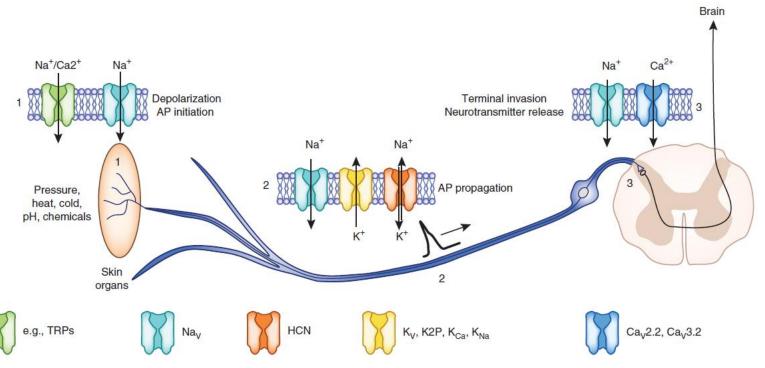
# In Vitro DRG Assay Development Using Multi-well MEAs

John Graef, Ph.D. Bristol Myers Squibb

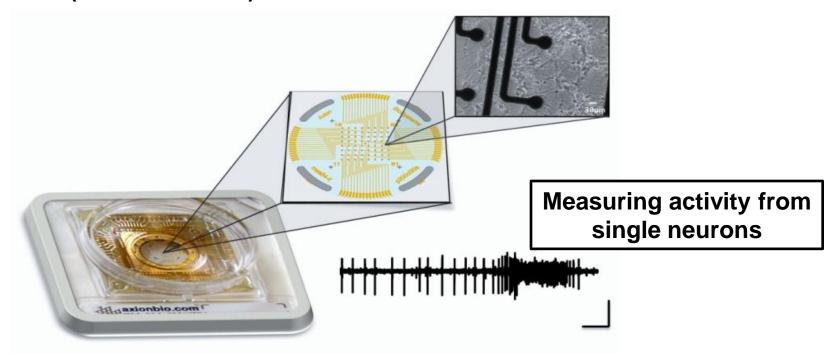
# **Dorsal Root Ganglion (DRG)**

- DRG neurons transmit sensory information from periphery to central nervous system
  - Little spontaneous activity under normal conditions in vitro
  - Hyperexcitable following nerve injury or inflammation



# Multielectrode arrays (MEAs)

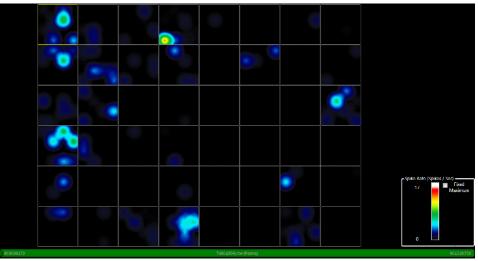
- Tightly spaced grid of electrodes allowing for multi-site recording and stimulation of primary cultures and tissues
  - Culture primary neurons directly onto grid investigate spontaneous and evoked electrical activity within cellular networks over long periods of times (weeks to months)



#### **MEAs: Increased throughput**

- Multi-well MEA system from Axion Biosystems with 12 and 48 well MEA plates
  - 768 electrodes divided among the wells
    - 48 well plate 16 electrodes per well
    - 12 well plate 64 electrodes per well





#### **Custom Analysis Program**

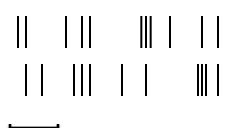


Load File											
Experiment Info Active Electrodes Delect time to analyze Select time to analyze											
Date	Threshold for						Select Metric			Entire File	
File	Active Electrodes	Condition				# of Acti	# of Active Electrodes			Selected time range	
riie	Spikes/min	1	2	3	4	# of Acti	ive Electrodes			From (s): To (s):	
Plate ID	Min # Active Electrodes per Well	A				Average	Spikes per M	inute		.,	
Notes		В				Average Spike Duration (ms)  Crosscorrelation Probability				Sort Spikes by Well	
		С									
	Electrodes	Mean Std								Enter cross-correlation parameters	
Choose Burst Detection Method		n				Average Bursts per Minute				Bin Size (ms) 1	
Network Burst Detection					_	Average Burst Length (s)  # Spikes per Burst  Cross-Correlation Window (s) 1					
	Total Spikes in a Burst (%)										
Rate Method	Spike Frequency in Burst (Hz)										
Min Rate (Hz) 100 ISI	48 Well Plate				Average Interburst Interval (s)						
Bin Size (s) 0.05 Mi	in Spikes 10						Select Metric				
Min IBI (s) 0.2		Condition									
Min IBI (s) 0.2		1	2	3	4	5	6	7	8	# of Active Electrodes	
Apply	Apply	A	_	_			_	-	_	Calculate	
		В									
Single Channel Burst Detection		С								Export Stats to Excel	
<ul><li>Surprise Method</li></ul>	Interval Method	D									
Min Surprise 3	SI (s) 0.1	E F									
	Min Spikes 5	Mean									
Min Spikes 3	- Spines	Std									
ISI limit (s) Default		n									
Apply	Apply										
1.66.1											

#### **Network Analysis**

- Synchronous activity
  - Average peak cross correlation between all active electrodes in a well
  - Quantification of network bursts

#### Raster Plot

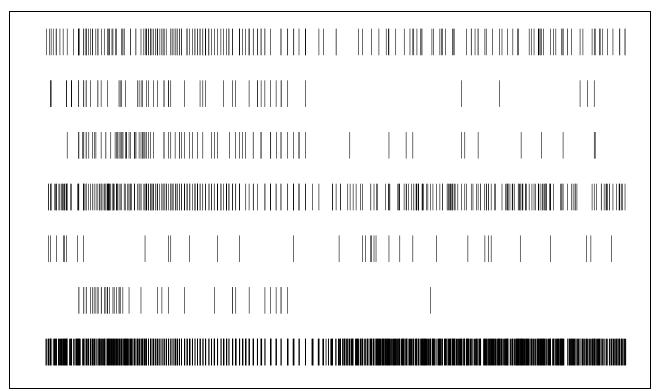


500ms window

SAMPLE CROSSCORRELOGRAMS

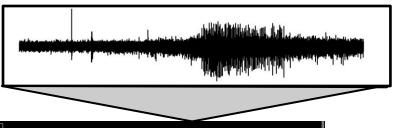


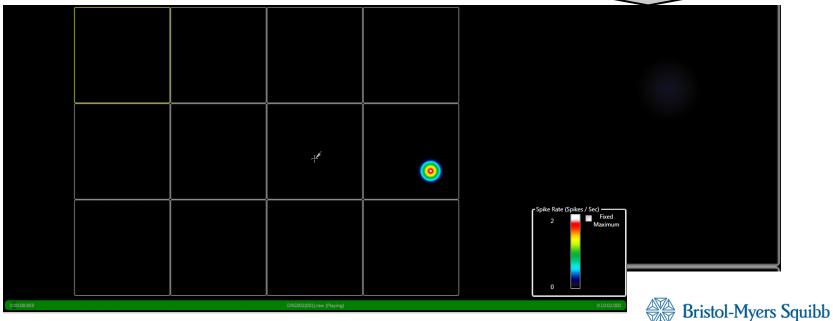




# Early attempts at DRG activity

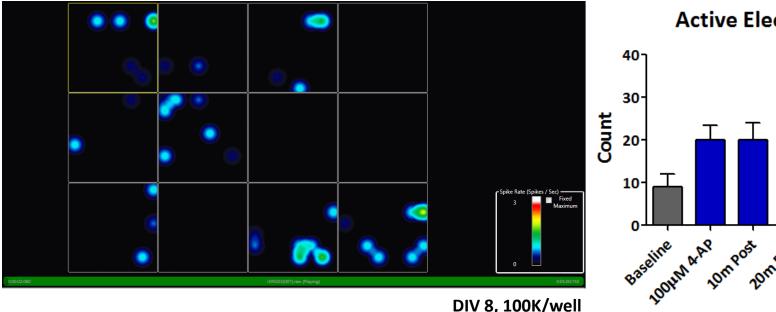
- No spontaneous activity at several different plating densities (50K, 100K, 150K, 250K)
  - Only evoked activity with 1µM capsaicin, 200µM menthol
- 100ng/ml NGF added to media
  - Small amounts of variable spontaneous activity

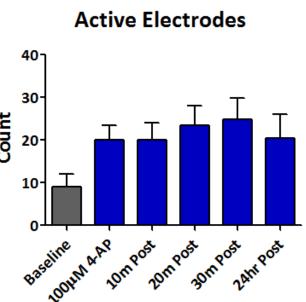




#### Increasing spontaneous activity

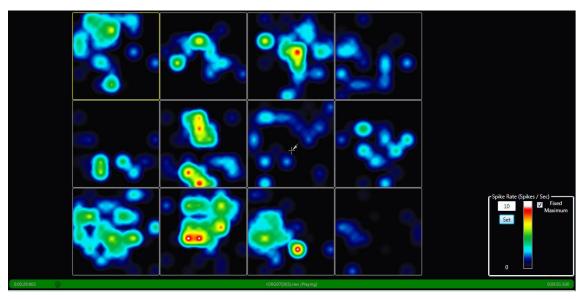
- Addition of anti-mitotic agents increased the number of active electrodes (> 5 spikes/min)
- 100µM 4-AP lead to an increased and sustained increase in the number of active electrodes and spikes per minute





#### **Optimized culture conditions**

 Optimization of culture media and conditions significantly increases spontaneous activity



Rat DRG @ 150K

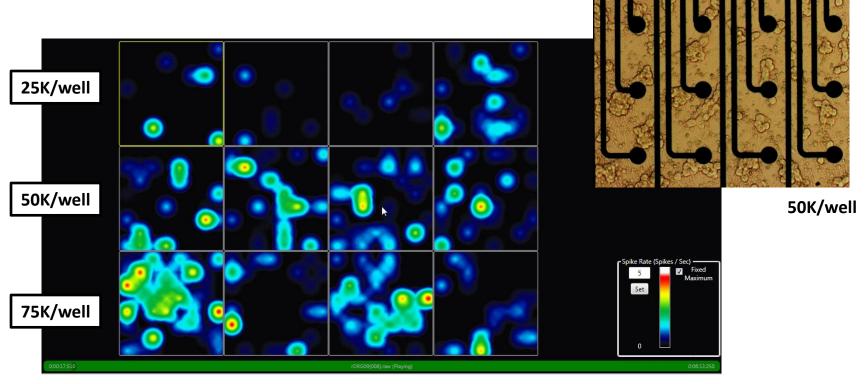
NB (Optimized)

NB (Optimized)

**DIV 4, 100K/well** 

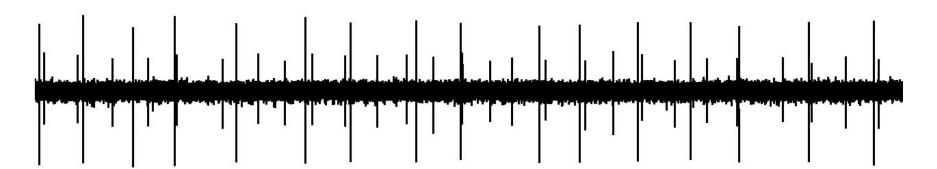
# **Optimized conditions allows for** lower plating densities

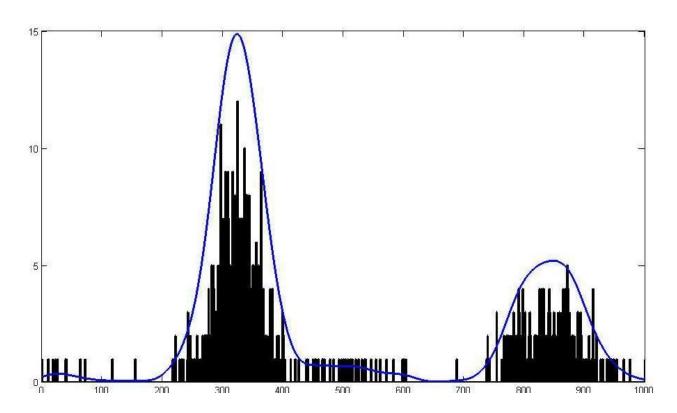
Increasing amounts of spontaneous DRG activity starting at 25K cells per well



**DIV 11** 

# **DRG Spike Sorting Based on Amplitude**



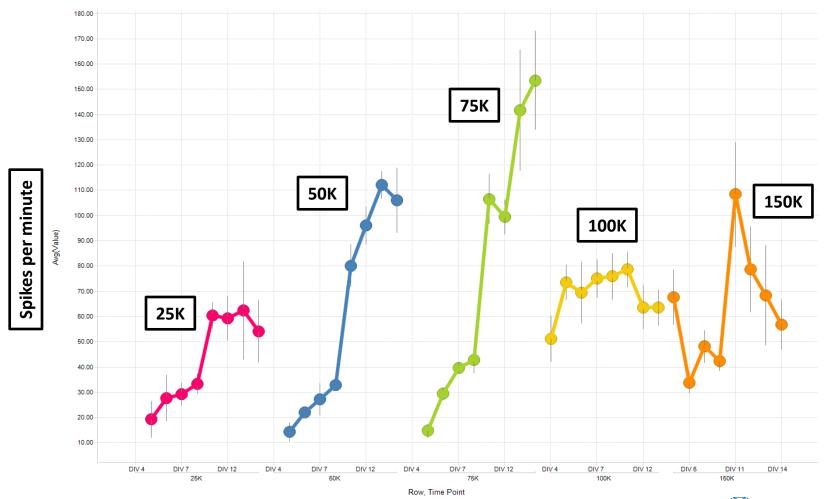




1s

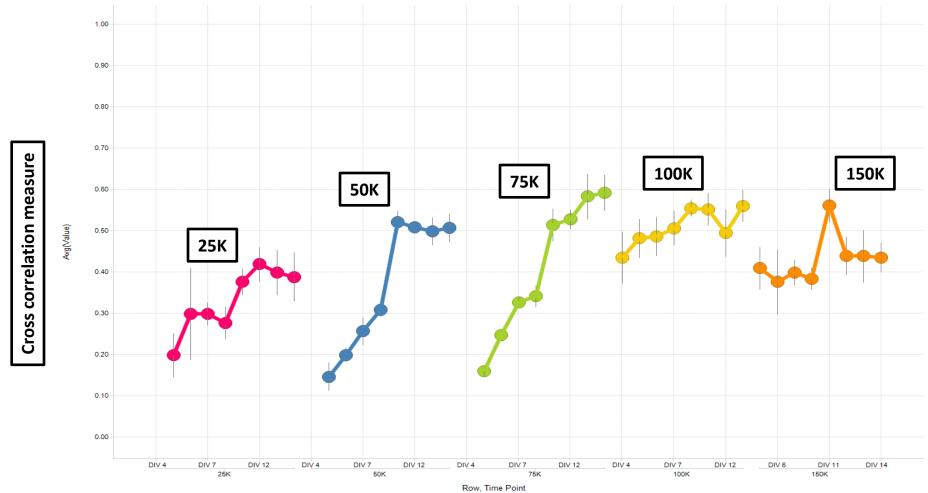
# **Density-dependent activity levels**

Average spontaneous activity starting at DIV 4 through DIV 14



#### Time-dependent network formation

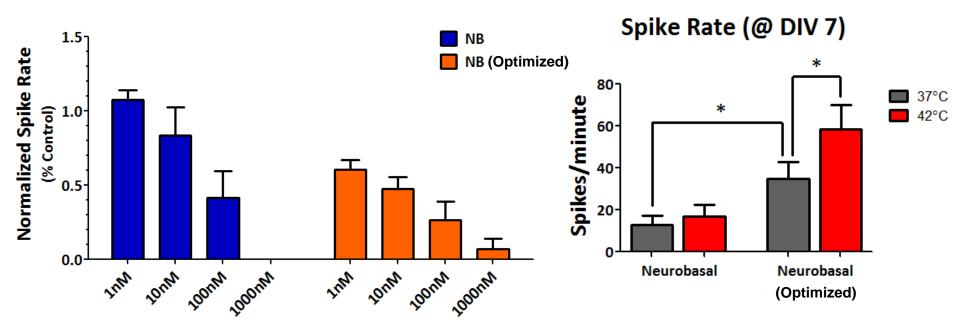
Average peak cross correlation measure (DIV 4 through DIV 14)



#### **DRG** characterization

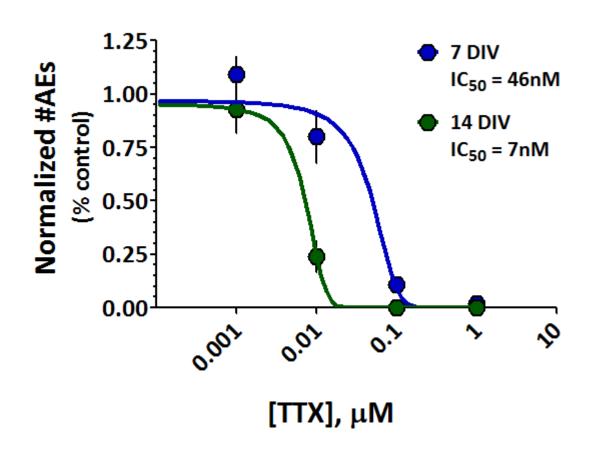
- Concentration dependent reduction in spike rate with TTX
- Increased spike rate in response to higher temperatures

TTX Effects on Spike Rate (@ DIV 7)



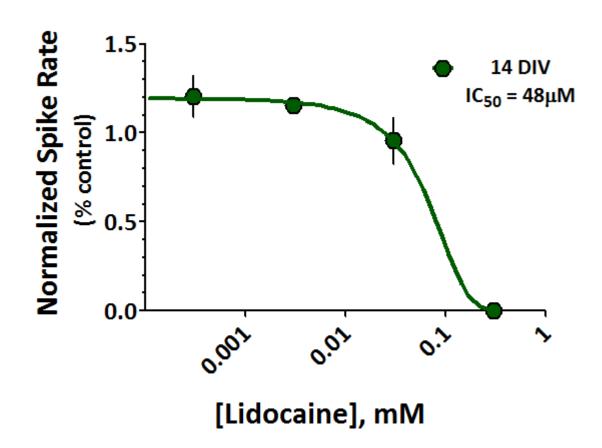
#### **DRG** characterization

Increased sensitivity to TTX in older cultures



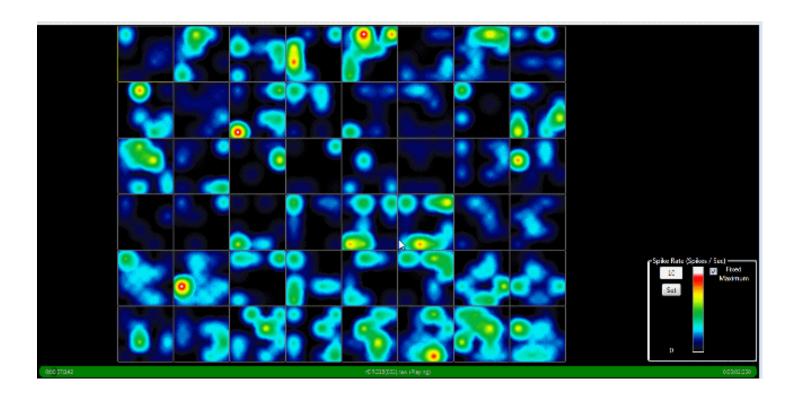
#### **DRG** characterization

Effect of Lidocaine on spontaneous activity at DIV 14



#### **Culture Conditions Scale to 48 well Plate**

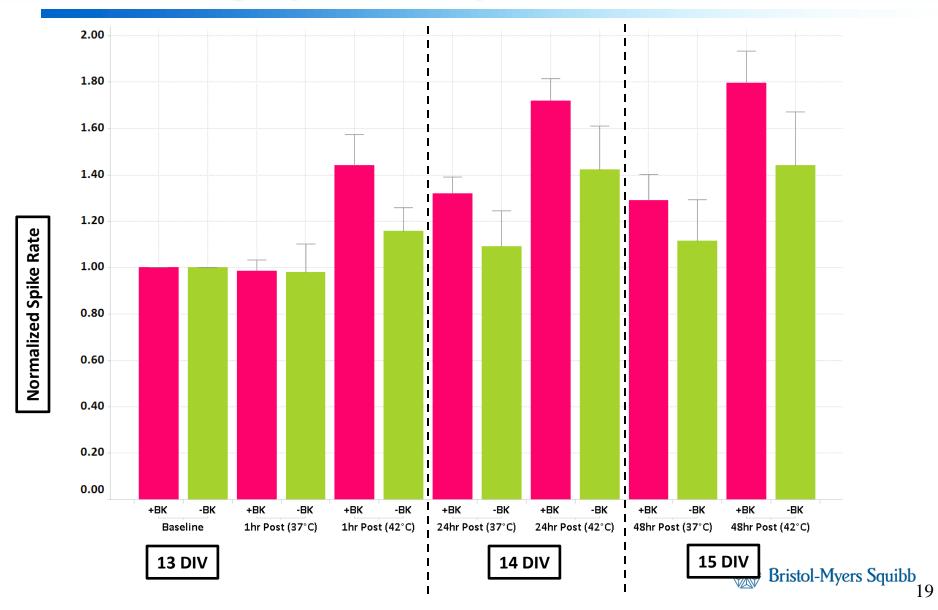
Similar spontaneous activity to 12 well plates



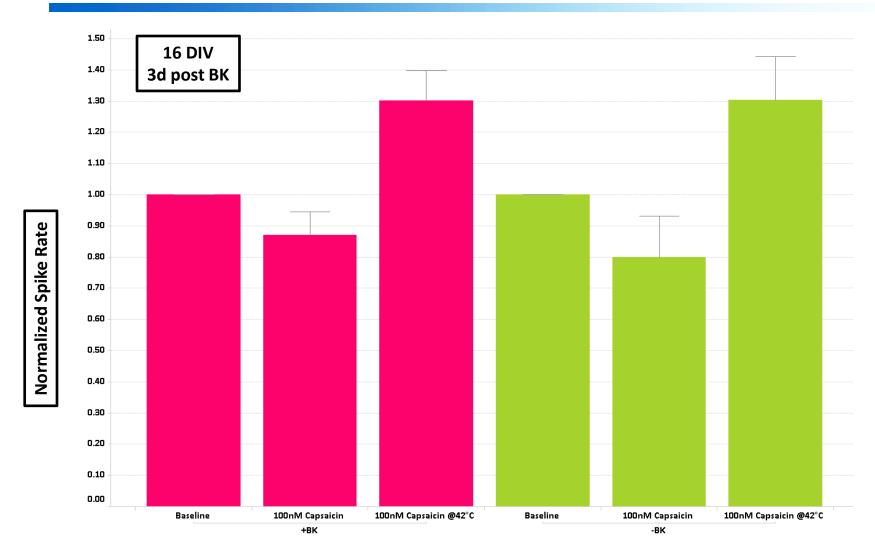
#### In vitro Pain Model?

- NGF and bradykinin have been shown to sensitize isolated DRGs to noxious stimuli such as capsaicin and heat (Zhu et al. 2004, J Neurophysiol)
- Optimized culture conditions with NGF increases activity and heat responses in MEA
- Can bradykinin sensitize further?

# No significant sensitization to heat following 1µM bradykinin treatment



# No change in response to capsaicin 3d after bradykinin treatment



#### Summary

- Robust levels of activity can be induced in DRG cultures with optimized culturing conditions in both 12 and 48 well plates
- DRGs show optimal network activity between 11 and 14 DIV when plated at 50-75K cells/MEA
- DRGs show increased activity levels in response to increased temperatures
- Older cultures show greater sensitivity to TTX
- Bradykinin doesn't appear to further sensitize spontaneously active DRG cells

#### Acknowledgements

- Kim Newberry
- Shuya Wang
- Axion Biosystems
  - Daniel Millard