NC3Rs

National Centre
for the Replacement
Refinement & Reduction
of Animals in Research

HESI.

Accelerating the acceptance of mathematical models as evidence in safety and efficacy decision making

14 & 15 September 2016

Posters

Pioneering Better Science

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Beyond Heart Rate Variability: Using attractor reconstruction with all the data in a blood pressure signal for feature extraction

Professor Philip Aston

University of Surrey

The multitude of heart rate variability (HRV) methods have dominated the analysis of heart data (blood pressure, ECG, etc.) for over two decades. All these methods start by extracting the heart beats from the data and this reduced time series is then analysed in many ways. However, this approach discards most of the data before commencing any analysis and so cannot detect any changes in the waveform shape. In contrast, our approach analyses *all of the data* from a physiological signal using attractor reconstruction. The naturally occurring baseline variation is removed by projecting the attractor onto a plane. Quantitative measures can be derived from the reconstructed attractor and traced out as a time window moves through the data. This approach can detect changes in the shape of the waveform that HRV methods cannot detect. We have applied this approach to two problems:

1. Detection of contractility changes using blood pressure data. Excellent agreement has been obtained when compared with changes in left ventricular pressure.

2. The early detection of sepsis for which our results are significantly better than those obtained using systolic or diastolic blood pressure.

Beyond Heart Rate Variability: Using Attractor Reconstruction With All The Data in a Blood Pressure Signal for Feature Extraction

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Introduction

- Blood pressure (BP) and ECG data at high sampling frequency is available over long periods of time
- Detection of subtle changes in the data can indicate early onset of disease offering the opportunity for early clinical intervention
- Can we extract diagnostic information from the data?

Attractor Reconstruction Method

- We use *all the data*, not the beat-to-beat (RR) intervals
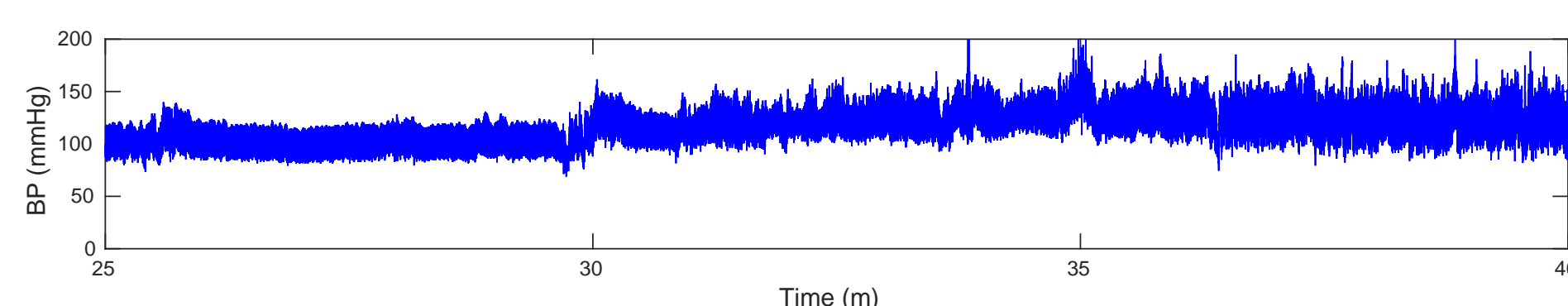- So we can detect changes in the *shape of the waveform*

Figure 1: Blood pressure data.

Our method consists of four steps:

1. Reconstruct the attractor using all the data

- Little information can be gleaned from a compressed plot of blood pressure against time
- It would be better to plot the data in phase space
- Problem: We only have one variable!
- Solution: Use Takens' method for reconstructing attractors using delay coordinates [1]
- If the blood pressure signal is $x(t)$, we define

$$y(t) = x(t - \tau), \quad z(t) = x(t - 2\tau)$$

where $\tau > 0$ is a fixed time delay

- We can now plot the trajectory $(x(t), y(t), z(t))$ in a three-dimensional phase space (see Fig. 2)
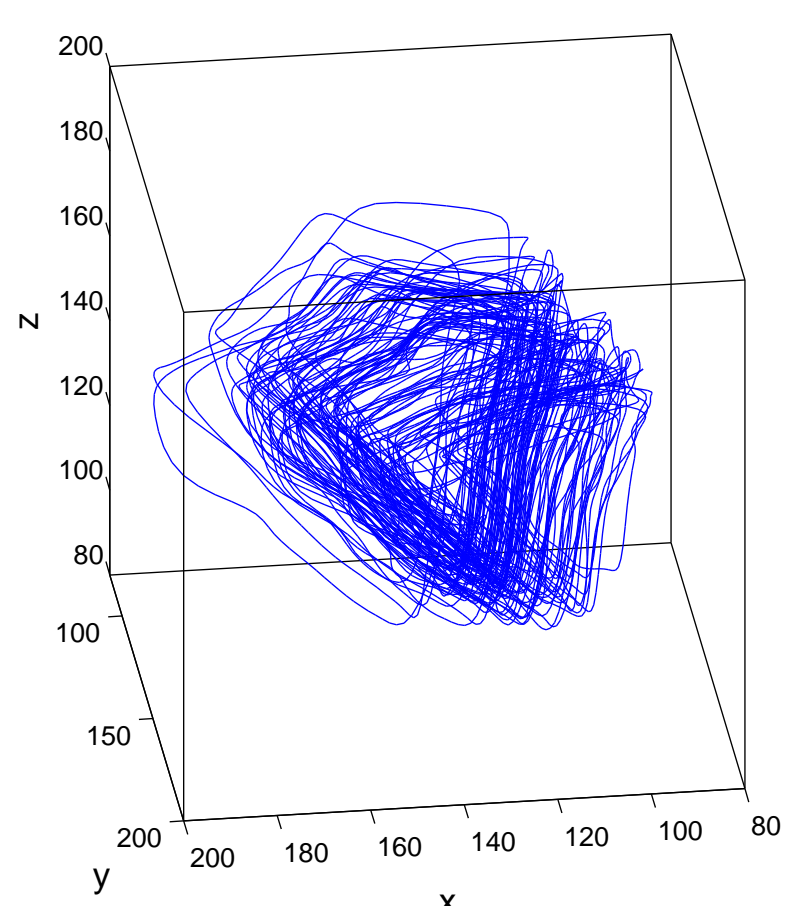

Figure 2: Plot of the data in phase space using $\tau = 31$ms.

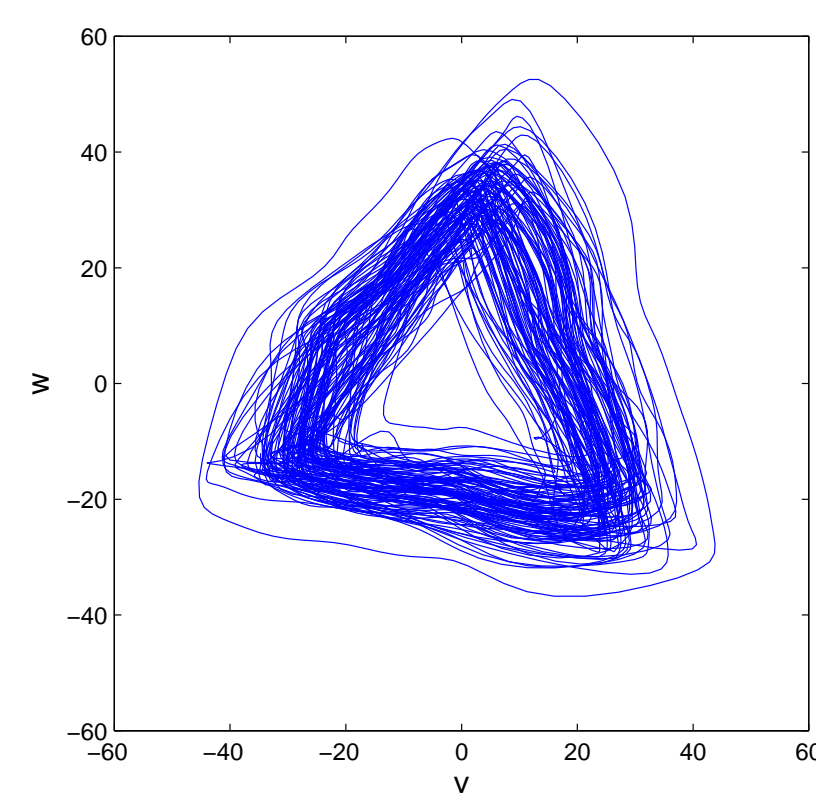Figure 3: Plot of the attractor in Fig. 2 in the (v, w) plane.

2. Remove baseline variation

- There is natural variation in the blood pressure which we want to remove
- If $x(t) \to x(t) + c$ then there is a similar shift in y and z
- In the phase space, we then have

$$(x(t), y(t), z(t)) \to (x(t) + c, y(t) + c, z(t) + c)$$
$$= (x(t), y(t), z(t)) + c(1, 1, 1)$$

- We define the new variables

$$u = \tfrac{1}{3}(x + y + z), \quad v = \tfrac{1}{\sqrt{6}}(x + y - 2z), \quad w = \tfrac{1}{\sqrt{2}}(x - y)$$

- The (v, w) plane is perpendicular to the vector $(1, 1, 1)$, which is the direction associated with vertical movement in the signal (see Fig. 3)

3. Construct a density

- We construct a density in the (v, w) plane since this is more useful than a blur of lines (see Fig. 4)
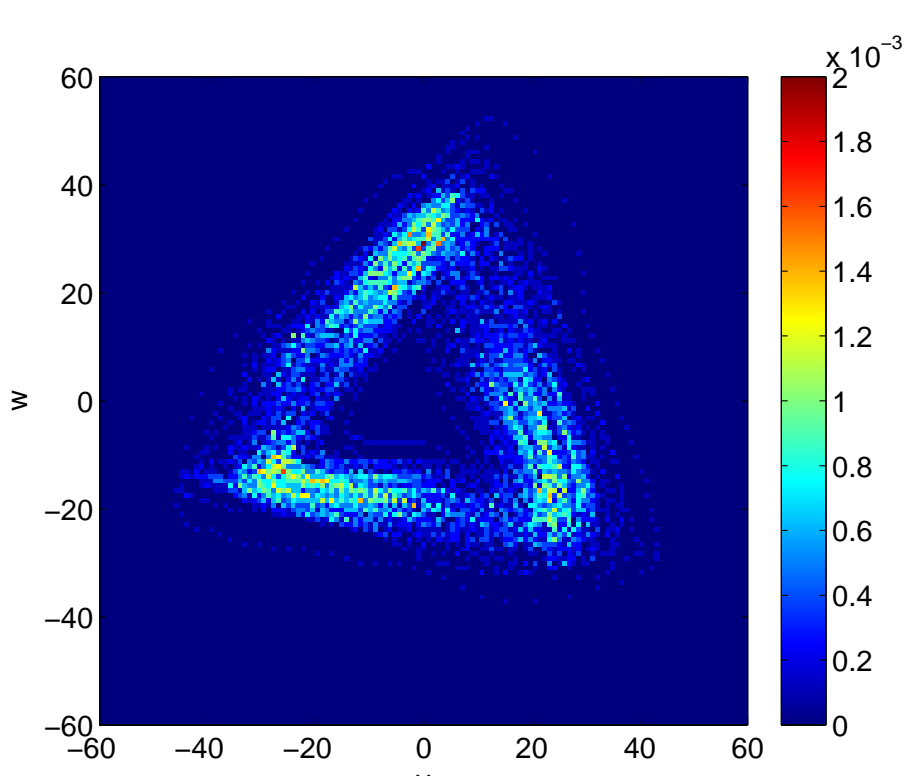

Figure 4: The density generated from the attractor in Fig. 3.

4. Generate time traces

- The density is constructed using a 10s time window
- Various scalar measures are derived from the density
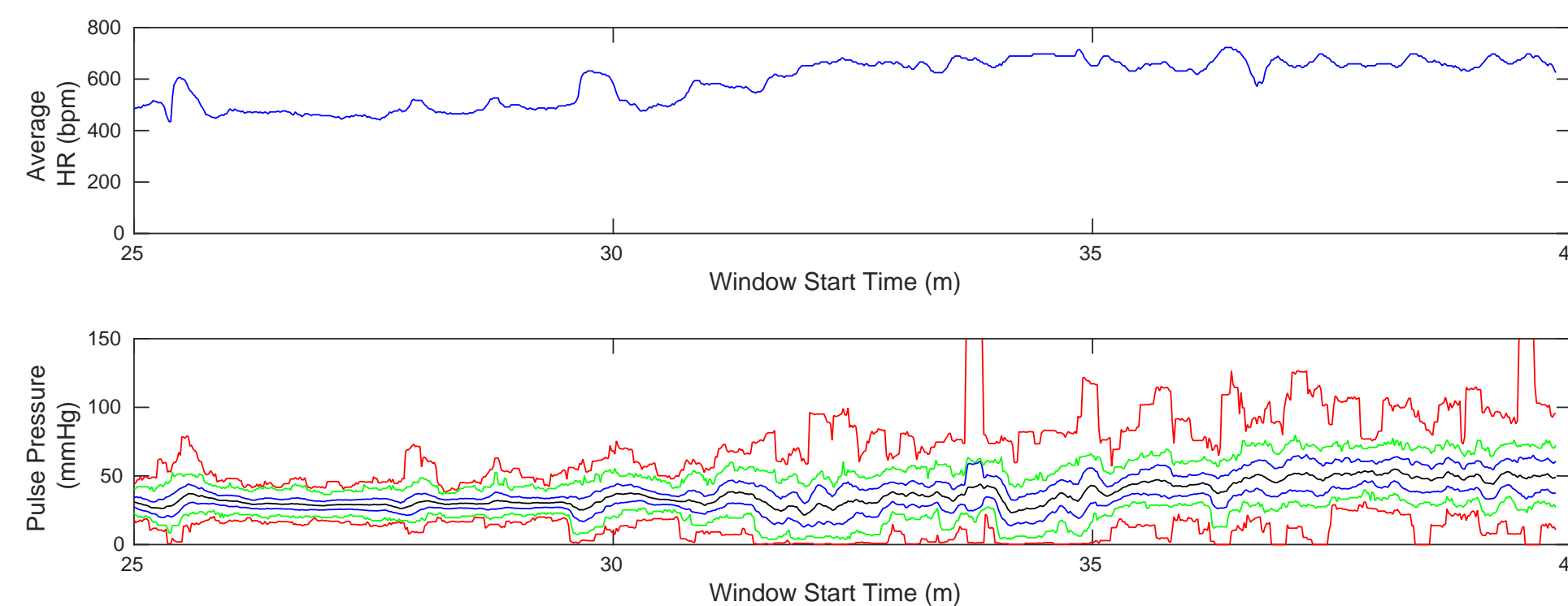- The time window is moved through the data to generate a collection of time traces of these measures

Figure 5: Time traces derived from the density.

## Comparison with Heart Rate Variability

HRV:

- HRV reduces the large volume of data by considering only the beat-to-beat (RR) intervals
- There are many ways of analysing this reduced time series and using it to diagnose various diseases [3]
- All the data regarding the shape of the waveform is discarded which may contain useful diagnostic information
- Accurately determining the peaks in the data can be difficult

Attractor Reconstruction:

- Our method uses *all the available data*
- It can detect changes in the *shape of the waveform* that HRV cannot detect
- We can detect both cardiac and vascular changes
- The method is robust as artefacts in the data have little effect on the density
- Removal of baseline variation is very simple
- This approach can be used for any approximately periodic signal e.g. ECG, PPG, respiratory waveform
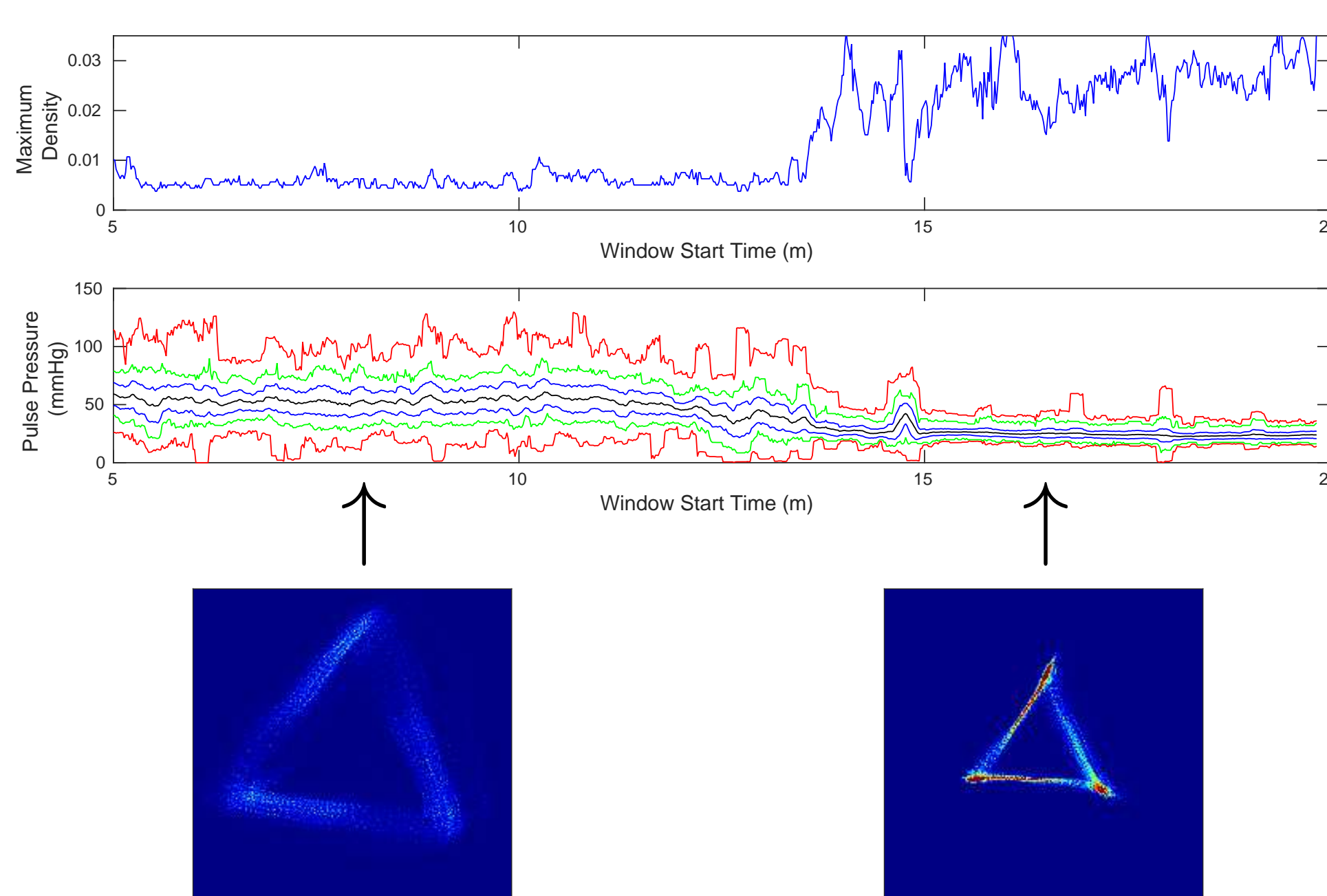
Example
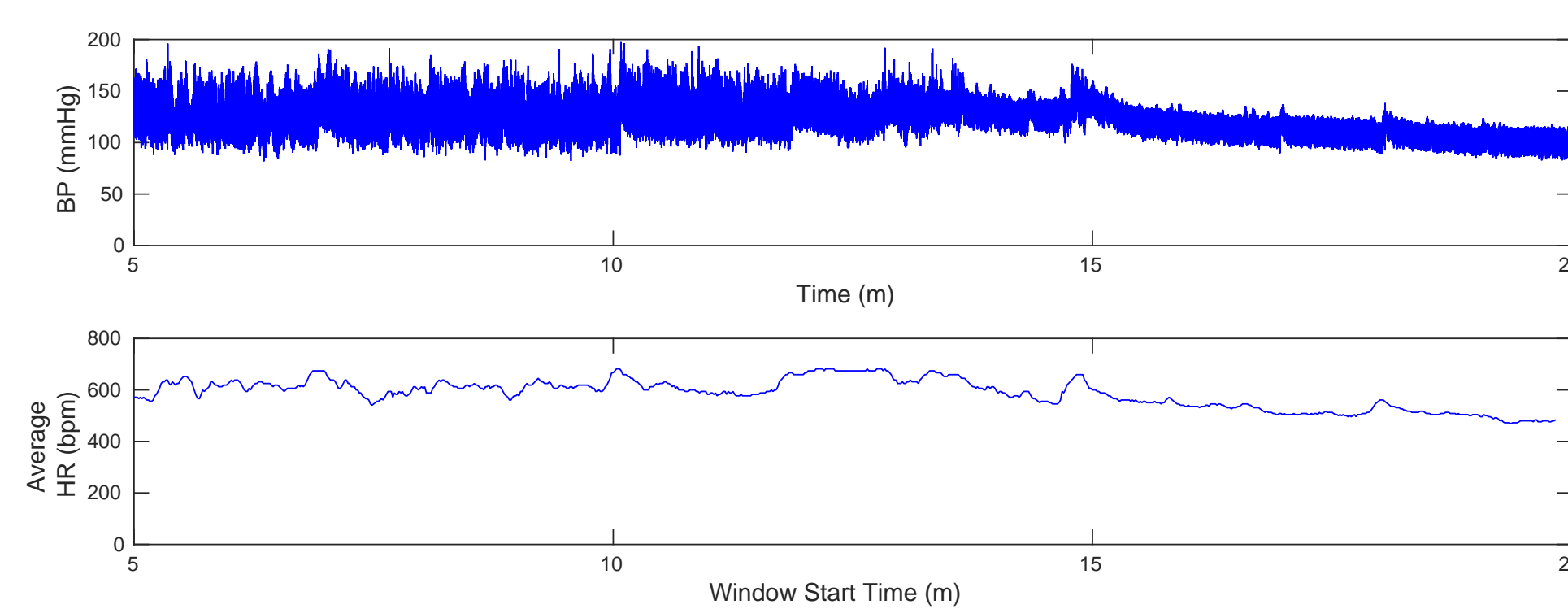
For this data, there is no significant change in heart rate over the 15 minute time interval

HRV:
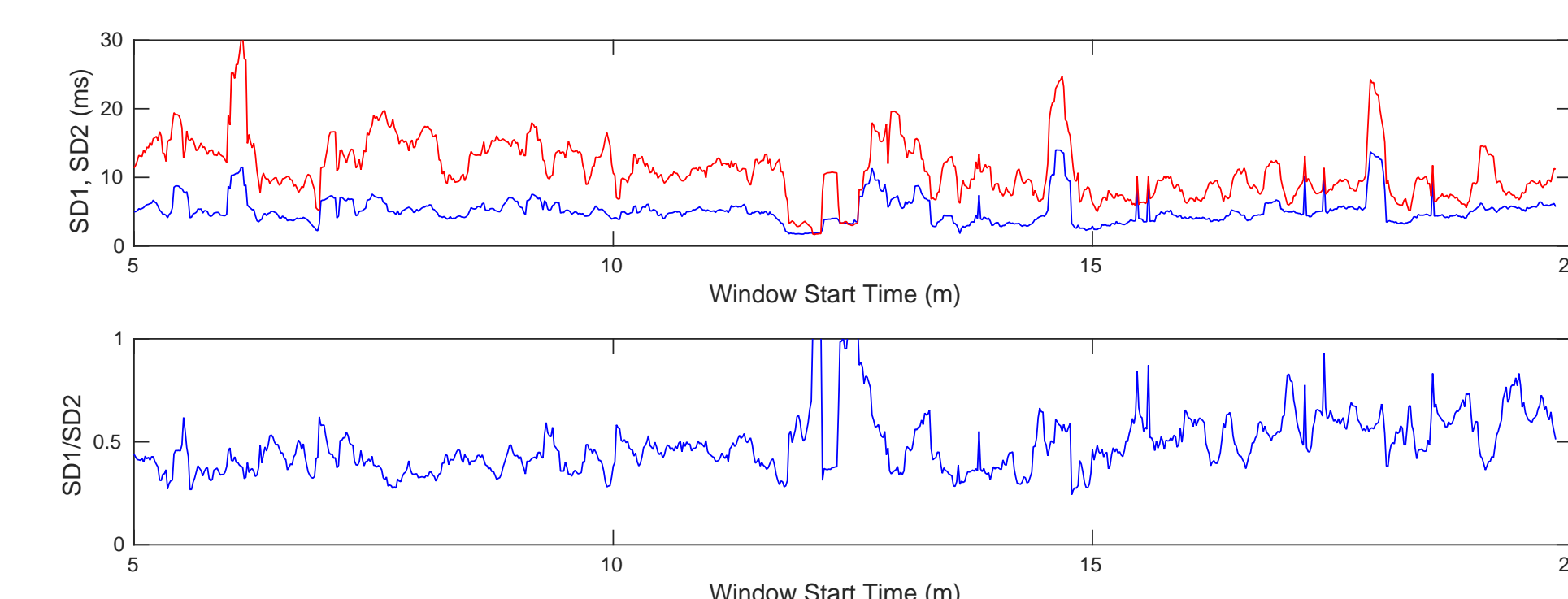
AR:

Conclusions

- There is a significant change in the data at 13 minutes
- This change is *not detected* by the HRV parameters
- The change is *very clearly detected* by the AR maximum density parameter
- The AR pulse pressure measures also show a significant change in the variability and the magnitude of the pulse pressure (amplitude) at the same point
- The blood pressure data changes from quite variable to almost periodic. This variability (or lack of it) is often associated with health (or disease)
- *This significant change in the data is detected by the AR parameters, but is not detected by the HRV parameters*

Contractility

- Drugs can cause unwanted changes in the contractility of the heart which can result in failure of a new drug
- Changes in contractility can be characterised by changes in the left ventricular pressure (LVP) [4] measured with a probe in the heart

> **Q:** Can drug induced changes in LVP be detected using a peripheral blood pressure (BP) signal?

- A trace from an adaptation of our method has been compared with the data for LV dP/dt_{max}
- Results have been obtained for 4 animals with either high dose itraconazole (negative inotrope) or high dose pimobendan (positive inotrope)

Results

Average correlation coefficients for the 4 animals are:

Itraconazole	Pimobendan
0.9784	0.7624

A linear transformation can be used to compare our results with the LVP data

Itraconazole

Pimobendan
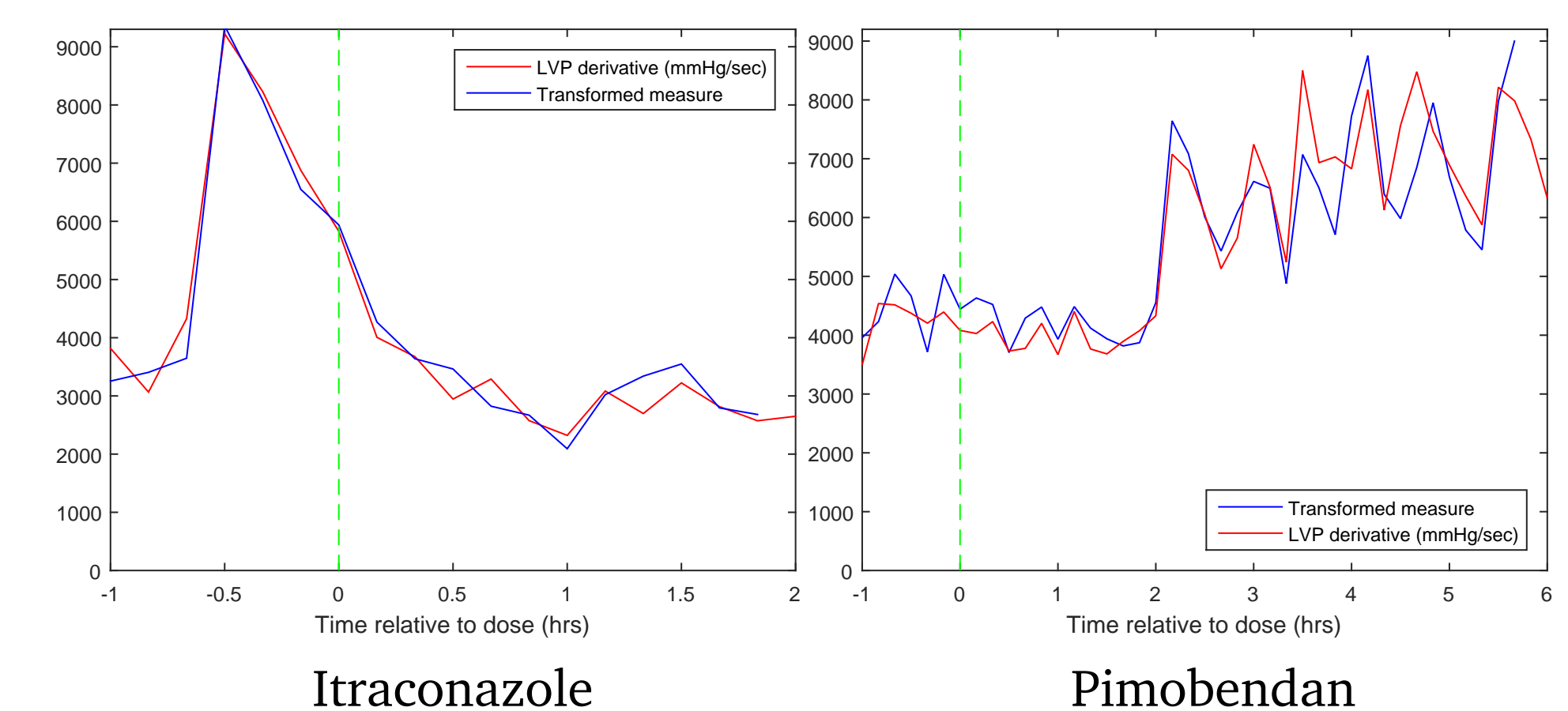
Conclusion

- Changes in LV dP/dt_{max} *have good correlation* with our measure extracted from blood pressure data for both a positive and a negative inotrope

3Rs Impact

- **Replacement:** Blood pressure data from past experiments can be re-analysed to obtain new results
- **Reduction:** Blood pressure data is inherently noisy and so extracted features are often noisy too. Our traces are less variable and show a larger drug related change than traditional measures and so a drug effect can be determined using fewer animals
- **Refinement:** One aim of our work is to provide an early diagnosis of disease. Early detection means that animal experiments can be run for a shorter time and terminated at a less severe time point

References

[1] F. Takens. Detecting strange attractors in turbulence. Dynamical Systems and Turbulence, Warwick 1980, Lecture Notes in Mathematics 898, 366–381, 1981.

[2] P.J. Aston, M. Nandi, M.I. Christie and Y.H. Huang. Beyond Heart Rate Variability: Using attractor reconstruction with all the data in a blood pressure signal for feature extraction. In preparation.

[3] U.R. Acharya, K.P Joseph, N. Kannathal, C.M. Lim and J.S. Suri. Heart rate variability: a review. Med. Bio. Eng. Comput. 44, 1031–1051, 2006.

[4] B.D. Guth *et al*. The evaluation of drug-induced changes in cardiac inotropy in dogs: Results from a HESI-sponsored consortium. J. Pharm. Tox. Meths 75, 70–90, 2015.

Cardiovascular Modelling to Support the 3Rs

Dr Michael Chappell

University of Warwick

Mean arterial pressure (MAP) and heart rate (HR) are important factors in assessing the safety of novel compounds. While MAP and HR are measured simultaneously, and are known to be interrelated; the impact on each variable is typically quantified with two separate drug effects.

Hemodynamic modelling incorporates the feedback from MAP on other cardiovascular variables, and thus captures the impact on both endpoints through a single mechanism of action. This can potentially improve the predictivity of safety testing, by capturing the behaviour of homeostasis with physiological parameters.

The complexity of such models places a greater burden on the need for a *priori* testing of structural identifiability, and the robustness of parameter estimates in practice.

We present structural identifiability analysis and parameter estimation results for three hemodynamic models. The analysis clarifies the suitability of models for different experimental conditions, and the parameters that must be fixed to ensure identifiability. Appropriate use of hemodynamic models has the potential to reduce animal use in cardiovascular safety testing, and in developing effective strategies to manage undesired effects.

Structural Identifiability analysis of Cardiovascular Feedback Models

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[2]AstraZeneca, Cambridge, UK

WARWICK
THE UNIVERSITY OF WARWICK

Introduction

Drugs influence the cardiovascular system in a wide variety of ways; either as part of an intended treatment or as an undesired side effect. Mean arterial pressure (MAP) and heart rate (HR) are important factors in assessing the safety of novel compounds. While MAP and HR are usually measured simultaneously, and are known to be interrelated; a drug's effect on each variable is typically quantified separately. There have been a few attempts to model the combined drug effect on hemodynamic variables by using homeostasis models with MAP negative feedback (Francheteau et al. 1993; Snelder et al. 2014).

The advantage of these models are an improved understanding of effects on the cardiovascular system earlier in preclinical development, and the potential to better anticipate the magnitude effects in humans. However, the complexity of such models places a greater burden on the need for *a priori* testing of structural identifiability, and the robustness of parameter estimates in practice.

Structural identifiability analysis determines whether it is possible to uniquely estimate model parameters, based on the structure of the model and experimental observations available; assuming noise-free data.

Models of the Cardiovascular System

Haemodynamic variables are fundamentally related as follows:

CO = HR.SV

MAP = TPR.CO

Where *HR* is Heart Rate, *CO* is Cardiac Output (blood flow), *SV* is Stroke Volume (volume of blood ejected in each heart beat), *TPR* is Total Peripheral Resistance and *MAP* is Mean Arterial Pressure.

Several feedback mechanisms respond to changes in MAP and act to maintain blood pressure homeostasis over the short and medium term. Within their own limits, heart rate responds to maintain blood pressure, and TPR responds via blood vessels dilating and contracting. There is a need for simplified models represent this complex feedback in aggregate terms.

Snelder et al. (2014)

- Linked turnover equations
- Proportional feedback on HR, TPR and SV. E.g:

$$\frac{dHR}{dt} = kinHR * (1 - FB * MAP) * (1 + E) - koutHR * HR$$

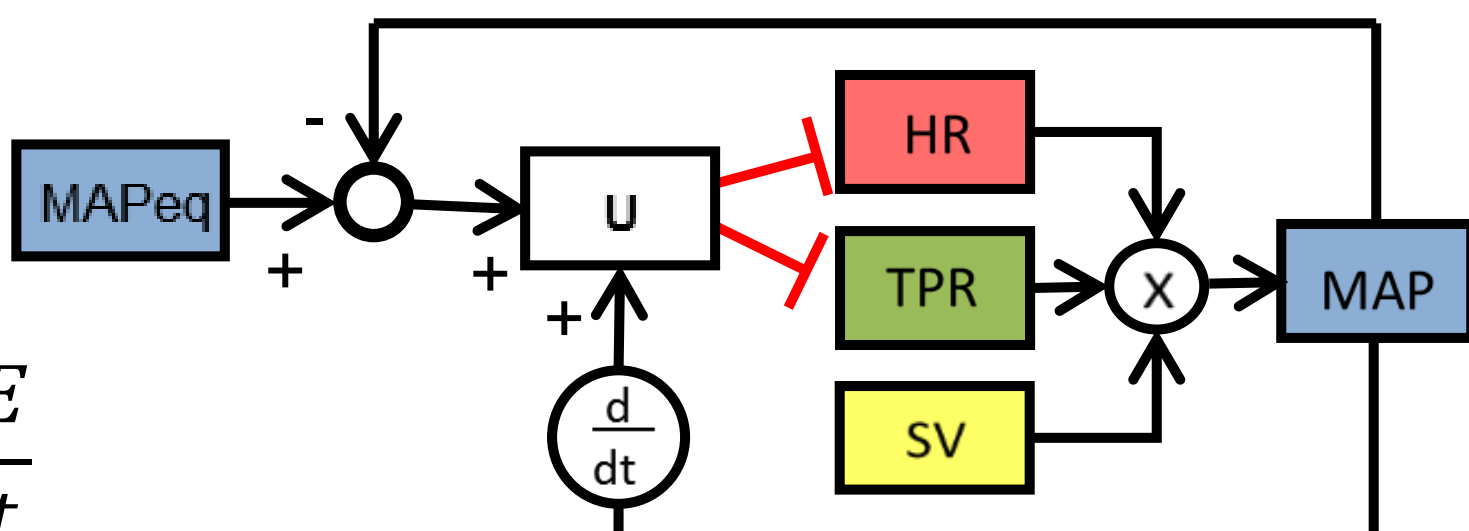$$kinHR = koutHR * HReq, \qquad E = drug\ effect$$
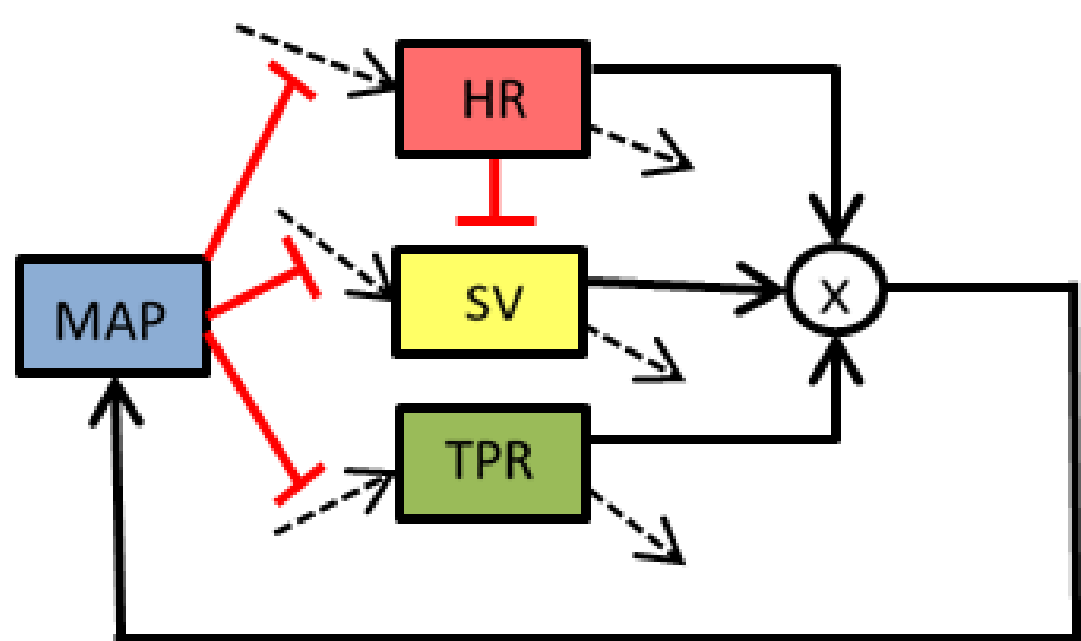
Francheteau et al (1993) & Cheung et al. (2012)

- Proportional and derivative feedback
- Baseline MAP as a setpoint.
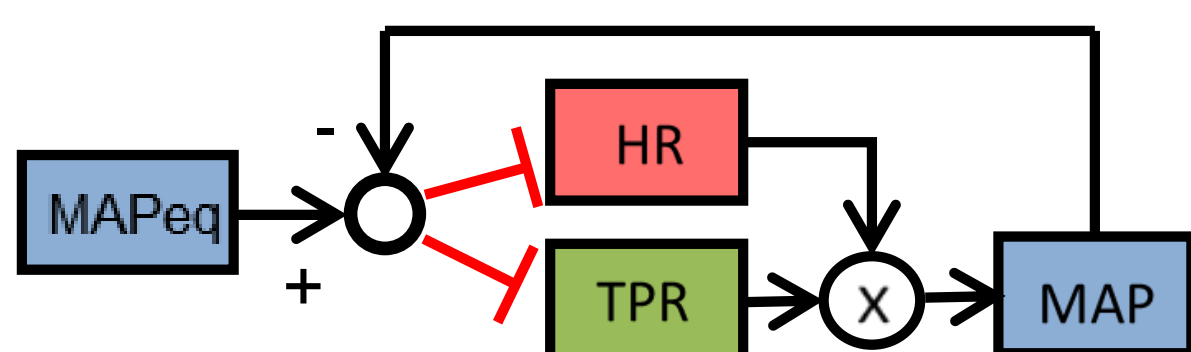- External control state *U*. E.g.

$$\frac{dHR}{dt} = \frac{HReq * (1 - \alpha * U - E - HR)}{\tau_{HR}} - \frac{dE}{dt}$$

$$\frac{dU}{dt} = \frac{ac * (MAP - MAPeq) + \frac{dMAP}{dt} - U}{\tau_U}$$

Reduced Model

- Based on Cheung et al (2012).
- Proportional feedback
- Baseline MAP as a setpoint
- No external control state. E.g.

$$\frac{dHR}{dt} = \frac{HReq * (1 - \alpha * (MAP - MAPeq) - E - HR)}{\tau_{HR}} - \frac{dE}{dt}$$

Results

Structual identifiability analysis of the four models was performed using a differential algebra approach (Evans et al. 2013) and the exact algorithm rank approach (Karlsson and Anguelova 2012). The model of Snelder et al. (2013) requires measurement of cardiac output to be structurally globally identifiable (SGI). Francheteau et al. (1993) was confirmed to be SU, and Cheung et al. (2012) to be SGI, conditional on fixing the value of stroke volume. The model should be used with an awareness of this assumption

Model	Observations	
	HR & MAP	HR, MAP & CO
Francheteau 1993	SU	-
Cheung 2012	SGI*	-
Reduced Model	SGI	-
Snelder 2013	SU	SGI
Snelder 2014	SGI	SGI

*SGI with a fixed value of SV or TPR_{EQ}, otherwise SU
SGI: Structurally globally identifiable, SU: Structurally unidentifiable

Parameter estimation was performed for SGI models on HR and MAP data from rat telemetry (n=8) Nifidipine (0, 3 and 30mg/kg) and Amphetamine (0, 0.3 and 3mg/kg) studies.

All three SGI models could be fitted to the data. Some system parameters could not be robustly determined in the Snelder 2014 model, without fixing some parameters (baseline SV and TPR were fixed due to their clear physiological meaning). This suggests that it could be difficult to distinguish effects on SV and TPR in practice from HR and MAP data alone.

Discussion

The reduced model had the fewest parameters and state equations, and was able to adequately describe the available MAP and HR data. It could potentially offer an improved choice of model in terms of identifiability and parameter estimation.

The inclusion of rate-sensitive feedback, as in the Cheung et al. (2012) model may provide a more accurate physiological representation, and be preferred in certain situations.

However, both Cheung et al. (2012) and the reduced model require *a priori* the value of SV to be fixed, meaning that they cannot represent or estimate the effects on SV. (Alternatively, if a drug is known to effect SV, the value of TPR could be fixed instead of SV). The Snelder et al. (2014) model is more ambitious in including SV and TPR simultaneously. Well parameterised, this model could serve a range of applications in quantifying, translating and predicting hemodynamic effects.

Different strategies may be preferred in the models to implement circadian baseline functions in MAP and/or HR. For example, a cosine function can be used on the MAP equilibrium set point in Cheung et al. (2012) and the reduced model, and in Snelder et al. (2014) this instead amplifies the production rate of HR. The circadian rhythm could not be distinguished from background variability in this case. This is not an ideal scenario for parameter estimation, but reflects a common situation ,with MAP and HR evaluated at hourly timepoints.

The feedback models provided better fits than using two separate drug effects on MAP and HR.

3Rs Impact

Cardiovascular safety is one of the leading reasons for drug attrition and withdrawal. By improving confidence in hemodynamic models, this work has the potential to improve their predictive power. Making better use of experimental data enables the reduction and refinement of animal use in cardiovascular safety testing, and in developing treatments to manage hemodynamic effects.

References: Cheung et al. (2012). Eur. J. Pharm. Sci., 46, 4, 259–271., Evans et al. (2013). Automatica, 49, 1, 48–57., Francheteau et al. (1993). J. Pharmacokinet. Biopharm., v21, 5, 489–514., Karlsson and Anguelova (2012). 16th IFAC Symposium on System Identification, 941-946., Snelder, et al. (2013). Br. J. Pharmacol., 169, 1510–24., Snelder, (2014). Br. J. Pharmacol., 171, 5076–92.

Development and application of evidencing and argumentation tools in the NC3Rs CRACK IT Virtual Infectious Disease Laboratory and Virtual Fish Ecotoxicology Laboratory Projects

Professor Mark Coles
SimOmics Ltd / University of York

Mathematical and computational models have the potential to accelerate medical research and reduce and replace the use of animals in therapeutic discovery and development and toxicology testing. This is because simulations provide a platform to rapidly predict outcomes of complex phenomena (e.g. immune function) without the limitations inherent in animal experiments. Models permit testing of a large number of variables and capacity to provide key evidence for clinical trial design and regulatory approval. However, simulations are inherently dependent on processes and assumptions that underpin their design, these are frequently unclear and leads to a loss of confidence in the intended users. One method to address this challenge is to utilise a robust methodology to evidence the models in an open and transparent format that is accessible to all potential users, from basic research scientists, regulators and modeller to corporate management. This mitigates risk, provides a platform to understand the model as scientific understanding of complex biological systems evolve. Through taking a robust approach to the evidencing and argumentation process it is possible to convince key stakeholders that a given computational / mathematical model is appropriate and have confidence in the relationship between simulation outcomes and real-world data.

Evidenced-based Virtual Laboratories to Support the 3Rs

Paul Andrews, Adam Nellis, Ed Clark, Mark Coles, Jon Timmis

SimOmics

leishsim.simomics.com - www.simomics.com – vfetl.simomics.com

Background: SimOmics is funded by NC3Rs and InnovateUK to develop virtual laboratories to reduce and replace animals in pharmaceutical development and testing. SimOmics is developing software tools and modelling technologies to assist in evidence-based decision making in areas including infectious disease and ecotoxicology risk assessment. SimOmics technologies include software to generate argumentation structures, automated generation of diagrams to reveal model structures and automated tools for the detailed analysis of computational and mathematical models, all of which can be linked via a novel web-based user interfaces for high degrees of transparency.
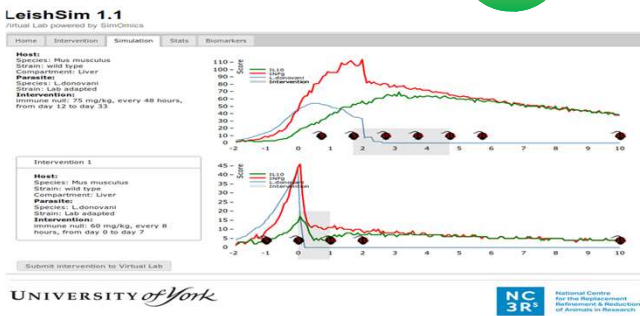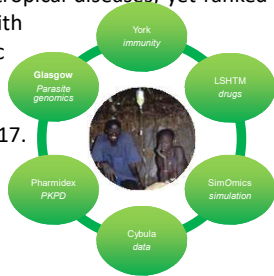
2. Virtual Fish Ecotoxicology Laboratory (VFETL)

VFETL Virtual Lab Virtual Fish Eco-toxicology Laboratory SimOmics

VFETL   All Substances   Models   Experiments   Arguments   Search for a substance...   Search

3Rs and fish toxicology: All new active pharmaceutical ingredients (API) must undergo an environmental risk assessment (ERA) before being authorised. Currently tens of thousands of fish are used worldwide as part of API ERAs. Development of predictive in silico models already has the potential to significantly reduce animal use (3Rs) and reduce R&D costs around the ERA of pharmaceuticals. **VFETL:** Our approach will characterise the movement of an API from the patient, through the wastewater and river systems, into fish tissues and predict the apical and non-apical effects on individuals and populations. By understanding the pathway from patient to effect, it will be possible to develop an optimum experimental testing strategy for an API. Working closely with AstraZeneca and University of York to deliver commercial product by 2018.

3. Virtual Infectious Disease Laboratory (VIDL)

LeishSim: Part of a wider team developing a virtual platform that models infection and the host response to the Leishmania pathogen for basic research and enhances new target development in infectious diseases. Leishmania, one of the most neglected tropical diseases, yet ranked 9th in analysis of global burden of disease, with an associated mortality amongst parasitic infections second only to malaria. A free virtual lab, focussed on Leishmania, will be available to the community by mid 2017.

York immunity

Glasgow Parasite genomics

LSHTM drugs

Pharmidex PKPD

SimOmics simulation

Cybula data

LeishSim 1.1
/virtual Lab powered by SimOmics

Home   Intervention   Simulation   Stats   Biomarkers

Host:
Species: Mus musculus
Strain: wild type
Compartment: Liver
Parasite:
Species: L.donovani
Strain: Lab adapted
Intervention:
Immune null: 75 mg/kg, every 48 hours, from day 12 to day 33

Intervention 1

Host:
Species: Mus musculus
Strain: wild type
Compartment: Liver
Parasite:
Species: L.donovani
Strain: Lab adapted
Intervention:
Immune null: 60 mg/kg, every 8 hours, from day 0 to day 7

Submit Intervention to Virtual Lab

UNIVERSITY of York

NC3Rs National Centre for the Replacement Refinement & Reduction of Animals in Research

Leshsim: Model-driven Experimentation, combining 'omics' with simulation

4. Membrane Domain Specific Language (MDSL)

A modelling language developed to support mechanistic modelling of cellular and/or intracellular processes with support for membrane bound molecules using a concise grammar.

```
#on surface reaction
IFNg around redpulp_macrophage binds IFNg_receptor on redpulp_macrophage modifier IFNg_on <=> IFNg_bound under redpulp_macrophage modifier IFNg_off
IL-4 around redpulp_macrophage binds IL-4_receptor on redpulp_macrophage modifier IL-4_on <=> IL-4_bound under redpulp_macrophage modifier IL-4_off
IL-6 around redpulp_macrophage binds IL-6_receptor on redpulp_macrophage modifier IL-6_on <=> IL-6_bound under redpulp_macrophage modifier IL-6_off
IL-10 around redpulp_macrophage binds IL-10_receptor on redpulp_macrophage modifier IL-10_on <=> IL-10_bound under redpulp_macrophage modifier IL-10_off
IL-1 around redpulp_macrophage binds IL-1_receptor on redpulp_macrophage modifier IL-1_on <=> IL-1_bound under redpulp_macrophage modifier IL-1_off
TNFa around redpulp_macrophage binds TNFa_receptor on redpulp_macrophage modifier TNFa_on <=> TNFa_bound under redpulp_macrophage modifier TNFa_off
LPS around redpulp_macrophage binds LPS_receptor on redpulp_macrophage modifier LPS_on <=> LPS_bound under redpulp_macrophage modifier LPS_off    #LPS_
TGFb around redpulp_macrophage binds TGFb_receptor on redpulp_macrophage modifier TGFb_on <=> TGFb_bound under redpulp_macrophage modifier TGFb_off
```

An extensive toolset ensures that even large and complex models are transparent and easily interrogated with auto-diagram generation allowing easy visual inspection structure of the model and animation of the simulation data giving quick visual feedback on the dynamics.

Redpulp Macrophage Intracellular Model   **Inspect The IL-10 Pathway**   **IL-10 Producers and Consumers**
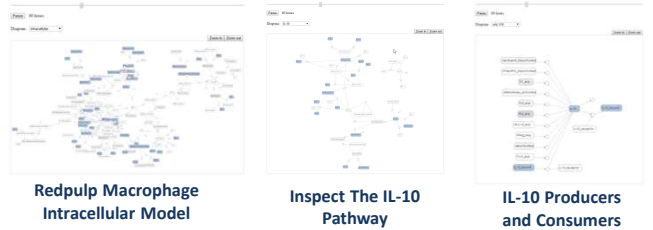
5. Evidence and Transparency Tools

Underpinning our virtual laboratories is a clear evidence base that can be summarised using a variant of Goal Structuring Notation (GSN) adapted from critical systems engineering. This allows for the construction of logical arguments underpinning evidence relating to data , assumptions in models and assessment of risk. Users will be able to develop their own evidence base, and critical, assess those of others.

Artoo
User
User: jon Or
Current:
File
New Save Load
Export Manage
Move
Zoom-In Zoom-Out Center
Zoom-To-Fit Snap
Build
Claim Strategy Evidence
Context Assumption Justification
Diagram Connection
Display

Model is fit for purpose

Appropriate Process
Argue over the science, engineering and results

Biological Domain
Appropriate scoping has been undertaken to establish the biological domain

Sound Engineering
Appropriate use has been made of software engineering and mathematical models

Appropriate Results
Results are representative of the biological domain.

ArtooPro: Use of logical arguments based on safety systems engineering to increase confidence in models http://artoo.simomics.com

Leishmania   Implementation   LeishSim   Results   Arguments

Overview
Entities
Research Context
Kupffer Cell Infection

<< Observed >>
Granuloma formation around infected Kupffer Cells

<< Expected >>
Infiltration of leucocytes into Kupffer Cell microenvironment

<< Expected >>
Aggregation of leucocytes around infected Kupffer Cells

MHCII-Antigen   CD4+ (Th0)   Th1
iNKT   CD1d-αGalCer   Kupffer Cell   Th2   IFNg
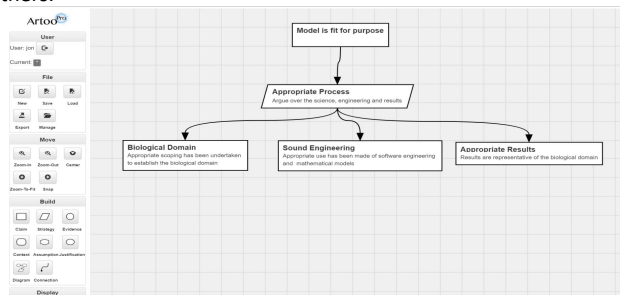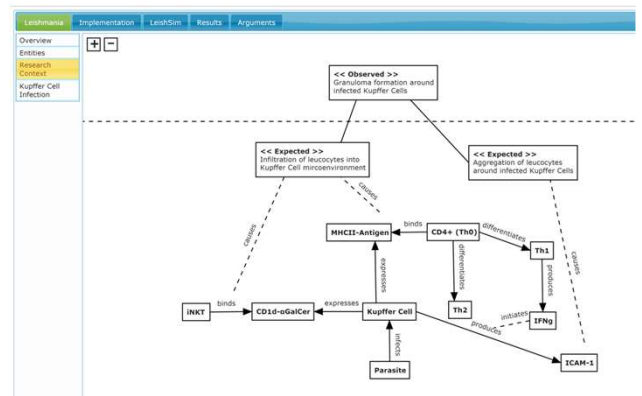Parasite   ICAM-1

SimOmics Evidence

6. Summary

- We are developing an integrated web-based system for the intelligent analysis of APIs in the environment and assessment of drug therapeutics for infectious disease
- Focus is on developing commercially viable 3Rs technologies that can be translated to a wide variety of 3Rs challenges

Investigating the role of lactate and proton efflux in extracellular acidification using a pH dependent *in silico* model of hepatic glycolysis

Ross Kelly
Liverpool John Moores University

Extracellular flux analysis (EFA) is gaining momentum as a versatile high throughput method of assessing cellular bioenergetics for a plethora of biological points of interest, boasting inclusion in over 1500 peer reviewed publications. Commonly used as a method of investigating drug induced mitochondrial toxicity/dysfunction in a wide variety of different cellular systems, EFA using any of the XF analysers (Seahorse machines) is accomplished by measuring rates of oxygen consumption and proton efflux. The majority of investigations centre on changes cellular respiration, measured via changes oxygen consumption rate (OCR), with the concomitant extracellular acidification rate (ECAR) measurements, indicative of glycolytic flux, often considered a secondary measurement likely due to the ambiguous nature of ECAR.

In order to investigate lactic acid as the predominant driving force of ECAR during extracellular flux analysis, a mathematical model of hepatic glycolysis that is pH-dependent with respect to reaction equilibria and enzyme kinetics, capable of computing a dynamic pH time course as a function of glycolytic flux was constructed. Recognising that lactic acid exists as its lactate anion and a proton at physiological pH prompted ECAR measurements to be simulated using the liver specific monocarboxylate transporter 1 (MCT1) flux. The model was then aligned with *in vitro* EFA data using HepG2 cells, assessing the changes in ECAR as a function of extracellular glucose concentrations illustrating a relationship between the GLUT2 transporter V_{max} parameter and ECAR. Finally, the model was used to simulate the effects of extracellular pH on ECAR highlighting the need for care when using compounds that may alter extracellular pH.

pH – Dependent Model Of Human Hepatic Glucose Metabolism

Ross Kelly[1], Amy Chadwick[2], Joe Leedale[2] Andy Harrel[3], Steven Webb[1].

[1]Applied Mathematics, Liverpool John Moores University, UK. [2]MRC Centre for Drug Safety Science, University of Liverpool, UK. [3]GlaxoSmithKline, R&D Ware, UK.
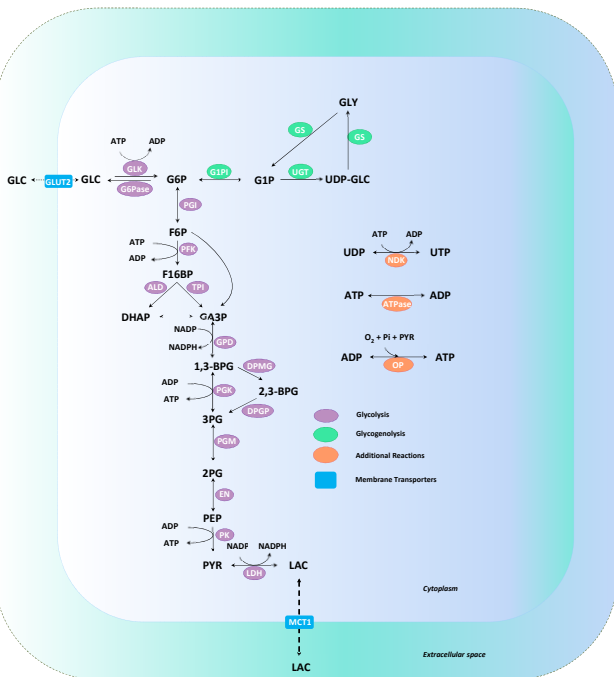
Introduction

Glucose metabolism (glycolytic flux) accounts for a significant portion of cellular bioenergetics as a direct source of ATP, and as a molecular precursor source for other energy producing systems such as the mitochondria via the TCA cycle. As a result, perturbations of cellular respiration, often as a result of "off-target" drug induced mitochondrial toxicity, may be observed by compensatory effects on glycolytic flux [1].

- *In vitro*, extracellular acidification rate (ECAR) is the predominant method of measuring glycolytic flux, with lactic acid postulated to be the source main of acidification [1].
- As a biological system of high importance, particularly for drug toxicity, *in silico* models of hepatic glucose metabolism already exist [2].
- However, a comprehensive pH-dependent model that includes dynamic proton buffering and metal cation binding within hepatic cellular respiration and glucose metabolism has yet to be presented.

Aims

- Construct pH-dependent mathematical model that includes dynamic proton buffering and metal cation binding
- Illustrate the models ability to replicate *in vitro* hepatic glucose metabolism

Hepatic Glucose Model

- Illustration of model components. The model includes the major metabolic pathways glycolysis, gluconeogenesis and glycogenolysis, as well as additional reactions for ATP synthesis and production by oxidative cellular respiration (OP).
- Model was constructed using Matlab (2015b)

Proton & Ion Binding

The state with *i* protons bound to reactant A is referred to as $[AH_i]$. Considering only proton binding, the total concentration of **L** is given by (1.0) and the concentration of A with a single protonation is given by (1.1). Here, k_1 denotes the dissociation constant. Iteratively accounting for N protonations leads to the general equation (1.2) [3-5].

$$[A] = \sum_{i=0}^{N}[AH_i] \qquad [AH_1] = [AH_0]\frac{[H^+]}{k_1} \qquad [AH_i] = [AH_0]\frac{[H^+]^i}{\prod_{j \leq i} k_j}$$
(1.0) (1.1) (1.2)

Relating the total concentration with the least protonated form of the reaction gives us (1.3). This conversion is known as the binding polynomial **P**. Using this method, the model state variables correspond to the *reactants*, the sum of all species. Therefore, the amount of protonated ATP can be computed using (1.4), where [ATP] corresponds to the model state variable [4-5].

$$[A] = [AH_0]P_A([H^+]) \qquad [HATP^{3-}] = \frac{[ATP]}{PATP([H^+])}\frac{[H^+]}{k_H^{ATP}}$$
(1.3) (1.4)

Thermodynamically Derived Equilibrium Constant K_{eq}

The equilibrium constant for a reaction may be derived using the $\Delta_r G^0$ the Gibbs free energy change for the reaction. For reaction (2.0), the expression for K_{eq} may be written in terms of $\Delta_r G^0$ (2.1), in terms of reactant concentrations (2.2) using their binding polynomials, allowing inclusion of respective proton and ion binding [5].

$$ATP^{4-} + H_2O \leftrightarrow ADP^{3-} + HPO_4^{2-} + H^+ \quad (2.0)$$

$$K_{eq} = e^{\frac{-\Delta_r G^0}{RT}} \quad (2.1) \qquad K_{eq} = \left(\frac{[ATP^{3-}][PI][H^+]}{[ATP]}\right)eq, \frac{[P_{ATP}]([H^+])}{[P_{ADP}]([H^+])[P_{PI}]([H^+])} \quad (2.2)$$

Sensitivity Analysis

Model Parameter Sensitivity

Extracellular [H$^+$] Sensitivity (ECAR)

- Model parameter sensitivity is expressed as the % mean change of variable time course solutions altering all 118 model parameters within the range of -99 to +400% of their original values.
- Parameters that induce >10% sensitivity are classed as sensitive.

- The parameters Keq LACT and Keq LDH were found to be the most sensitive with respect to extracellular pH changes using the same % mean sensitivity analysis.

Biochemical Reactions

Abbreviation	Reaction
GLUT2	Glucose Transporter
GLK	Glucokinase
G6Pase	Glucose-6-phosphatase
PGI	Phosphoglucose Isomerase
PFK	Phosphofructokinase
ALD	Aldolase
TPI	Triosephosphate Isomerase
GPD	Glyceraldehyde-3-phosphare Dehydrogenase
PGK	Phosphoglycerate Kinase
PGM	Phosphoglycerate Mutase
EN	Enolase
PK	Pyruvate Kinase
LDH	Lactate Dehydrogenase
MCT1	Monocarboxylate Transporter 1
G1PI	Glucose-1,6-phosphate Isomerase
UGT	Glucose-1-ppsphate uridyltransferase
GS	Glycogen Synthase
NDK	Nucleoside Diphosphokinase
ATPase	ATP Hydroylsis
OP	Cellular Respiration

Biochemical Species

| Abbreviation	Species
GLC	Glucose
ATP	Adenosine Triphosphate
ADP	Adenosine Diphosphate
AMP	Adenosine Monophosphate
G6P	Glucose-6-phosphate
F6P	Fructose-6-phopsate
F16BP	Fructose-1,6-bisphosphate
DHAP	Dihydroxyacetonephosphate
GA3P	Glyceraldehyde-3-phosphate
1,3-BPG	1,3-bisphosphoglycerate
2,3-BPG	2,3-bisphosphoglycerate
3PG	3-phosphoglycerate
2PG	2-phosphoglycerate
PEP	Phosphoenolpyruvate
PYR	Pyruvate
LAC	Lactate
G1P	Glucose-1-phosphate
GLY	Glycogen
UTP	Uridine Triphosphate
Pi	Inorganic Phosphate

In Vitro Glucose Metabolism
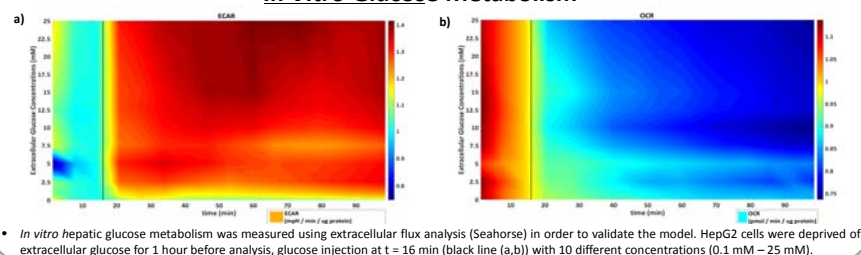
- *In vitro* hepatic glucose metabolism was measured using extracellular flux analysis (Seahorse) in order to validate the model. HepG2 cells were deprived of extracellular glucose for 1 hour before analysis, glucose injection at t = 16 min (black line (a,b)) with 10 different concentrations (0.1 mM – 25 mM).

Discussion

- The pH-dependent hepatic glucose metabolism model simulates the fundamental characteristics of cellular hepatic bioenergetics.

- This model is able to simulate ECAR via the MCT1 flux (Figure c), lending credibility to Lactic acid efflux as the driving factor of ECAR.

- Readjusting the MCT1 flux by fitting to *in vitro* data allows the model to be used synergistically with extracellular flux analysis, providing an enhanced platform from which to predict and assess drug induced bioenergetic impairment, reducing the usage of animal models.

ECAR Sensitivity
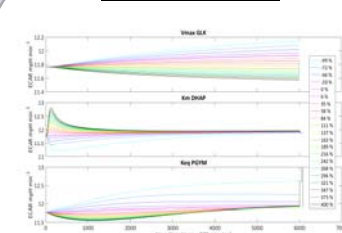
- Changes in model ECAR after parameter perturbations in the form of sensitivity analysis. Vmax GLK, Km DHAP and Keq PGYM were changed from -99 to 400 % and Changes in ECAR were measured.

ECAR Simulation
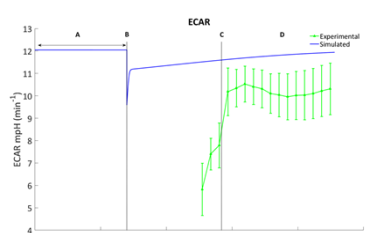
- Experimental design: A) Cell seeding incubation -720 min, B) Media removal – 60 min, C) glucose injection (5 mM), D) ECAR measurement / simulation – 90 min.

NC3Rs Impact

- Approximately 18000 rodent animal models were used for assessing toxicity, 13000 of which were used by pharmaceutical industries.

- Evidently, there is a clear need to develop alternative methods for the assessment of toxicity.

- This research coupled with the existing EFA toxicological data, could be used as a screening tool in early development. It can also be used as part of a weight of evidence approach in risk assessment.

- Perhaps the key use of this research is to glean more information from *in vitro* toxicity assessment via the *in silico* coupling, thereby increasing the efficiency of experimental design thus reducing the use of animal models.

Conclusions

- A pH-dependent *in silico* model of human hepatic glucose metabolism that accounts for proton buffering and rapid metal cation binding was successfully constructed.
- Coupling detailed thermodynamically driven, charge and mass balanced mathematical models with *in vitro* extracellular flux analysis has the potential to yield mechanistic insight into "off-target" toxicities such as drug induced mitochondrial dysfunction.
- *In silico* methods combined with *in vitro* techniques allow intelligent prediction and assessment of drug toxicity, facilitating the reduction of animal models.

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[1] Kamalian, L. , Chadwick, A. E. and Kevin Park, B. (2015), The utility of HepG2 cells to identify direct mitochondrial dysfunction in the absence of cell death. Toxicology *in vitro*.
[2] Konig, M. and Holzhutter, H. G. (2012) Quantifying the contribution of the liver to glucose homeostasis. PLoS Comput Biol 8(6), e1002577
[3] Beard, D. A. and Hong, Q. (2005) Thermodynamic-based computational profiling of cellular regulatory control in hepatocyte metabolism. Am J Physiol Endocrinol Metab 288: E633–E644
[4] Vinnakota, K. and Kushmerick, (2006) Dynamics of muscle glycogenolysis modelled with pH time course computation and pH-dependent reaction equilibria and enzyme kinetics. Biophysical Journal Volume 91
[5] Vanlier, J. and Beard, D. A. (2009) BISEN: Biochemical simulation environment. Bioinformatics

New possibilities in Mathematical Toxicology

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Bayesian Networks have recently been advocated to predict hazard and potency class of chemicals in the context of skin sensitization by using only animal-free assays. We have used developments in probability and statistics to both simplify and generalize these initial results. Our new approach provides robust procedures for constructing Bayesian Networks, avoiding earlier pitfalls involving data imputation, loss of information and cross-validation while maintaining (or improving) the accuracy of hazard and potency prediction.

Importantly, our methods use Machine learning, avoiding need for mechanistic knowledge of the biological processes in question. This gives us hope that Bayesian Networks may be successful in general toxicity prediction using only Machine Learning and animal-free assays. In this spirit preliminary results for liver carcinogenicity, where the underlying biological mechanisms are much less understood, will be discussed.

By extending this probabilistic thinking, we have also developed a rigorous framework to derive optimal integrated testing strategies for toxicity assessment using animal-free test alone. We combine a population model (accounting for individual-level differences in exposure and in reaction to that exposure) with an explicit cost structure (including both testing and misclassification costs) to derive optimal integrated testing strategies based on the powerful mathematical machinery of Markov Decision Problems. It turns out that, even in the simplest set-ups, optimal policies turn are typically adaptive. In other words, our mathematics demonstrates that one-size-fits-all testing policies cannot possibly be optimal.

Exploring the use of Bayesian statistical models to reduce the number of animals in control groups

Ros Walley and John Sherington

UCB

Many of the *in-vivo* models used in pre-clinical pharmaceutical research are run repeatedly over a period of time with a consistent protocol and control group(s) to test different compounds. Typically, historic data from the control group of previous studies are ignored when running a new study. We have used Bayesian statistical models to incorporate information from the historical controls into a new study, down-weighting this information to allow for variation between studies. This Bayesian methodology can allow the number of control animals in the current study to be reduced or, in some cases, a whole control group to be excluded. Alternatively, more statistical precision can be obtained with the same number of animals. We have successfully piloted this approach and present two case studies from immunology and CNS research areas.

Reference: Walley R, Sherington J, Rastrick J, Detrait E, Hanon E and Watt G Using Bayesian analysis in repeated preclinical *in vivo* studies for a more effective use of animals. *Pharmaceutical Statistics* 2016; 15(3):277-285, DOI: 10.1002/pst.1748

Exploring the use of Bayesian statistical models to reduce the number of animals in control groups

Ros Walley, John Sherington, Joe Rastrick, Alex Vugler, Gill Watt

Inspired by **patients.**
Driven by **science.**

Introduction

- Many of the in-vivo models used in pre-clinical pharmaceutical research are run repeatedly over a period of time with a consistent protocol and control group(s) to test different compounds.

- Typically, historic data from the control group of previous studies are ignored when running a new study.

- We have used Bayesian statistical models **to incorporate information from the historical controls into a new study, down-weighting this information to allow for variation between studies**.

- This Bayesian methodology can allow the number of control animals in the current study to be reduced or, in some cases, a whole control group to be excluded. Alternatively, more statistical precision can be obtained with the same number of animals.

- We have successfully piloted this approach and present two case studies from immunology and CNS research areas.

How useful is the historical information?

Imagine we have 10 historic experiments and are about to run experiment 11.

Intuitively, the relevance of the historic controls depends on the size of the study to study variation.

Low expt-to-expt variation

High expt-to-expt variation
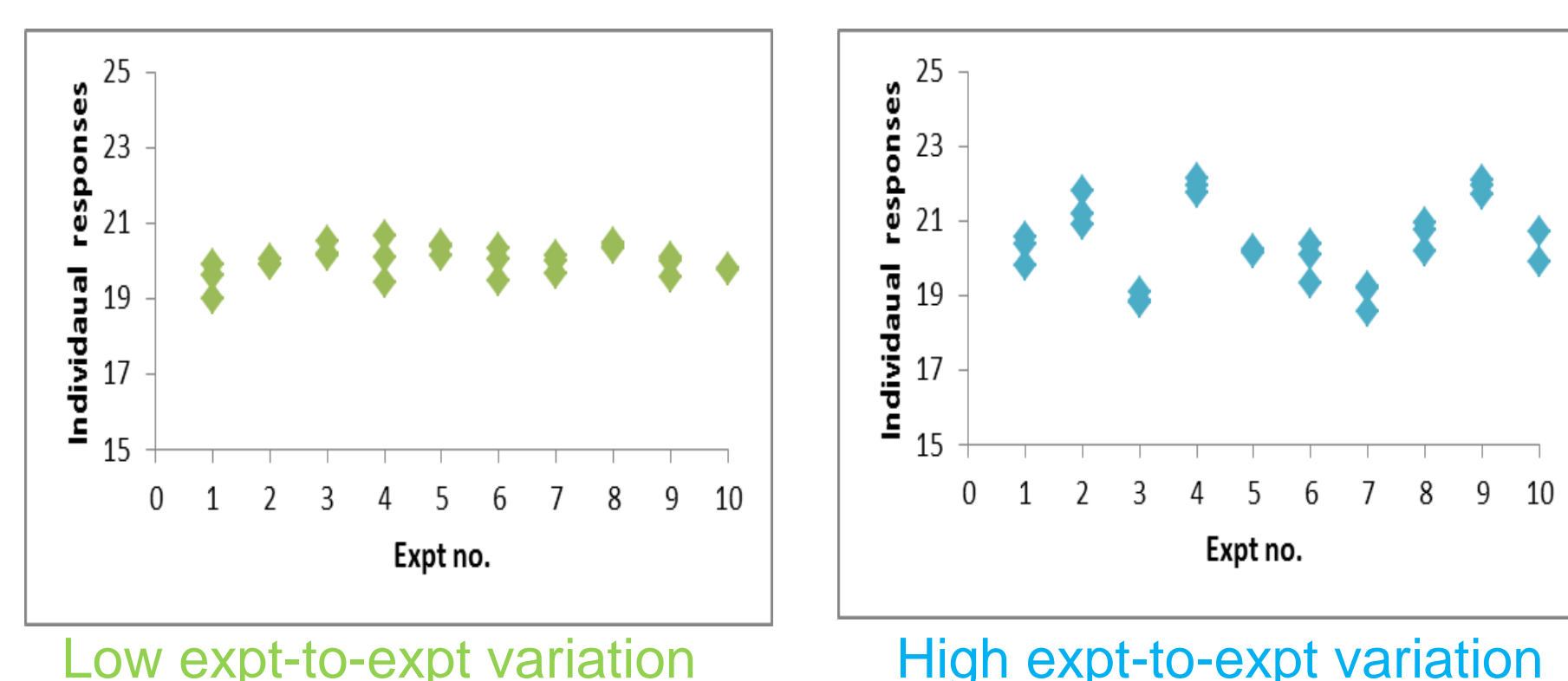
Overview of Bayesian methods used

1. Analyse historic control data, excluding the last study.

   - Bayesian meta-analysis, based on methodology in Neuenschwander et al., Clin Trials 2010 7: 5

2. Analyse the last study

   - Show what would have happened if we had "bought into" the Bayesian approach; omitting animals if necessary

3. Possible options for future studies:

   - Omit all/some animals from all/some control groups.

   - Use historic data as prior information combined with observed data in a Bayesian analysis.

   - Use historic data to give a predictive distribution for control group. i.e. don't include that treatment group in current study.

Two approaches for different controls

1. Control group **not** used in formal comparisons.

- Examples of uses: To ensure challenge is working in a robust reproducible manner; to establish a "window"; to check consistency with previous studies; to convert values to %.

- Approach: Replace control group with a range from a predictive distribution. This is illustrated for a 'No challenge' group in Case study 1.

### 2. Control group used for formal comparison vs. test compounds/doses

- Examples of uses: Comparison in t-tests; confidence intervals of differences between control vs. treated etc…

- Approach: Combine down-weighted historic data with the current experiment. This is illustrated for the Vehicle treated group in Case study 2.

> "Using a Bayesian approach will lead to more rapid and more economical drug development without sacrificing good science"
>
> Berry DA. Bayesian clinical trials. Nature Reviews 2006; 5: 27-36.

Case study 1: Lipopolysaccharide (LPS)-induced endotoxaemia

- The LPS-induced model of endotoxaemia is a short term in vivo pharmacology model used for the initial assessment of novel inhibitors on pro-inflammatory pathways.

- Mice were pre-treated with either an anti-inflammatory reference antibody; test compound(s); or vehicle - 5 and 30mins prior to LPS challenge.

- Mice were challenged with LPS (i.p; 25mg/mouse).

- There was an additional vehicle treated group that was not challenged

- At 3.5 hours post LPS challenge, mice were terminally anaesthetized, blood was collected and then plasma IL-6 levels determined using an ELISA-based multiplex platform (Mesoscale Discovery; N75012B-1).

- All studies were performed using male Balb/c mice (20-25g) obtained from Harlan (UK). All mice were housed in specific pathogen free conditions in standard cages with food and water supplied ad libitum. All experiments were performed in accordance with the UK Animals (Scientific Procedures) Act 1986.
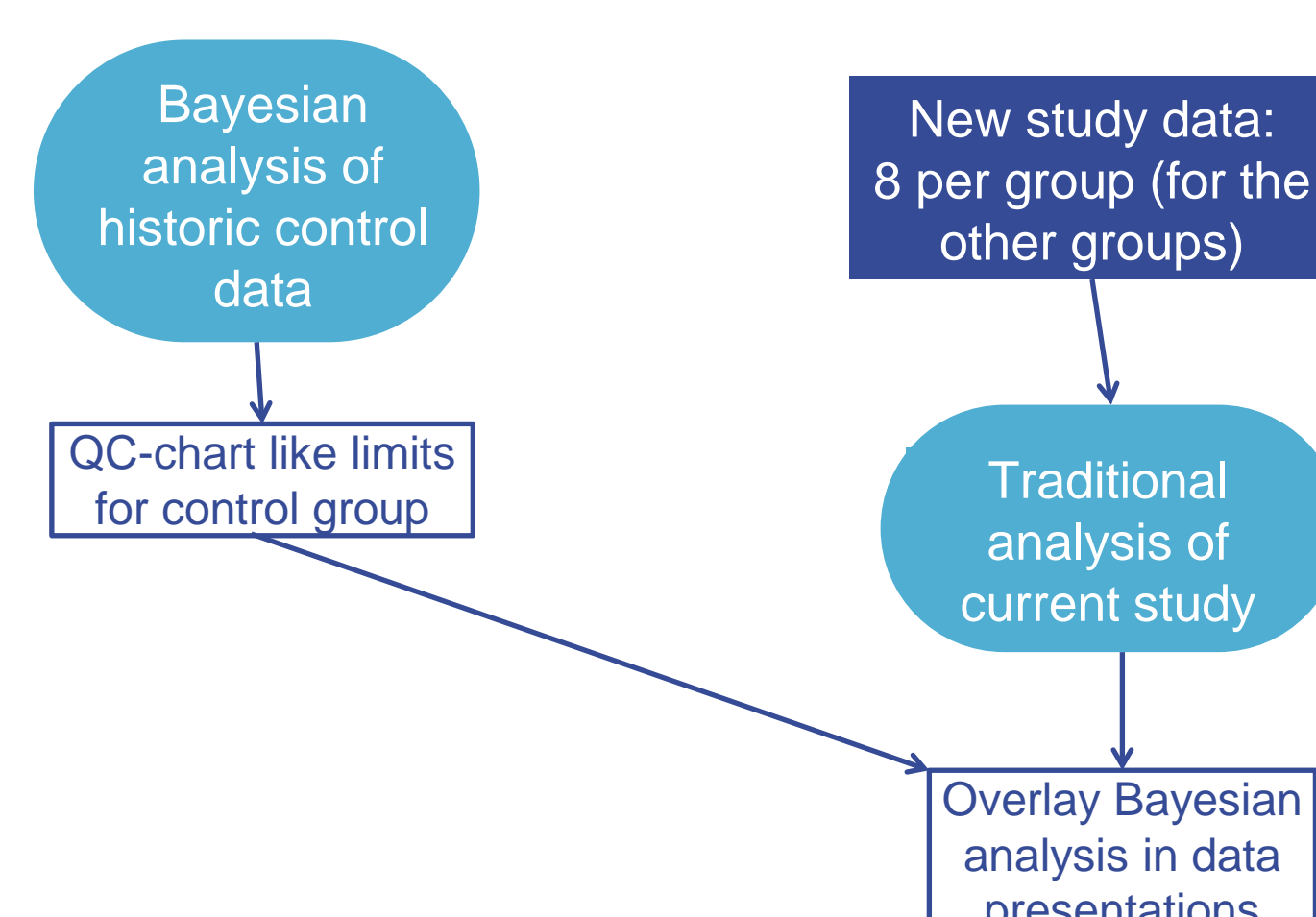
Data from the 'No Challenge' group from 25 previous studies was analysed to give a predictive distribution for a new study.

Conventional analysis of new study omitting the No Challenge group and replacing it with predictive limits is shown below.
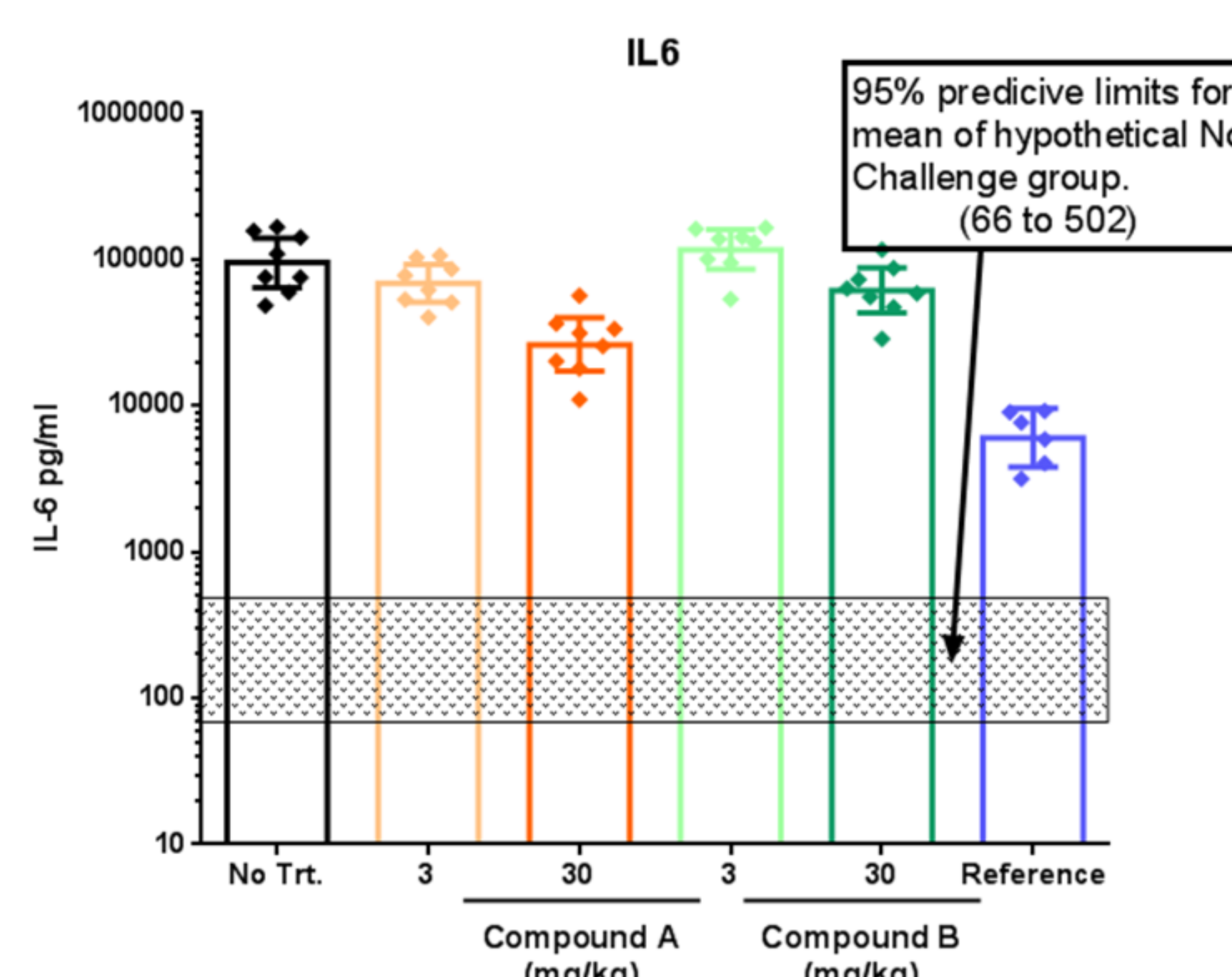
IL6

95% predictive limits for mean of hypothetical No Challenge group. (66 to 502)

Case study 2: Novel Object Recognition model

- Each experiment is performed in two trials:-

  - Acquisition trial – the rat is allowed to explore an arena containing two identical objects.

  - Retention trial – this is run a fixed time after the acquisition trial, and one of the two objects presented during the acquisition trial is replaced by an unknown (novel) object. With this delay, untreated animals poorly distinguish familiar and novel objects.

- An index of differential exploration is calculated as the difference in time spent exploring the new and familiar objects divided by the sum of both times, and is regarded as representative of the functionality of recognition.

- Data from Vehicle group from 17 previous studies were analysed to give prior information for the mean of this group.
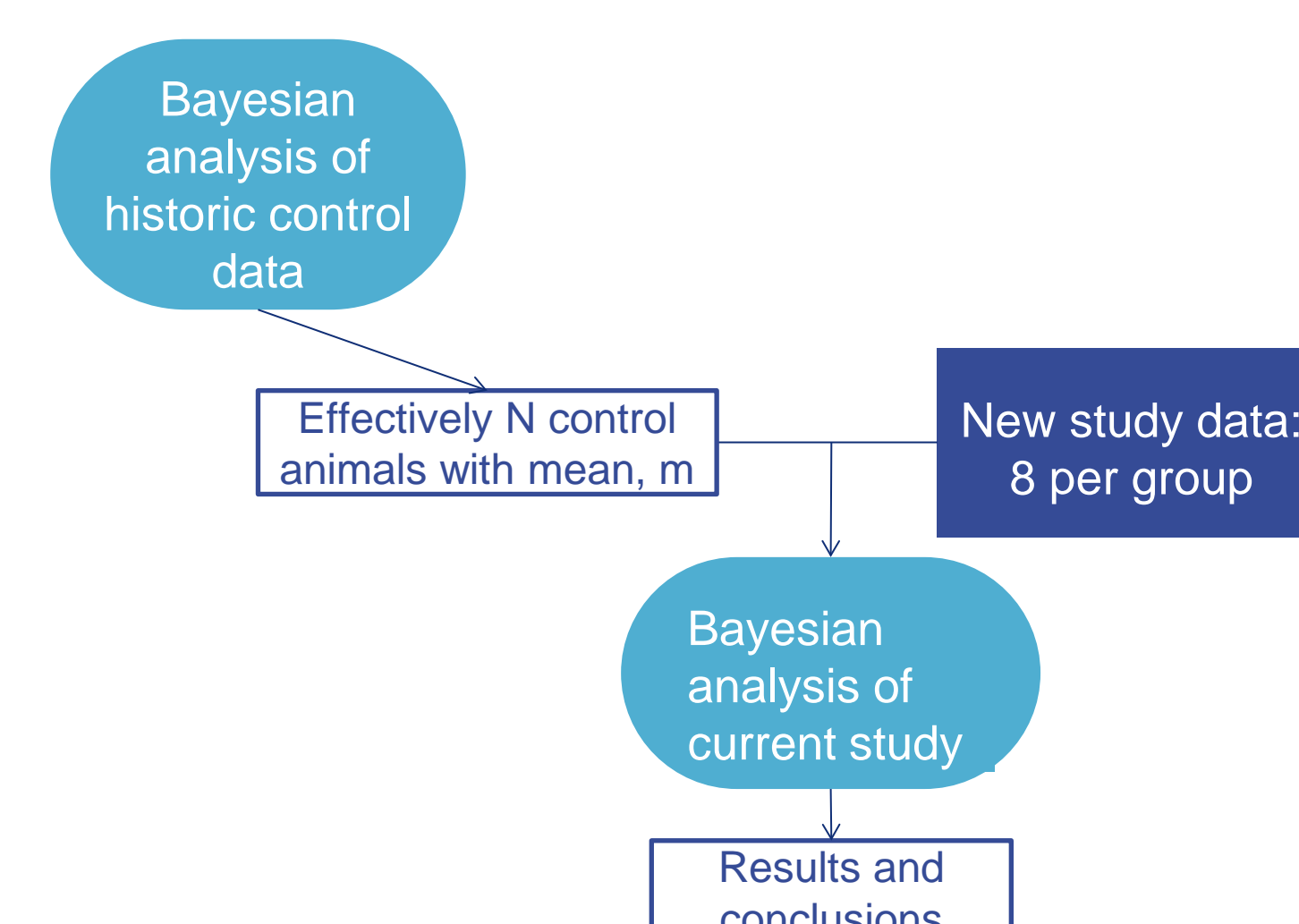
- Data from Vehicle group from 17 previous studies were analysed to give prior information for the mean of this group.

- This was then incorporated into a full Bayesian analysis of the latest study.

- The prior information was equivalent to have an additional 90 vehicle animals

The figure below shows interval estimates of the vehicle means and differences from vehicle, comparing a conventional ANOVA vs. the full Bayesian approach.

Vehicle means

Differences from Vehicle (with 95% Interval)

A = ANOVA
B = Bayesian
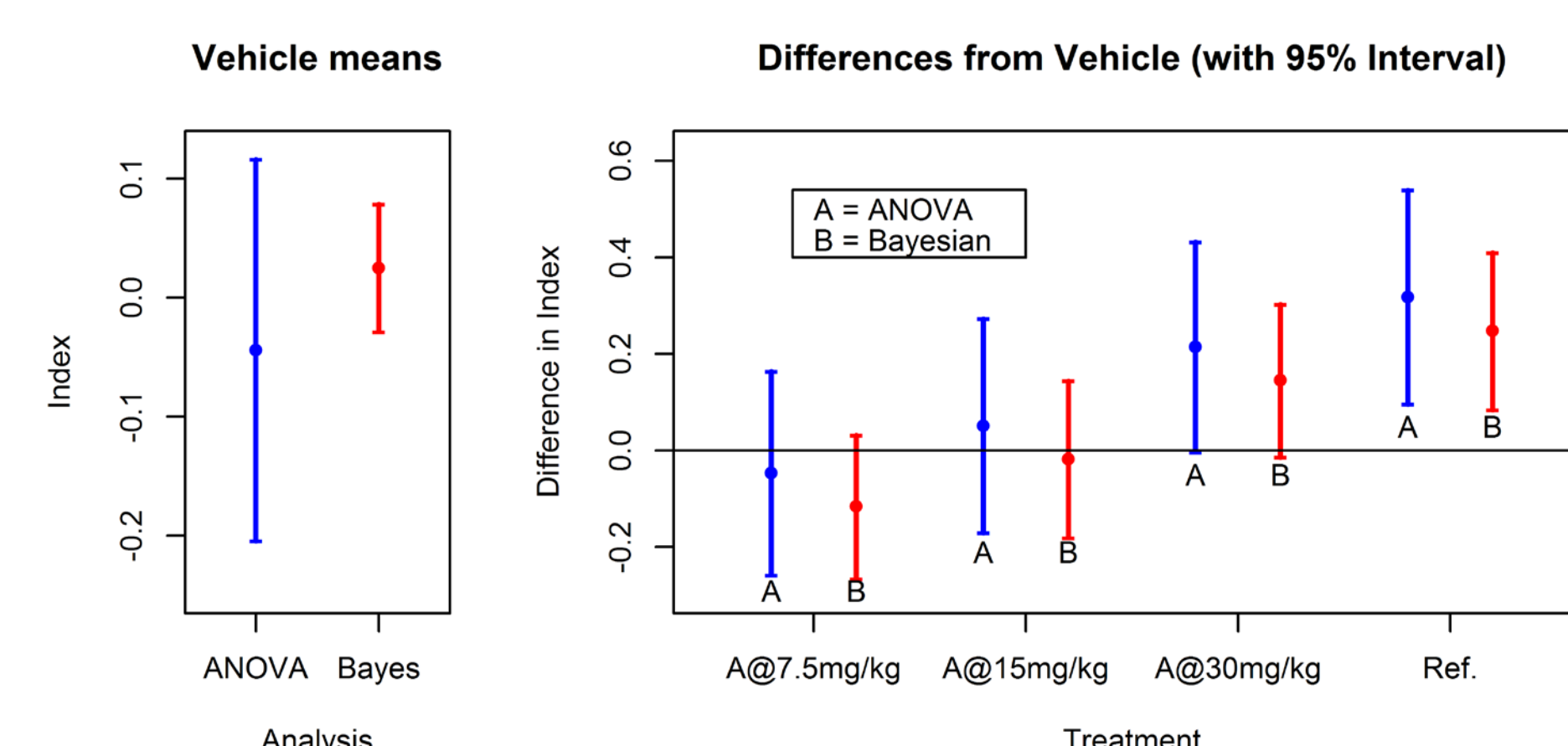
Conclusions

Bayesian methods for the statistical analysis of in-vivo studies have two potentially useful roles, both of which can reduce the use of animals in research :

- Predictive distributions can be used to replace a control group (one not used in formal statistical comparisons), as in the LPS model.

- A full Bayesian approach can be used to augment a control group with historical data, appropriately down-weighted, as in the NOR model

In terms of the 3Rs, Bayesian statistical models can reduce the numbers of control animals used in studies.

- While the reduction of numbers of animals in a single study may be modest, for repeated assays the reductions significantly accumulate over time.

- For example, if the number of control animals is reduced from 12 to 6 in an assay run 25 times per year, this amounts to 150 animals per year.