High density MEA recording of primary rat neuron cultures and human iPSC-derived neuron cultures growing at low density on astrocyte feeder layers

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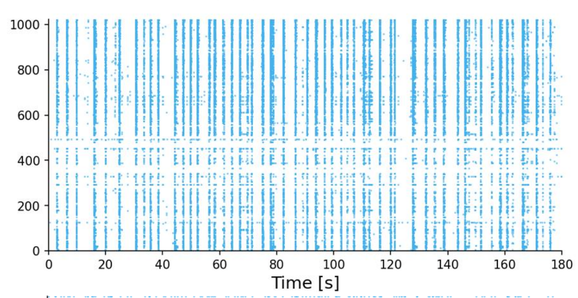
Abstract

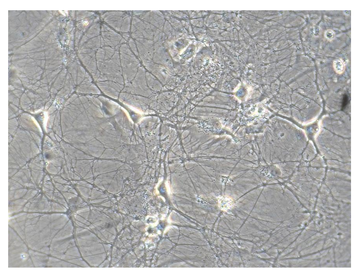
Primary rodent neuronal cell cultures are used for both mechanism of action studies and validation of gene targets expressed in their native environment. Drug discovery projects rely on primary rodent neuronal cell cultures to interrogate the efficacy and potency of novel therapeutic molecules. Functional electrophysiological properties of neurons measured are by patch clamp recording. Neurons growing at low density on a monolayer of astrocytes are an ideal model system for developing the mature neuronal phenotype *in vitro*. Astrocytes provide the optimum substrate for neuronal survival and differentiation. Neurons express a full repertoire of voltage-gated and ligand-gated ion channels as well as GPCRs that modulate neuronal excitability. Both intrinsic and synaptic excitability can be recorded in these cultures. However, the data throughput of patch clamp recording is limited to 1 or 2 neurons at a time. High density microelectrode arrays (MEAs) from MaxWell promise to greatly increase the data throughput of functional endpoints to 100s to 1,000s of neurons at a time. Conventionally, MEA data has been generated from neuronal cultures plated at very high density (2,000-3,000 cells/mm2). We present MEA data recorded from low density rat cortical neuron cultures (25-100 cells/mm2) plated on astrocytes. These cultures conditions more closely resemble the conditions used for patch clamp recording. Neuronal activity (number of active electrodes, firing rate, bursting) increase with development time *in vitro*. In addition, we also present data from human iPSC derived NGN2 neurons at low density on a substrate of rat astrocytes.

Methods

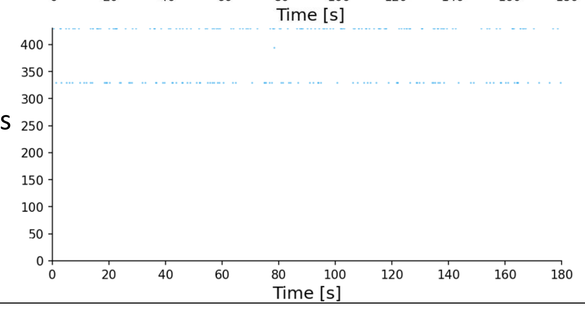
Primary neuron and astrocyte cultures were prepared from embryonic day 18 rat cortex. Cortical tissue was dissociated by enzymatic (papain) and mechanical (trituration) means. Cells were plated in T75 flasks in MEM medium containing 10% FBS to grow astrocytes for 4-6 weeks. Purified astrocytes were re-plated onto MEA plates at 200 cells/mm2 and grown for ~2 weeks to establish confluent monolayers. E18 neurons were plated on astrocyte monolayers in Neurobasal/B27 medium. Human iPSC-derived NGN2 neurons were thawed and plated at low density on astrocytes. Low density neuron culture exhibit robust activity at 17 div while higher density neuron cultures did not. Photomicrographs were taken at 14 days in vitro from sister cultures.

Low density on astrocytes
(56 cells/mm2)





High density w/o astrocytes
(1,000 cells/mm2)



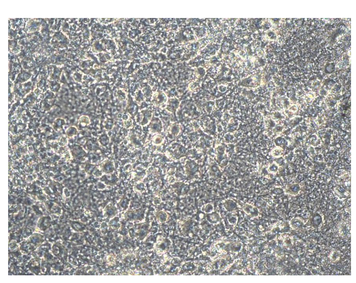


Fig 1 - Rat cortical neurons on astrocytes reliably exhibit more activity over time

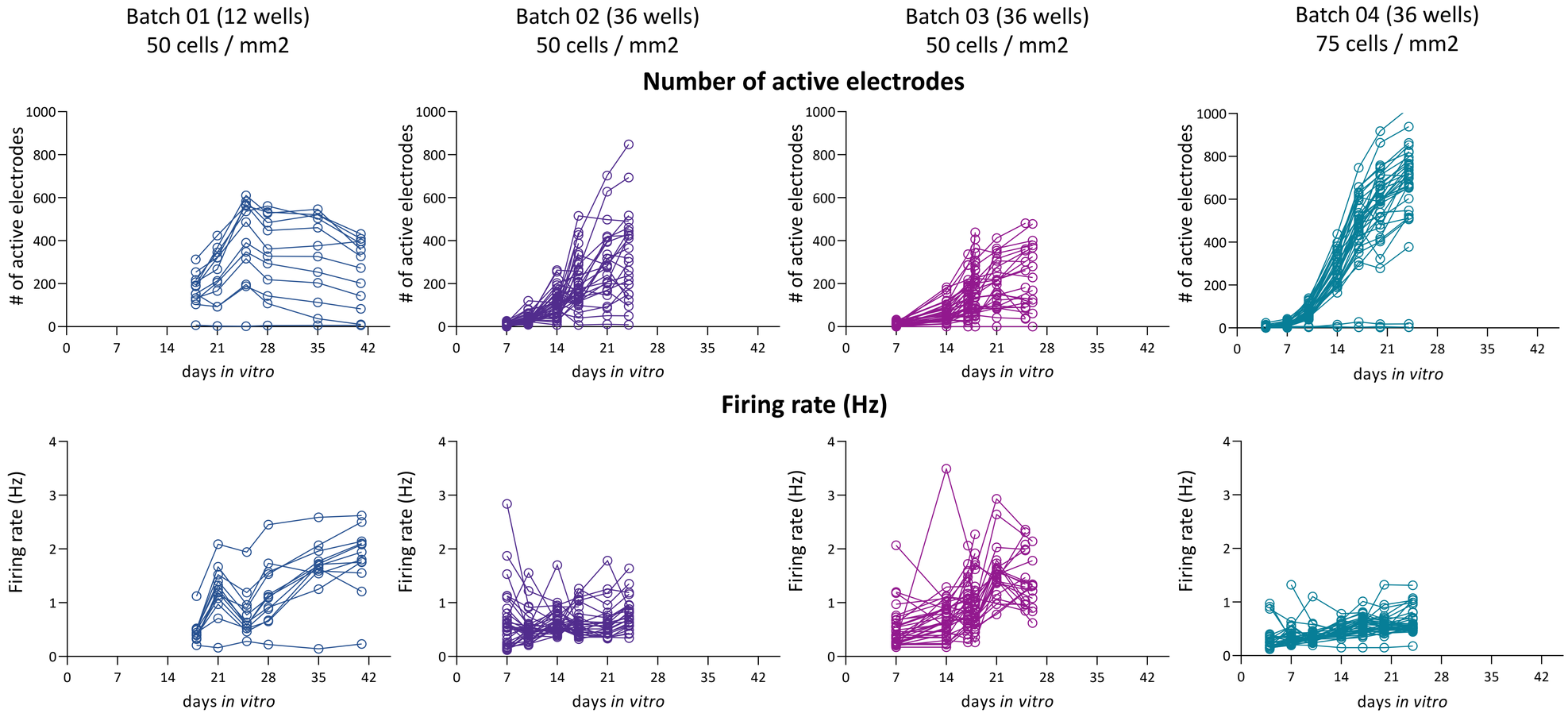


Fig 2 - Rat cortical neurons plated without astrocytes exhibit very little activity

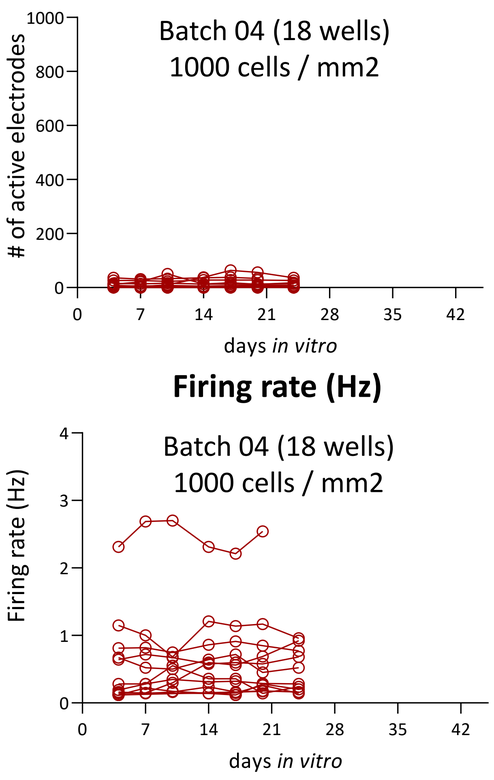


Fig 3 - NGN2 iPSC derived neurons exhibit more activity over time

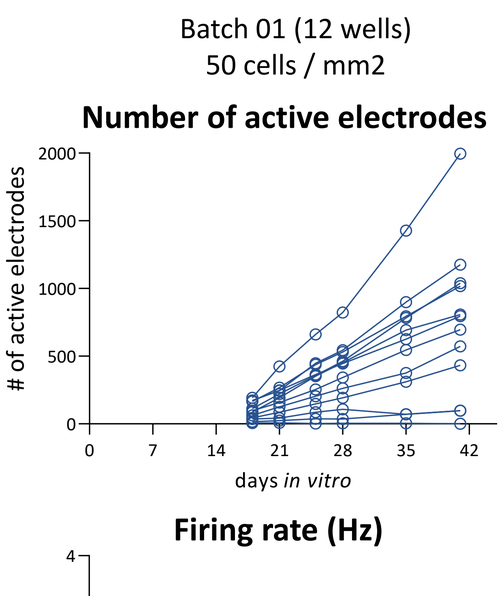
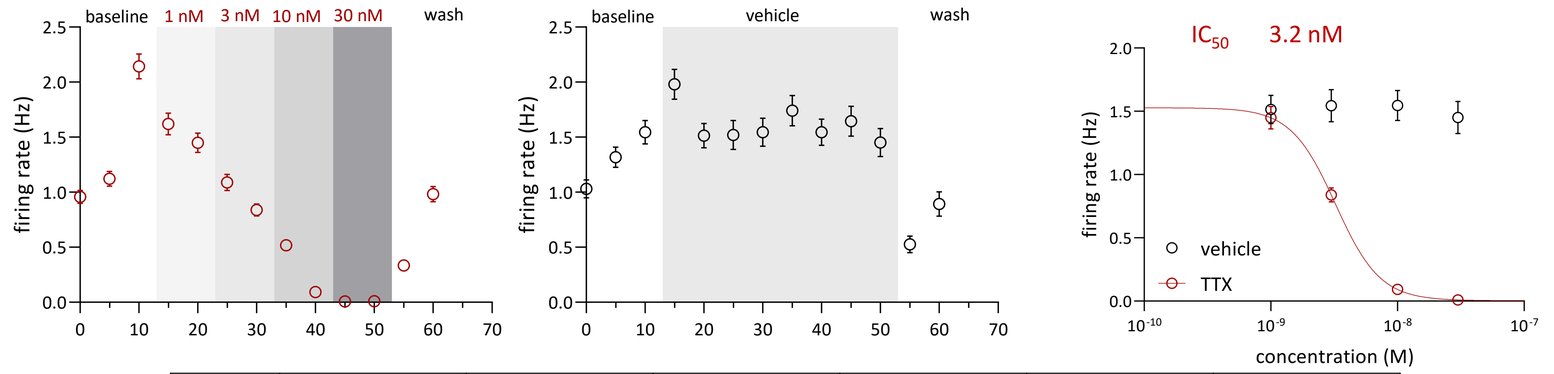
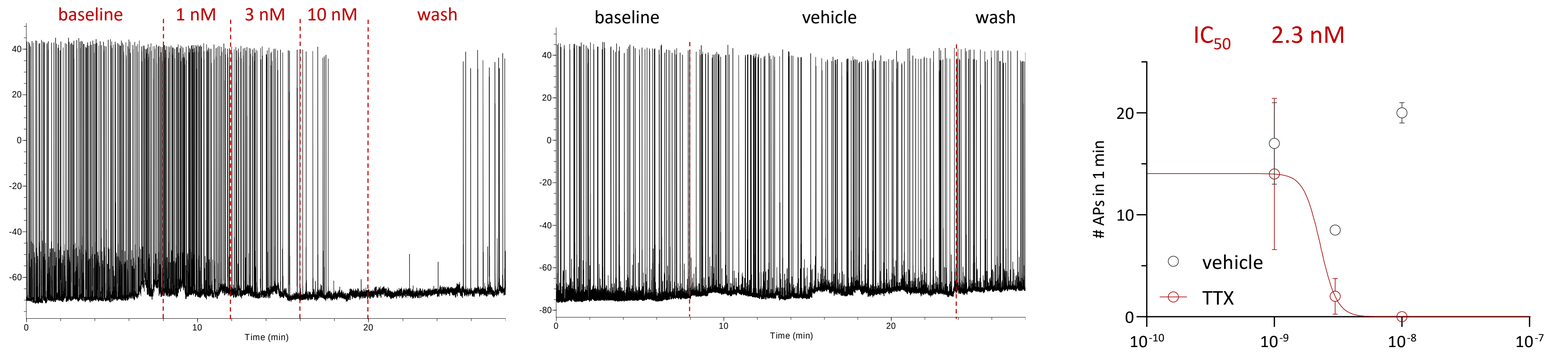


Fig 4 - Rat cortical neuron pharmacology assay preview:



Firing rate (Hz)	Baseline (LP1 10 min)	1 nM TTX (LP2 20 min)	3 nM TTX (LP3 30 min)	10 nM TTX (LP4 40 min)	30 nM TTX (LP5 50 min)	Wash (LP6 60 min)
Vehicle	1.5 ± 0.1 (416)	1.5 ± 0.1 (416)	1.5 ± 0.1 (416)	1.5 ± 0.1 (416)	1.5 ± 0.1 (416)	0.9 ± 0.1 (416)
TTX	2.1 ± 0.1 (659)	1.4 ± 0.1 (659)	0.8 ± 0.1 (659)	0.1 ± 0.0 (659)	0.0 ± 0.0 (659)	1.0 ± 0.1 (659)



	Baseline (LP1 8 min)	1 nM TTX (LP2 4 min)	3 nM TTX (LP3 12 min)	10 nM TTX (LP4 16 min)	Wash (LP6 24 min)
Vehicle	15 ± 3 (2)	17 ± 4 (2)	9 ± 1 (2)	20 ± 1 (2)	18 ± 2 (2)
TTX	36 ± 9 (5)	14 ± 7 (5)	2 ± 2 (5)	0 ± 0 (5)	17 ± 4 (5)

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