Preparation of 1000X Dilution Series

Test Material: Bis 1 Bisindolylmaleimide Lot: D00150457

Solvent: DMSO Lot: SHBC06617V

MW: 412.5

									1000X Stock
1.	1.0	mg of	Bis1	+	242	µL of solvent	→	10	mM
2.	36	µL of	1	+	84	µL of solvent	→	3.0	mM
3.	40	µL of	2	+	80	µL of solvent	→	1.0	mM
4.	36	µL of	3	+	84	µL of solvent	→	0.3	mM
5.	40	µL of	4	+	80	µL of solvent	→	0.1	mM
6.	36	µL of	5	+	84	µL of solvent	→	0.03	mM
7.	40	µL of	6	+	80	µL of solvent	→	0.01	mM
8.		µL of		+		µL of solvent	→		mM
9.		µL of		+		µL of solvent	→		mM
10.		µL of		+		µL of solvent	→		mM

Initials KAW Date: 4.7.11

TSS 2/4/11

Preparation of 1000X Dilution Series

Cortical Culture 4.2.14

Test Material: Sodium Orthovanadate Lot: 9.8.7 200mM

Solvent: H_2O Lot: 9.20.13

								1000X Stock
1.	75	µL of	Stock	+	425	µL of solvent →	30	µM
2.	150	µL of	1	+	300	µL of solvent →	10	µM
3.	150	µL of	2	+	350	µL of solvent →	3.0	µM
4.	150	µL of	3	+	300	µL of solvent →	1.0	µM
5.	150	µL of	4	+	350	µL of solvent →	0.3	µM
6.	150	µL of	5	+	300	µL of solvent →	0.1	µM
7.		µL of		+		µL of solvent →		µM
8.		µL of		+		µL of solvent →		µM
9.		µL of		+		µL of solvent →		µM
10.		µL of		+		µL of solvent →		µM

m

form mod. KAW 4.2.14

KAW
KAW
Initials KAW

Date: 4.2.14
4.7.14
4.11.14

TSS 4/4/14

Plate Map for 50X 96 Well Dilution Plate

	1	2	3	4	5	6	7	8	9	10	11	12
A	1000X Stock											
B	30	10	1.0	30	30	30						
C	10	3.0	0.3	10	10	10						
D	3.0	1.0	0.1	3.0	3.0	3.0						
E	1.0	0.3	0.03	1.0	1.0	1.0						
F	0.3	0.1	0.01	0.3	0.3	0.3			NA			
G	0.1	0.03	0.003	0.1	0.1	0.1						
H	0	0	0	0	0	0						

Acetaminophen
Bis 1
Domoic Acid
Loperamide
Mevastatin
Vanadate

Plate: MW 1007-27

Plate:

Pipet 5 µL of test Chemical 1000X into the appropriate wells. Then add 95 µL of NB/B27 Medium to each well.

Transfer 10 µL of the 50X Stock to the appropriate wells of a MEA 48 well plate with a pipet.

Preparation Date: 4.2.14 4:10pm INI: KAW

Preparation Date: 4.7.14 3:00pm INI: KAW

Preparation Date: 4.11.14 8:30am INI: KAW

TJS
4/14

Grounded Electrode Map MW 1007-27 Cortical Culture 4.2.14

DIV 2

~~4.14.14~~ 4.4.14
KAW

	1	2	3	4	5	6	7	8
A	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	(14) 24 34 44 13 23 33 43 (12) 22 32 42 11 21 31 41
B	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41
C	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41
D	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41
E	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41
F	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 (13) 23 (33) 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41

DIV 5

4.7.14
KAW

	1	2	3	4	5	6	7	8
A	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 (12) 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	(14) 24 34 44 13 23 33 43 (12) 22 32 42 11 21 31 41
B	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41
C	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41
D	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41
E	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41
F	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41

DIV 7

4.9.14
KAW

	1	2	3	4	5	6	7	8
A	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	(14) 24 34 44 13 23 33 43 (12) 22 32 42 11 21 31 41
B	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41
C	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41
D	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 (42) 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41
E	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41
F	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 (13) 23 (33) 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41

TJD
[illegible]

Grounded Electrode Map

Cortical Culture 4.2.14

DIV 9
4.11.14
KAW

	1	2	3	4	5	6	7	8
A	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	**14** 24 34 44 13 23 33 43 **12** 22 32 42 11 21 31 41
B	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41
C	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41
D	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 **42** 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41
E	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 **44** 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41
F	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 **13** 23 **33** 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41

DIV 12
4.14.14
KAW

	1	2	3	4	5	6	7	8
A	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	**14** 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41
B	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41
C	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41
D	14 24 34 44 **13** 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 **42** 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 **41**
E	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41
F	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 **13** 23 **33** 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41

DIV

	1	2	3	4	5	6	7	8
A	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41
B	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41
C	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41
D	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41
E	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41
F	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41	14 24 34 44 13 23 33 43 12 22 32 42 11 21 31 41

Cortical Culture 4.2.14 MW 1007-27

	1	2	3	4	5	6	7	8
A	Acetaminophen 0	0.1	0.3	1.0	3.0	10	30	0
B	Bis I 0	0.03	0.1	0.3	1.0	3.0	10	0
C	Domoic Acid 0	0.003	0.01	0.03	0.1	0.3	1.0	0
D	Loperamide 0	0.1	0.3	1.0	3.0	10	30	0
E	Mevastatin 0	0.1	0.3	1.0	3.0	10	30	0
F	Vanadate 0	0.1	0.3	1.0	3.0	10	30	0

13μL 50mM Bic + 507μL NB/B-27

Initials KAW

Date 4.2.14

TJS 11/4/14

The CellTiter Blue® Cell Viability Assay

The CellTiter-Blue® Cell Viability Assay provides a homogeneous, fluorometric method for estimating the number of viable cells present in multiwell plates. It uses the indicator dye resazurin to measure the metabolic capacity of cells as an indicator of cell viability. Viable cells retain the ability to reduce resazurin into resorufin, which is highly fluorescent. Nonviable cells rapidly lose metabolic capacity, do not reduce the indicator dye, and thus do not generate a fluorescent signal. Resazurin is dark blue in color and has little intrinsic fluorescence until it is reduced to resorufin, which is pink and highly fluorescent (544 nm Ex/590 nm Em).

Primary rat cortical neuronal cells were seeded on to 48-well MEA plate(s).

Culture Date: 4·2·14

Freshly-prepared solutions of test materials were made in NB/B-27 medium then 500 µl of test material dilutions were added to the appropriate wells of the 48-well MEA plate(s).

DIV 0 Treatment Time and Date: 4:10 pm 4·2·14

DIV 5 Treatment Time and Date: 3:00 pm 4·7·14

DIV 9 Treatment Time and Date: 8:30 am 4·11·14

The cells were incubated at 37°C, 5% CO_2 for 12days. The treatment medium was removed and replaced with 200 µl fresh NB/B-27 medium containing 1:10 dilution of CellTiter-Blue® Reagent.

Preparation of CellTiter-Blue® Reagent containing medium:

Volume of media 10.8 Amount of CellTiter-Blue® Reagent 1.2

Addition Time: 8:40 am

After 1- 4 hours incubation at 37°C, 5% CO_2, remove cultures from incubator and allow to come to room temperature. Transfer ~~100~~ 150 µl* from each well to 96-well plate. Measure the fluorescence Ex 544 nm and Em 590 nm on a Fluorostar Optima (A54895). The percent of viable cells determined and plotted in Excel.

Read Plate Time: 1:00 pm

* Form Mod KAW 4.14.14

Reagents:

NB/B-27 medium 3·20·14

Alamar Blue (Promega G8081) 13364

Recorded by KAW

Page# K

Date 4.14.14

TJS 11/2/14

User: MUNDY Path: C:\Program Files\BMG\FLUOoptima\Mundy\Data\ Test ID: 900
Test Name: CELL TITER BLUE Date: 4/14/2014 Time: 12:42:58 PM
Fluorescence (FI)

Raw Data (544, 590)

	1	2	3	4	5	6	7	8	9	10	11	12
A	59606	63910	61683	63265	64717	61054	61483	64158	1328	1325	1324	1309
B	64237	63901	64452	64207	65000	62643	9105	61636	1339	1340	1339	1305
C	65000	65000	65000	65000	64740	64764	64806	64601	1330	1357	1333	1316
D	64573	65000	65000	61009	60686	14834	4579	62751	1348	1343	1333	1313
E	60663	64952	62517	44764	42087	29140	25479	61733	1331	1355	1330	1319
F	64422	65000	64427	61994	63541	64743	52018	61047	1335	1337	1325	1309
G	4597	4642	4646	4653	4653	4706	4695	4700	1325	1349	1338	1321
H	1322	1322	3296	1325	1334	1357	1682	1327	1309	1344	1335	1288

T35 4/14/14

4.14.14 KAW

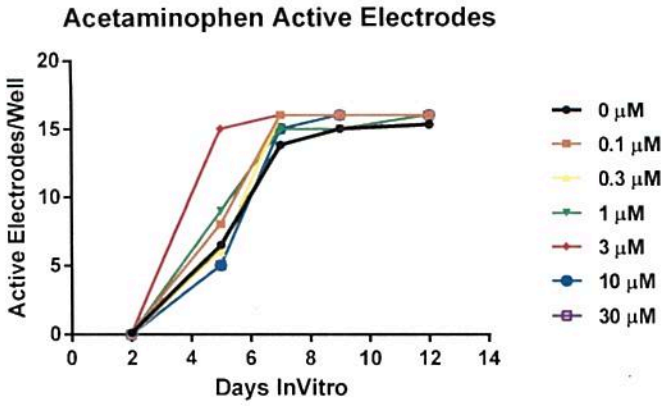
Summary of Results from 4/2/2014 Ontogeny MEA Plate 1007-27

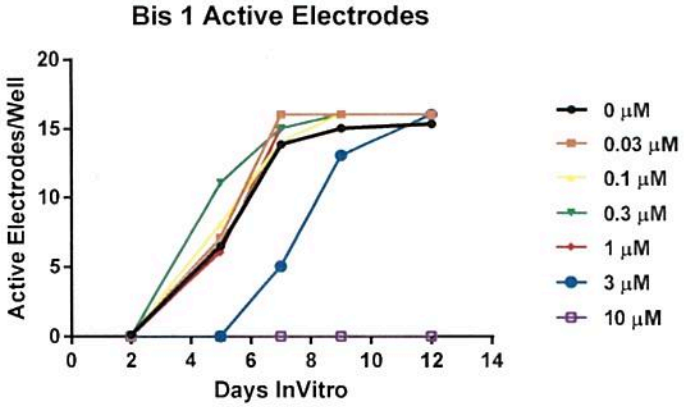
Chemical	Conc (μM)	DIV 02	DIV 05	DIV 07	DIV 09	DIV 12	BIC	Plate	Date	DIV
Acetaminophen	0	0	9	15	16	16	16	1007-27	4/2/2014	12
‹cetaminophen	0	0	9	13	14	14	15	1007-27	4/2/2014	12
Acetaminophen	0.1	0	8	16	16	16	16	1007-27	4/2/2014	12
Acetaminophen	0.3	0	6	16	16	16	16	1007-27	4/2/2014	12
Acetaminophen	1	0	9	15	15	16	16	1007-27	4/2/2014	12
Acetaminophen	3	0	15	16	16	16	16	1007-27	4/2/2014	12
Acetaminophen	10	0	5	15	16	16	16	1007-27	4/2/2014	12
Acetaminophen	30	0	5	15	16	16	16	1007-27	4/2/2014	12
Bisindolymaleimide 1	0	0	10	16	16	16	16	1007-27	4/2/2014	12
Bisindolymaleimide 1	0	0	5	16	16	16	16	1007-27	4/2/2014	12
Bisindolymaleimide 1	0.03	0	7	16	16	16	16	1007-27	4/2/2014	12
Bisindolymaleimide 1	0.1	0	8	14	16	16	16	1007-27	4/2/2014	12
Bisindolymaleimide 1	0.3	0	11	15	16	16	16	1007-27	4/2/2014	12
Bisindolymaleimide 1	1	0	6	15	16	16	16	1007-27	4/2/2014	12
Bisindolymaleimide 1	3	0	0	5	13	16	16	1007-27	4/2/2014	12
Bisindolymaleimide 1	10	0	0	0	0	0	0	1007-27	4/2/2014	12
Domoic Acid	0	0	4	11	16	16	16	1007-27	4/2/2014	12
Domoic Acid	0	0	5	14	16	16	16	1007-27	4/2/2014	12
Domoic Acid	0.003	0	12	16	16	16	16	1007-27	4/2/2014	12
Domoic Acid	0.01	0	5	16	16	16	15	1007-27	4/2/2014	12
Domoic Acid	0.03	0	8	14	16	16	16	1007-27	4/2/2014	12
Domoic Acid	0.1	0	7	13	15	16	16	1007-27	4/2/2014	12
)omoic Acid	0.3	0	2	8	16	16	16	1007-27	4/2/2014	12
Domoic Acid	1	0	5	11	16	16	16	1007-27	4/2/2014	12
Loperamide	0	0	4	11	15	15	15	1007-27	4/2/2014	12
Loperamide	0	0	6	16	16	15	15	1007-27	4/2/2014	12
Loperamide	0.1	0	5	15	16	16	16	1007-27	4/2/2014	12
Loperamide	0.3	0	7	15	16	16	16	1007-27	4/2/2014	12
Loperamide	1	0	0	1	15	16	16	1007-27	4/2/2014	12
Loperamide	3	0	0	0	0	0	1	1007-27	4/2/2014	12
Loperamide	10	0	0	0	0	0	0	1007-27	4/2/2014	12
Loperamide	30	0	0	0	0	0	0	1007-27	4/2/2014	12
Mevastatin	0	1	11	16	16	16	16	1007-27	4/2/2014	12
Mevastatin	0	0	7	15	16	16	16	1007-27	4/2/2014	12
Mevastatin	0.1	0	0	5	10	12	11	1007-27	4/2/2014	12
Mevastatin	0.3	0	4	11	14	14	14	1007-27	4/2/2014	12
Mevastatin	1	0	6	6	15	16	16	1007-27	4/2/2014	12
Mevastatin	3	0	2	1	2	11	11	1007-27	4/2/2014	12
Mevastatin	10	0	0	0	0	1	1	1007-27	4/2/2014	12
Mevastatin	30	0	0	0	0	0	0	1007-27	4/2/2014	12
Vanadate	0	0	2	6	7	11	11	1007-27	4/2/2014	12
Vanadate	0	0	6	16	16	16	16	1007-27	4/2/2014	12
Vanadate	0.1	0	0	5	9	12	11	1007-27	4/2/2014	12
Vanadate	0.3	0	9	14	16	16	16	1007-27	4/2/2014	12
Vanadate	1	0	8	14	15	16	16	1007-27	4/2/2014	12
Vanadate	3	0	0	1	11	12	10	1007-27	4/2/2014	12
Vanadate	10	0	6	14	14	14	14	1007-27	4/2/2014	12
Vanadate	30	0	5	7	16	16	16	1007-27	4/2/2014	12

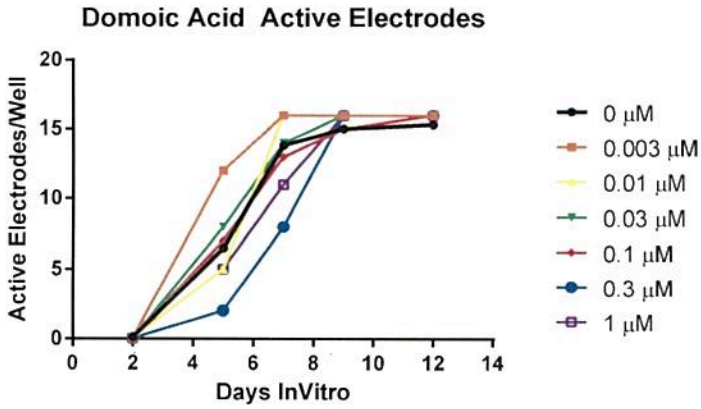
KHW 5.14.14

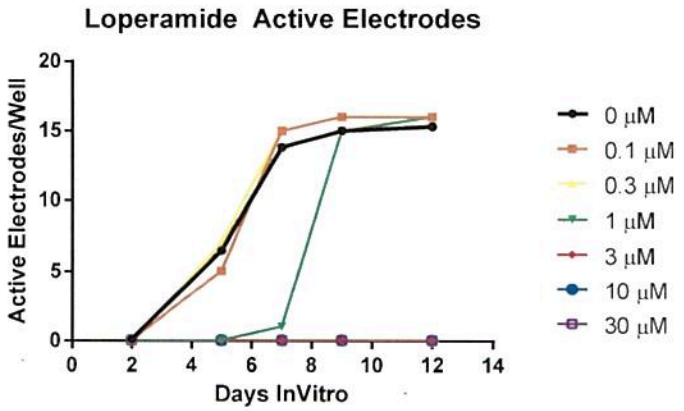
Chemical	Conc (μM	DIV 02	DIV 05	DIV 07	DIV 09	DIV 12	BIC	Survival
Acetaminophen	0	0	34	68	95	105	322	94%
Acetaminophen	0	0	13	49	84	81	230	102%
Acetaminophen	0.1	0	56	68	72	144	344	102%
Acetaminophen	0.3	0	18	78	84	137	269	98%
Acetaminophen	1	0	38	78	92	72	324	101%
Acetaminophen	3	0	48	59	64	105	198	103%
Acetaminophen	10	0	30	59	85	99	269	97%
Acetaminophen	30	0	72	50	76	100	141	98%
Bisindolymaleimide 1	0	0	30	40	57	91	112	102%
Bisindolymaleimide 1	0	0	41	52	75	73	136	98%
Bisindolymaleimide 1	0.03	0	48	39	74	73	186	102%
Bisindolymaleimide 1	0.1	0	49	61	71	43	239	103%
Bisindolymaleimide 1	0.3	0	30	69	69	90	277	102%
Bisindolymaleimide 1	1	0	45	79	78	102	502	104%
Bisindolymaleimide 1	3	0	0	17	30	57	216	100%
Bisindolymaleimide 1	10	0	0	0	0	0	0	8%
Domoic Acid	0	0	41	46	50	87	275	104%
Domoic Acid	0	0	42	80	79	98	270	103%
Domoic Acid	0.003	0	28	62	82	114	256	104%
Domoic Acid	0.01	0	81	37	73	86	160	104%
Domoic Acid	0.03	0	36	52	44	69	466	104%
Domoic Acid	0.1	0	57	48	47	52	320	103%
Domoic Acid	0.3	0	60	40	28	58	287	103%
Domoic Acid	1	0	26	22	29	85	253	103%
Loperamide	0	0	109	33	55	59	113	103%
Loperamide	0	0	53	51	82	71	139	100%
Loperamide	0.1	0	15	36	48	44	147	104%
Loperamide	0.3	0	28	42	50	47	75	104%
Loperamide	1	0	0	9	25	69	47	97%
Loperamide	3	0	0	0	0	0	5	96%
Loperamide	10	0	0	0	0	0	0	17%
Loperamide	30	0	0	0	0	0	0	0%
Mevastatin	0	10	94	60	95	58	194	96%
Mevastatin	0	0	52	35	66	28	93	98%
Mevastatin	0.1	0	0	107	47	57	117	104%
Mevastatin	0.3	0	32	67	46	59	212	99%
Mevastatin	1	0	41	115	37	67	164	69%
Mevastatin	3	0	24	151	56	41	78	64%
Mevastatin	10	0	0	0	0	96	69	42%
Mevastatin	30	0	0	0	0	0	0	36%
Vanadate	0	0	32	42	73	61	112	103%
Vanadate	0	0	128	59	87	103	235	97%
Vanadate	0.1	0	0	66	45	63	111	104%
Vanadate	0.3	0	78	45	92	81	199	103%
Vanadate	1	0	146	70	102	51	320	98%
Vanadate	3	0	0	322	34	63	143	101%
Vanadate	10	0	18	53	53	91	302	103%
Vanadate	30	0	47	50	57	71	241	81%

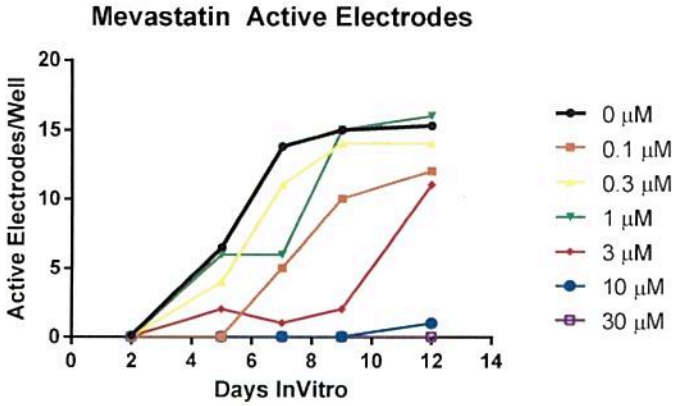
Cortical Culture 4.2.14

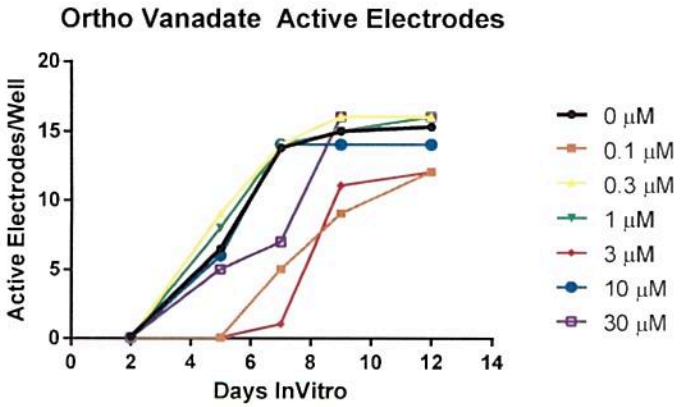












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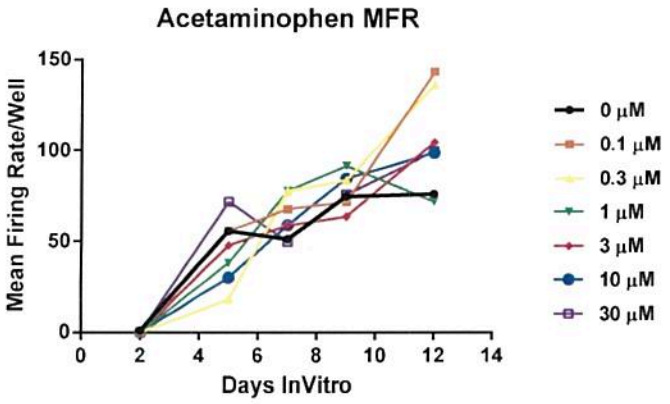
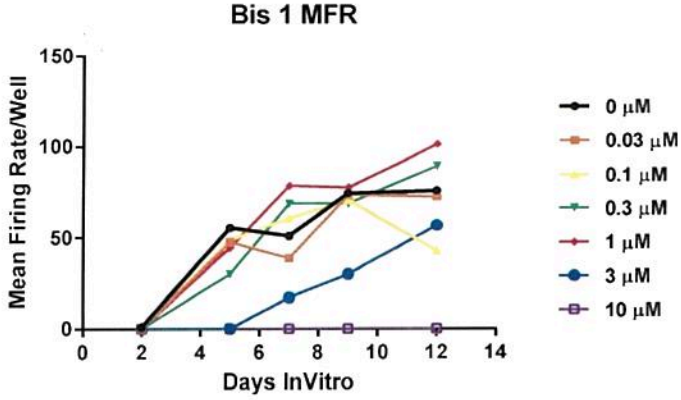
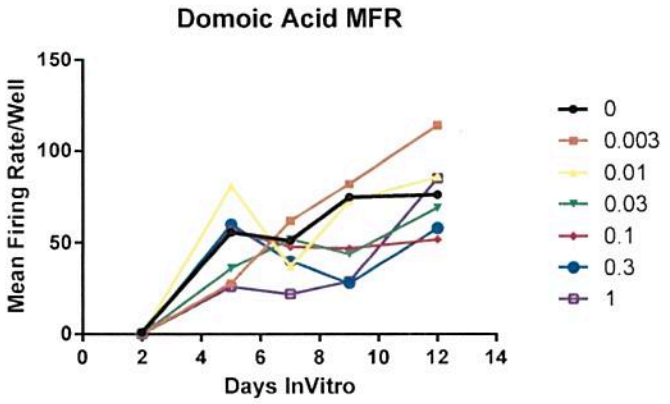
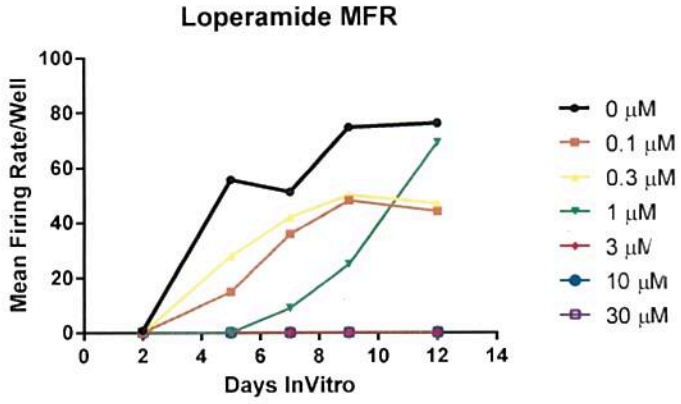
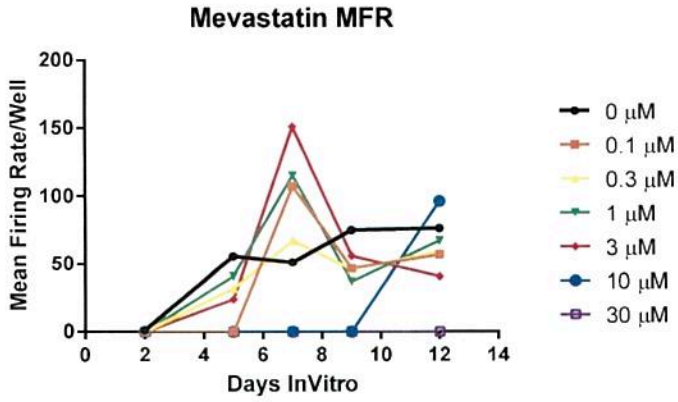
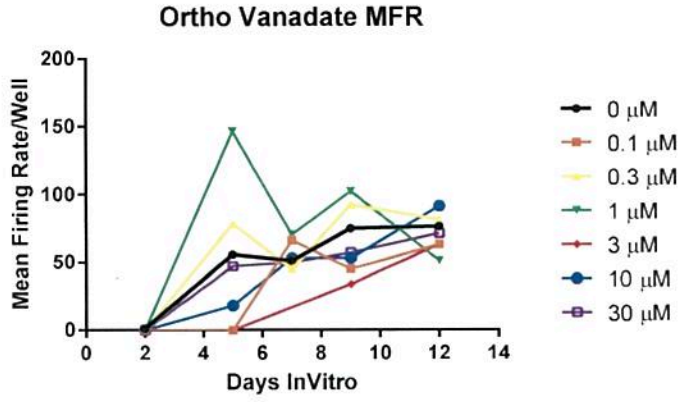

Acetaminophen MFR
Mean Firing Rate/Well
Days InVitro
0 µM
0.1 µM
0.3 µM
1 µM
3 µM
10 µM
30 µM


Bis 1 MFR
Mean Firing Rate/Well
Days InVitro
0 µM
0.03 µM
0.1 µM
0.3 µM
1 µM
3 µM
10 µM


Domoic Acid MFR
Mean Firing Rate/Well
Days InVitro
0
0.003
0.01
0.03
0.1
0.3
1

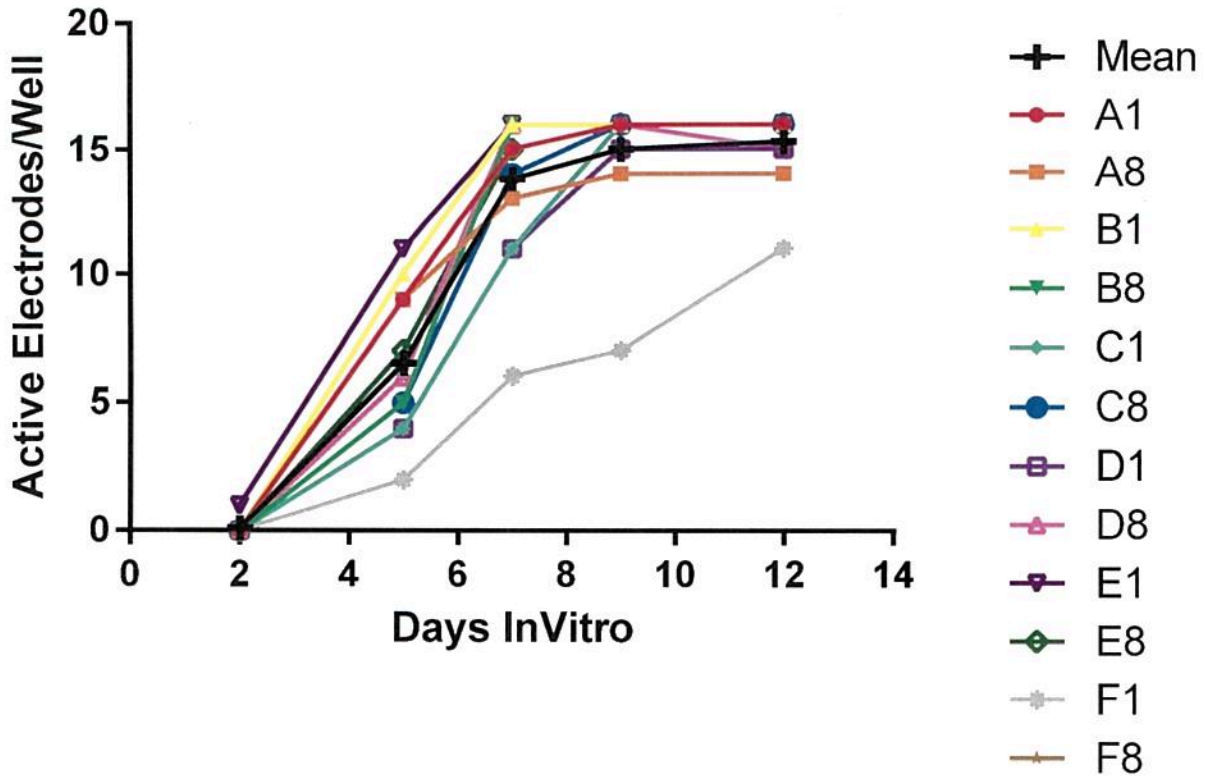

Loperamide MFR
Mean Firing Rate/Well
Days InVitro
0 µM
0.1 µM
0.3 µM
1 µM
3 µM
10 µM
30 µM


Mevastatin MFR
Mean Firing Rate/Well
Days InVitro
0 µM
0.1 µM
0.3 µM
1 µM
3 µM
10 µM
30 µM

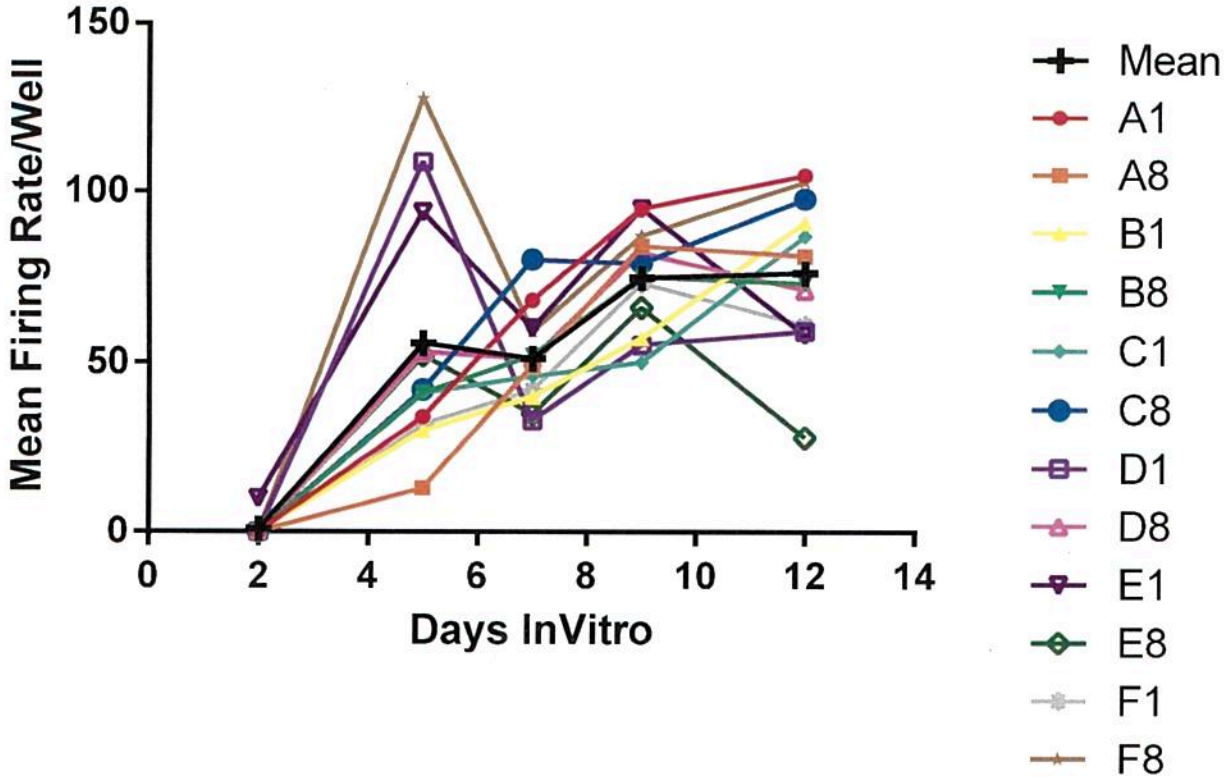

Ortho Vanadate MFR
Mean Firing Rate/Well
Days InVitro
0 µM
0.1 µM
0.3 µM
1 µM
3 µM
10 µM
30 µM

Cortical Culture 4.2.14

Negative Controls Active Electrodes



Negative Controls MFR



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