MaxWell HD-MEA assay development and pharmacological validation

Chen Tian

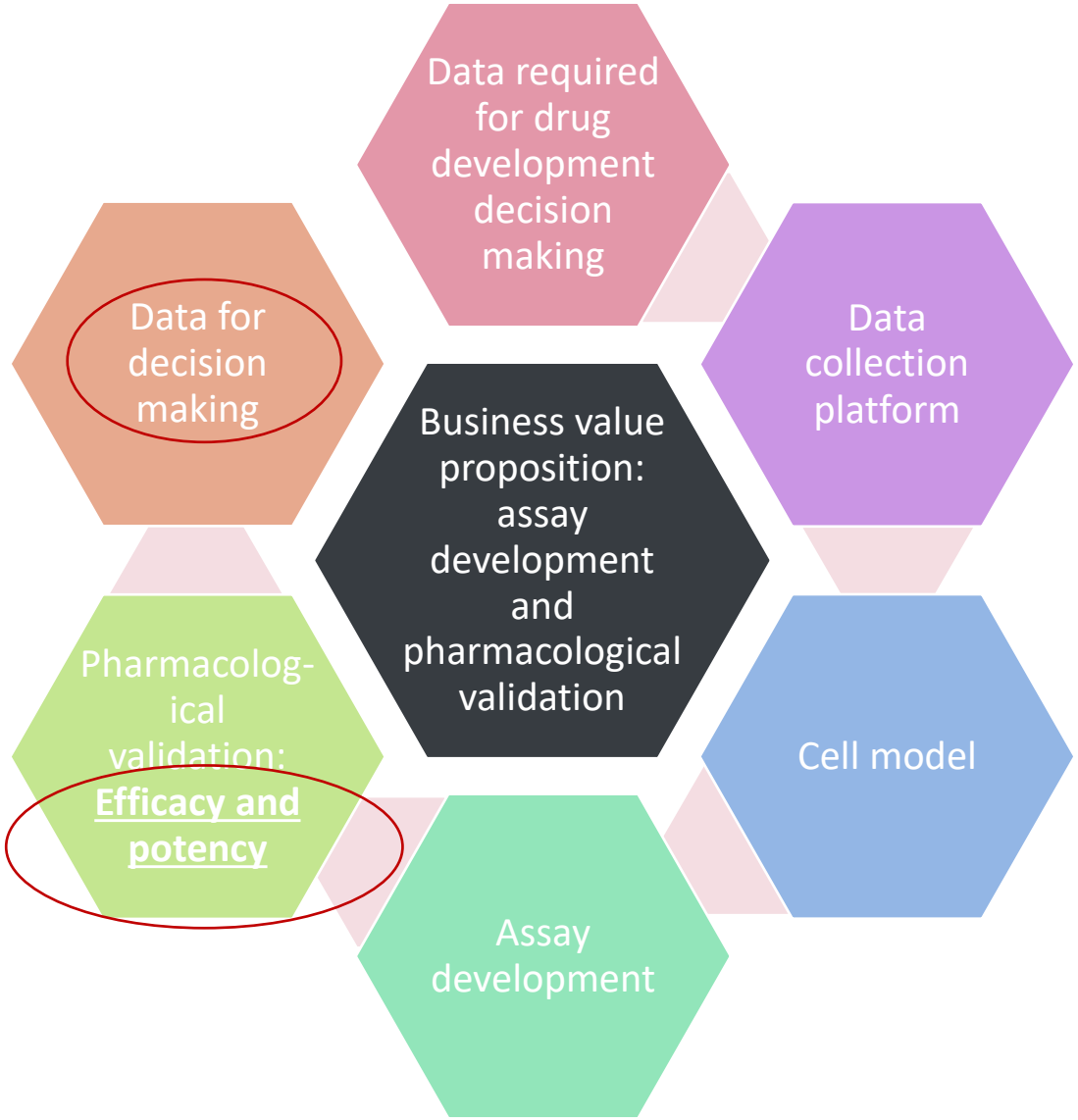
INNOVATION DAY

September 12, 2023

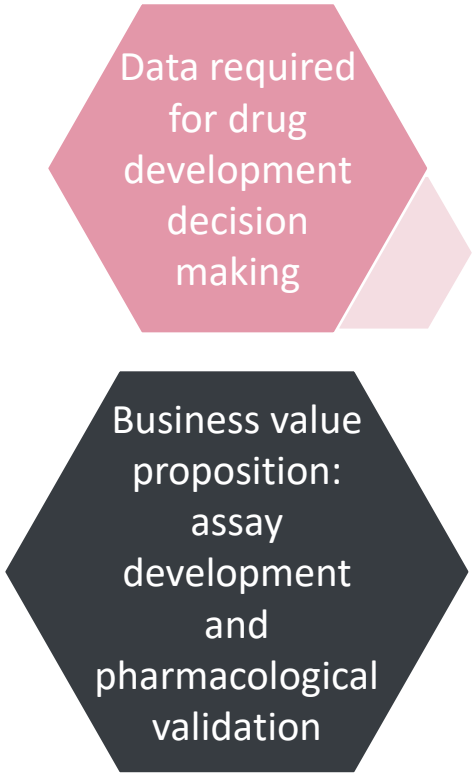
neuroservices ALLIANCE

Goal Assay development and pharmacological validation

Goal Assay development and pharmacological validation

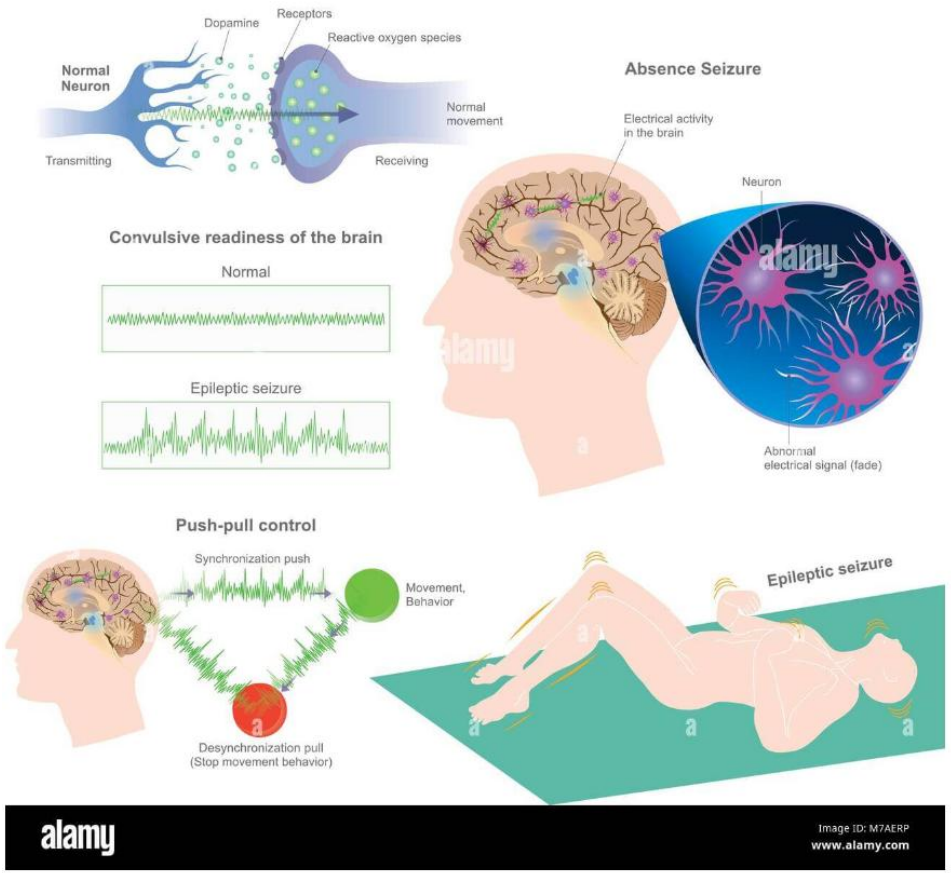


Goal Assay development and pharmacological validation

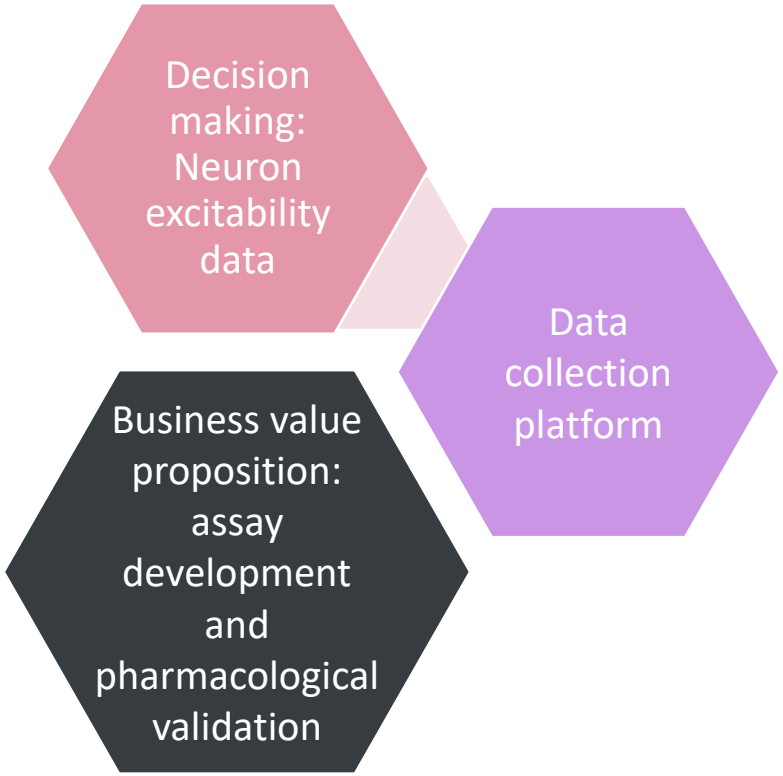


Neuron excitability is an essential endpoint in drug discovery

Excessive neuronal activity is associated with seizure

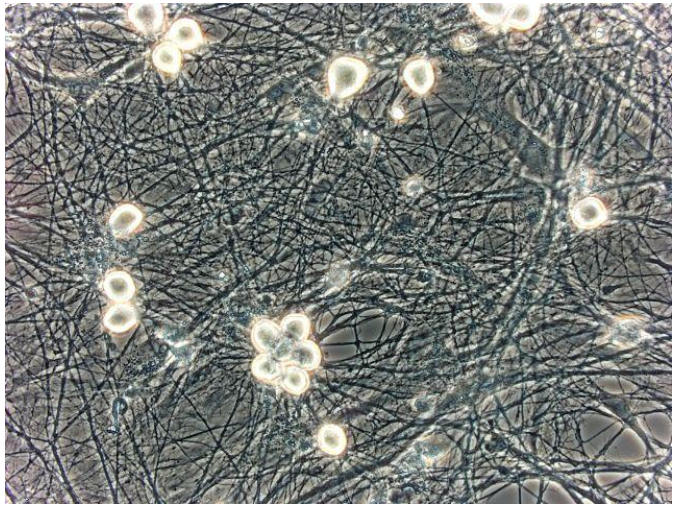


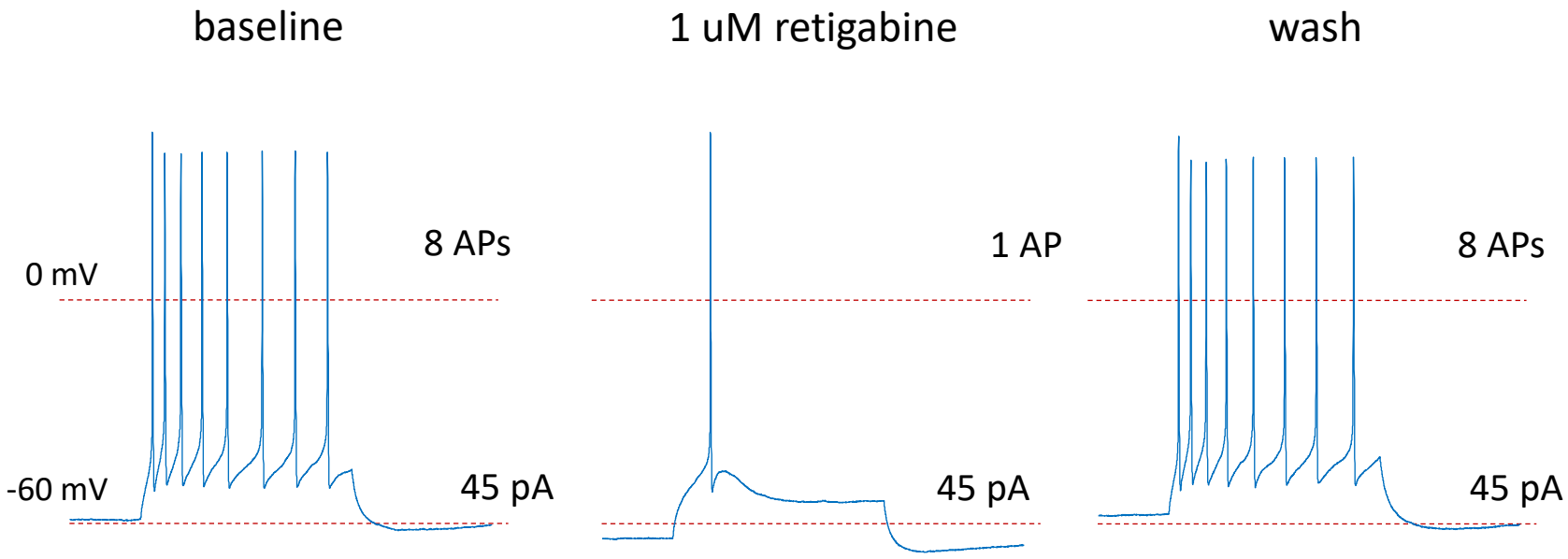
Goal Assay development and pharmacological validation



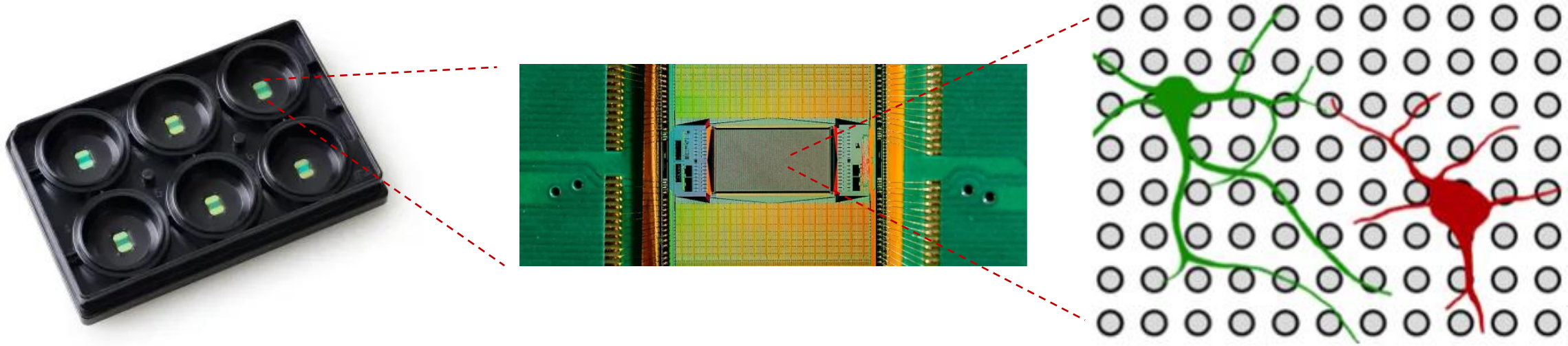
Neuron firing rate is currently measured using manual patch clamp

Human iPSC-derived neuron





High density (HD) MEA allows access to firing rate in >1000 neurons

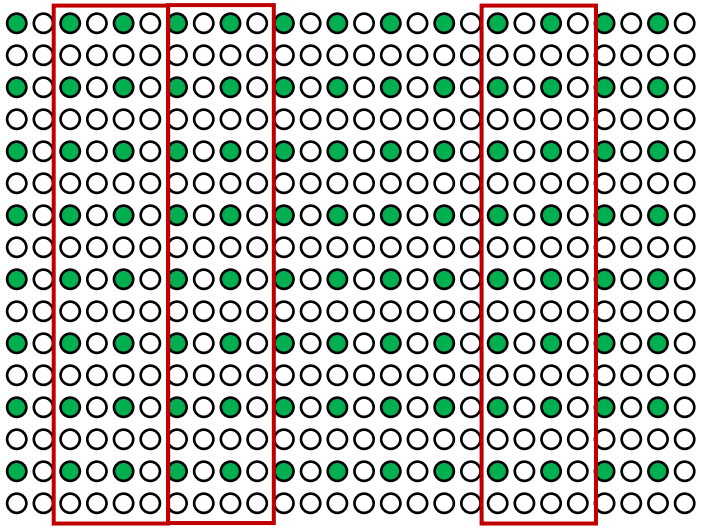


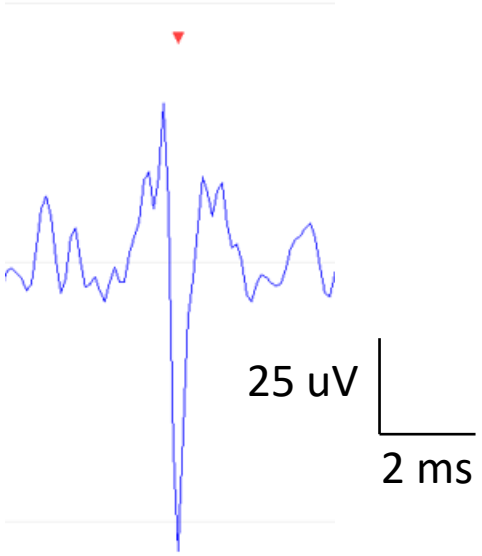
- 26,400 electrodes / well
- Non-destructive extracellular recording

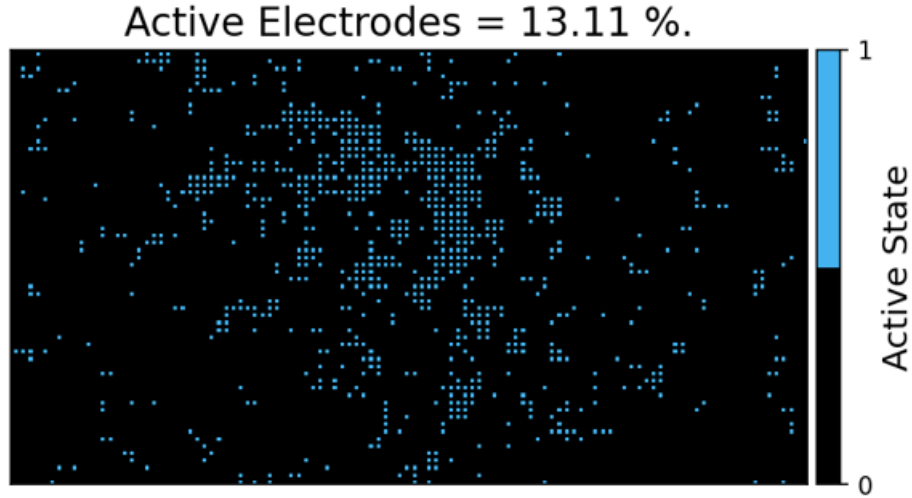
- Record up to 1020 electrodes / well at a time
- Record all wells simultaneously

Maxwell recording protocol

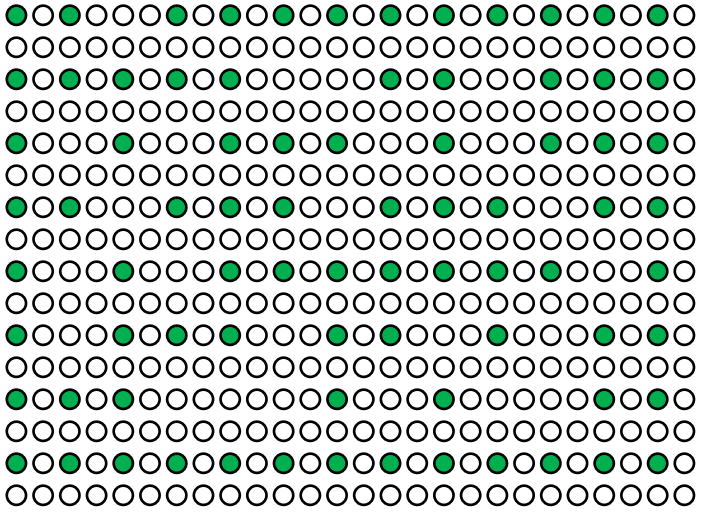
Activity scan

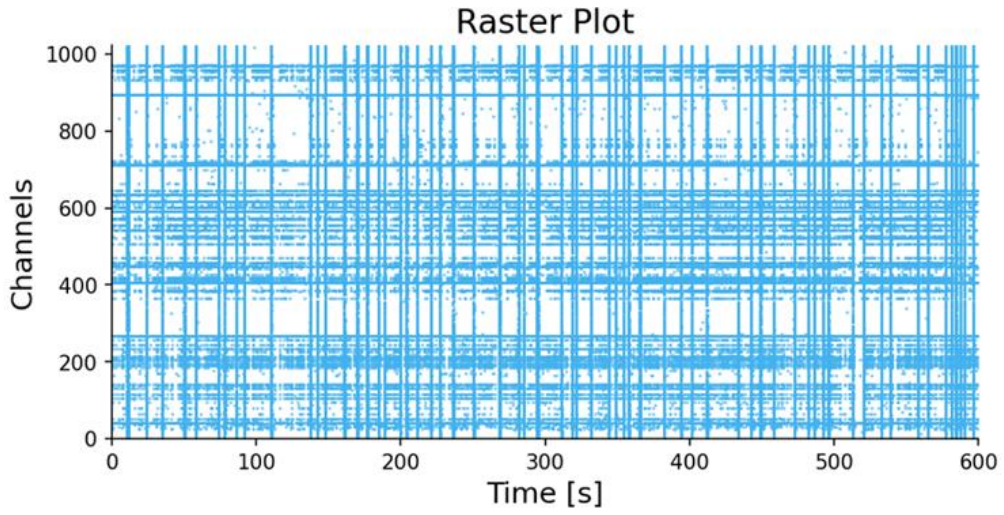




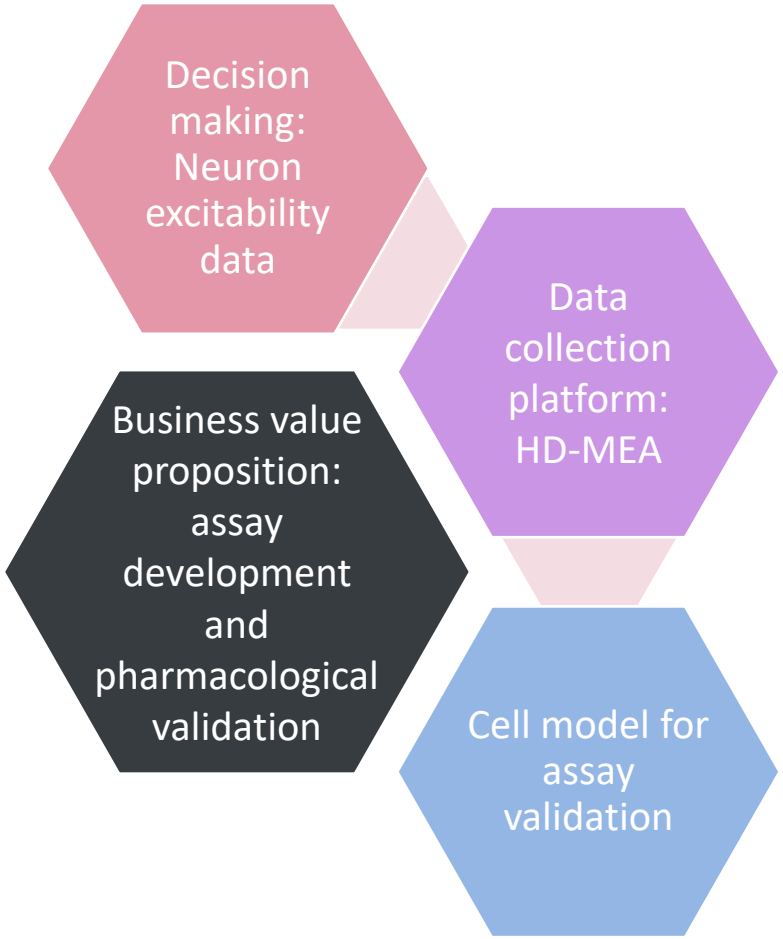


Network scan



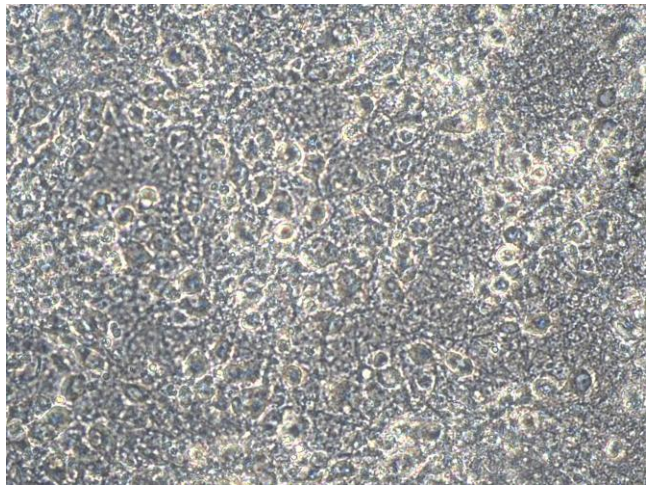


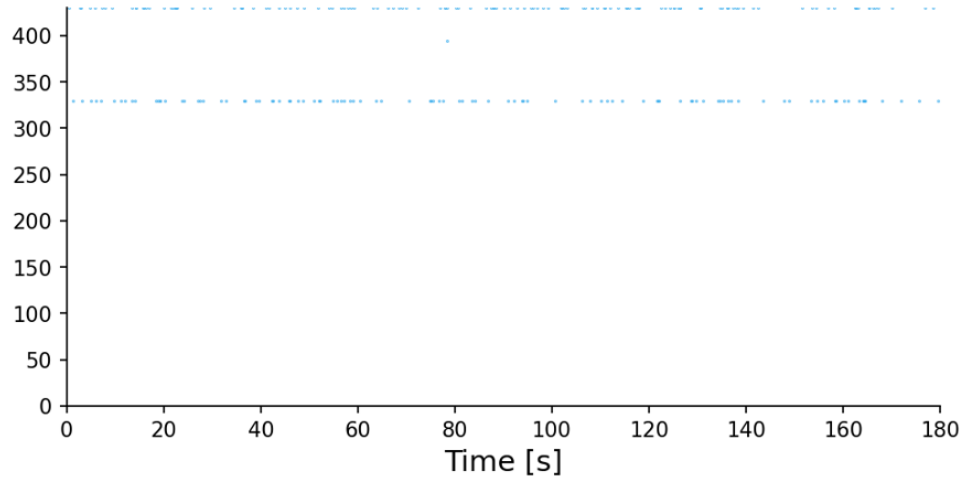
Goal Assay development and pharmacological validation



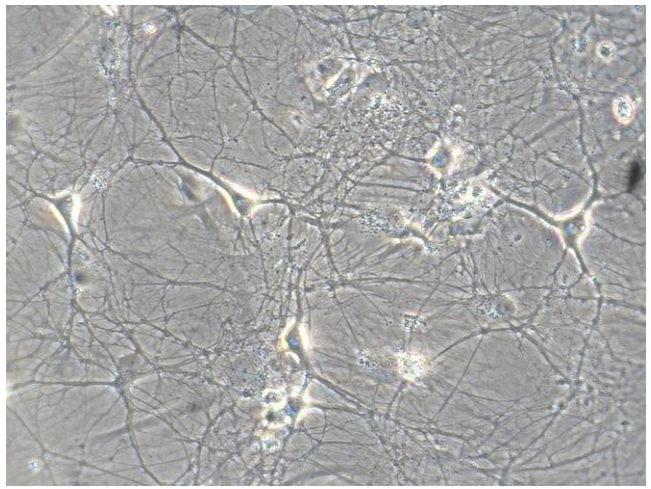
Neurons at low density on astrocytes exhibited more activity compared to neurons at high density without astrocytes

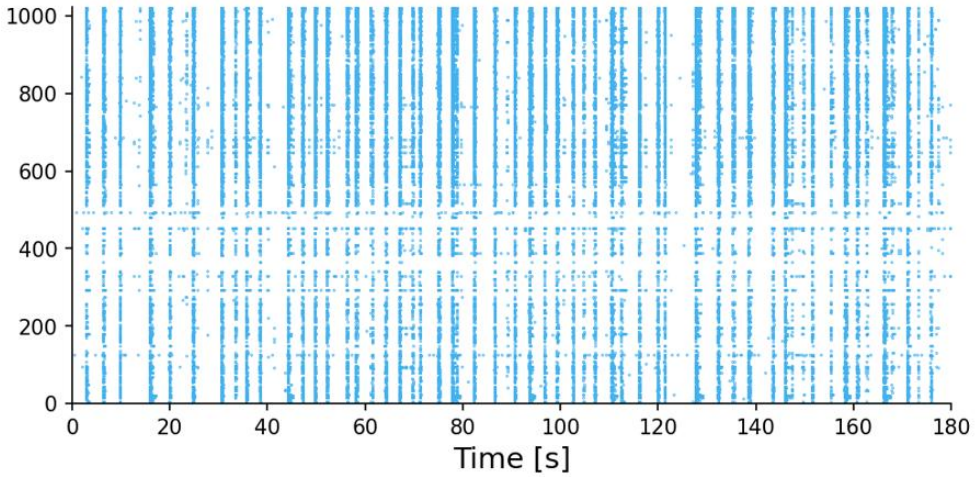
High density w/o astrocytes
(1,000 cells/mm2)



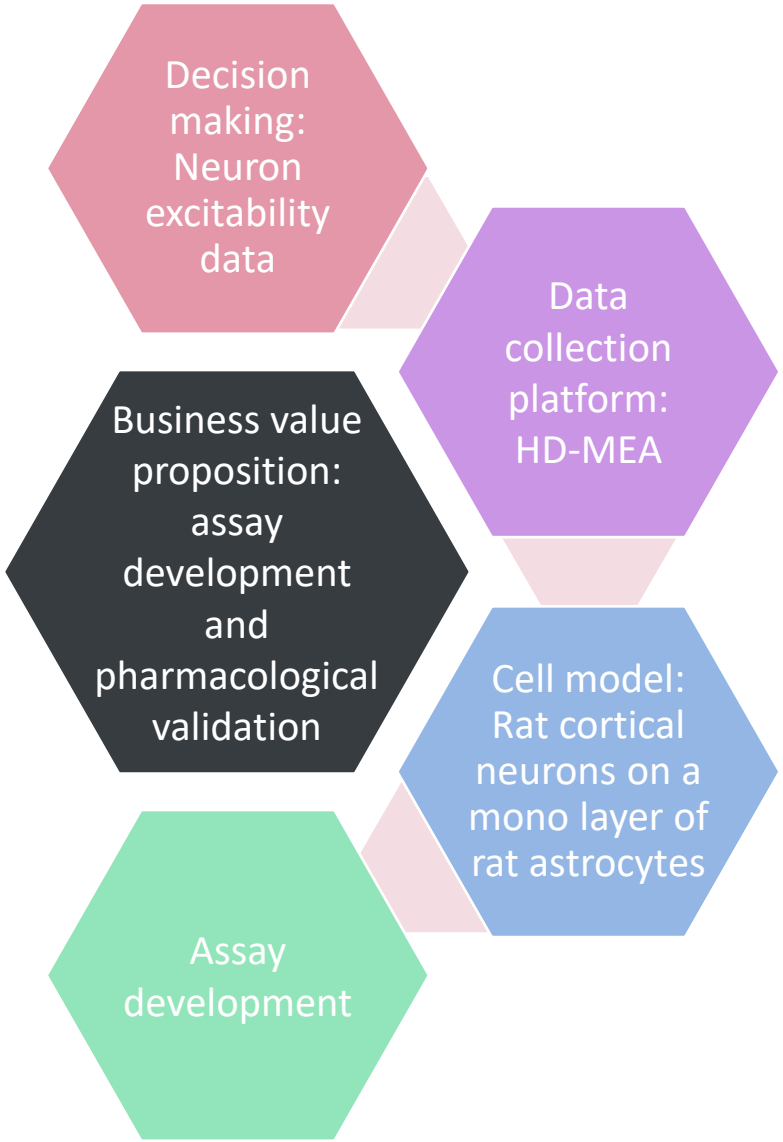


Low density on astrocytes
(56 cells/mm2)



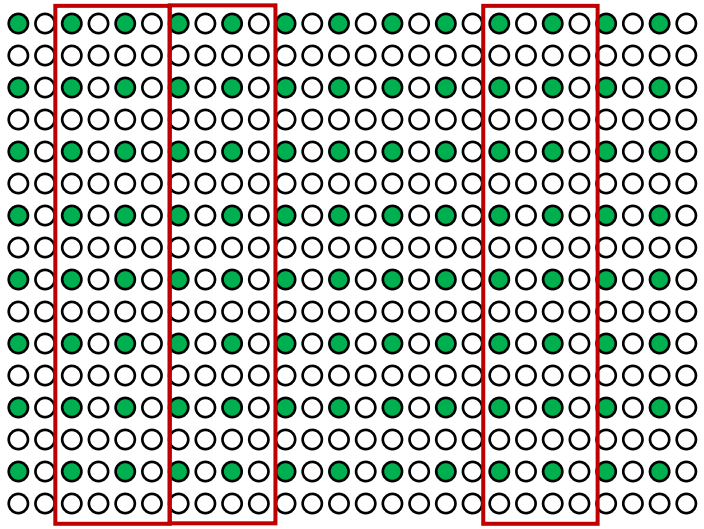


Goal Assay development and pharmacological validation

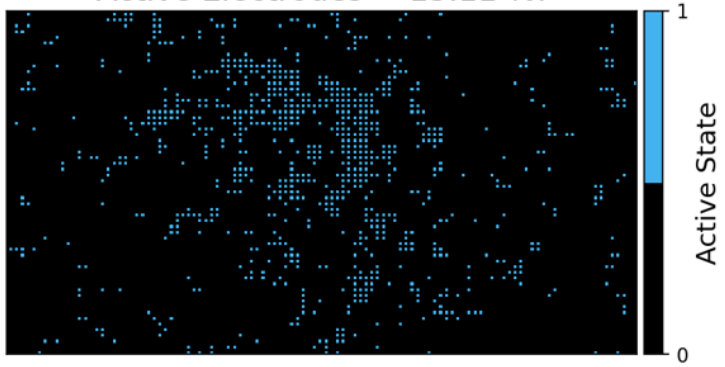


Rat cortical neurons exhibit high number of active electrode at 3-4 weeks in culture

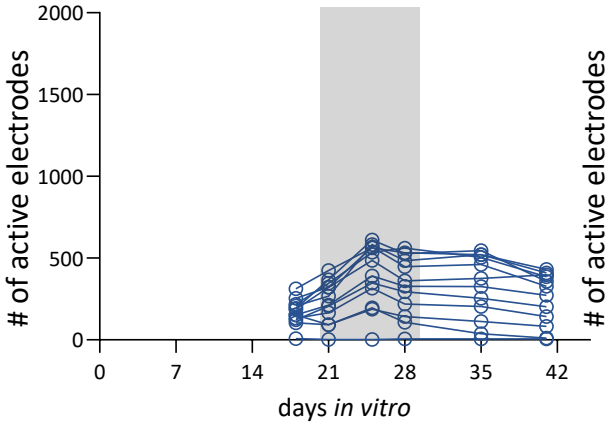
Activity scan



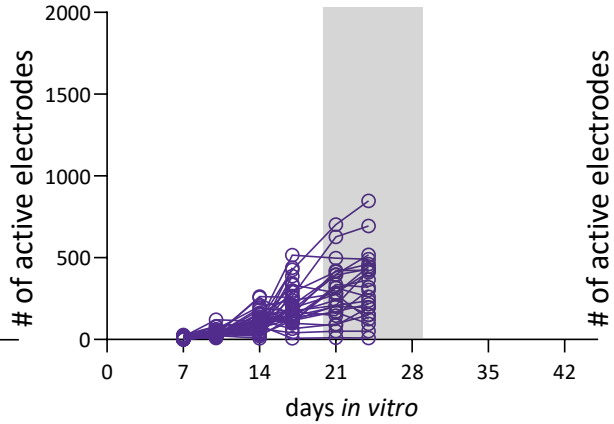
Active Electrodes = 13.11 %.



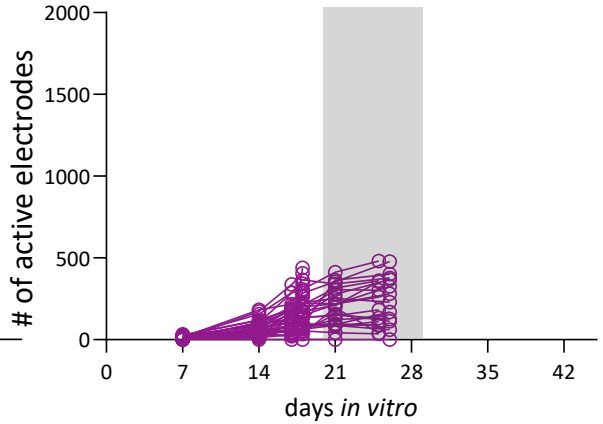
Batch 01 (12 wells)
50 cells / mm²



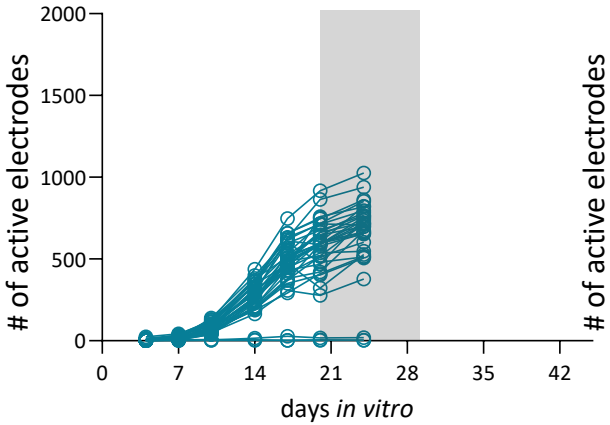
Batch 02 (36 wells)
50 cells / mm²



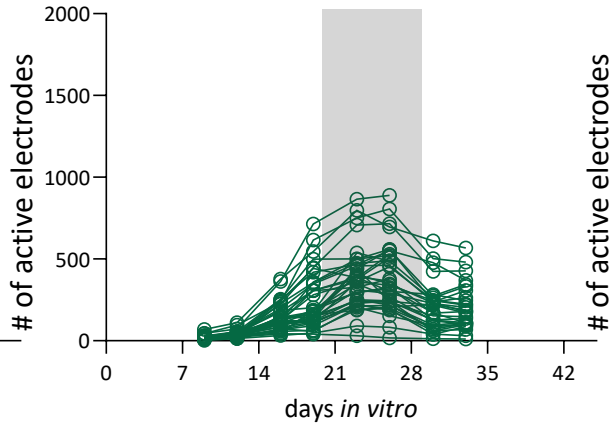
Batch 03 (36 wells)
50 cells / mm²



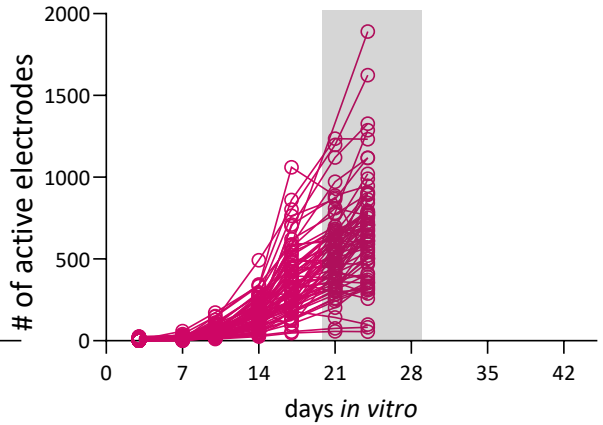
Batch 04 (36 wells)
75 cells / mm²



Batch 05 (36 wells)
75 cells / mm²

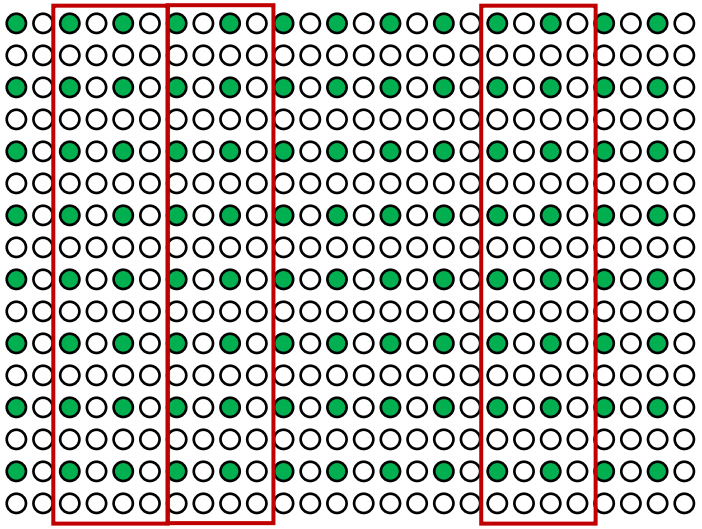


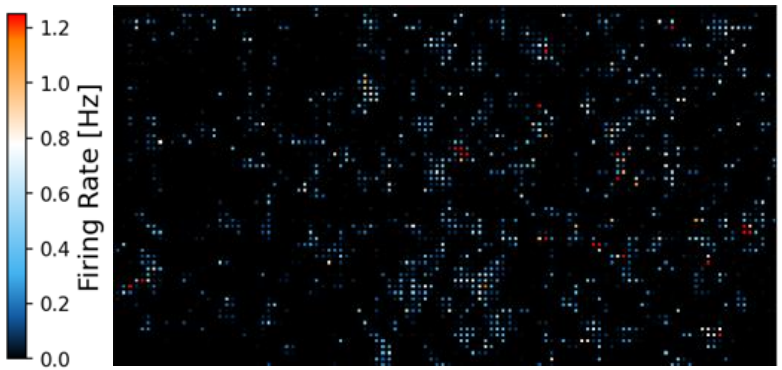
Batch 06 (72 wells)
75 cells / mm²



Rat cortical neurons exhibit sufficient firing rate at 3-4 weeks in culture

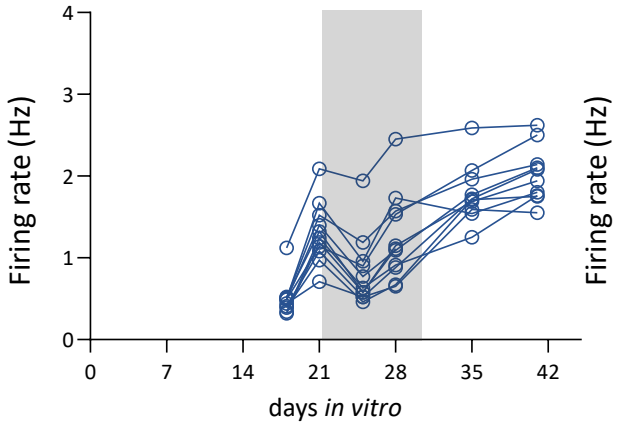
Activity scan



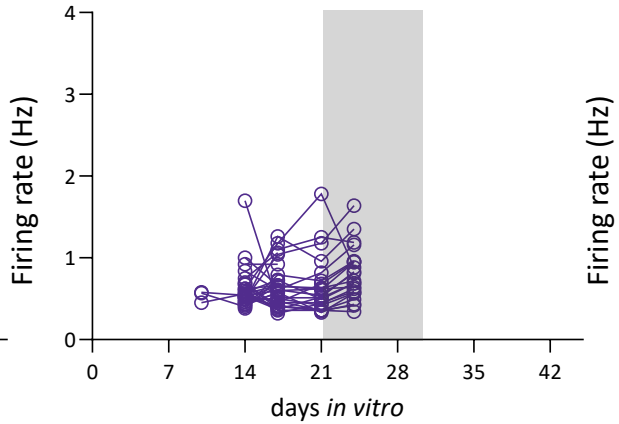


Wells with <50 active electrodes were excluded

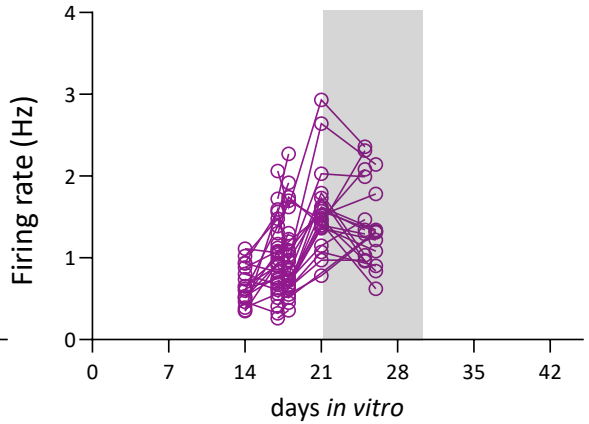
Batch 01 (12 wells)
50 cells / mm^2



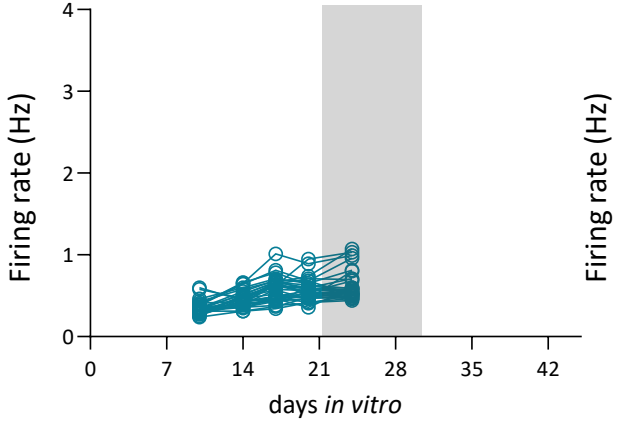
Batch 02 (36 wells)
50 cells / mm^2



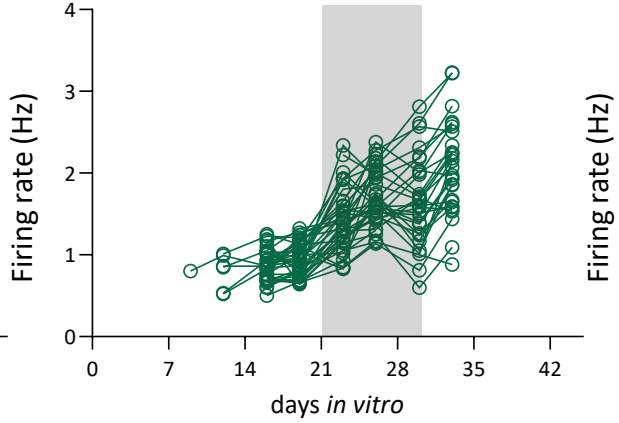
Batch 03 (36 wells)
50 cells / mm^2



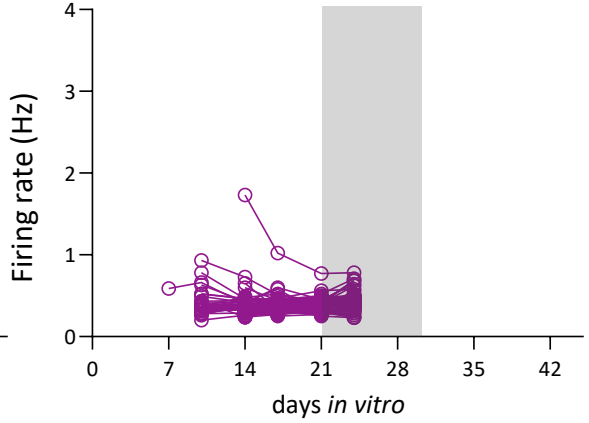
Batch 04 (36 wells)
75 cells / mm^2



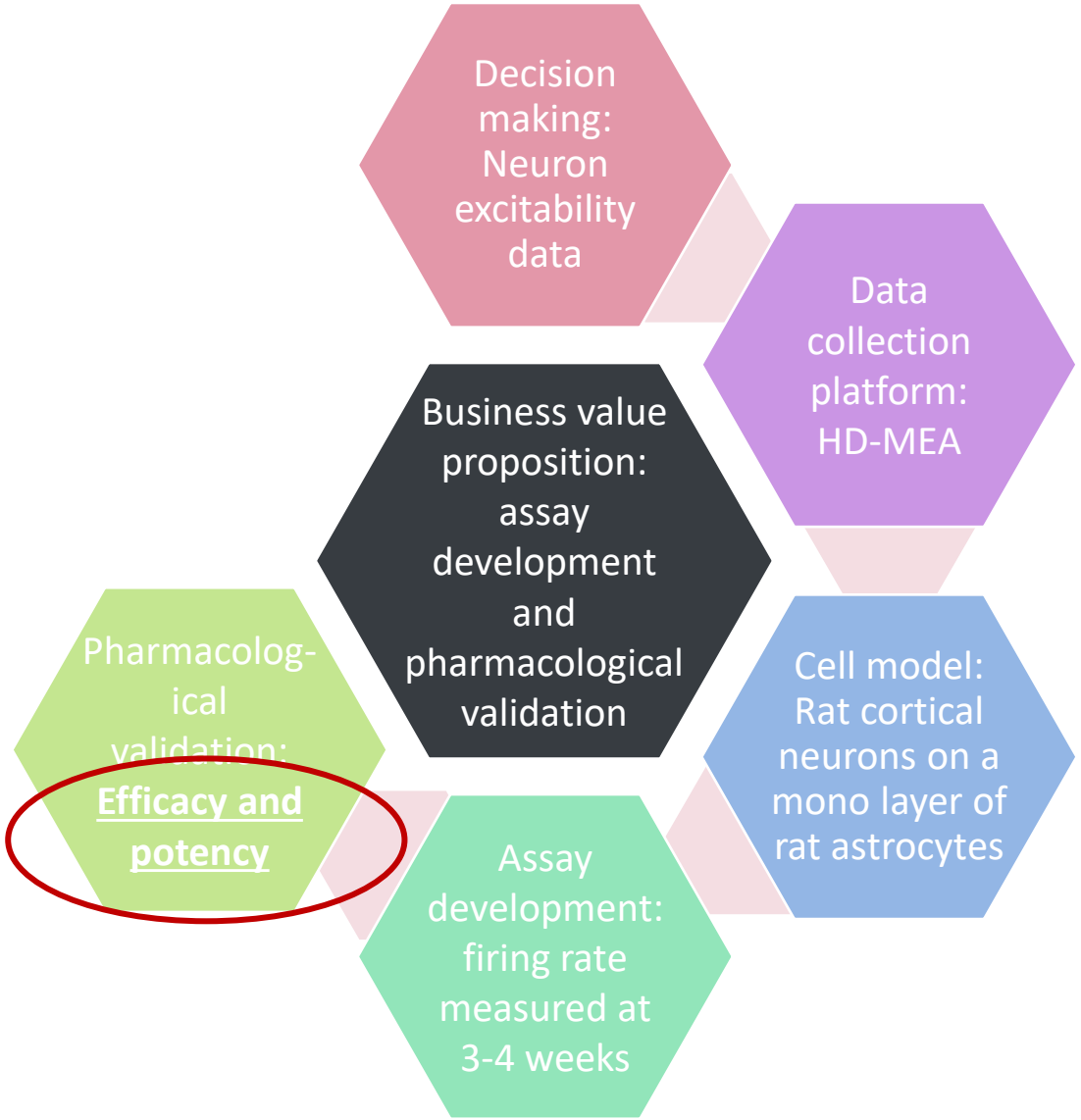
Batch 05 (36 wells)
75 cells / mm^2



Batch 06 (72 wells)
75 cells / mm^2



Goal Assay development and pharmacological validation

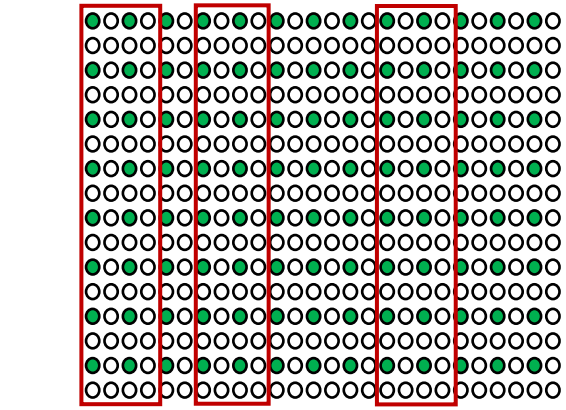


Workflow to develop pharmacological assay on rat embryonic cortical neurons

Plate embryonic rat cortical neurons at low concentration (75 cells / mm^2) on a mono layer of rat astrocytes

Culture the neurons for 3-4 weeks and closely monitoring neuron excitability and network activity

Identify active electrodes

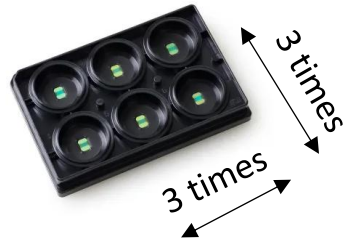


Record active electrodes and apply tool compound

3 mins BL 01

3 mins BL 02

3 mins BL 03



Add TTX conc 1

3 mins 1 nM TTX 01

3 mins 1 nM TTX 02

3 mins 1 nM TTX 03

Add TTX conc 2

3 mins 3 nM TTX 01

3 mins 3 nM TTX 02

3 mins 3 nM TTX 03

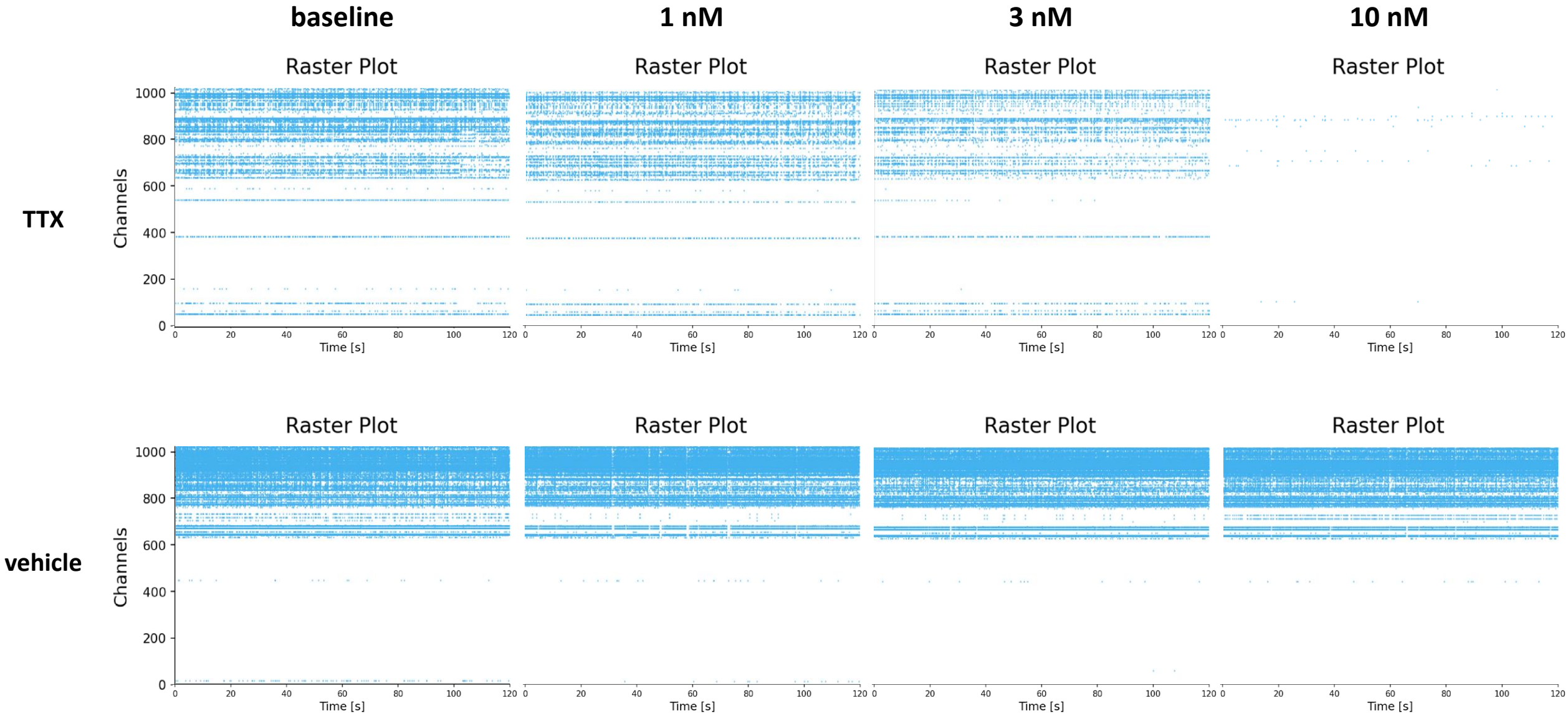
Add TTX conc 3

3 mins 10 nM TTX 01

3 mins 10 nM TTX 02

3 mins 10 nM TTX 03

TTX exhibited conc-dependent block of rat cortical neuron excitability

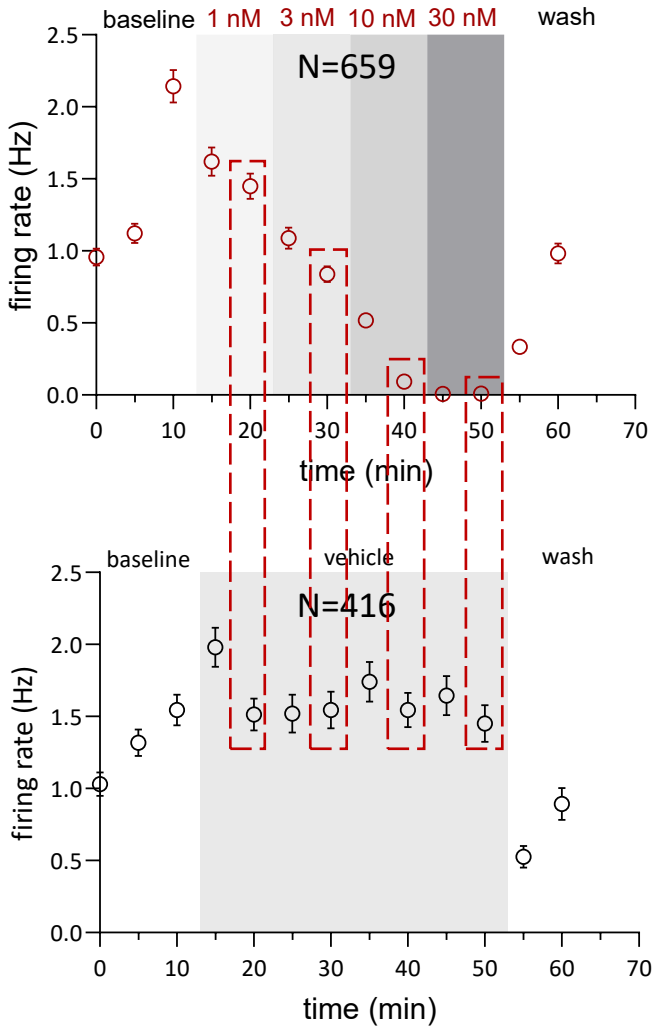


TTX exhibited conc-dependent block of rat cortical neuron excitability

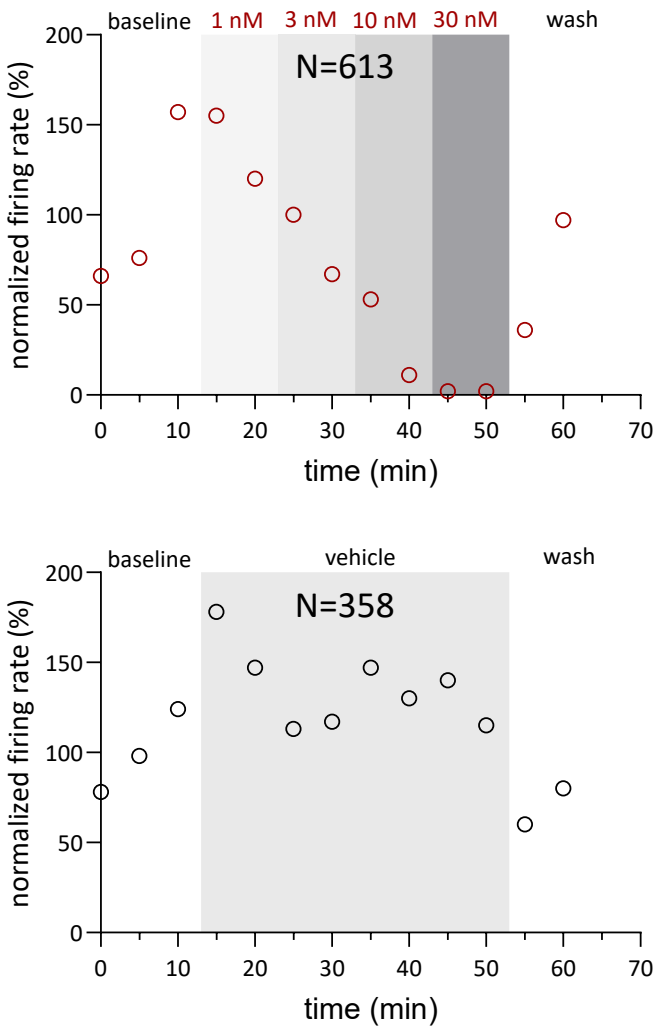
TTX

vehicle

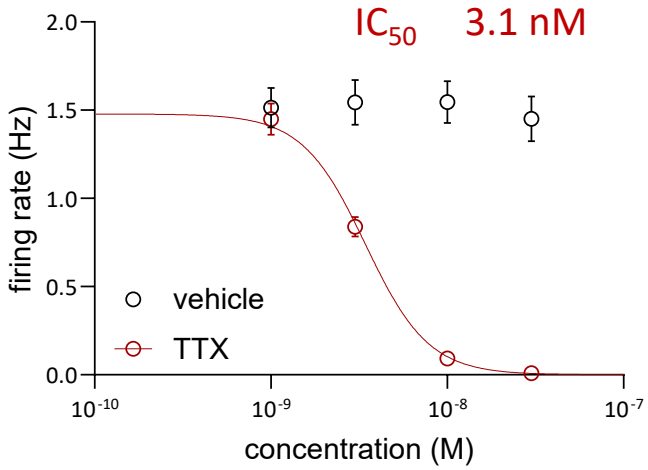
Firing rate (Hz)



Normalized firing rate (% baseline)



Concentration response curve



Note: In normalized firing rate, firing rate at each measurement were normalized to baseline average for each electrode. Electrodes with less than 0.01 Hz firing rate in baseline were excluded.

Adjustment of workflow to develop pharmacological assay on rat embryonic cortical neurons

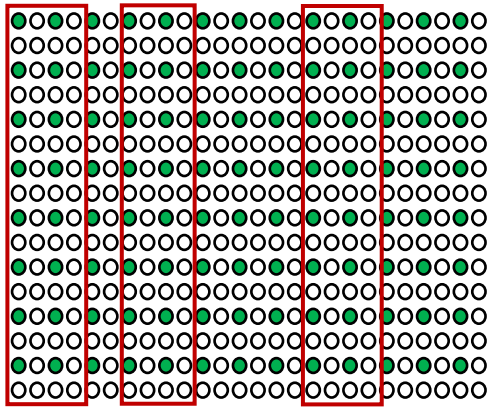
Plate embryonic rat cortical neurons at low concentration (75 cells / mm^2) on a mono layer of rat astrocytes

⇩

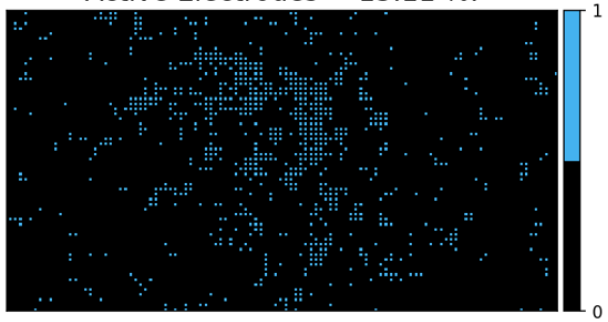
Culture the neurons for 3-4 weeks and closely monitoring neuron excitability and network activity

⇩

Identify active electrodes



Active Electrodes = 13.11 %.



⇩

Record active electrodes and apply tool compound

10 mins BL 01	10 mins BL 02	10 mins BL 03

⇩ Add drug conc 01, mix

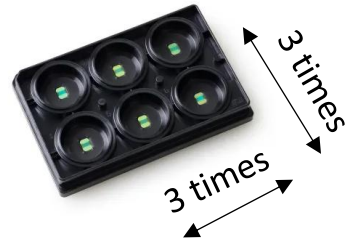
10 mins conc 01	10 mins conc 01	10 mins conc 01

⇩ Add drug conc 02, mix

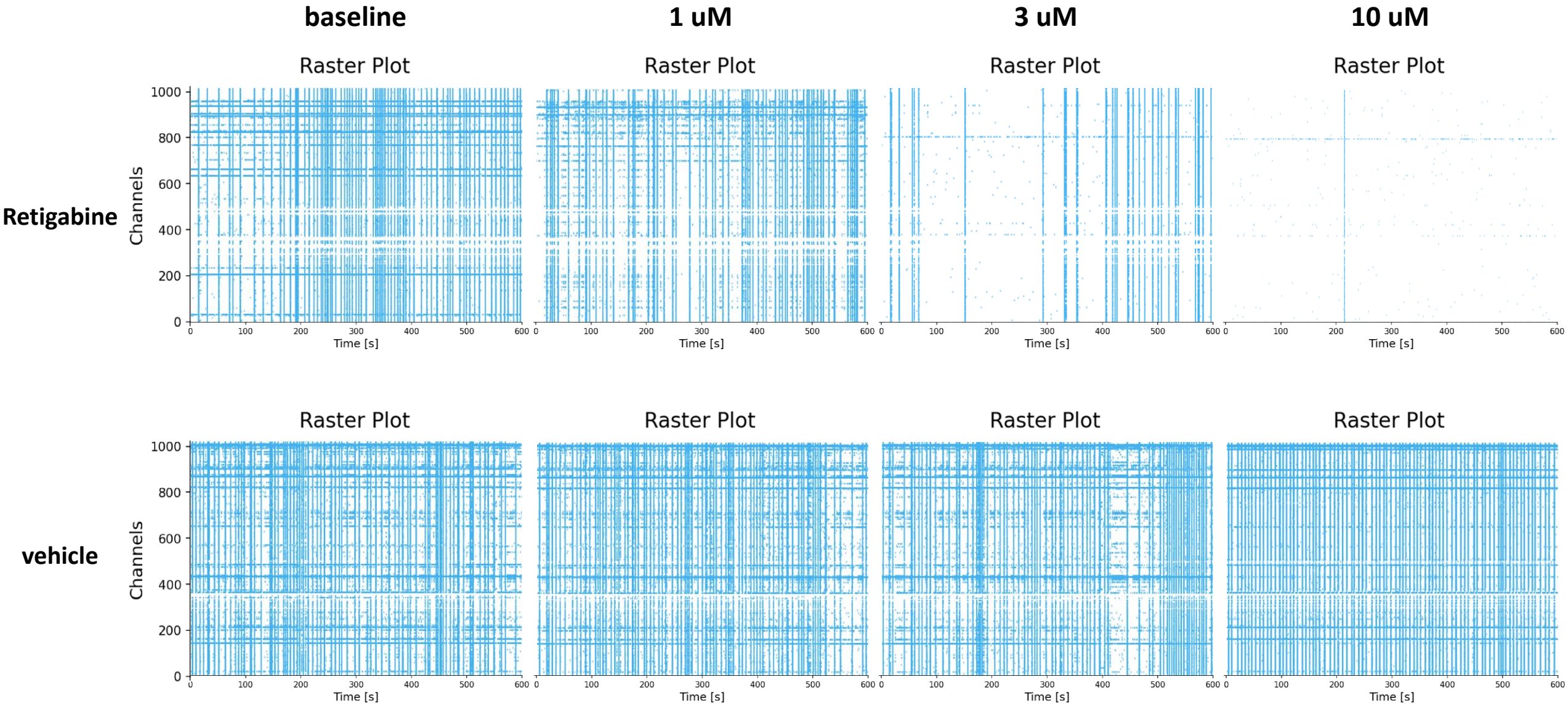
10 mins conc 02	10 mins conc 02	10 mins conc 012

⇩ Add drug conc 03, mix

10 mins conc 03	10 mins conc 03	10 mins conc 03



Retigabine exhibited conc-dependent block of rat cortical neuron excitability

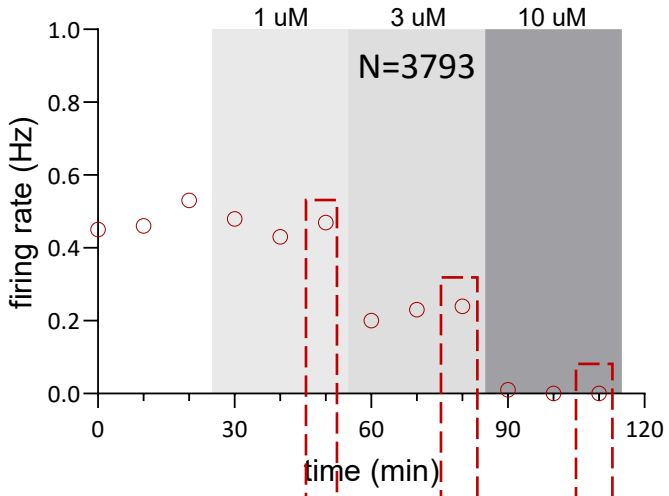


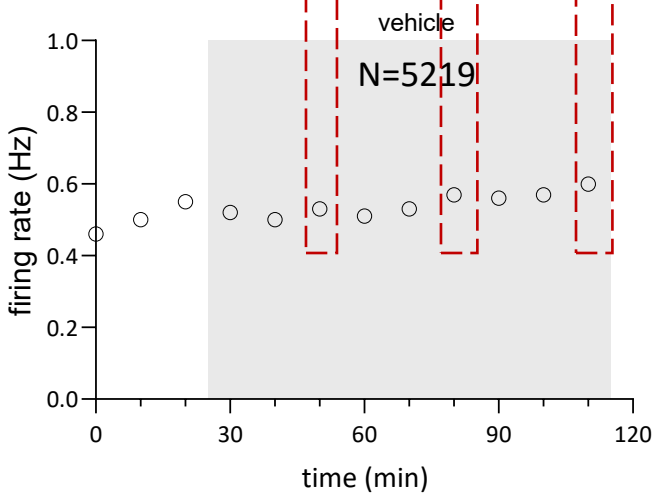
Retigabine exhibited conc-dependent block of rat cortical neuron excitability

Retigabine

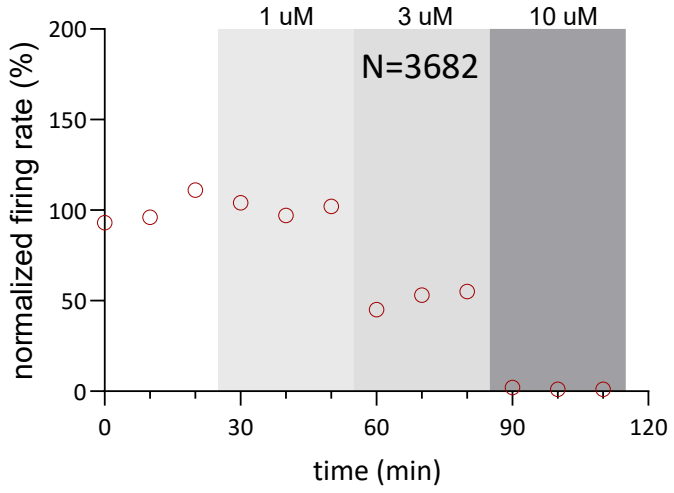
vehicle

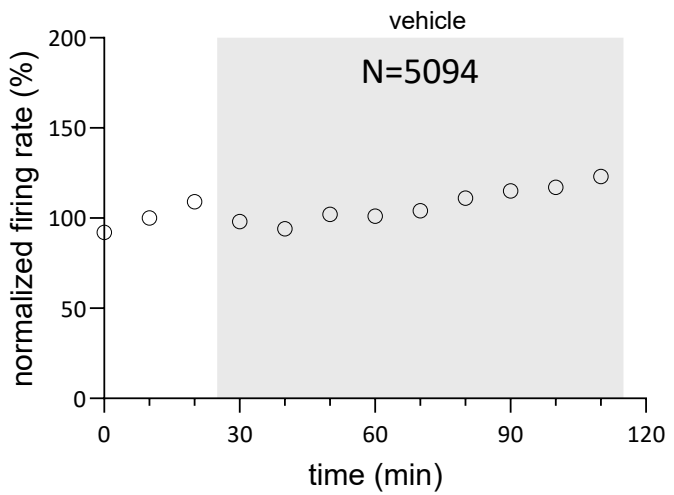
Firing rate (Hz)



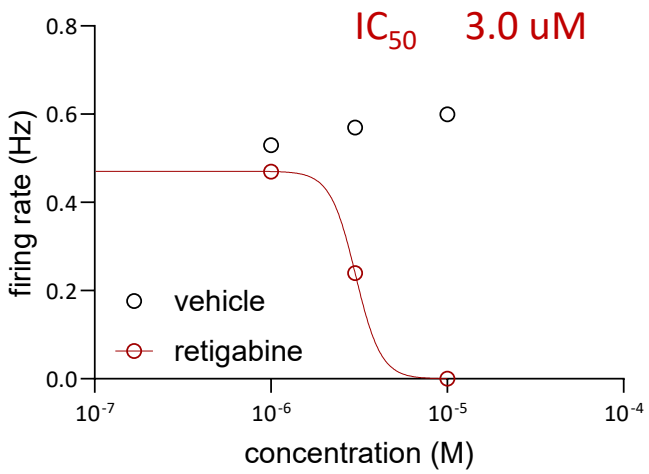


Normalized firing rate (% baseline)



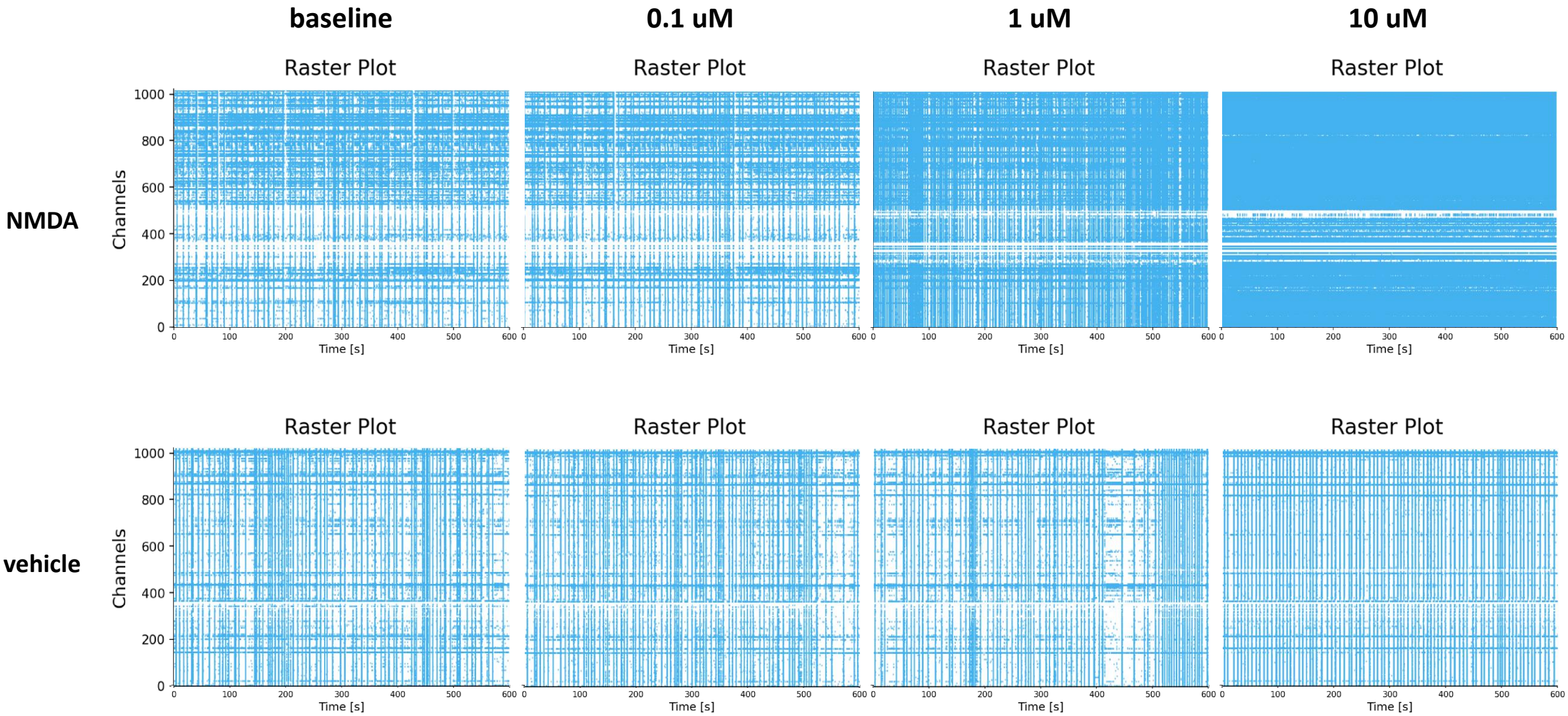


Concentration response curve



Note: In normalized firing rate, firing rate at each measurement were normalized to baseline average for each electrode. Electrodes with less than 0.01 Hz firing rate in baseline were excluded.

NMDA exhibited conc-dependent increase of rat cortical neuron excitability

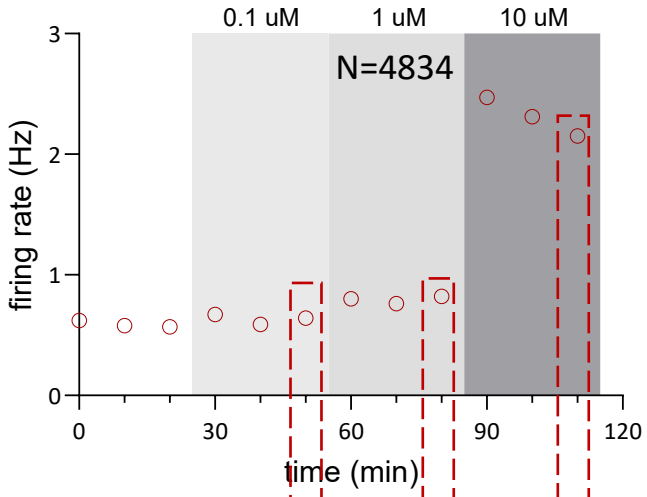


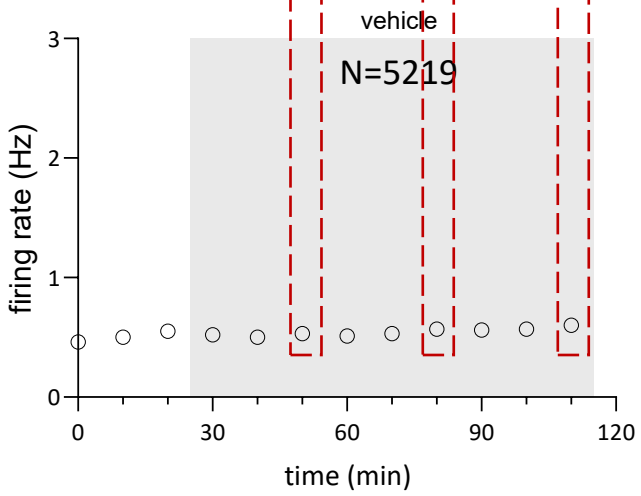
NMDA exhibited conc-dependent increase of rat cortical neuron excitability

NMDA

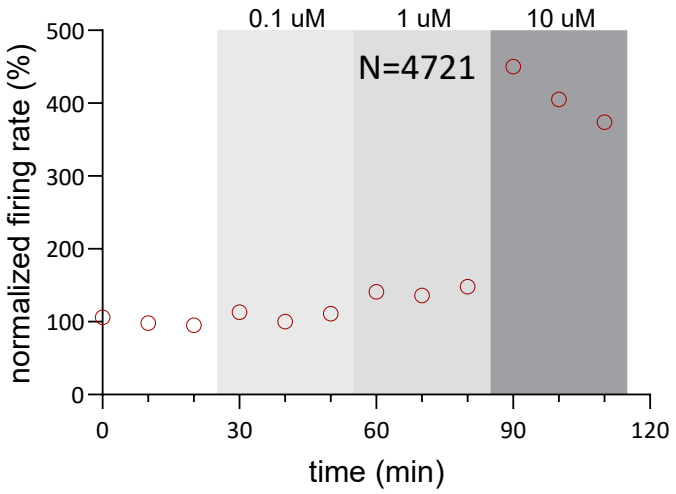
vehicle

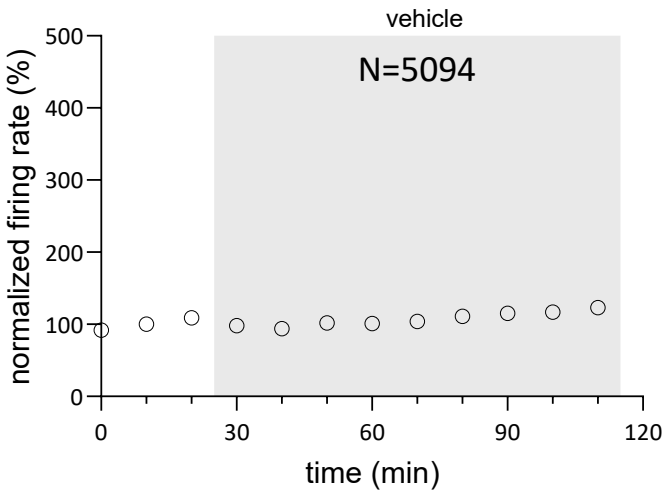
Firing rate (Hz)



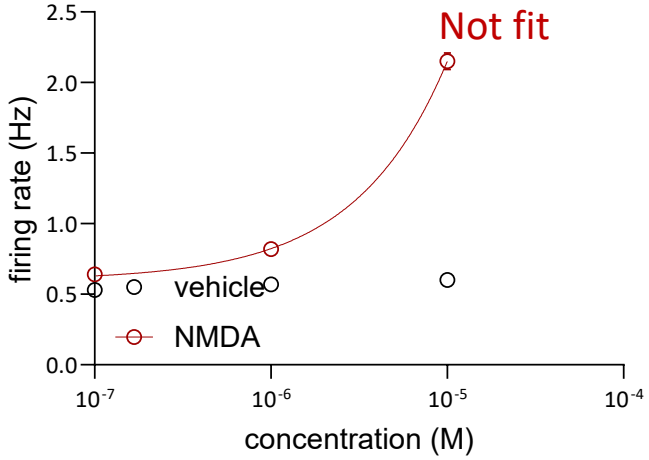


Normalized firing rate (% baseline)





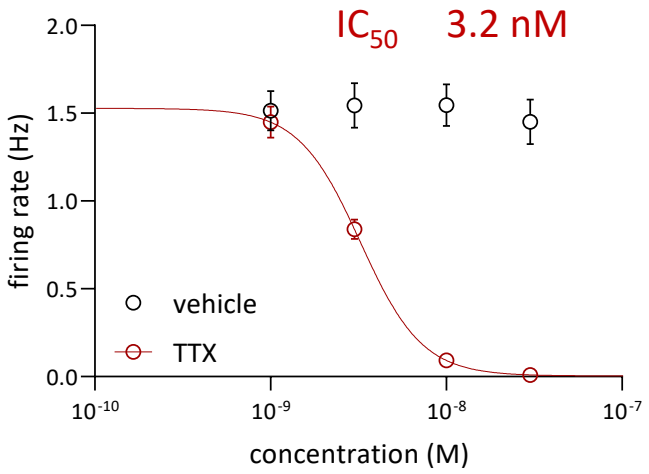
Concentration response curve



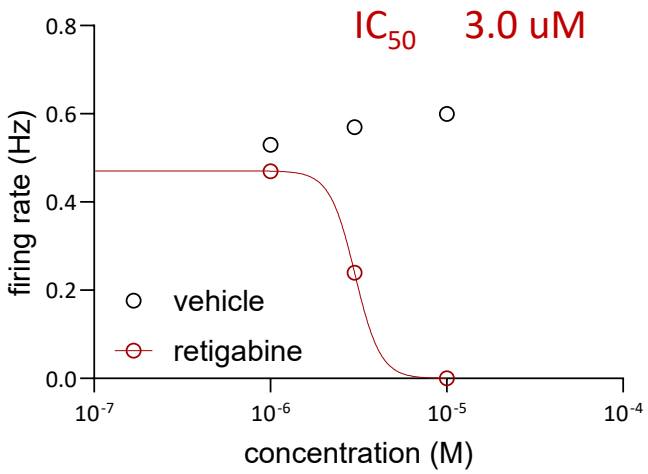
Note: In normalized firing rate, firing rate at each measurement were normalized to baseline average for each electrode. Electrodes with less than 0.01 Hz firing rate in baseline were excluded.

TTX, retigabine and NMDA exhibited effect on neuron excitability on MEA platform

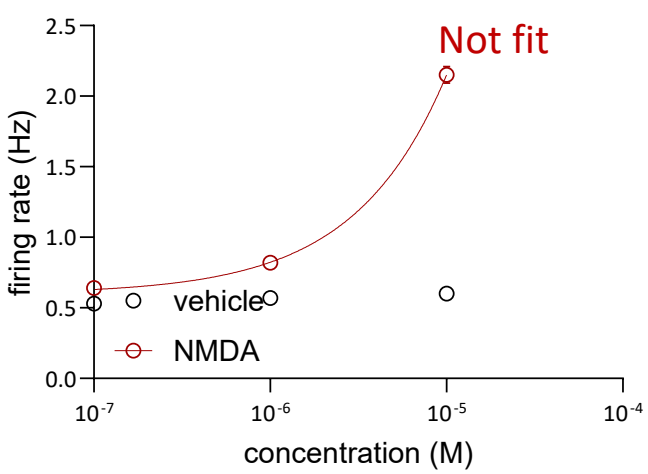
TTX
conc-resp curve



Retigabine
conc-resp curve



NMDA
conc-resp curve



Goal Assay development and pharmacological validation