CALCIUM IMAGING

Calcium imaging in primary rat neuron cultures

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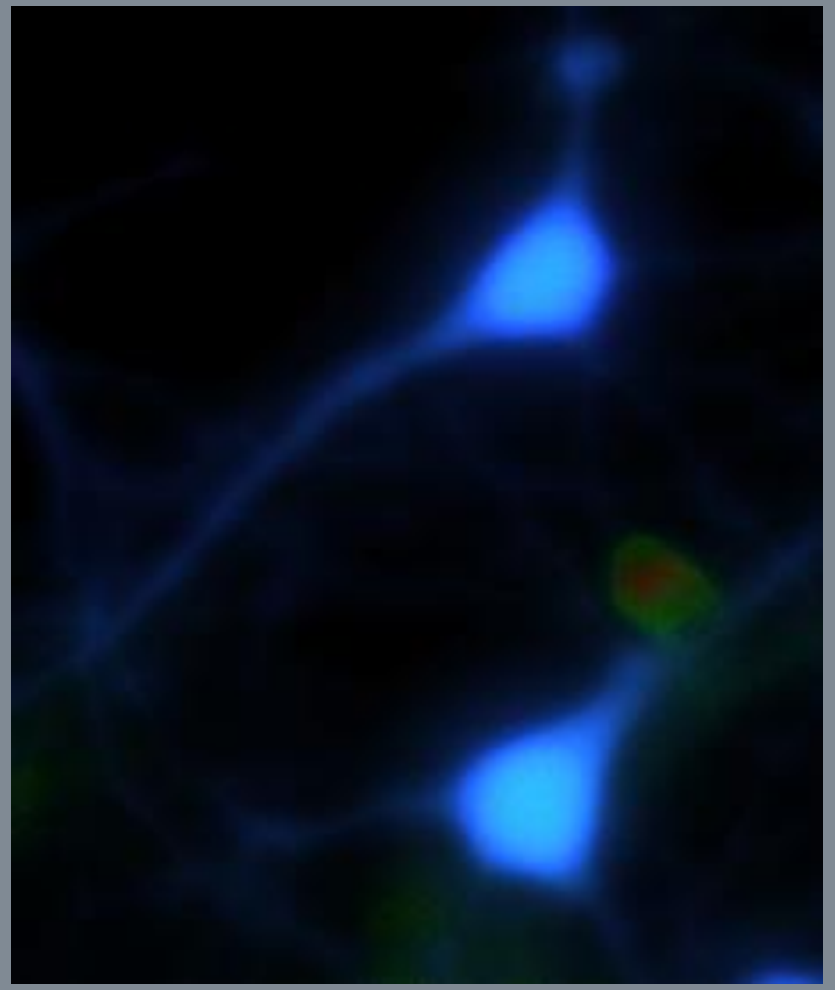
GLUTAMATE ELICITS CALCIUM FLUXES IN RAT PYRAMIDAL NEURONS

METHODS

- Neuron cultures were prepared from E18 rat cortex and plated on a monolayer of astrocytes growing on PDL-coated glass imaging dishes (MatTek).
- Calcium imaging was performed in Fura-2 AM loaded cells at 9 days *in vitro*.



Perfusion with 100 uM glutamate elicited an influx of calcium in E18 pyramidal neurons.

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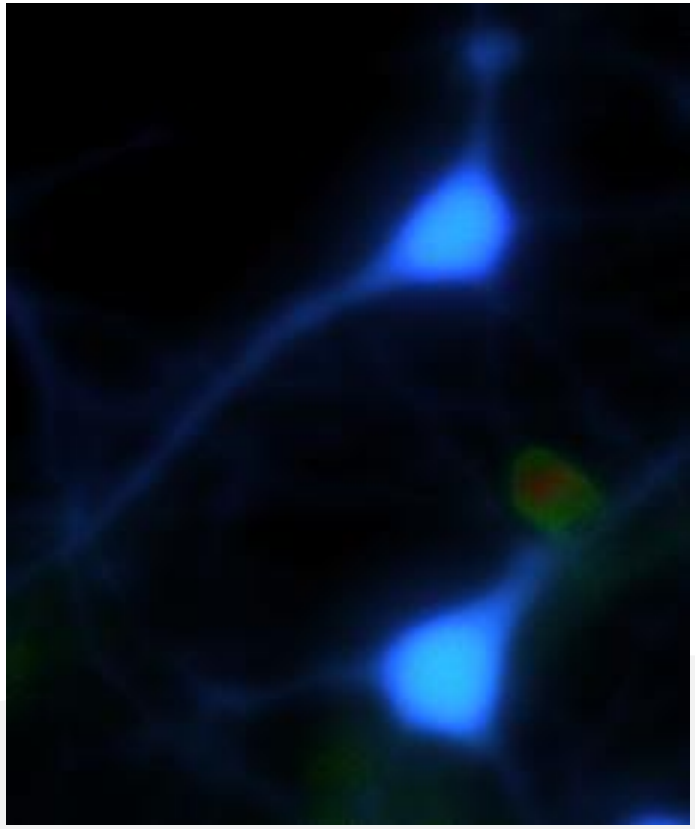
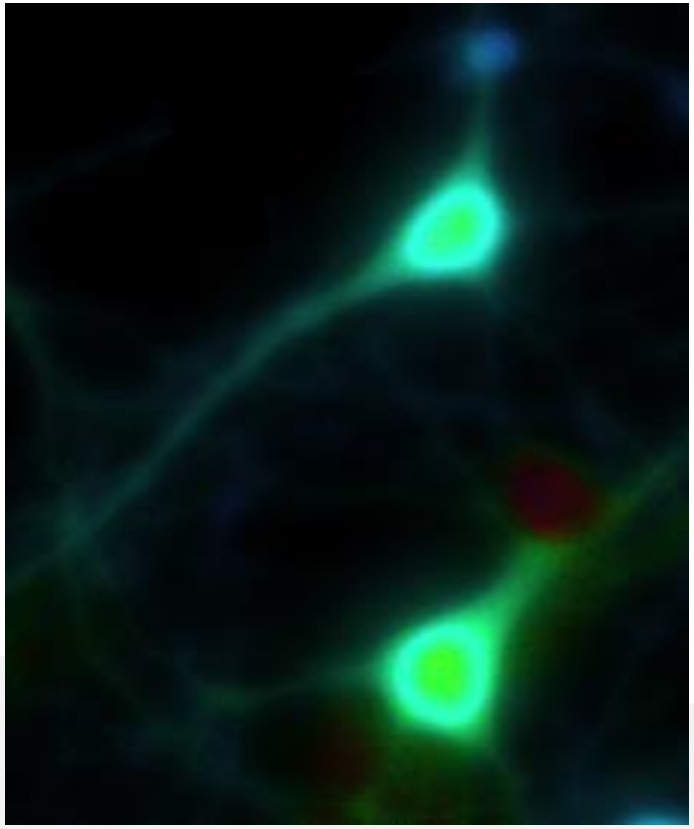
Baseline



100 µM glutamate



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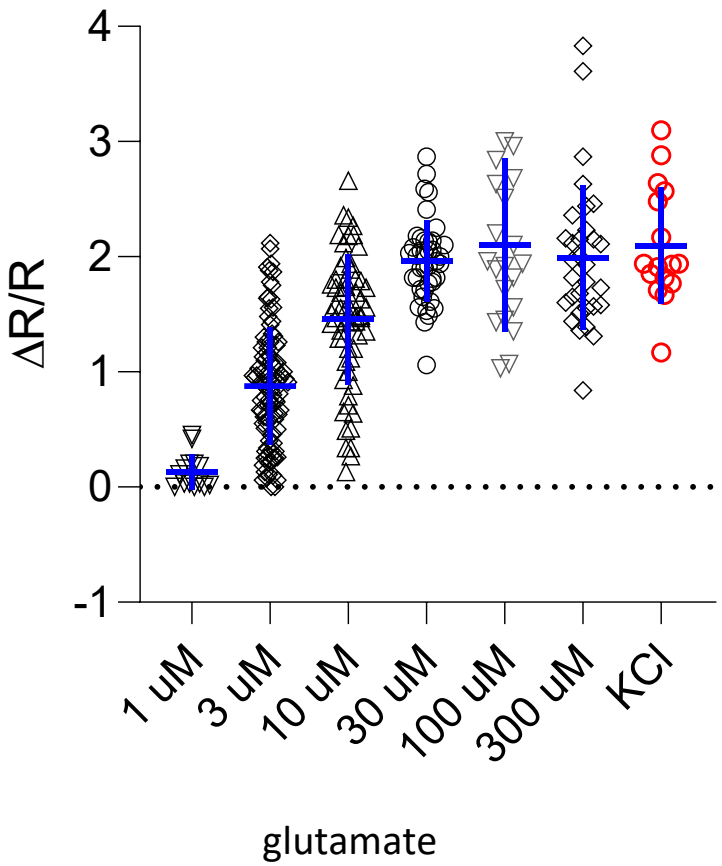
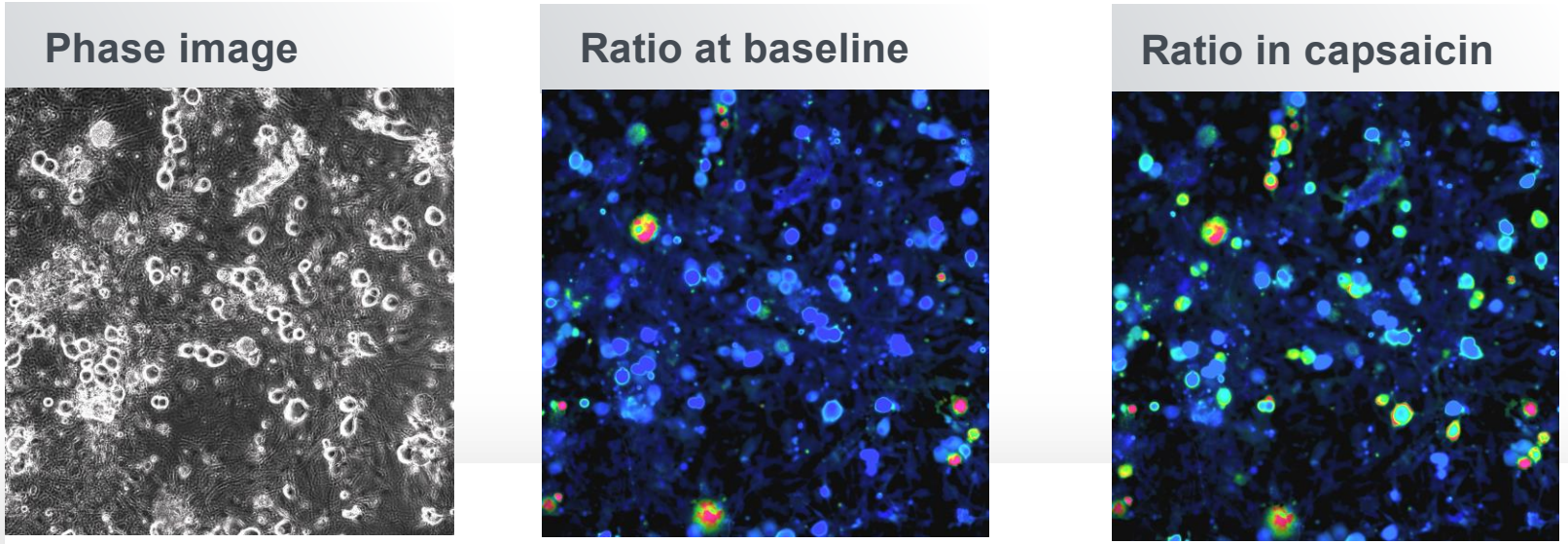


Calcium responses to glutamate in E18 pyramidal neurons were concentration dependent. Depolarization by 60 mM KCl also elicited a comparable calcium signal.

CAPSAICIN ELICITS CALCIUM RESPONSES IN RAT DRG SENSORY NEURONS

METHODS

- Sensory neuron cultures were prepared from adult rat dorsal root ganglia and plated on a monolayer of astrocytes growing on PDL-coated glass imaging dishes (MatTek).
- Calcium imaging was performed on Fura-2 AM loaded cells at 9 days *in vitro*.



Phase-contrast (left) and ratiometric images of Fura-2 loaded DRG sensory neurons. 300 nM capsaicin (TrpV1 agonist) elicited calcium responses in ~50% of sensory neurons.

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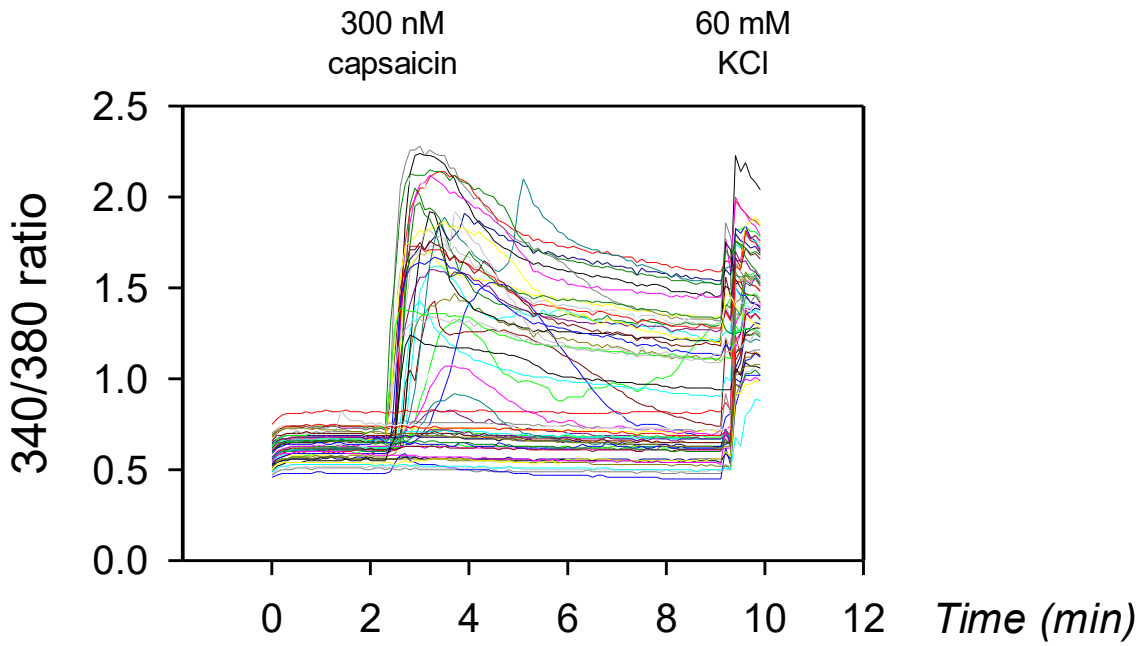
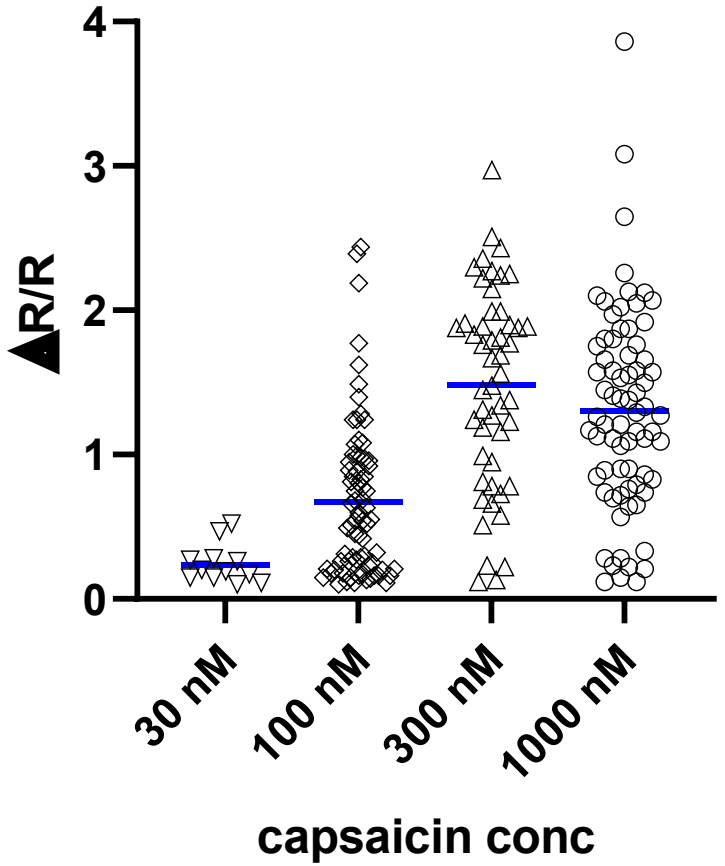


Time course of calcium responses in individual Fura-2AM loaded DRG sensory neurons indicated by colored lines.
Following a 2 min baseline period, perfusion for 1 min with 300 nM capsaicin (TrpV1 agonist) elicited calcium responses in ~50% of neurons expressing TrpV1. All cells responded to depolarization by 60 mM KCl indicating healthy, electrically excitable neurons.

Experiment #9 (12/6) DRG #8 (2 div) Capsaicin conc-response

Results

- Capsaicin elicited calcium responses in rat DRG sensory neurons in a concentration-dependent fashion
- The percent of responsive cells increased with increasing concentrations of capsaicin
- The magnitude calcium response (ΔR/R) increased with increasing concentrations of capsaicin
- Maximum responses were seen ≥300 nM



	30 nM	100 nM	300 nM	1 uM
% responders (ΔR/R >50%)	1% (1/84)	35% (42/119)	55% (46/84)	82% (64/78)
ΔR/R	24%	67%	148%	130%

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AITC elicits calcium response in rat DRG sensory neurons

- 50 uM AITC (1 min) elicits a calcium response in ≥50% of DRG sensory neurons
- 60 mM KCl used as positive control to identify healthy, excitable DRG sensory neurons