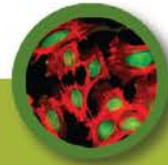
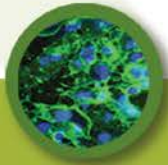




CELLular
Dynamics
international

xCELLigence Testing for RTI

06Apr2012



Methods Overview

- **Cell Culture**
- **Recording Layout**
- **Data Analysis**

Results

- **Controls**
- **JDTic**



Methods

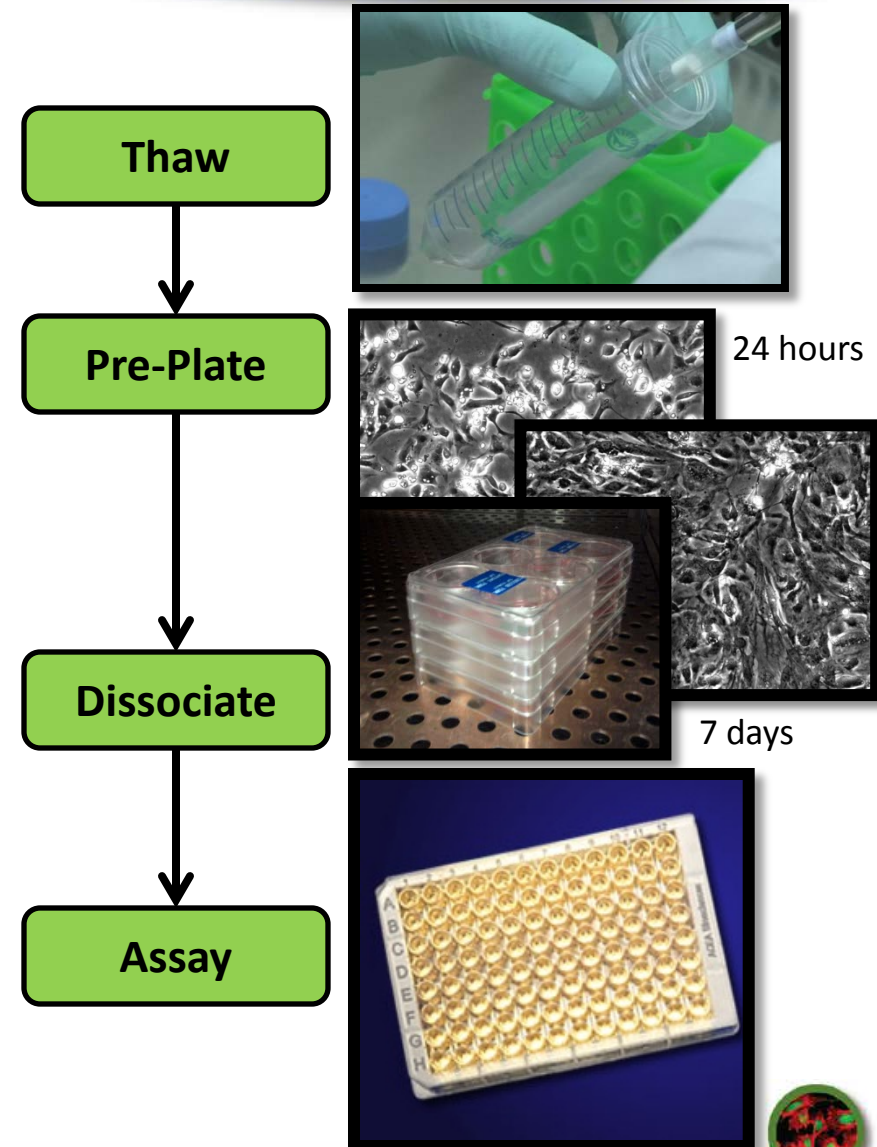


1. Overview

- a. JDTic and Terfenadine were provided in vehicle at 1000X. Vehicle alone also provided.
- b. JDTic and controls diluted according to RTI's instructions

2. Method

- a. Thaw cardiomyocytes according to User's Guide
 - i. 0.1% Gelatin coated 6-well dish at 750K plated cardiomyocytes/well
- b. Maintain in culture for 7 days according to User's Guide
- c. Dissociate using 0.1% Trypsin and re-plate on RTCA cardio plate at 40,000 viable cells/well
- d. Change the medium 48 hours after dissociation
- e. Allow fresh medium to equilibrate for at least 2 hours
- f. Record baseline, apply compounds for record according to RTI's requirements



1. **Baseline recorded before compound addition**
2. **Acute response recorded immediately after compound addition for 1 hour**
3. **Non-acute response recorded every hour for 23 hours**
4. **RTI requested baseline, 30min, 1hr, 4hr, and 16hr time points to be analyzed**

Recording Parameters

Time	Step Duration	Sweep Interval	Sweep Duration
Baseline	30 min	5 min	60 sec
Dosing	60 min	1 min	60 sec
Every Hour	22 hr	1 min	60 sec

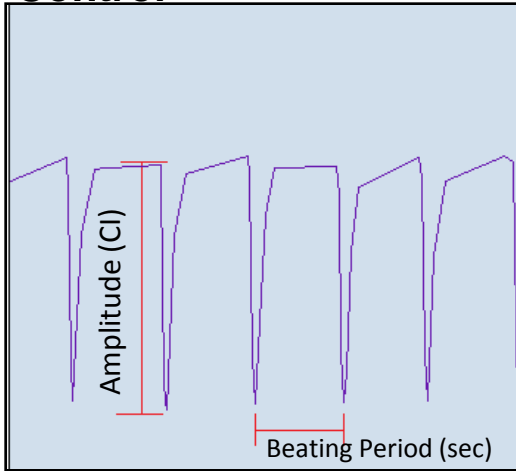
5. Plate Layout

- a. **8-point dose response curve, 10-fold dilution, n=4**
- b. **4 negative controls wells in vehicle (0.1% DMSO or water), 4-point E4031 dose response (3, 10, 30, 100nM n=3) and Terfenadine (1uM, 300nM, 100nM, 30nM) for positive control**
- c. **Add 20µl of a 10x solution to 180µl iCMM in bath. Mix 3 times**

	1	2	3	4	5	6	7	8	9	10	11	12
A	Media	Media	Media	Media	Media	Media	Media	Media	Media	Media	Media	Media
B	Media	JDTic Dose 1	JDTic Dose 2	JDTic Dose 3	JDTic Dose 4	JDTic Dose 5	JDTic Dose 6	JDTic Dose 7	JDTic Dose 8	Terf. 1uM	E4031 100nM	Media
C	Media	JDTic Dose 1	JDTic Dose 2	JDTic Dose 3	JDTic Dose 4	JDTic Dose 5	JDTic Dose 6	JDTic Dose 7	JDTic Dose 8	Terf. 300nM	E4031 30nM	Media
D	Media	JDTic Dose 1	JDTic Dose 2	JDTic Dose 3	JDTic Dose 4	JDTic Dose 5	JDTic Dose 6	JDTic Dose 7	JDTic Dose 8	Terf. 100nM	E4031 10nM	Media
E	Media	JDTic Dose 1	JDTic Dose 2	JDTic Dose 3	JDTic Dose 4	JDTic Dose 5	JDTic Dose 6	JDTic Dose 7	JDTic Dose 8	Terf. 30nM	E4031 3nM	Media
F	Media	DMSO 0.10%	DMSO 0.10%	Terf. 1uM	Terf. 300nM	Terf. 100nM	Terf. 30nM	Terf. 1uM	Terf. 300nM	Terf. 100nM	Terf. 30nM	Media
G	Media	DMSO 0.10%	DMSO 0.10%	E4031 100nM	E4031 30nM	E4031 10nM	E4031 3nM	E4031 100nM	E4031 30nM	E4031 10nM	E4031 3nM	Media
H	Media	Media	Media	Media	Media	Media	Media	Media	Media	Media	Media	Media

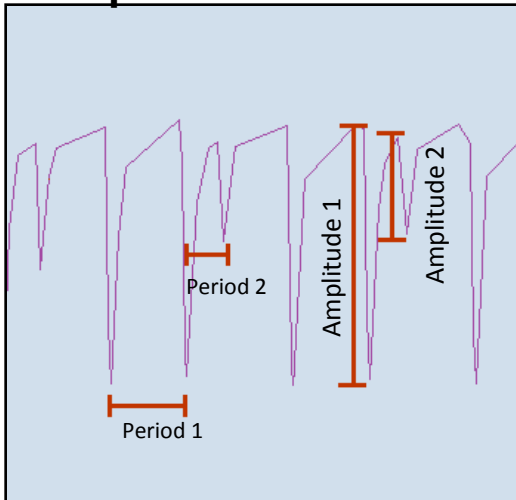


Control



- **Beating Period and Amplitude were measured with RTCA software.**
- **CV was calculated from RTCA values and standard deviations**
- **Beating Period was converted to Beating Frequency**
- **Beating Frequency and Amplitude were normalized to Baseline**

Compound



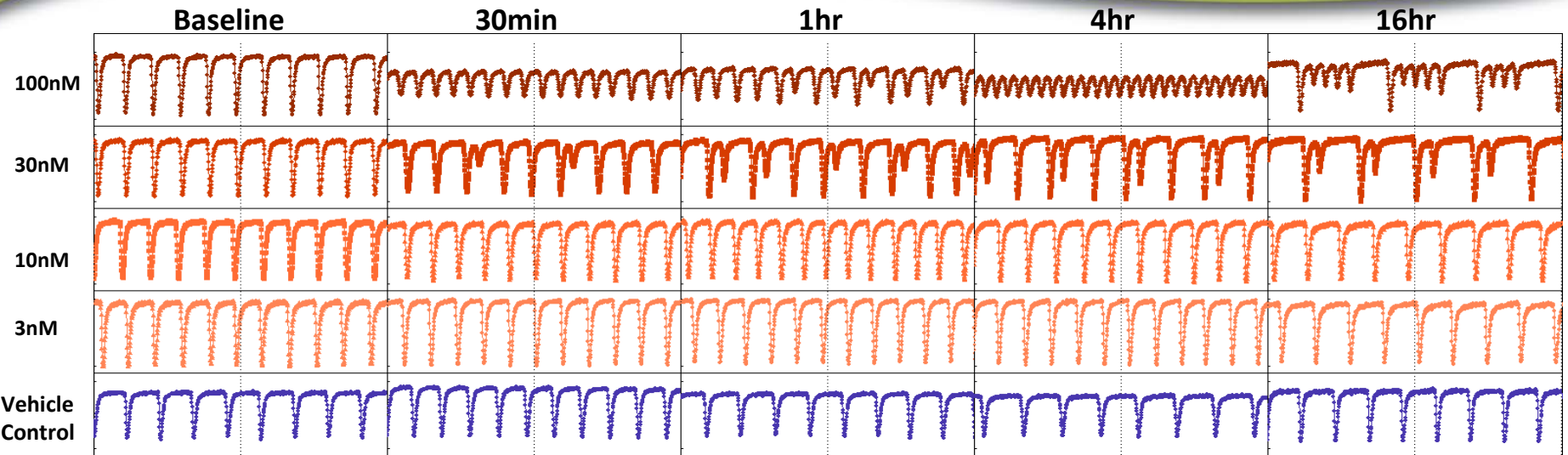
Example:

- **Small deflections are detected as a beat by RTCA software.**
- **The differences in Periods and Amplitudes increase the standard deviation, lowers the average, thus increasing CV.**
- **Alternatively Periods and Amplitudes can change to steady-state levels with little change in CV**
- **Drug effects can thus be detected by changes in CV and/or Mean values**



Results



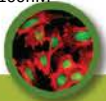
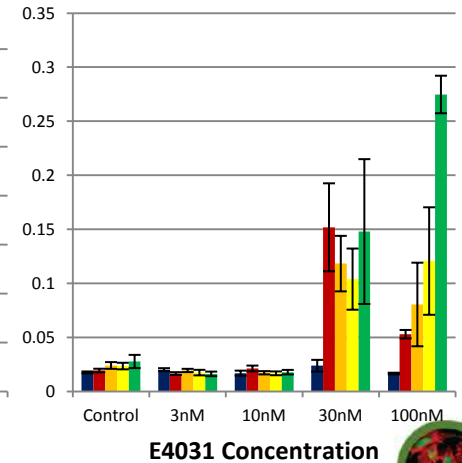
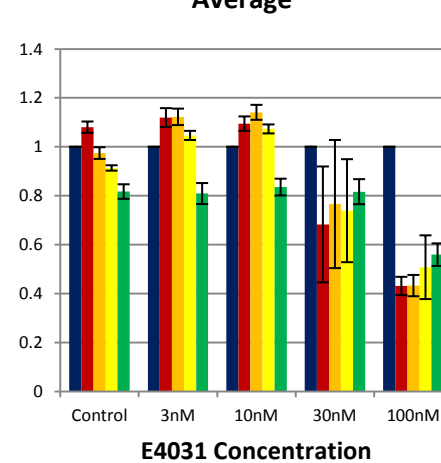
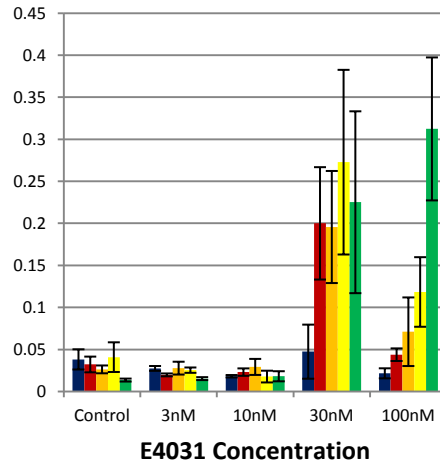
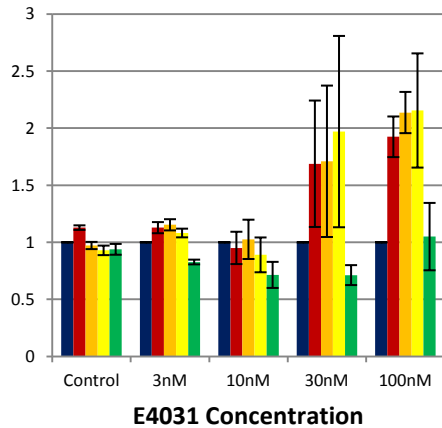


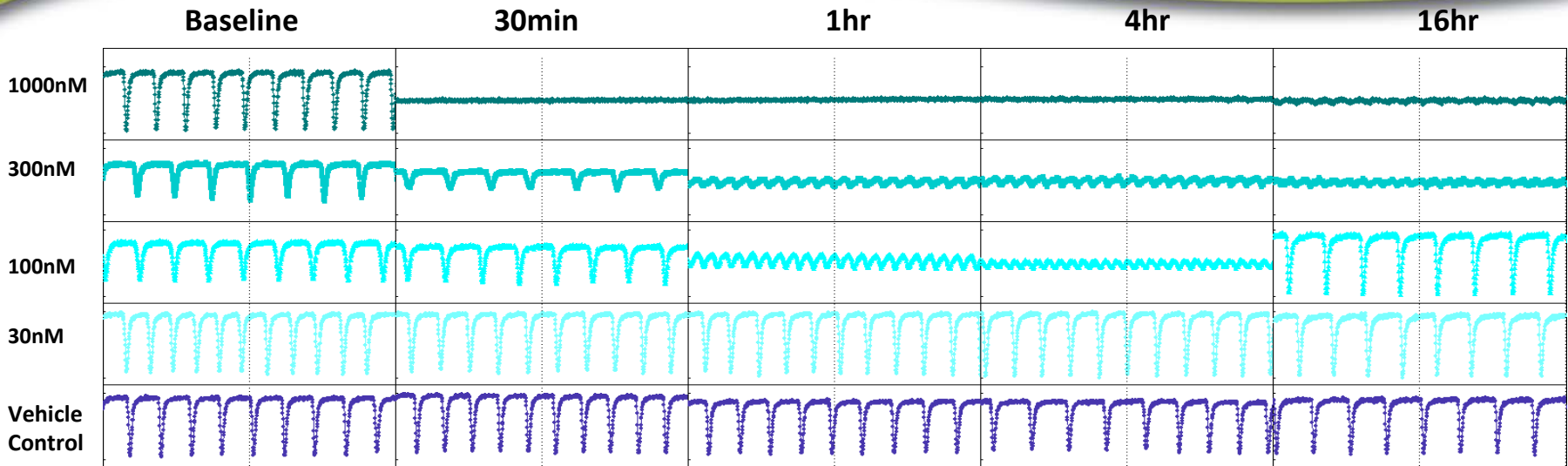
Beating Frequency (Hz):
Normalized Average

Beating Period (sec): CV

Amplitude: Normalized
Average

Amplitude: CV



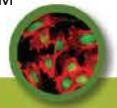
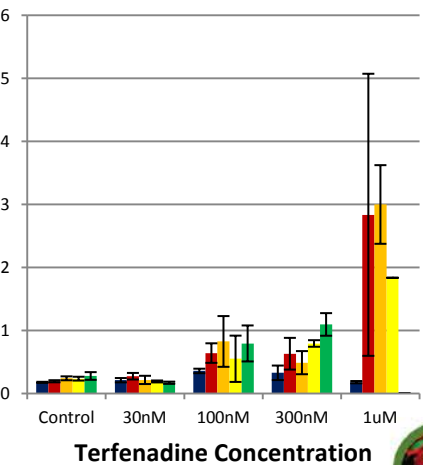
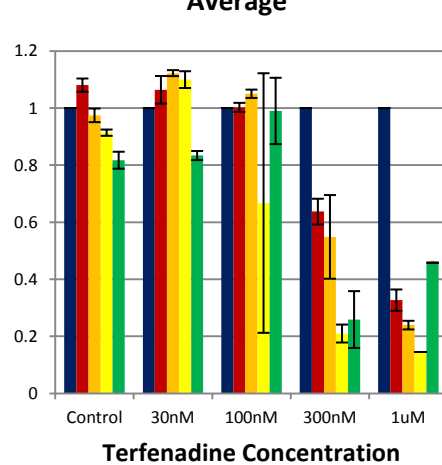
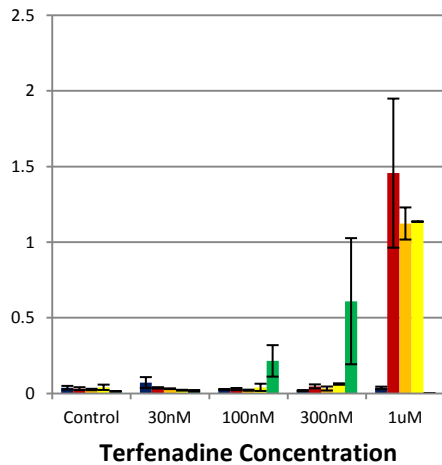
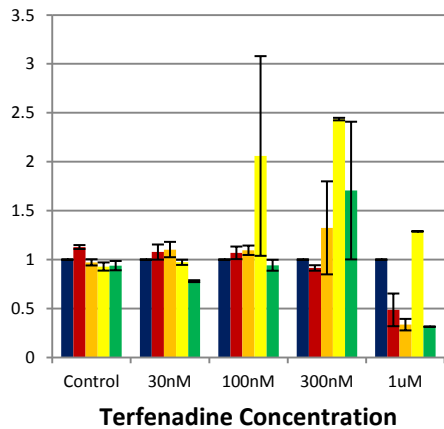


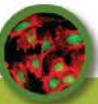
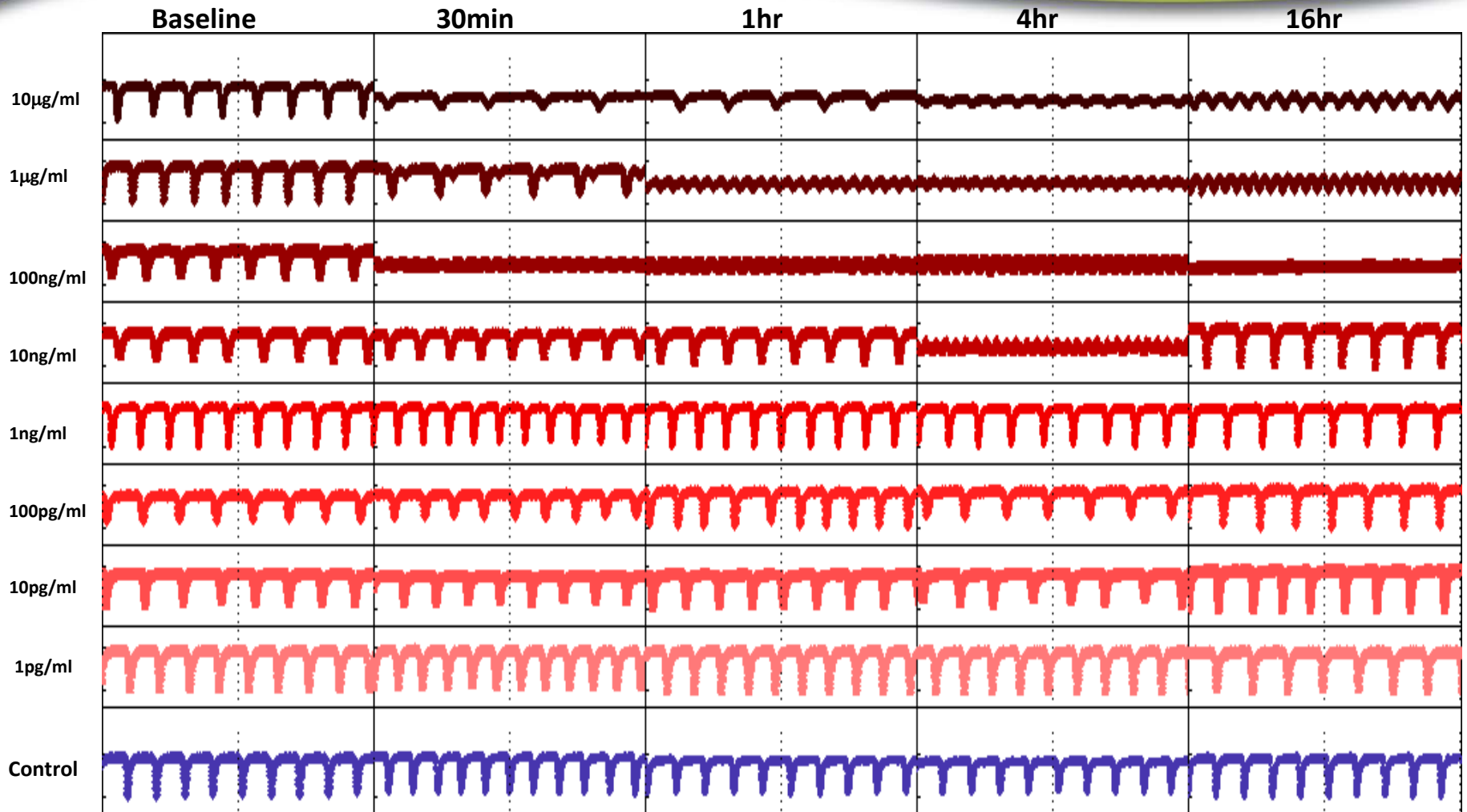
**Beating Frequency (Hz):
Normalized Average**

Beating Period (sec): CV

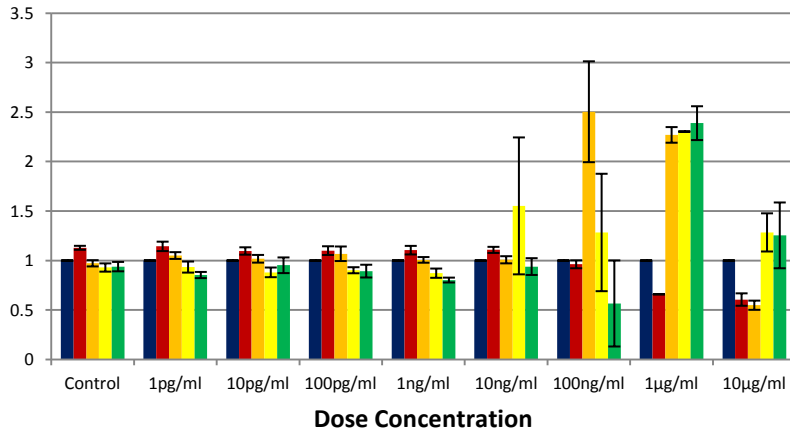
**Amplitude: Normalized
Average**

Amplitude: CV

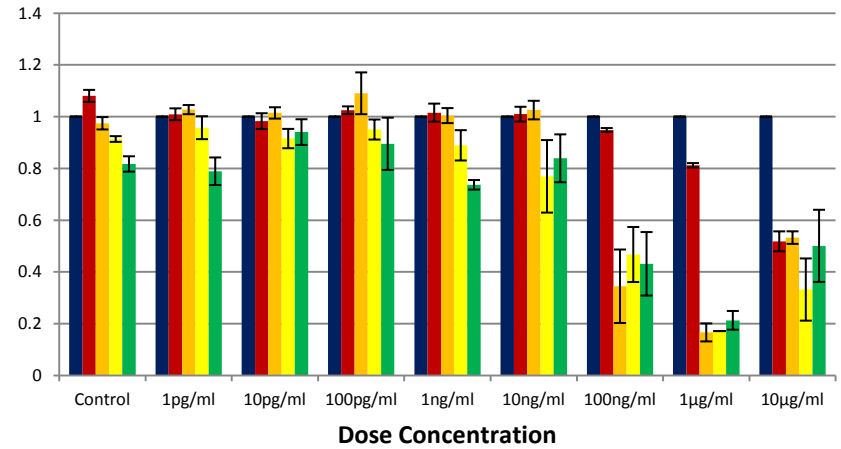




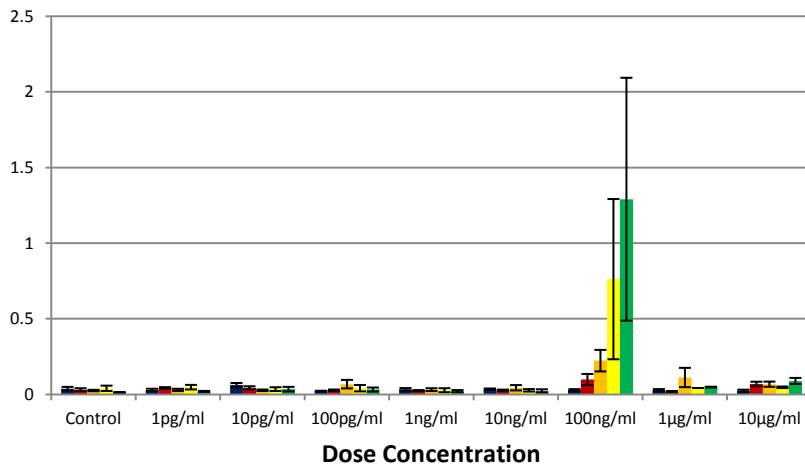
Beating Frequency (Hz): Normalized Average



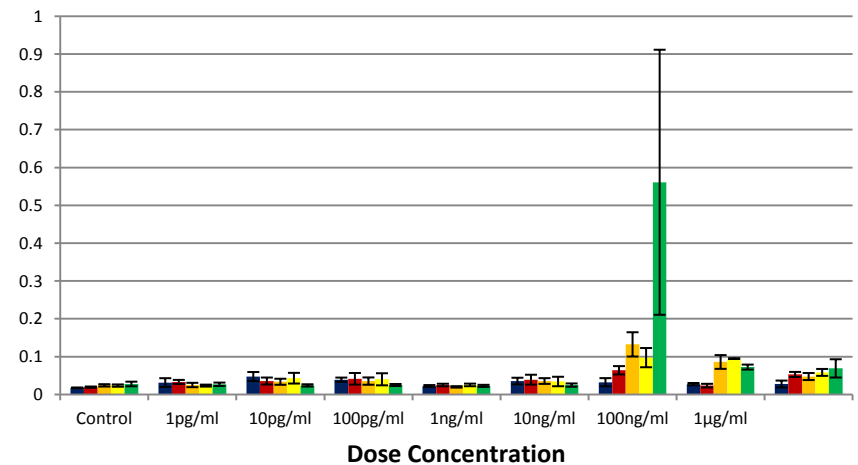
Amplitude: Normalized Average



Beating Period (sec): CV



Amplitude: CV



- **Positive and negative controls displayed expected responses**
 - **E4031: clear effects at 100 and 30 nM at all time points post drug addition**
 - **Terfenadine: clear effects at 1000, 300 and 100 nM**
 - 100 nM effects observed at 1 and 4 hours, but recovered by 16 hours
 - **Vehicle Control: no effects detected**
- **JDTic had a clear effect at high concentrations**
 - **Clear effects at 10 ug/mL, 1 ug/mL and 100 ng/mL at all time points**
 - **Response at 10ng/ml variable by time point**
 - **IB20**
 - Between 10 and 100 ng/mL at 30 minutes, 1 hour and 16 hour time points post dose application
 - Between 1 and 10 ng/mL at 4 hours post dose application

