In Vitro DRG Assay Development Using Multi-well MEAs

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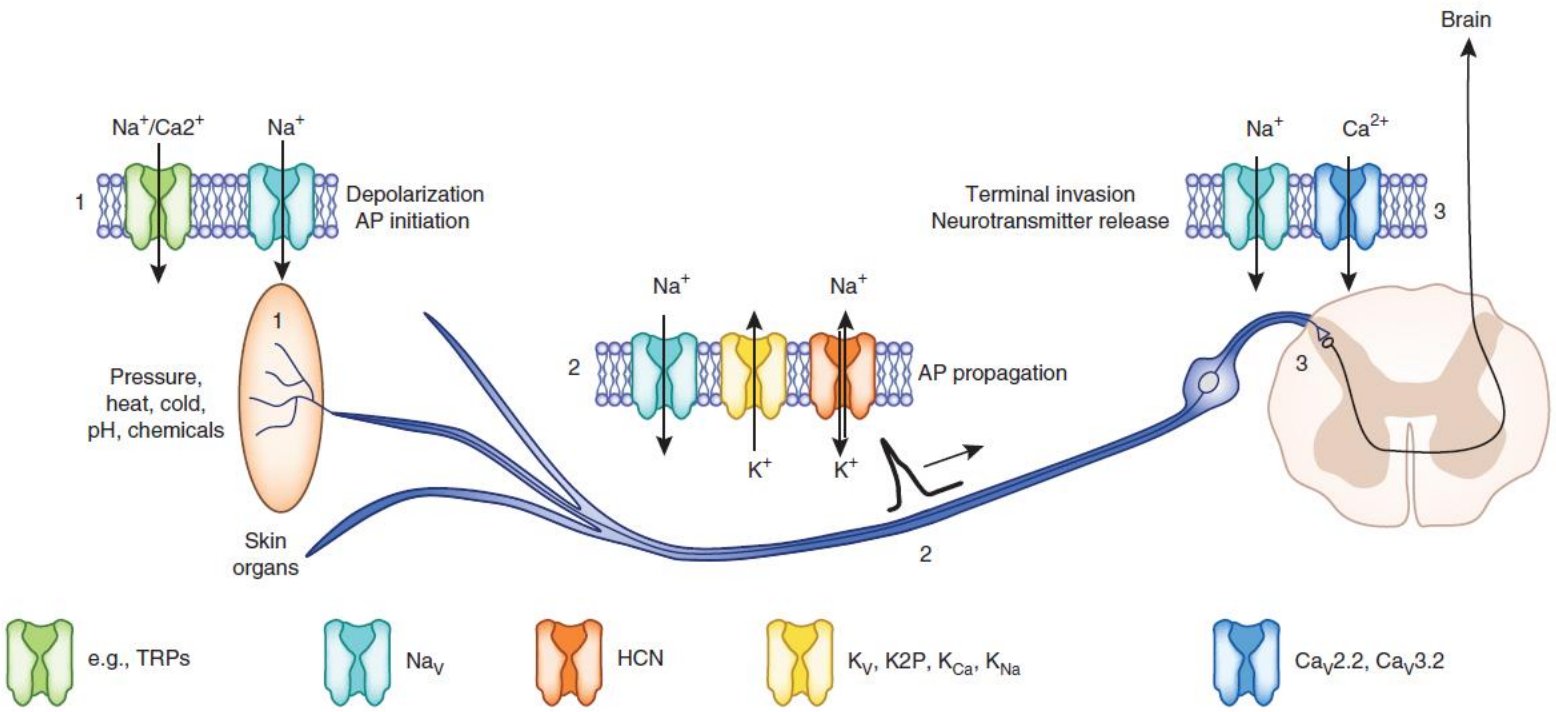
Dorsal Root Ganglion (DRG)

- **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**



Waxman and Zamponi, 2014, *Nat Neurosci*

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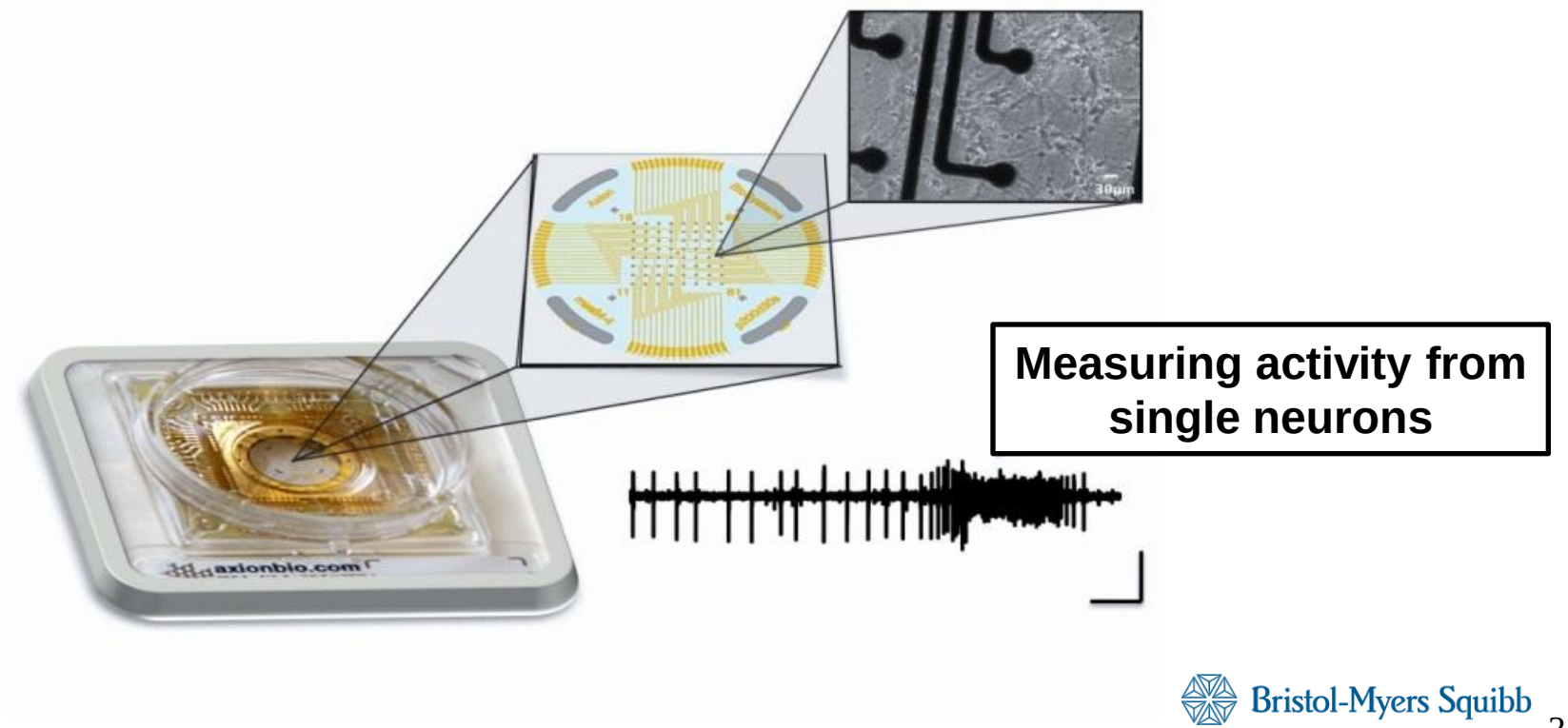
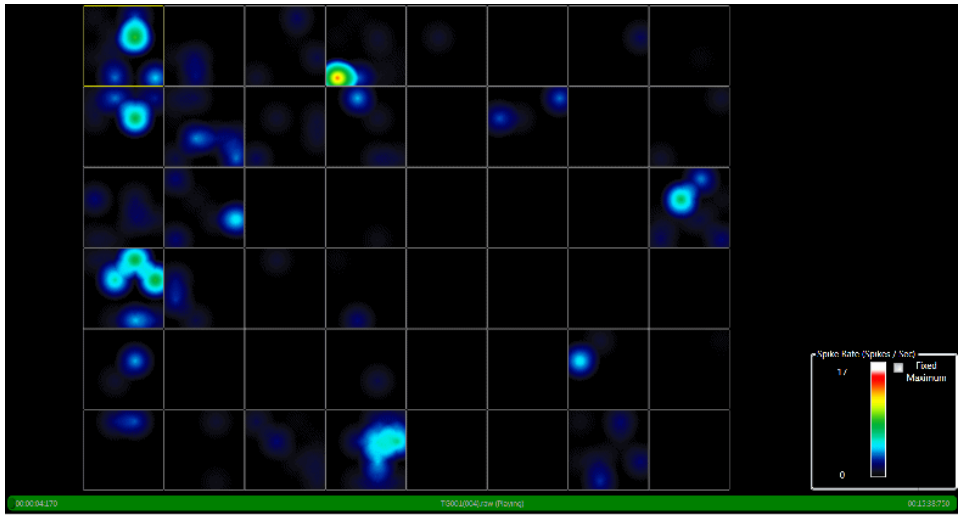
Multielectrode arrays (MEAs)

- **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



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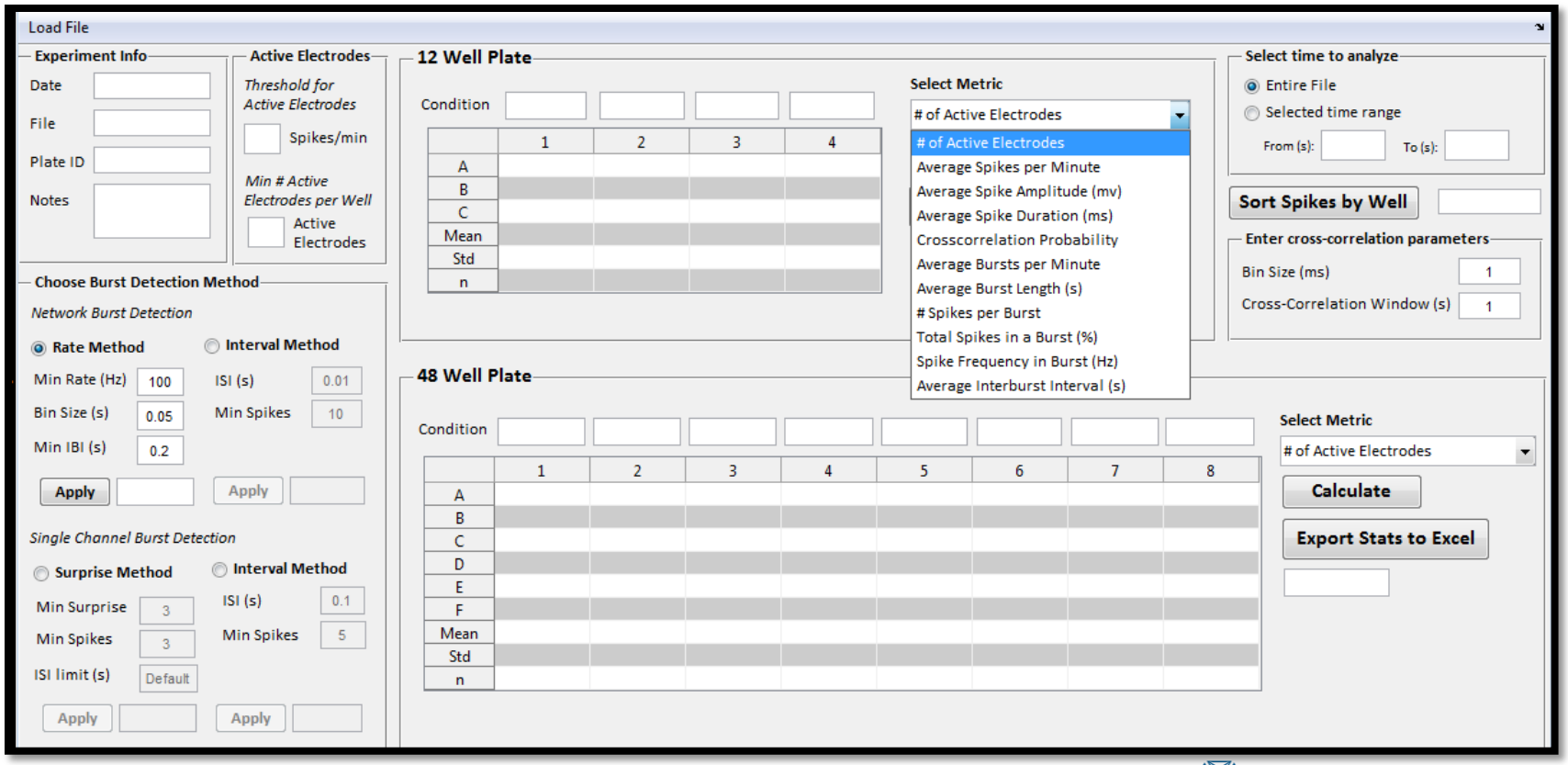
Custom Analysis Program



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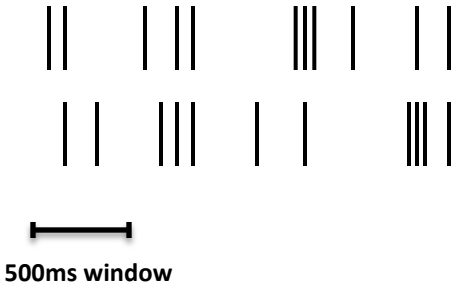
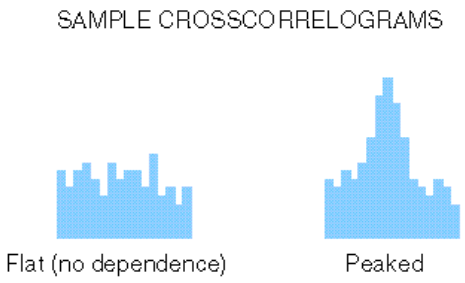
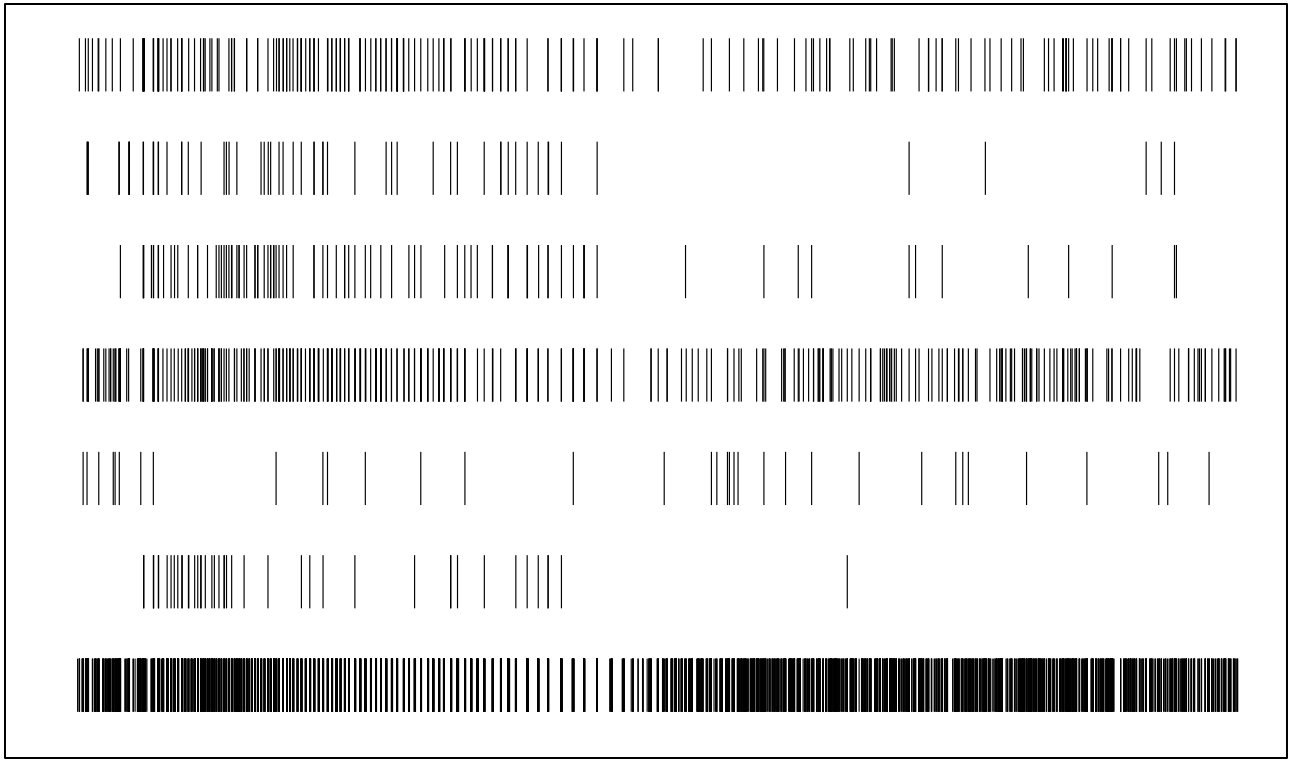
Network Analysis

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

Raster Plot







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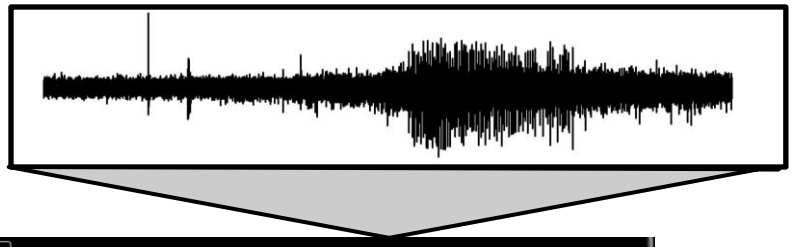
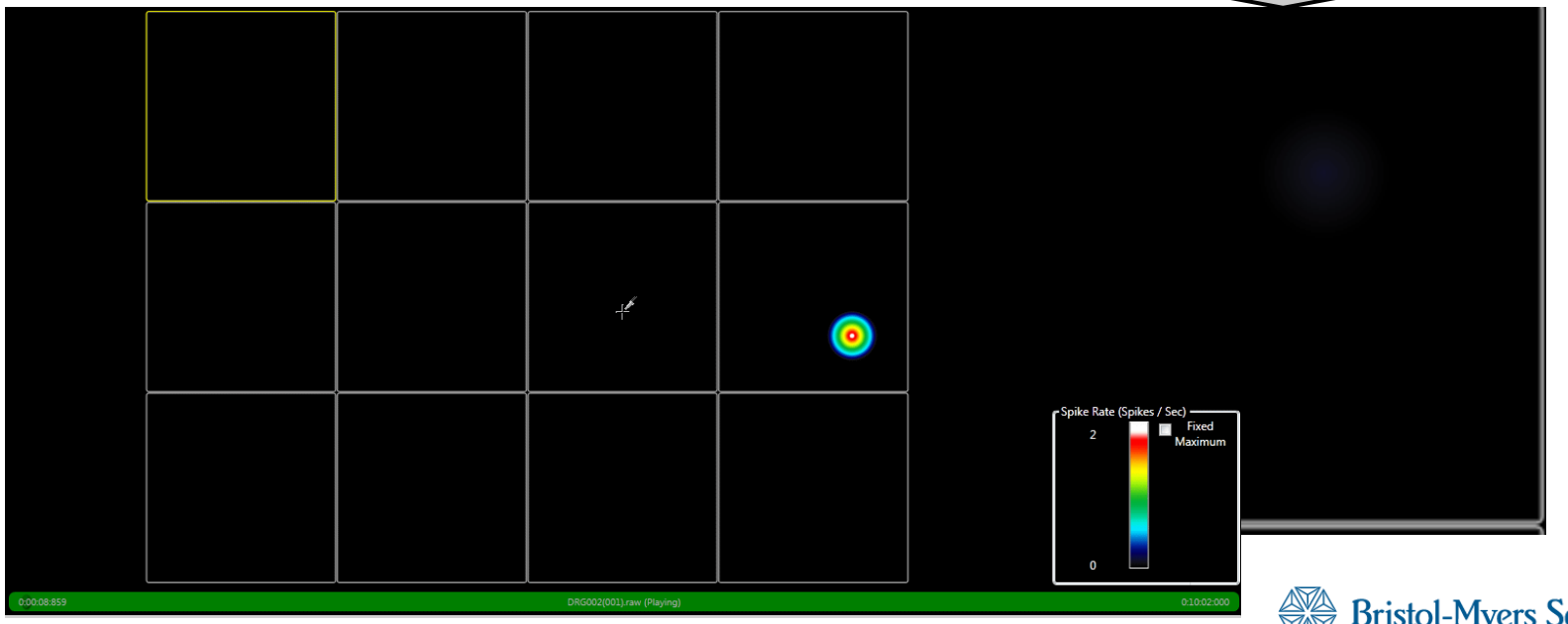
Early attempts at DRG activity

- 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





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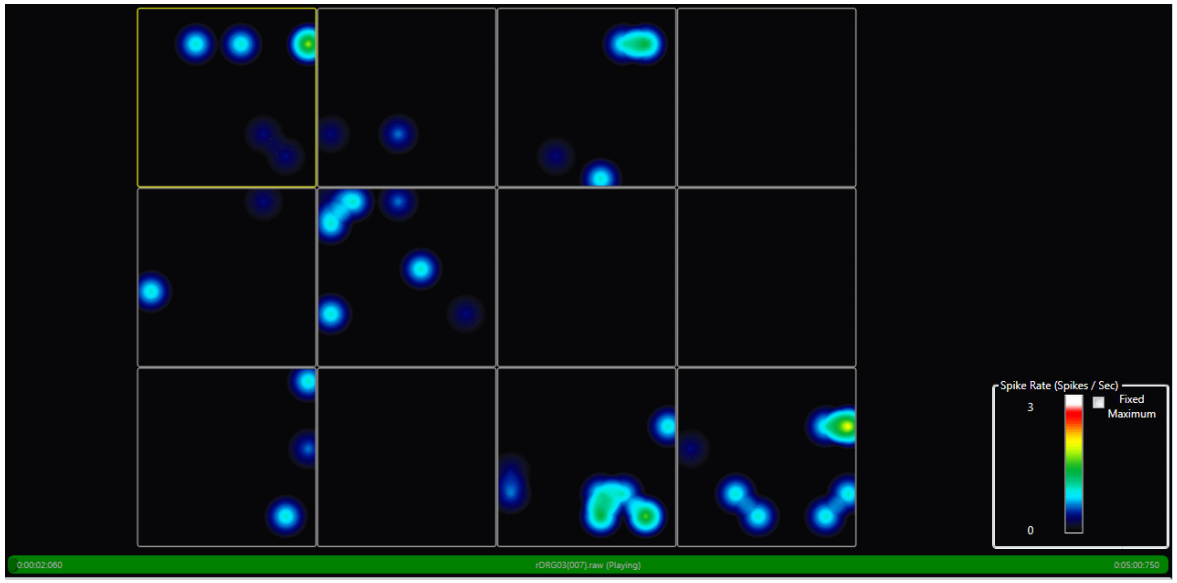
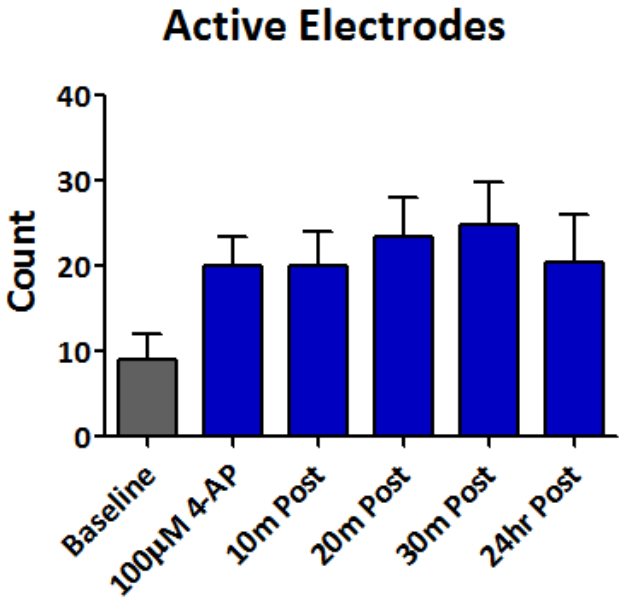
Increasing spontaneous activity

- 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

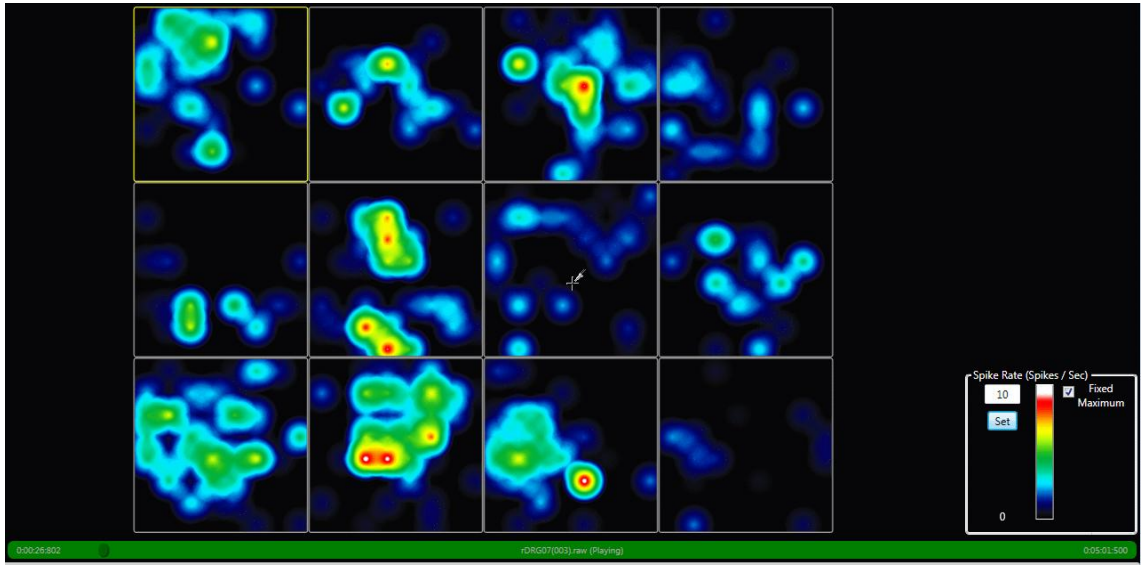


DIV 8, 100K/well

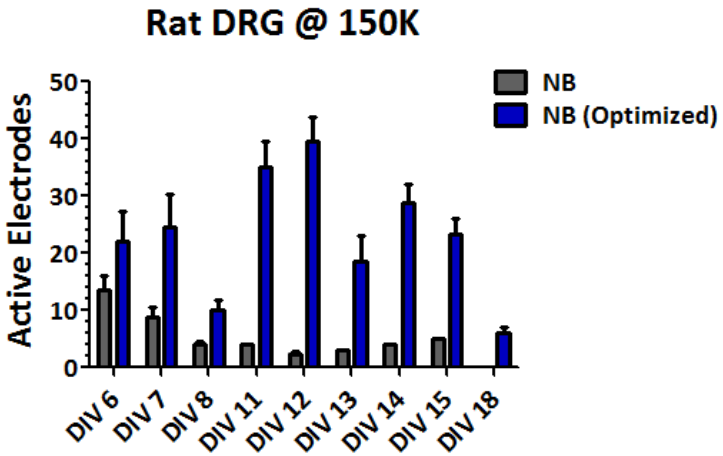


Optimized culture conditions

- **Optimization of culture media and conditions significantly increases spontaneous activity**



DIV 4, 100K/well



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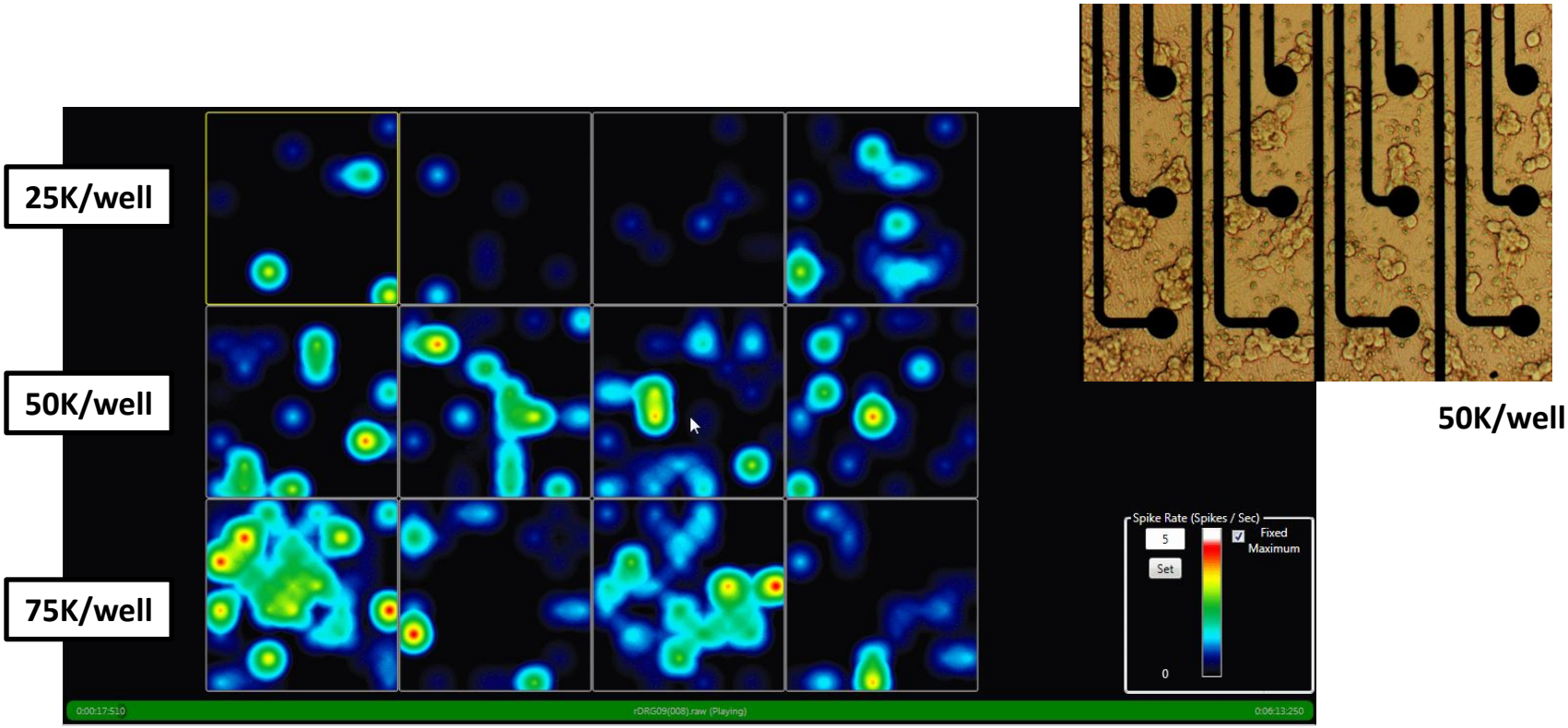
Optimized conditions allows for lower plating densities

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



50K/well

DIV 11

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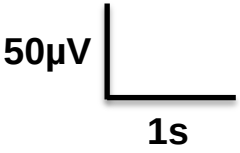
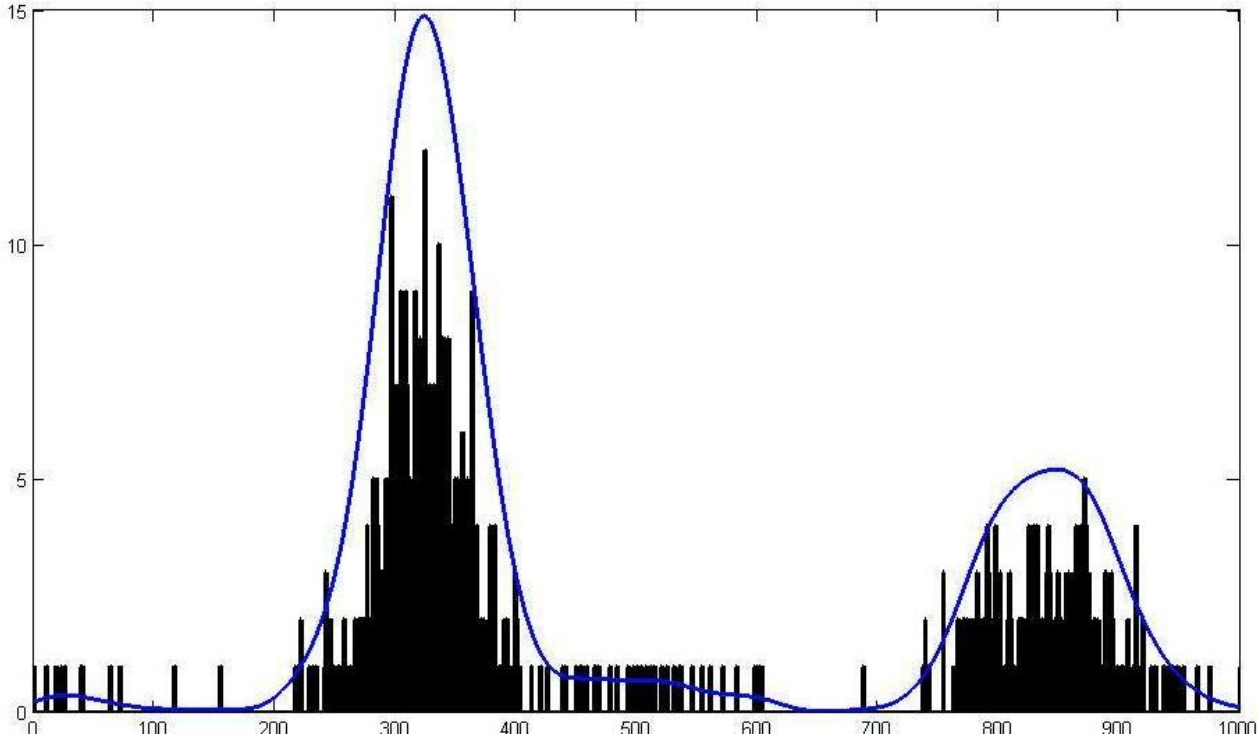
DRG Spike Sorting Based on Amplitude





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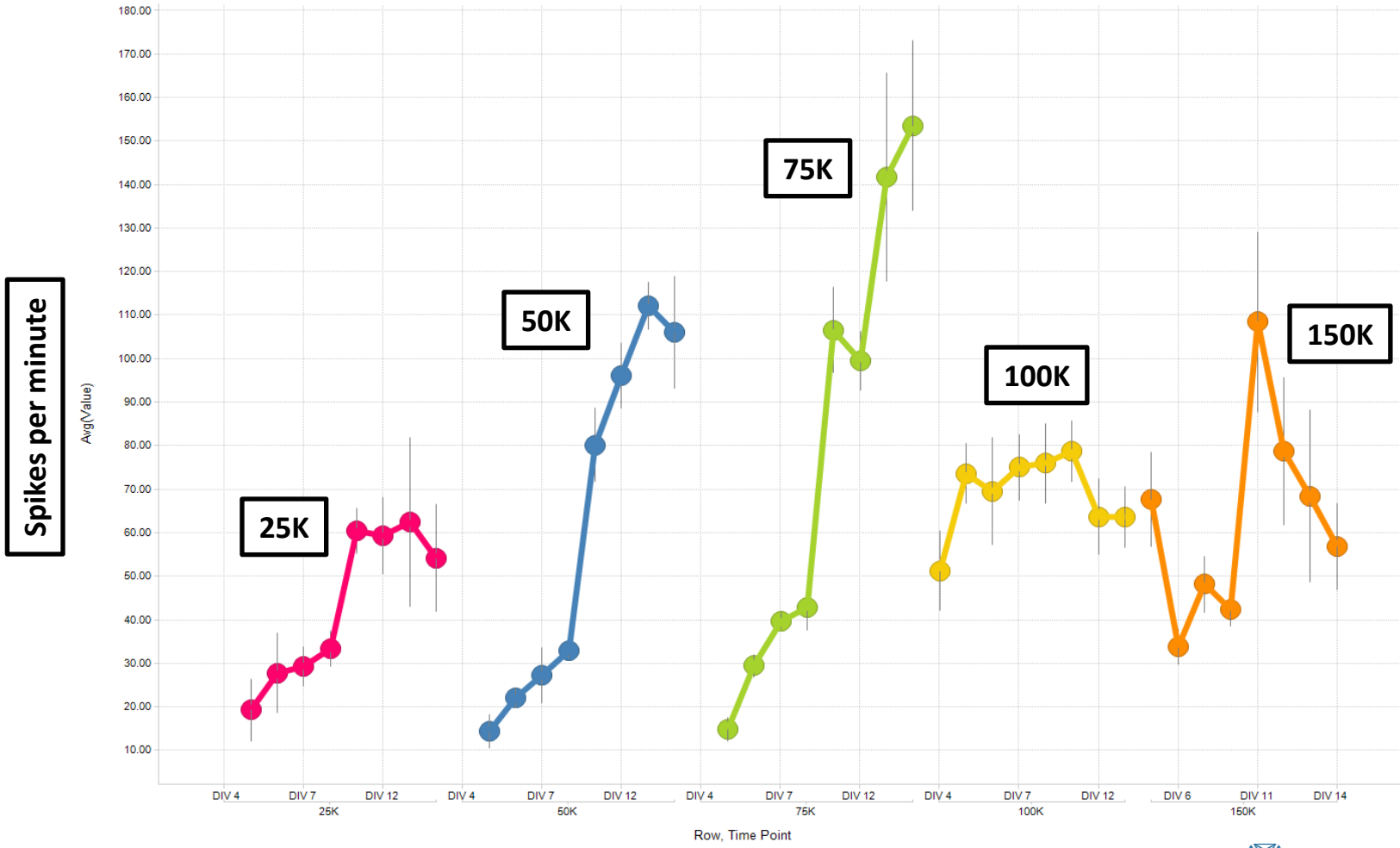
Density-dependent activity levels

- Average spontaneous activity starting at DIV 4 through DIV 14



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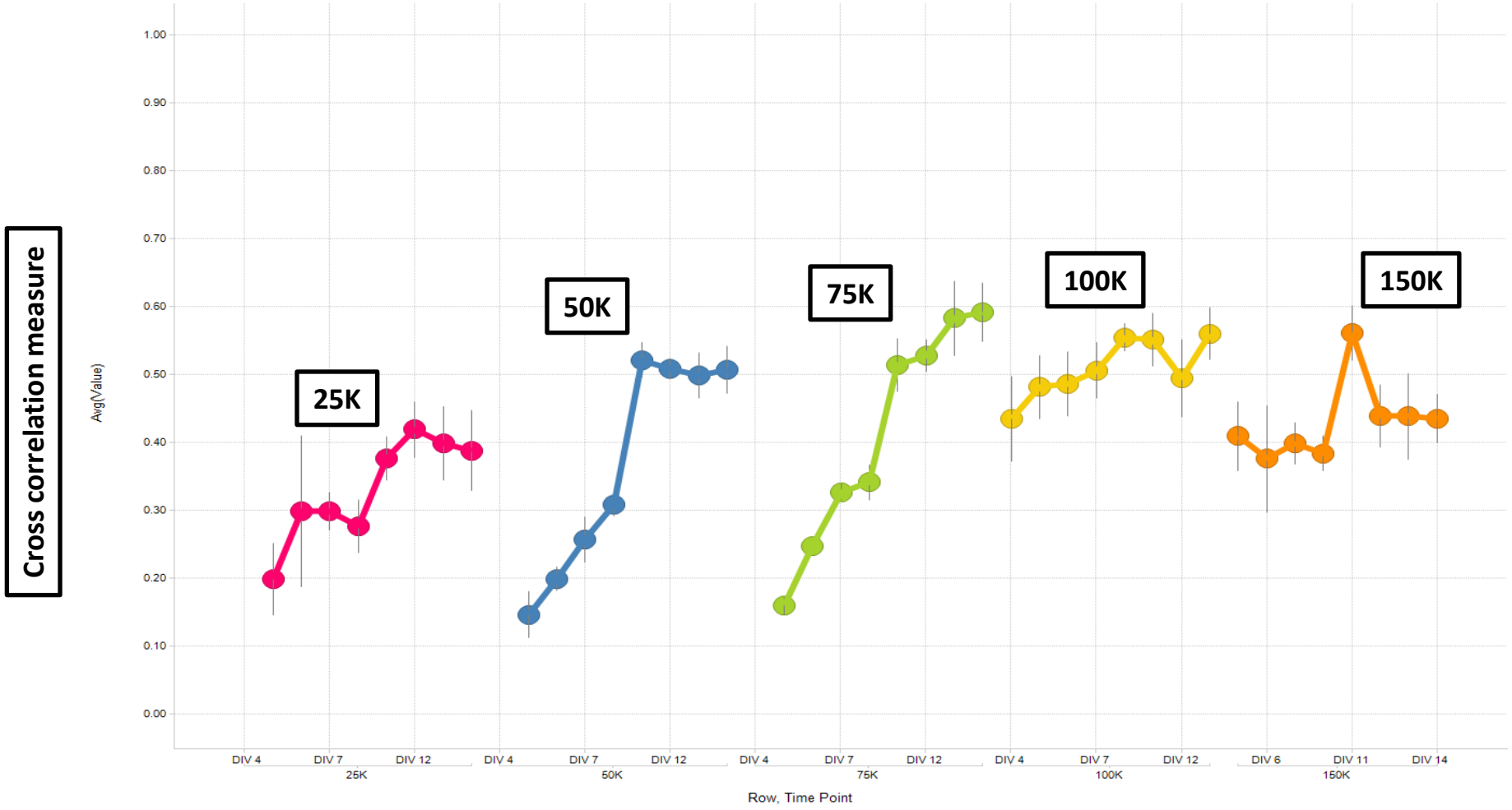
Time-dependent network formation

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



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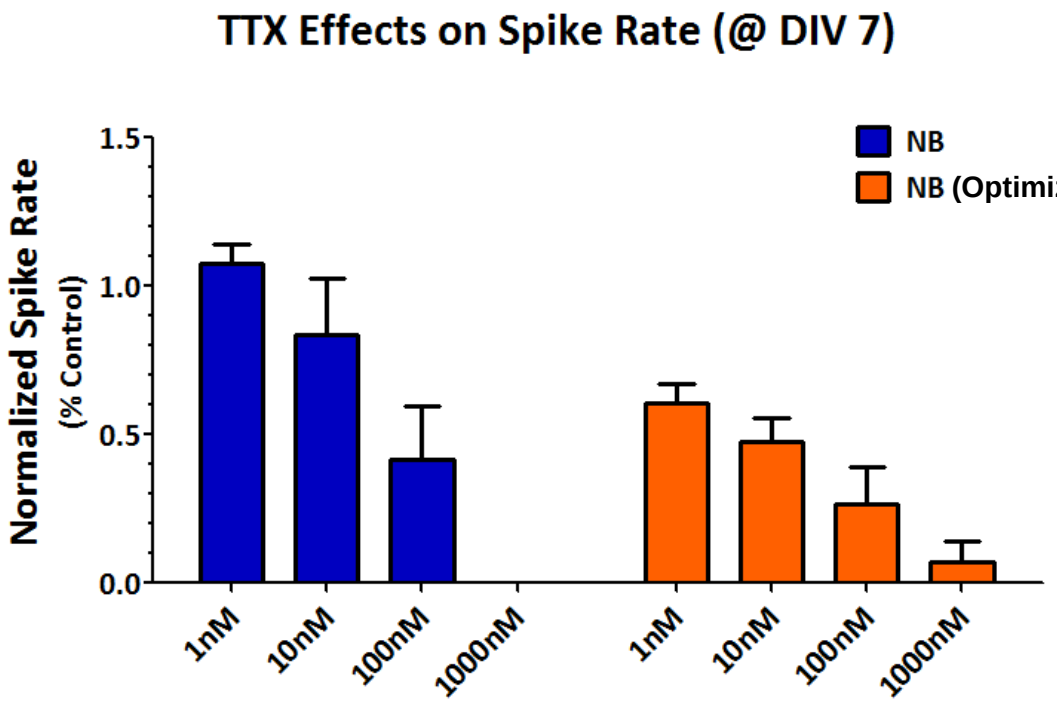
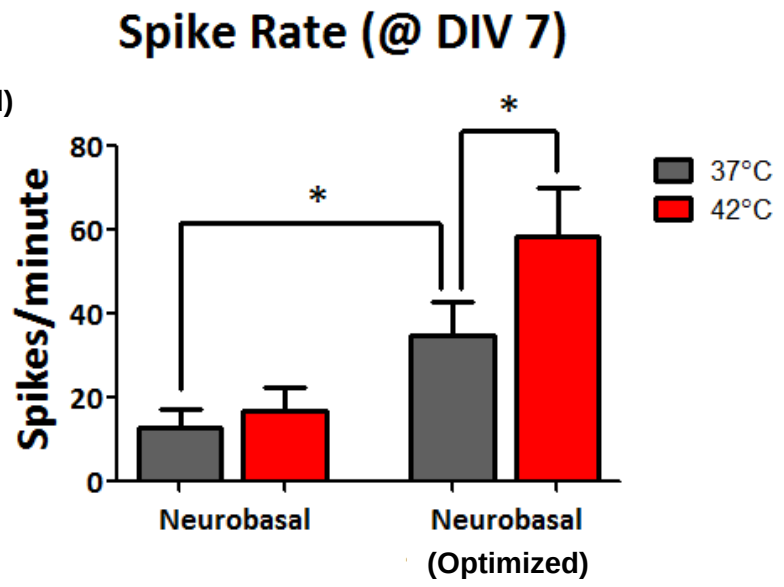
DRG characterization

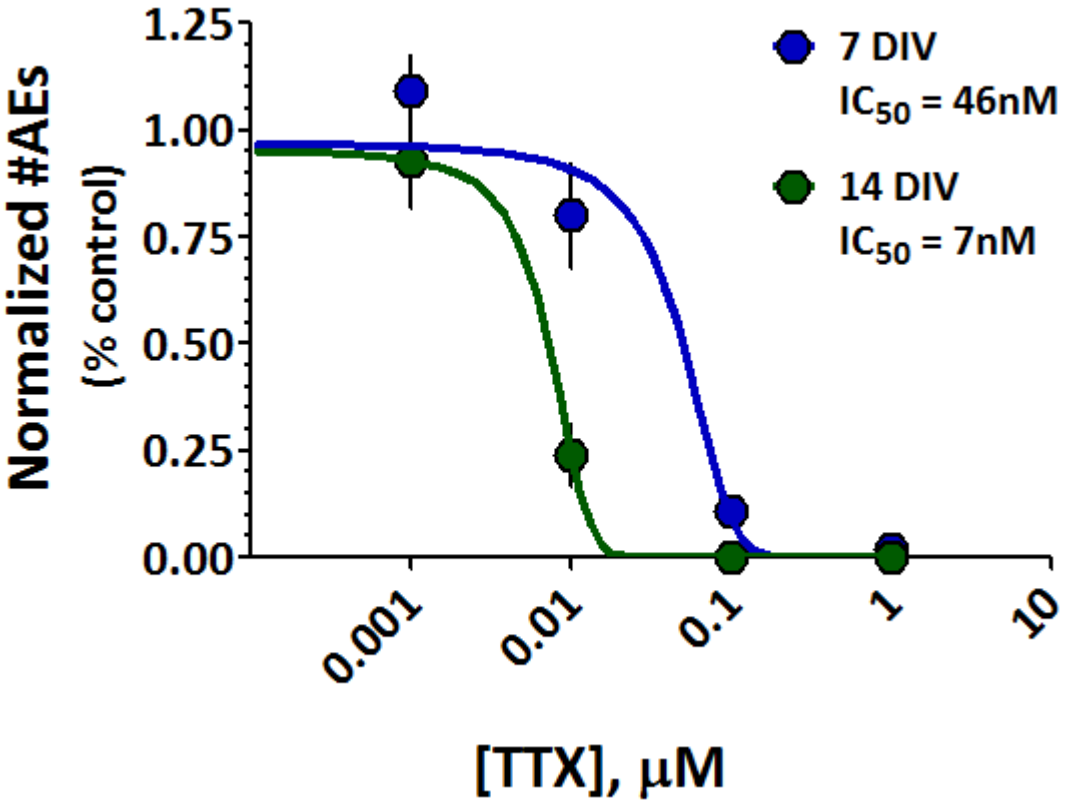
- Concentration dependent reduction in spike rate with TTX
- Increased spike rate in response to higher temperatures





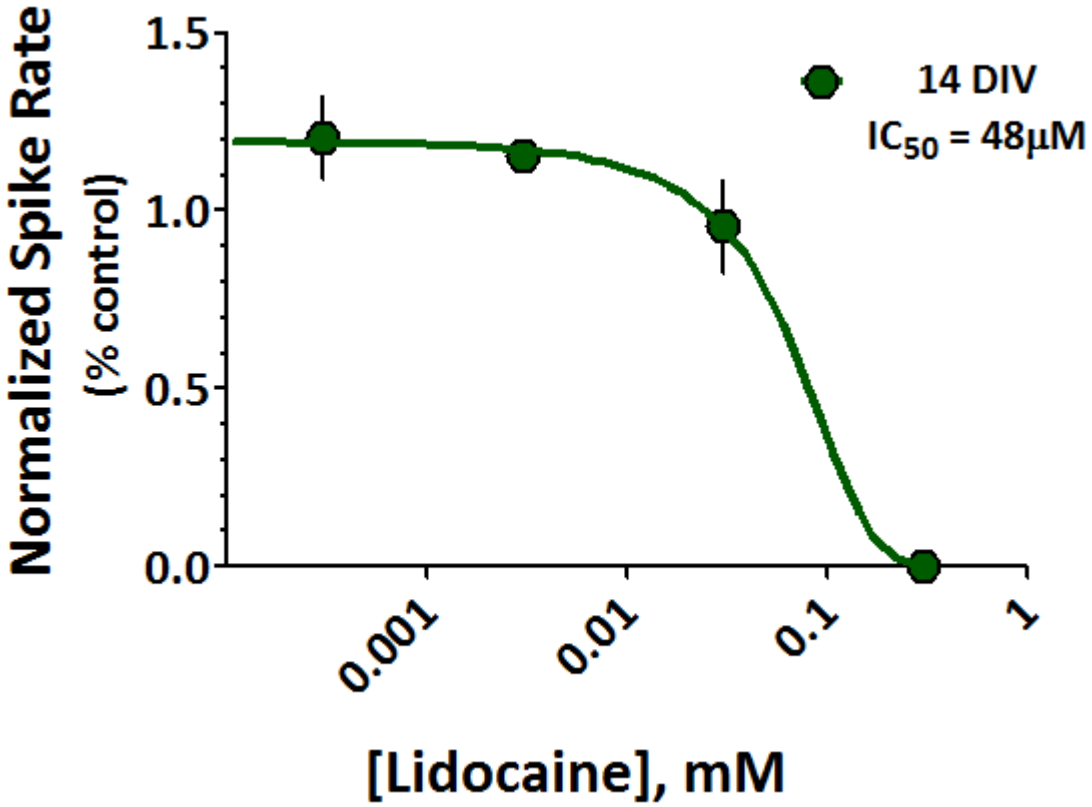
DRG characterization

- Increased sensitivity to TTX in older cultures



DRG characterization

- **Effect of Lidocaine on spontaneous activity at DIV 14**



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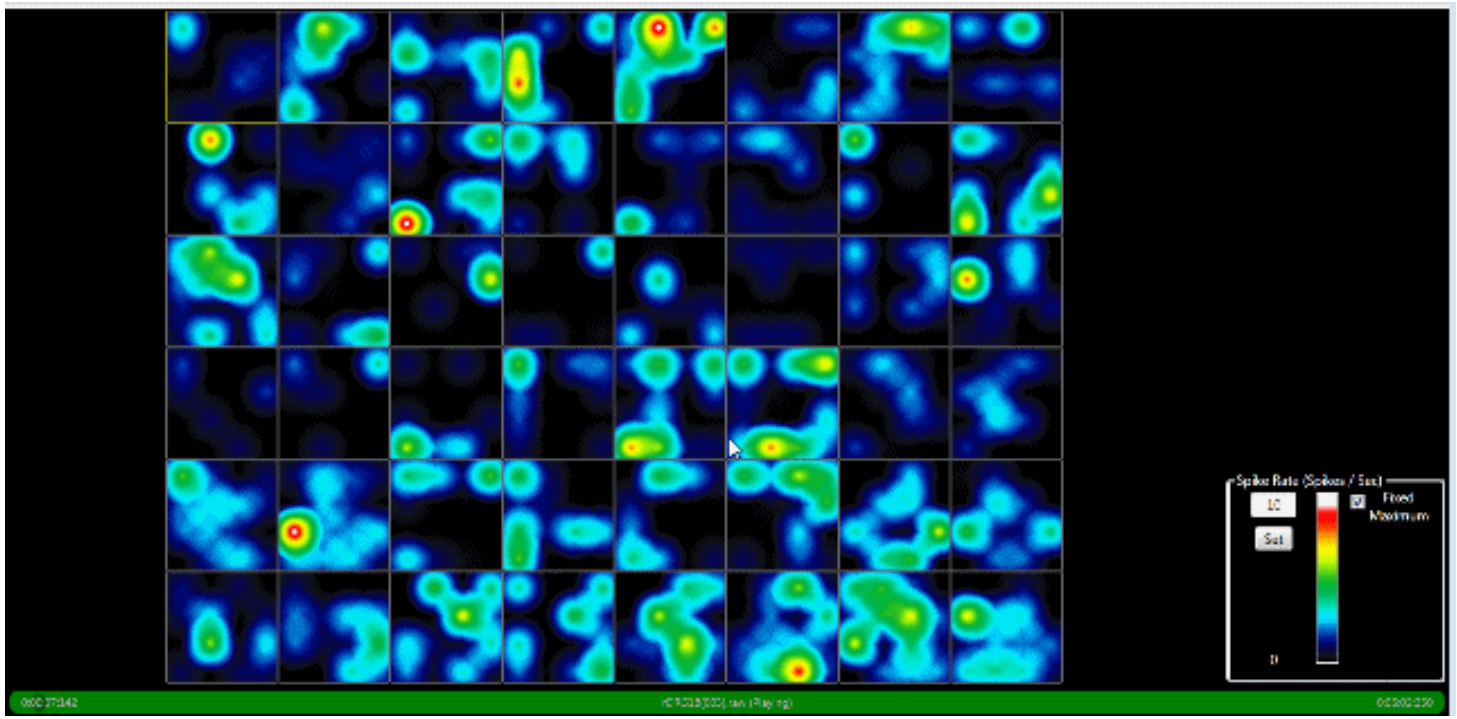
Culture Conditions Scale to 48 well Plate

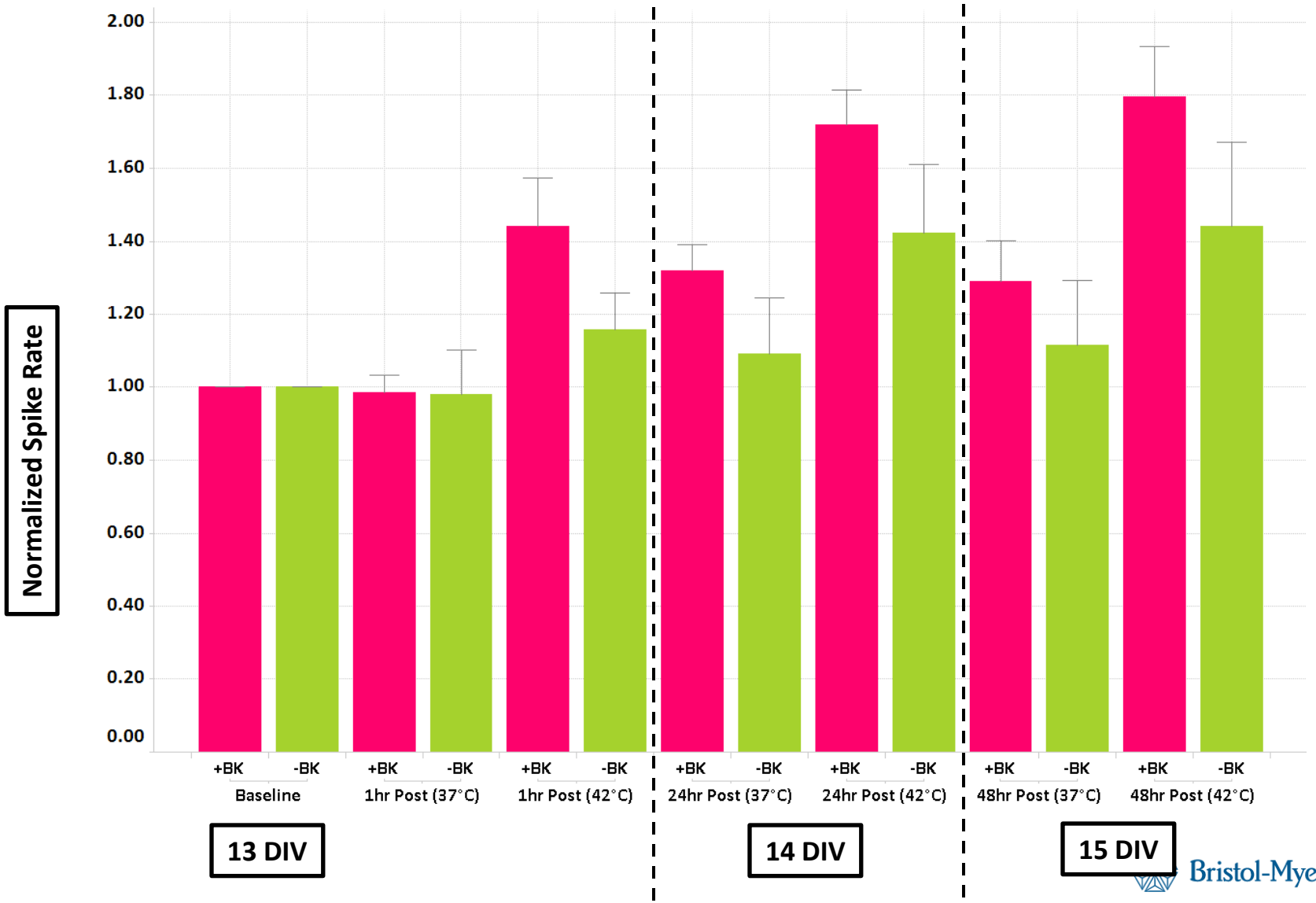
- Similar spontaneous activity to 12 well plates



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



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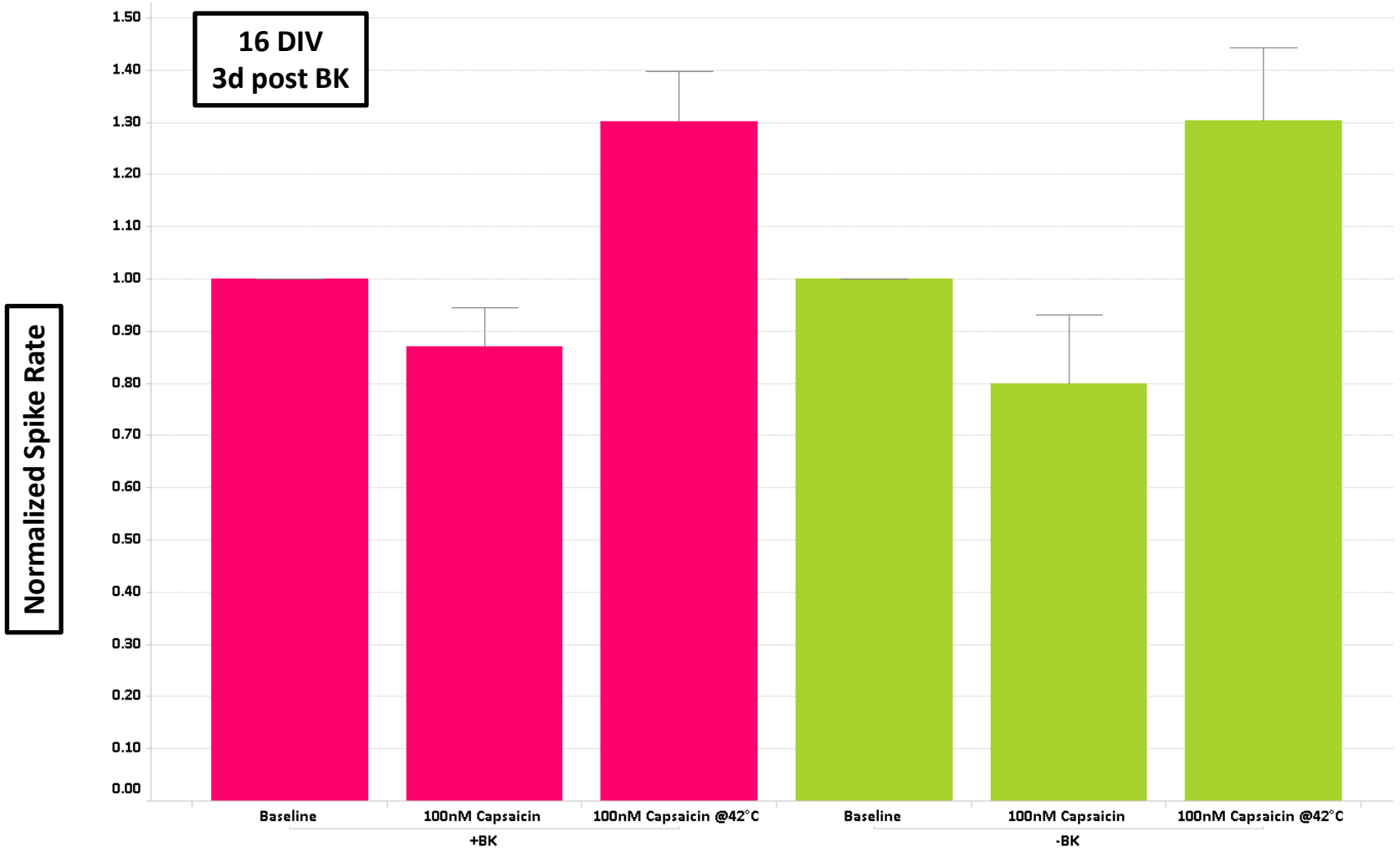
No change in response to capsaicin 3d after bradykinin treatment



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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
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