objects**

Fig.7

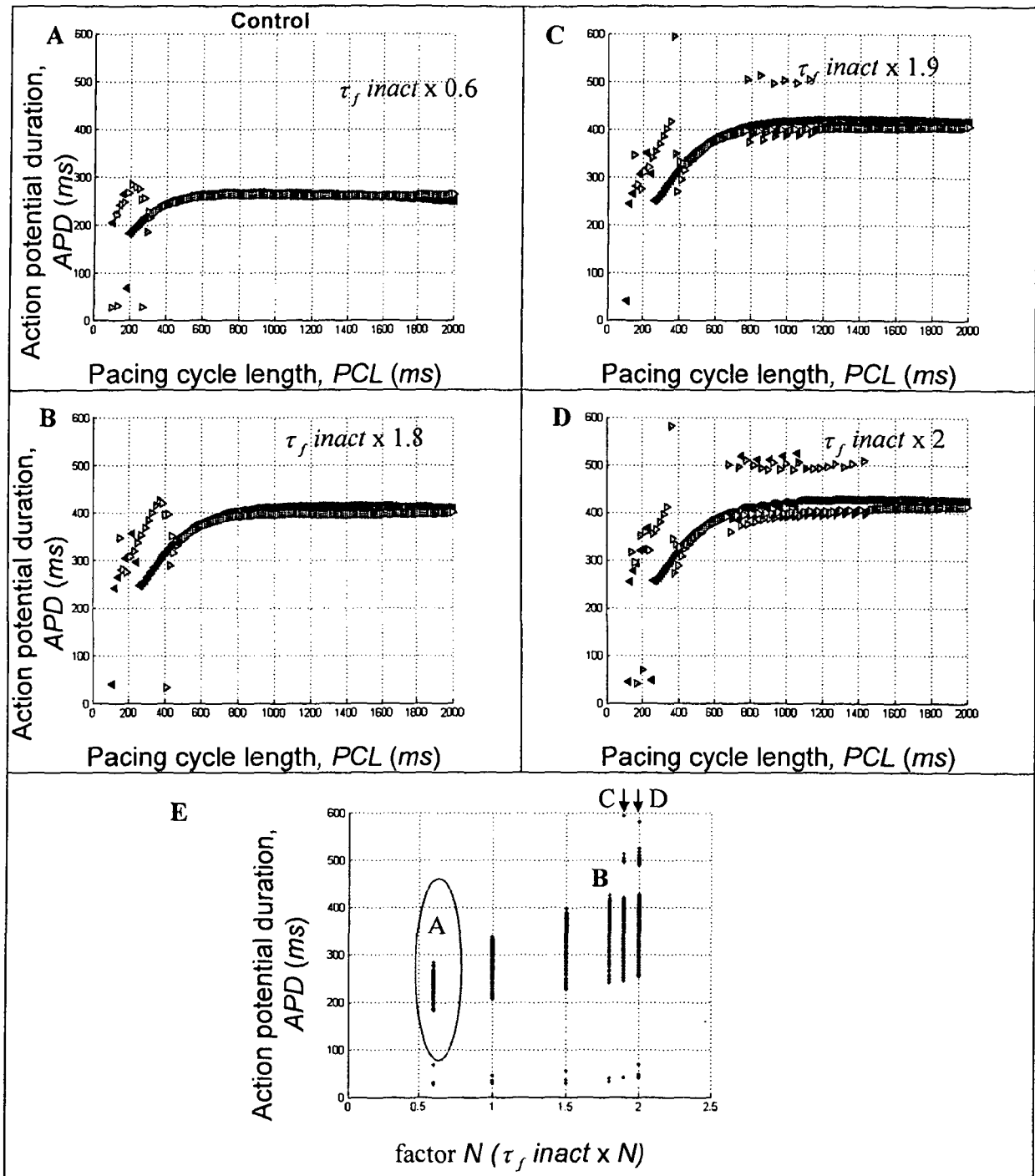


Fig 8

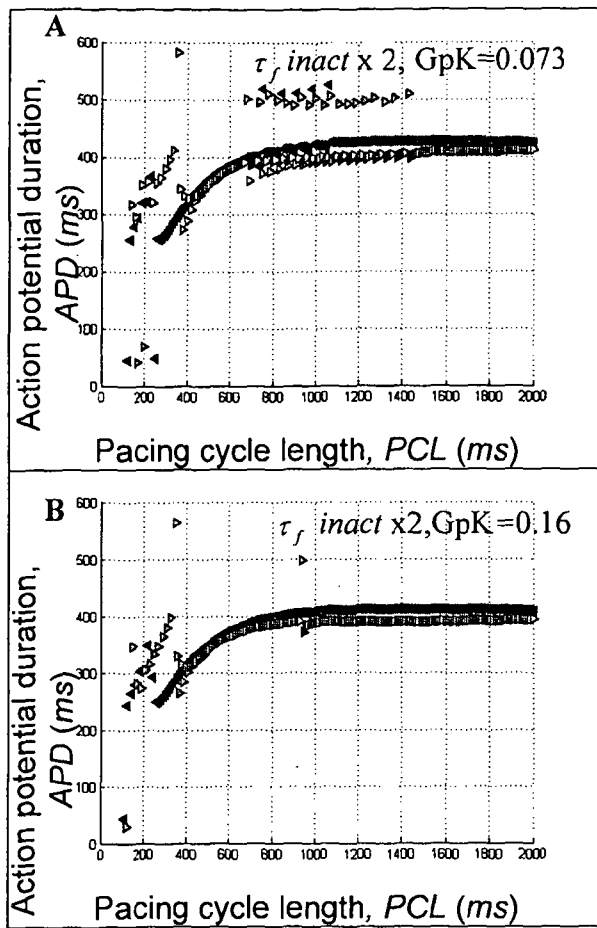
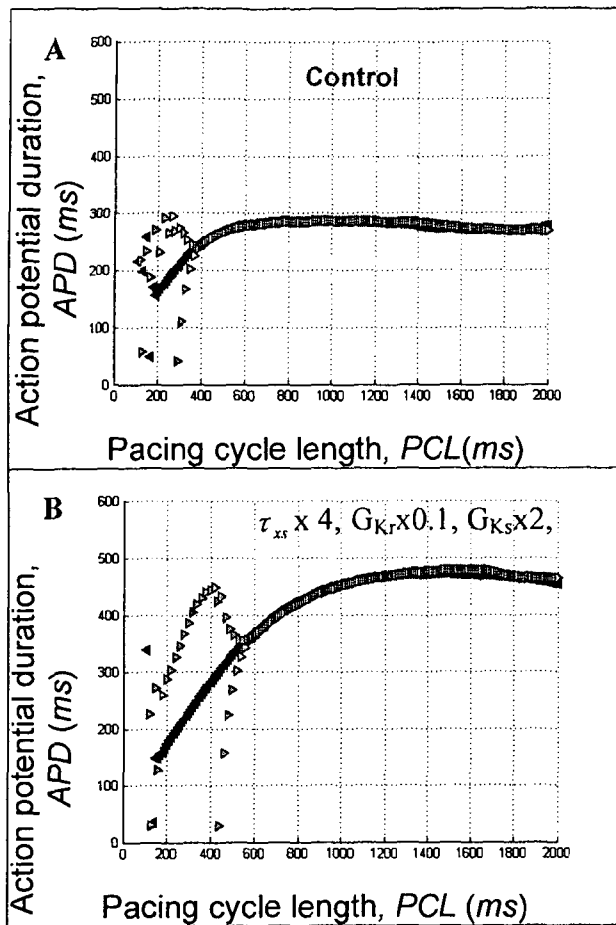


Fig. 9



INTERNATIONAL SEARCH REPORT

International application No

PCT/GB2011/000499

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61B5/0464 G01N33/68
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61B G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, CHEM ABS Data, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SUROVYATKINA E ET AL: "Multistability property in cardiac ionic models of mammalian and human ventricular cells", PROGRESS IN BIOPHYSICS AND MOLECULAR BIOLOGY, PERGAMON PRESS, OXFORD, GB, vol. 103, no. 1, 11 February 2010 (2010-02-11), pages 131-141, XP027181439, ISSN: 0079-6107 [retrieved on 2010-02-11] figure 1C	10-18
A	US 2004/015088 A1 (GRAY RICHARD A [US] ET AL) 22 January 2004 (2004-01-22) cited in the application paragraph [0026] - paragraph [0027]; figure 2 ----- -/-	1-23

☒ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents :

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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

28 June 2011

Date of mailing of the international search report

08/07/2011

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
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Authorized officer

Lanzrein, Markus

INTERNATIONAL SEARCH REPORT

International application No

PCT/GB2011/000499

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	TEN TUSSCHER K H W J ET AL: "Organization of ventricular fibrillation in the human heart: experiments and models", EXPERIMENTAL PHYSIOLOGY, vol. 94, no. 5, May 2009 (2009-05), pages 553-562, XP002643815, ISSN: 0958-0670 page 554, right-hand column, paragraph 2 - page 555, left-hand column, paragraph 2 -----	1-23

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/GB2011/000499

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2004015088	A1	22-01-2004	NONE
