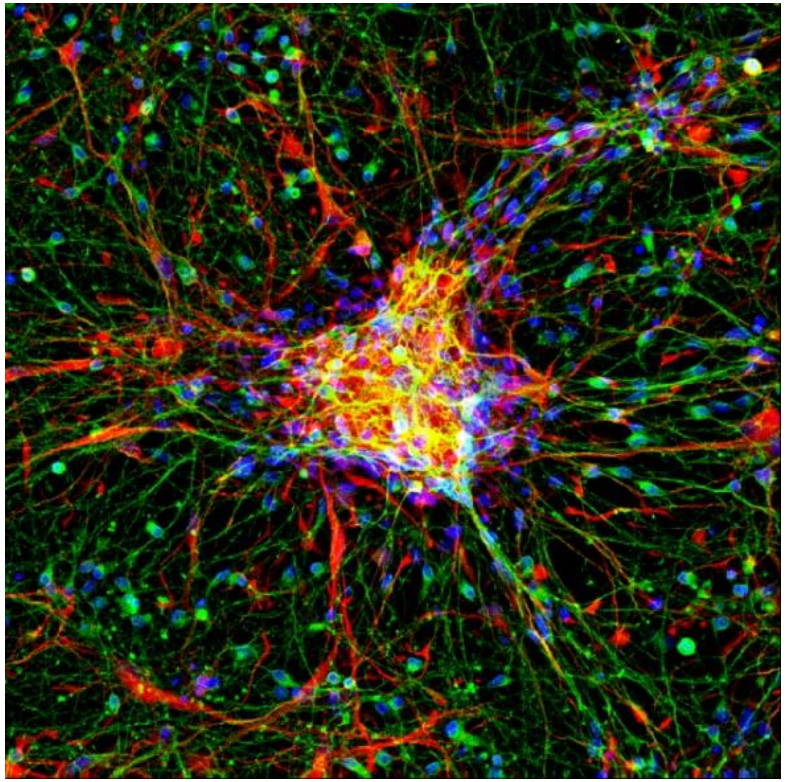
Utrecht University

April 15, 2021

Drug Discovery Toxicology Specialty Section
Society of Toxicology - (DDTSS-SOT)

In vitro approaches for neurotoxicity testing



Remco Westerink, PhD
Neurotoxicology Research Group, Toxicology Division,
Institute for Risk Assessment Sciences (IRAS)

Drug discovery and safety testing

- Increasing incidence of CNS-related disorders
- Many new drugs do not make it to the market
- Attrition rates high, especially for drugs targeting the CNS
  - Vulnerability of the CNS
  - Safety testing!

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- *In vivo* testing:

Drug discovery and safety testing

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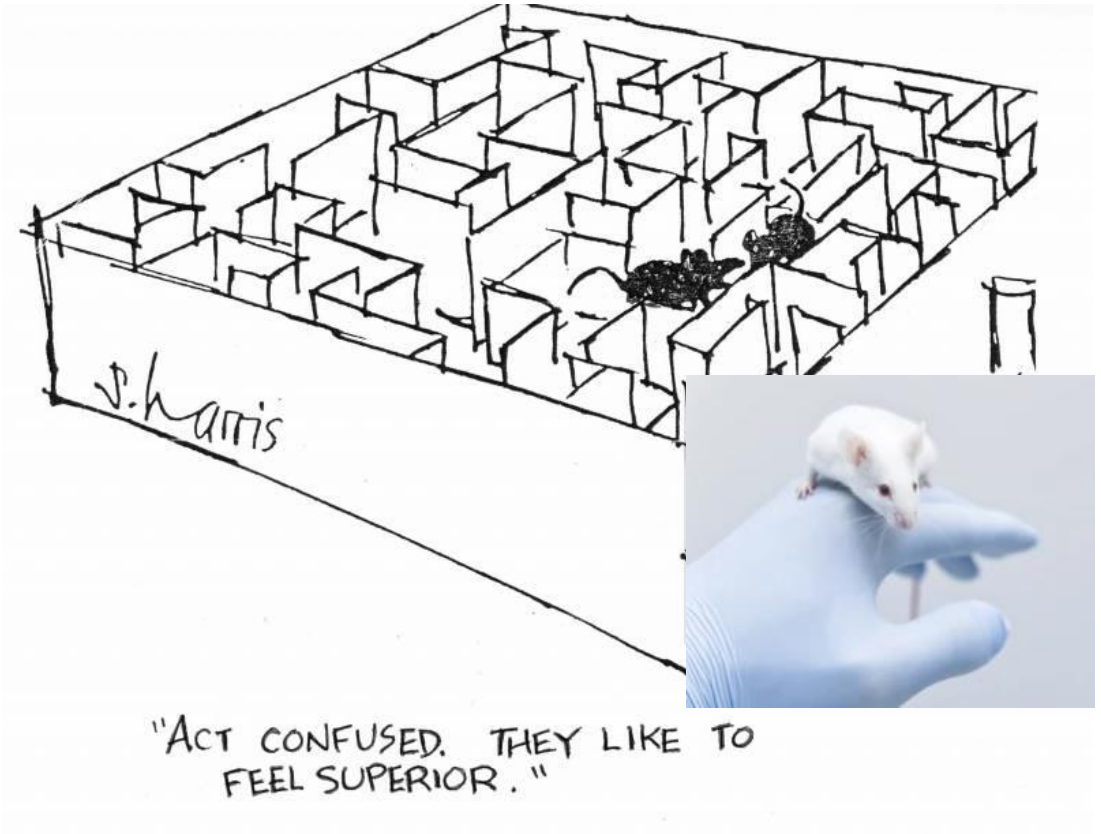
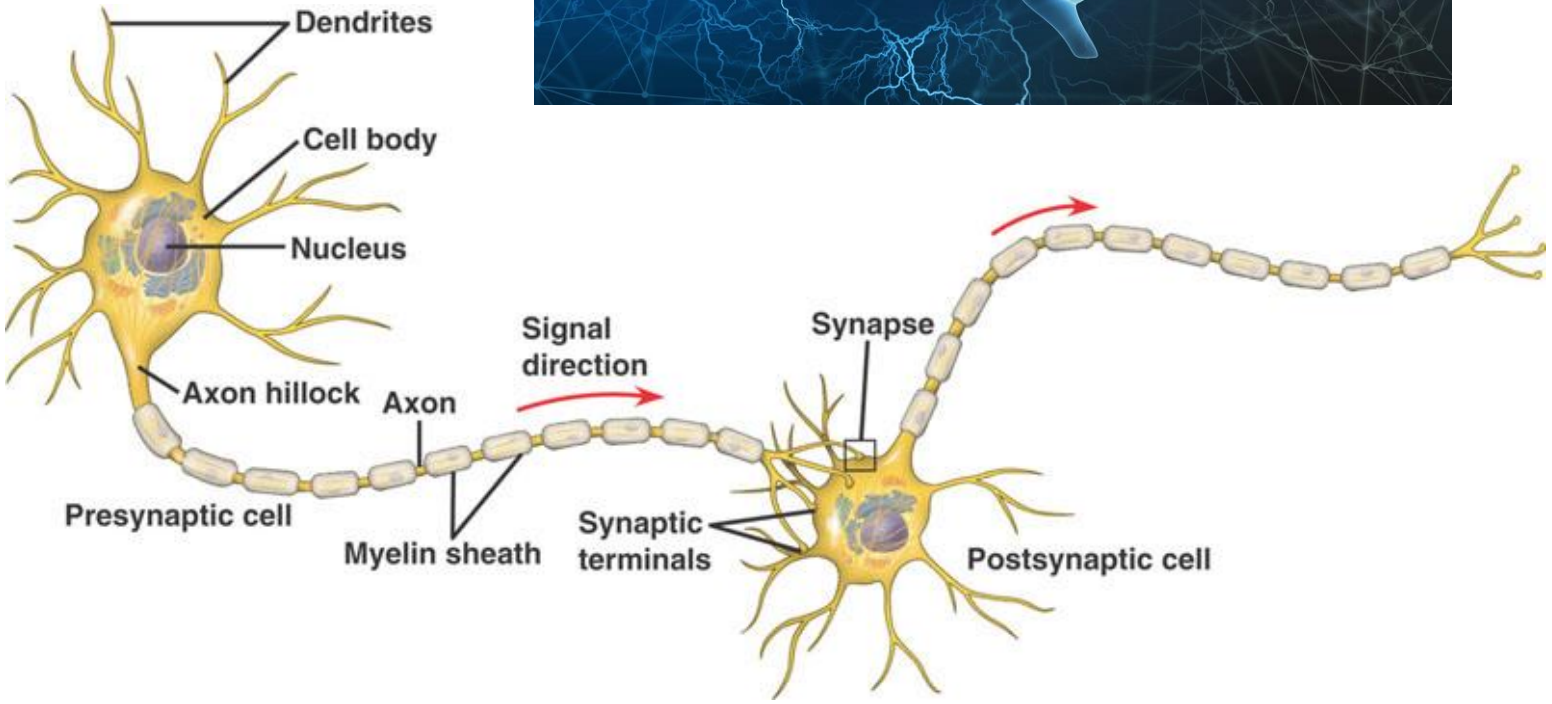
- Many new drugs do not make it to the market
- Attrition rates high, especially for drugs targeting the CNS
  - Vulnerability of the CNS
  - Safety testing!
- *In vivo* testing:
  - Expensive,
  - Ethically debated,
  - Not always predictive…



Drug discovery and safety testing

- Need for *in vitro* alternatives!
- Capture complexity of CNS processes
  - Sensitivity
  - Specificity
  - Translatability

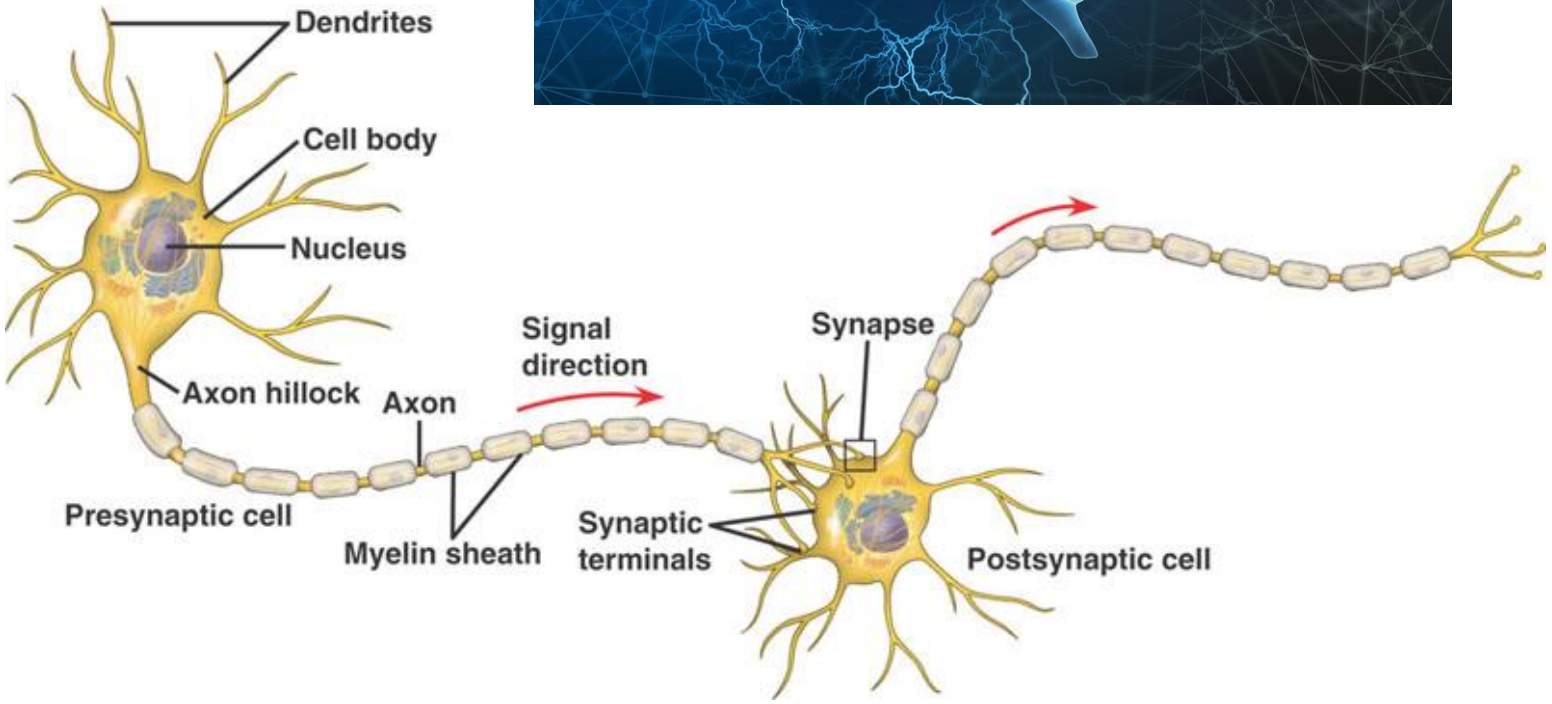


Drug discovery and safety testing

- Need for *in vitro* alternatives!

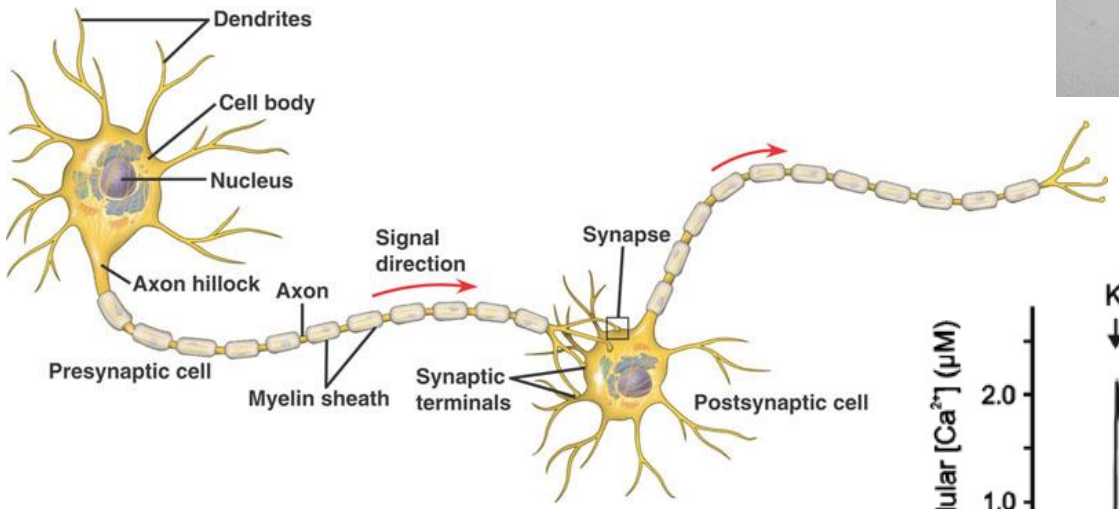
- Capture complexity of CNS processes
  - Sensitivity
  - Specificity
  - Translatability
  - Throughput

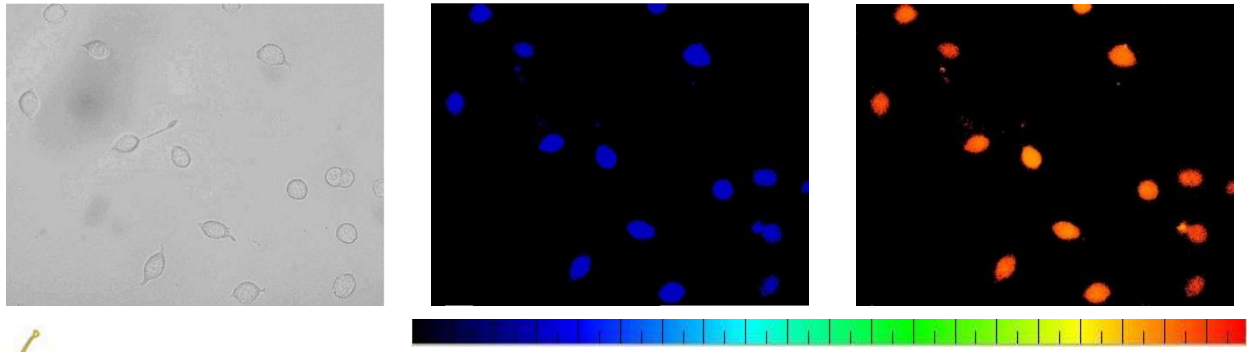
- Battery?
  - Cell viability
  - Ion channel function
  - Receptors
  - Transporters
  - Signalling (Ca^{2+})
  - .. .. ..

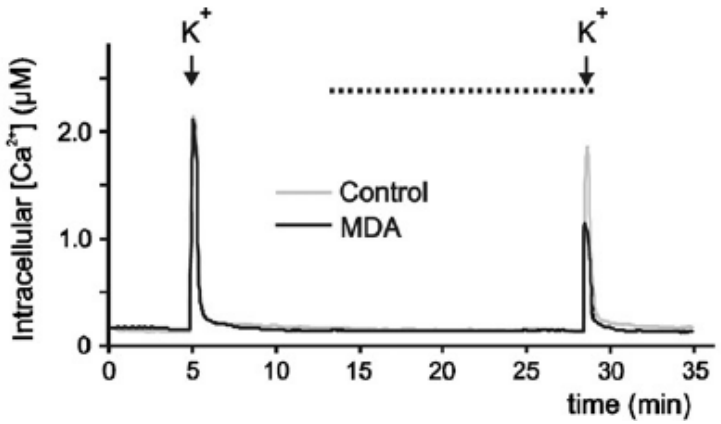


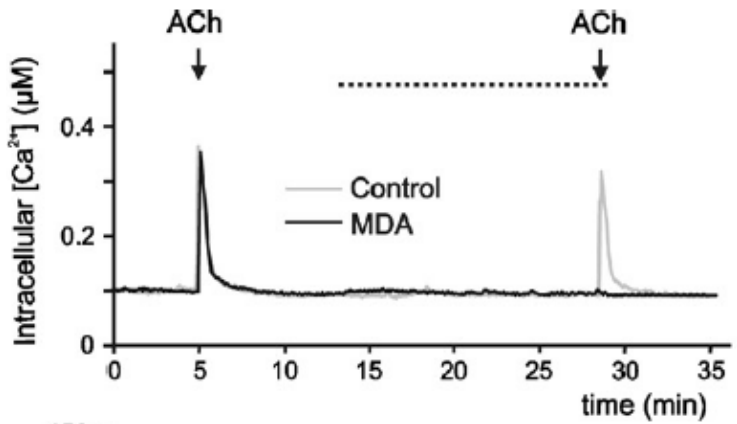
Extensive *in vitro* battery,..

- Calcium homeostasis, channels and receptors
  - Many (!) different types or receptors/channels!
  - Requires multiple cell types









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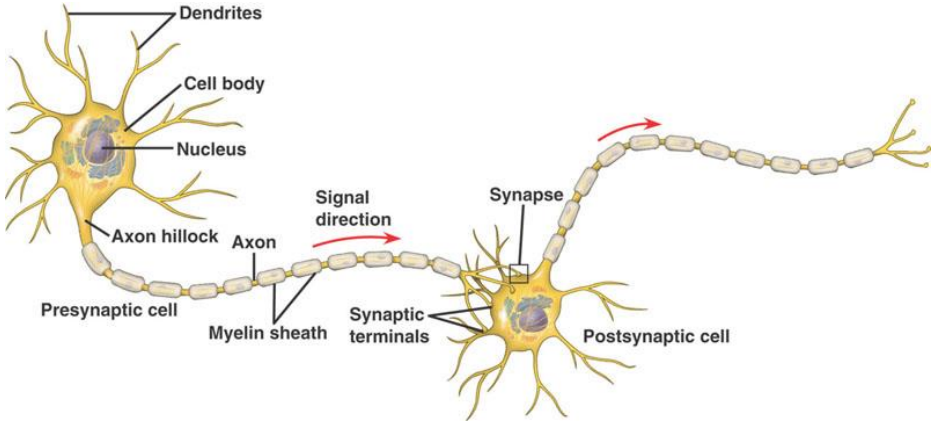
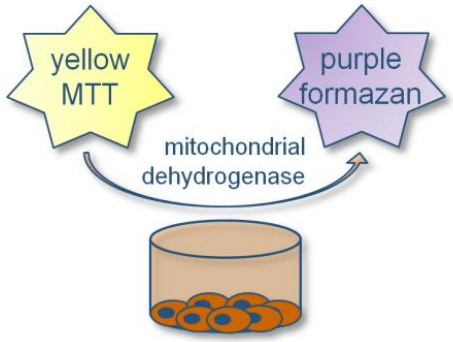
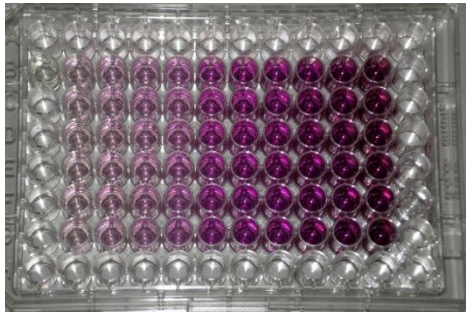
Hondebrink et al. (2012) NeuroToxicology

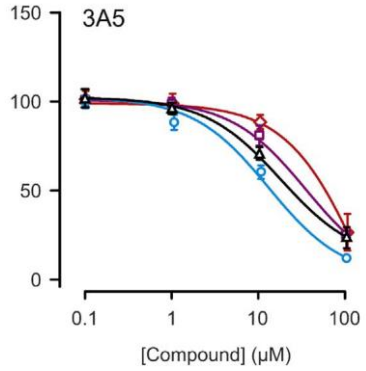
Extensive *in vitro* battery,..



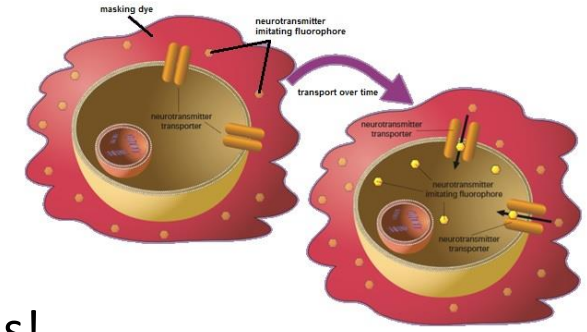


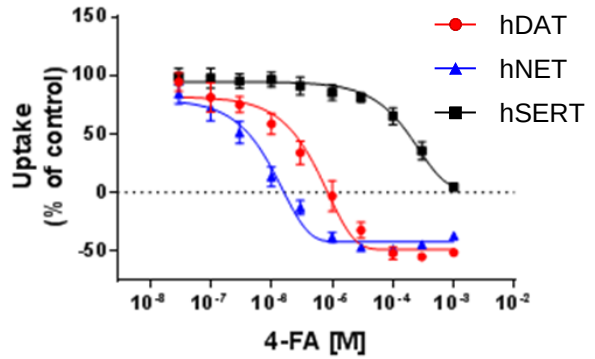
Heusinkveld et al. (2017) TIV



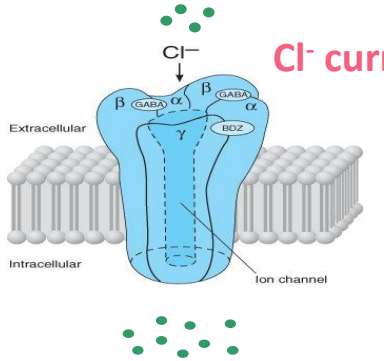


- Cell viability
- E-phys
- Transporter assays
  - Many (!) different types or receptors/channels!
  - Requires multiple cell types

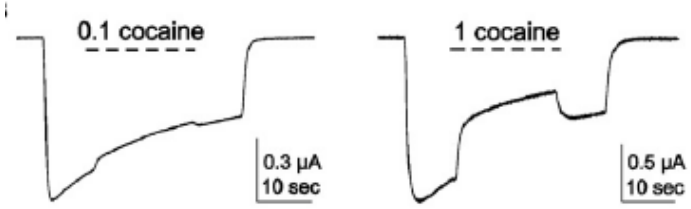




Zwartsen et al. (2017) TIV



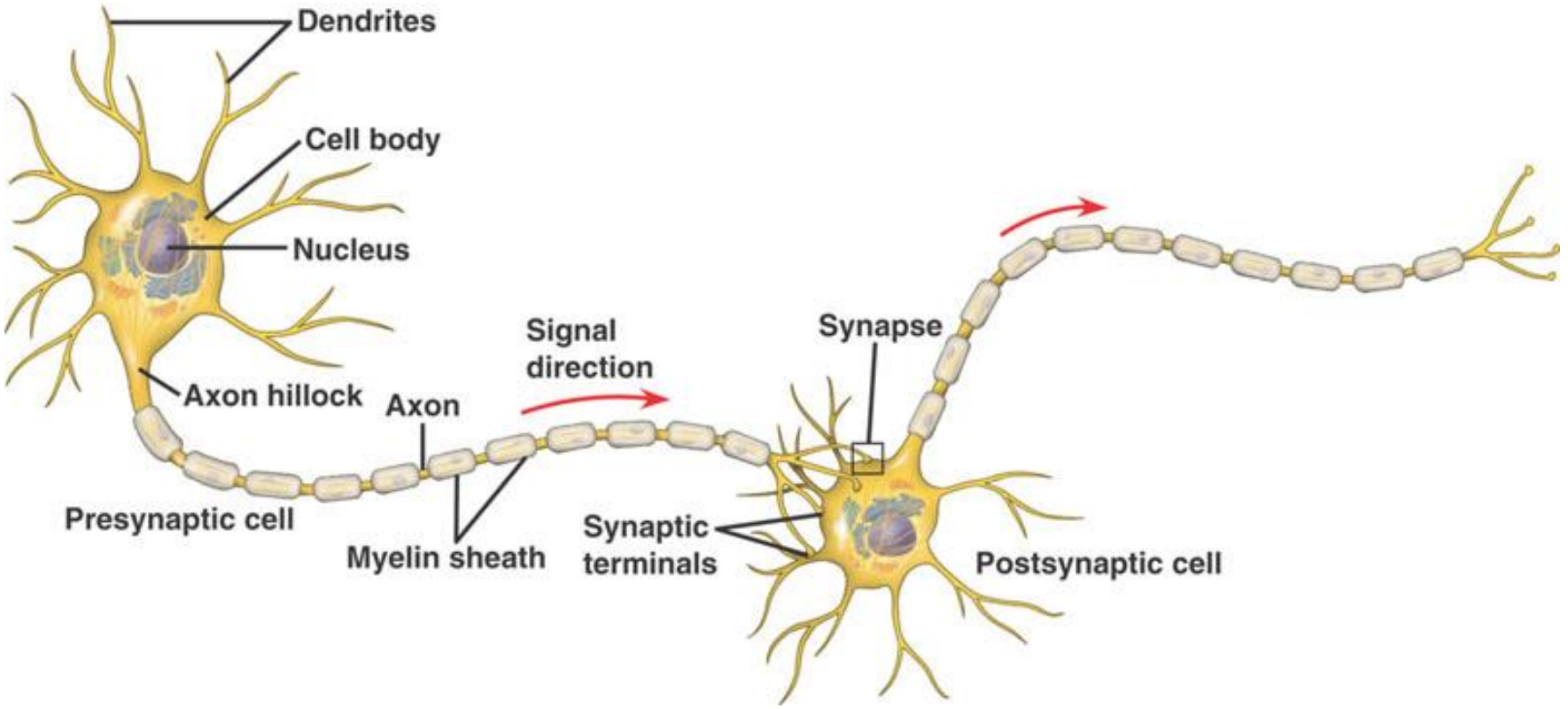
Cl⁻ current



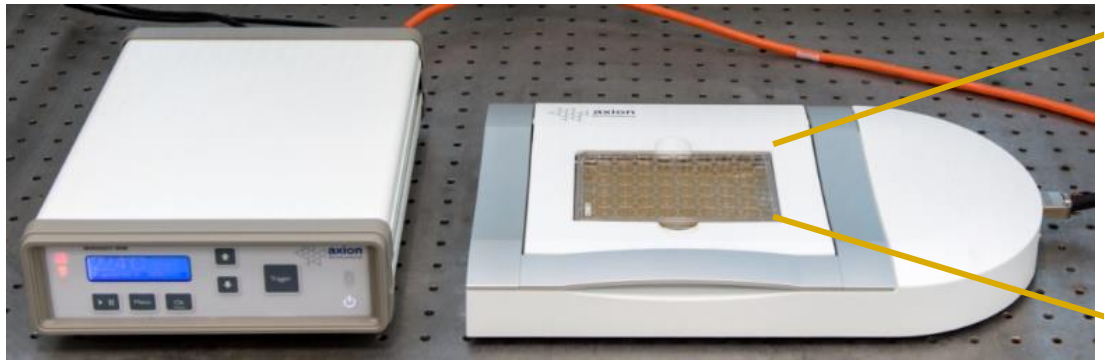
Hondebrink et al. (2013) NeuroToxicology

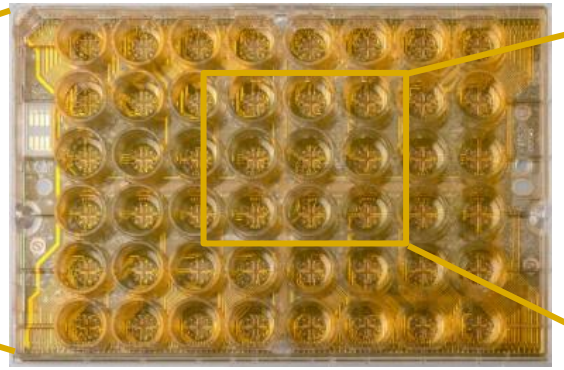
Integrated *in vitro* assay,..

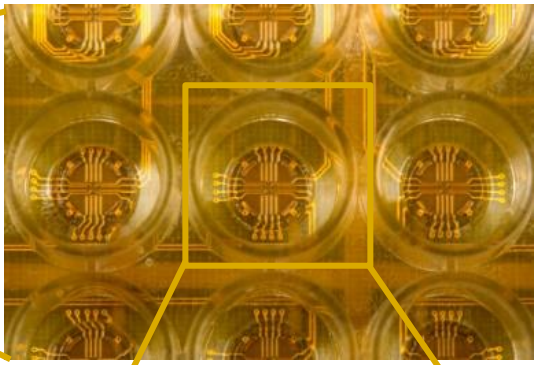
- Nerve cells form communicating networks that reflects combined effects on cell viability, network integrity, Ca^{2+} homeostasis, synaptic transmission, receptor and ion channel function!
- Measure neuronal network function!

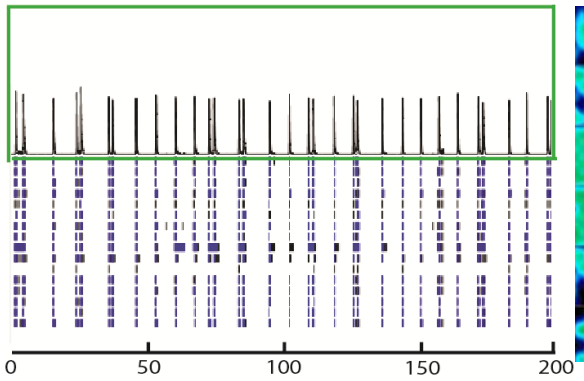


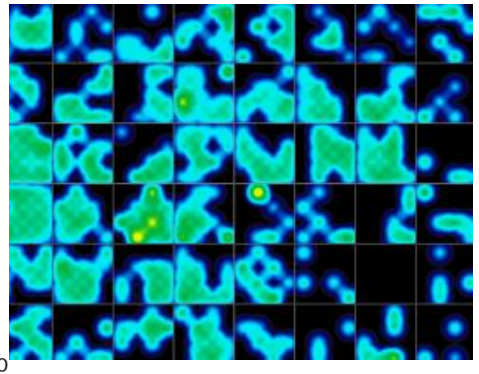
MicroElectrode Array (MEA) recordings

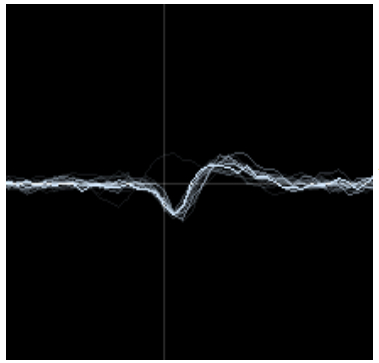


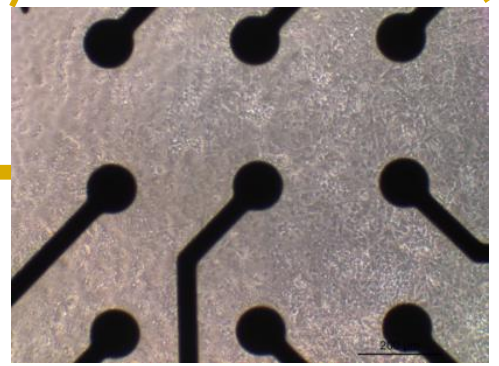










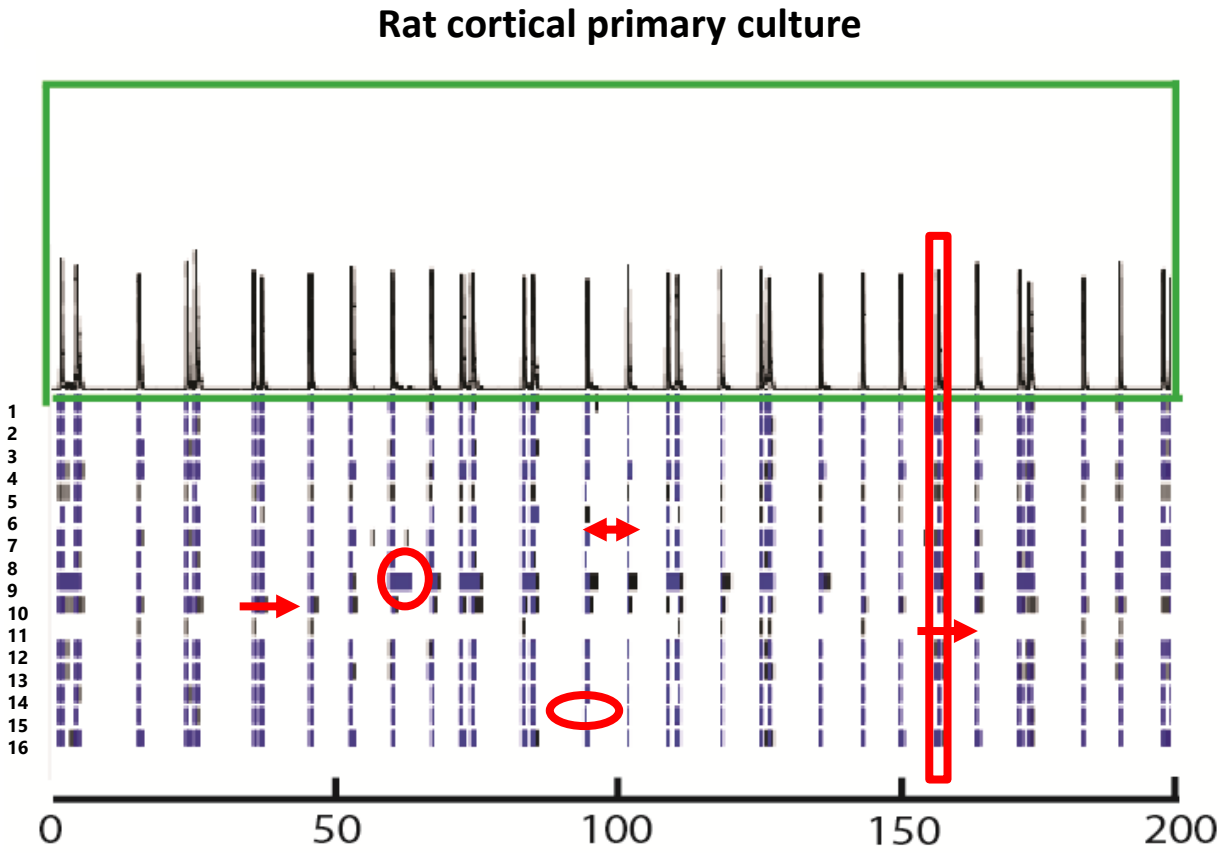


MicroElectrode Array (MEA) recordings

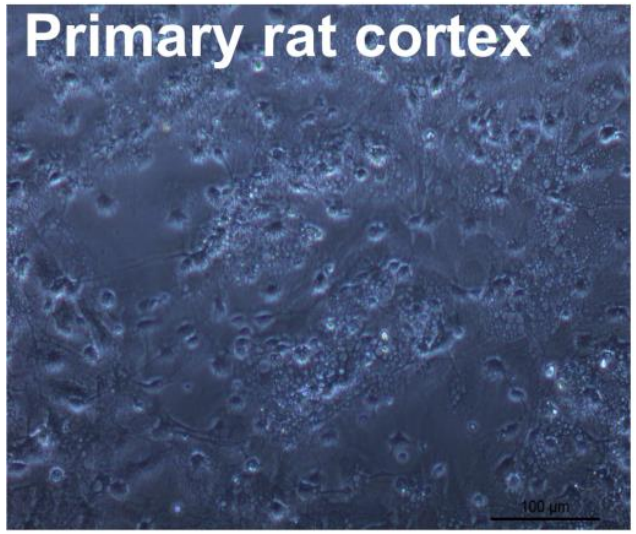
48-wells, each well 16 electrodes

Neuronal function reflected in:

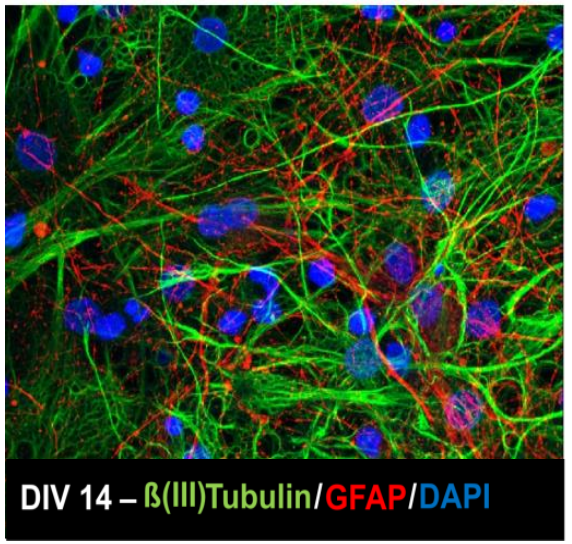
- Spikes
- Bursts
- Burst duration
- Inter-burst interval
- Network bursts
- Network burst duration
- Synchronicity



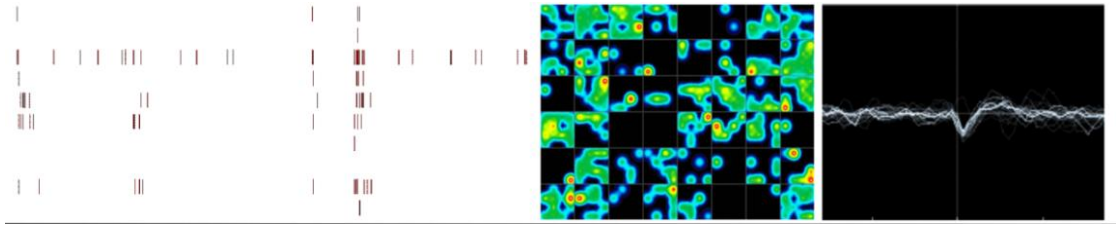
MEA recordings; rat primary cortex



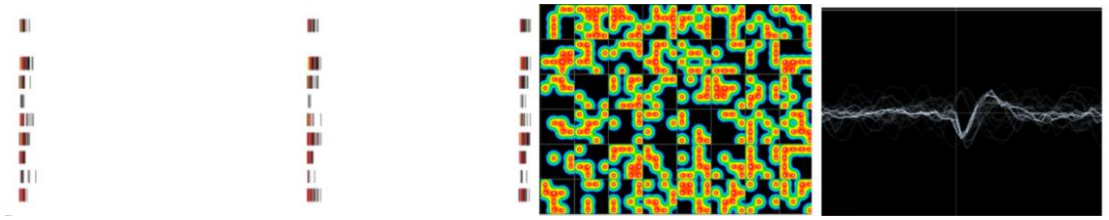
- Mixed (GABA/glutamate) neuronal cultures with ~45% astrocytes
- Develop into spontaneously active functional networks

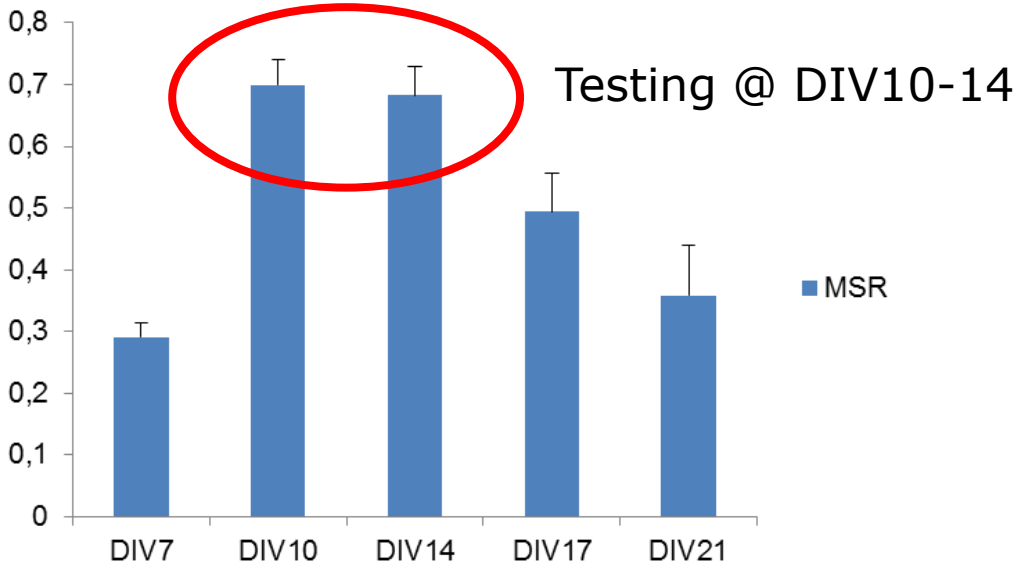


Primary rat cortical culture (DIV 7)



Primary rat cortical culture (DIV 11)



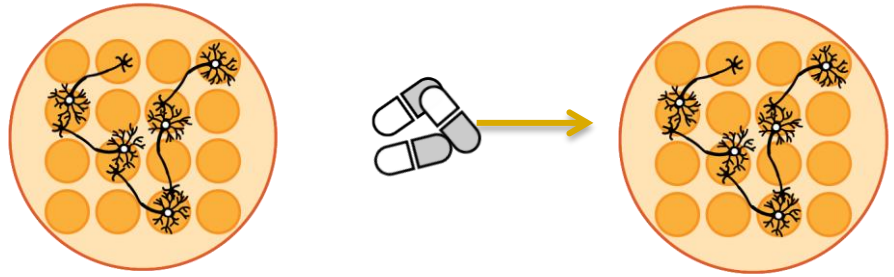


Testing @ DIV10-14

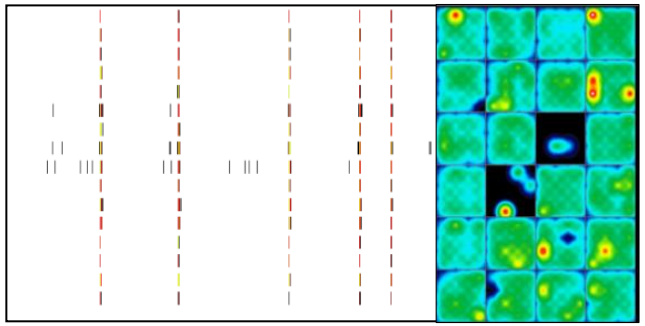
MEA recordings; rat primary cortex

Exposure to test compound

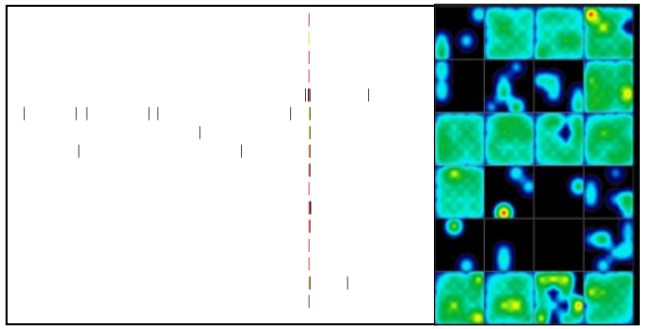
Baseline recording (30 min) → Acute exposure recording (30 min)



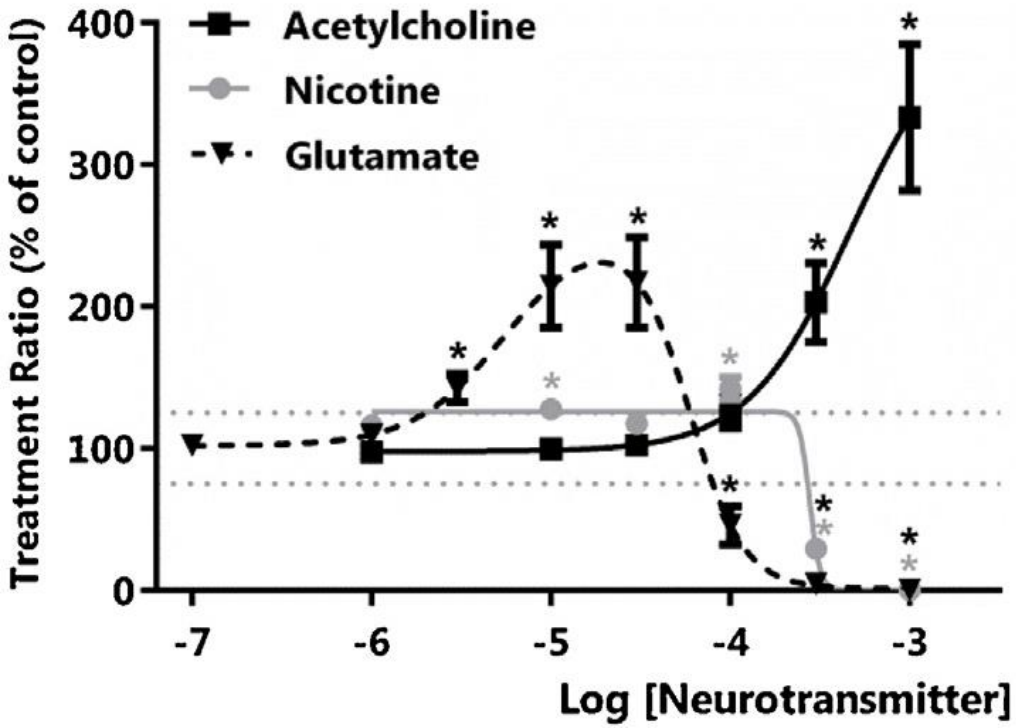
Before exposure



After exposure



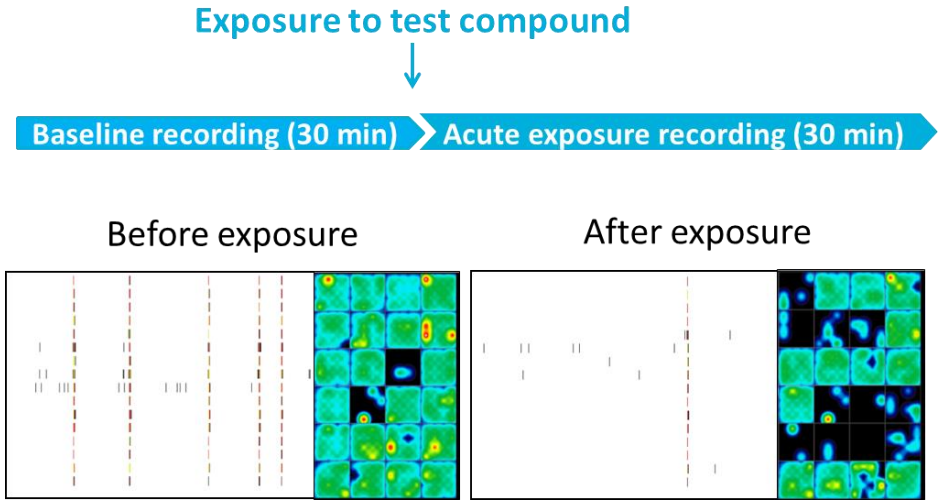
❖ Functional receptors & ion channels



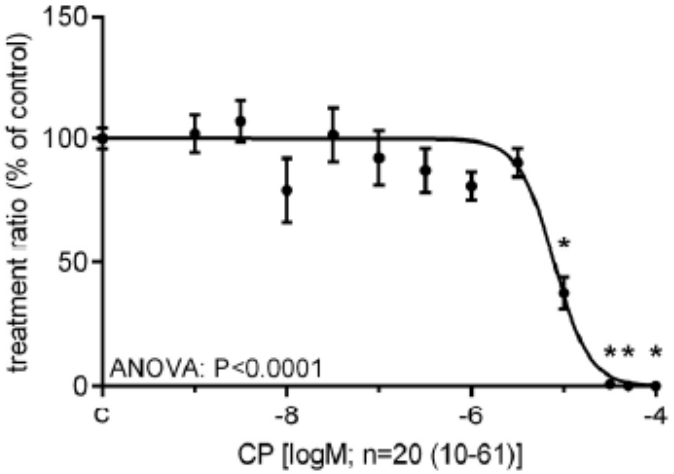
Average of treatment ratio ± SEM. n_{wells} = 14-27, * p ≤ 0.01
Dotted lines indicate biological variation in control wells (± 1x SD)

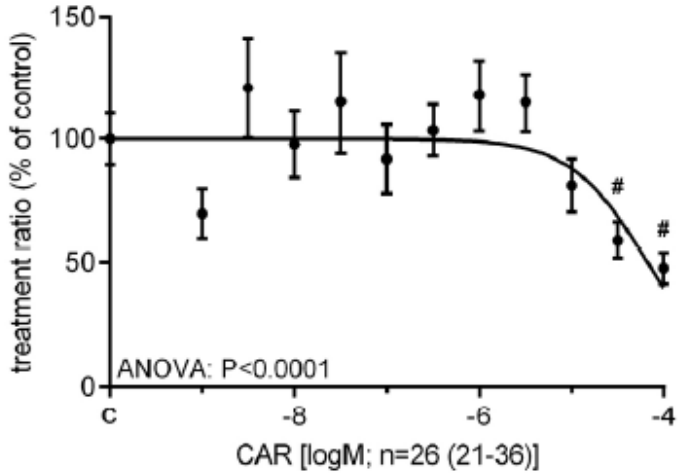
Hondebrink et al., NeuroToxicology, 2016

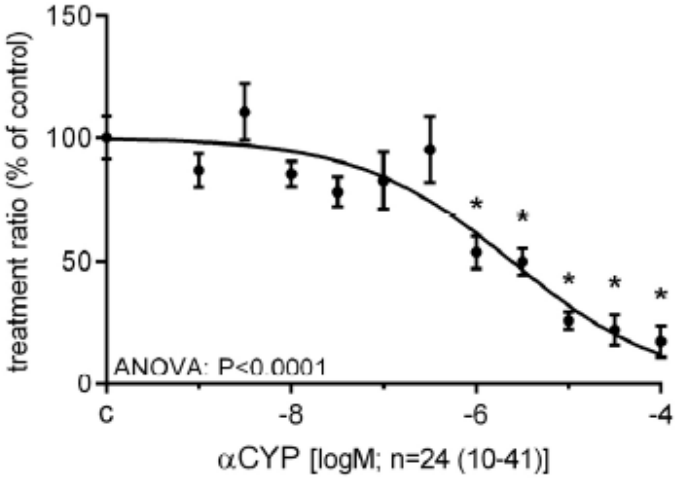
Acute effects of insecticides

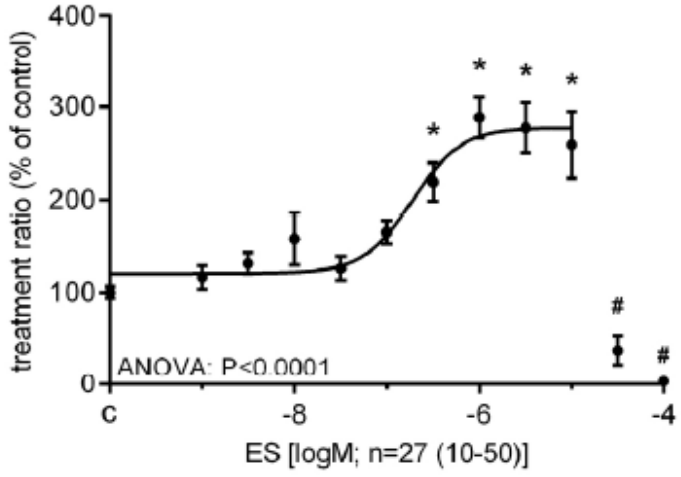


- Different classes of insectides induce inhibition of neuronal activity
- Known GABA-R antagonist endosulfan evokes neuronal hyperexcitation









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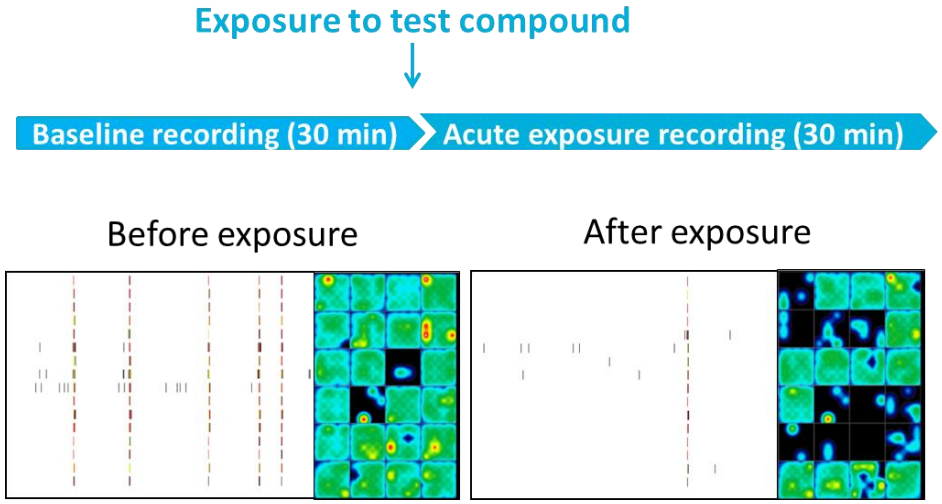
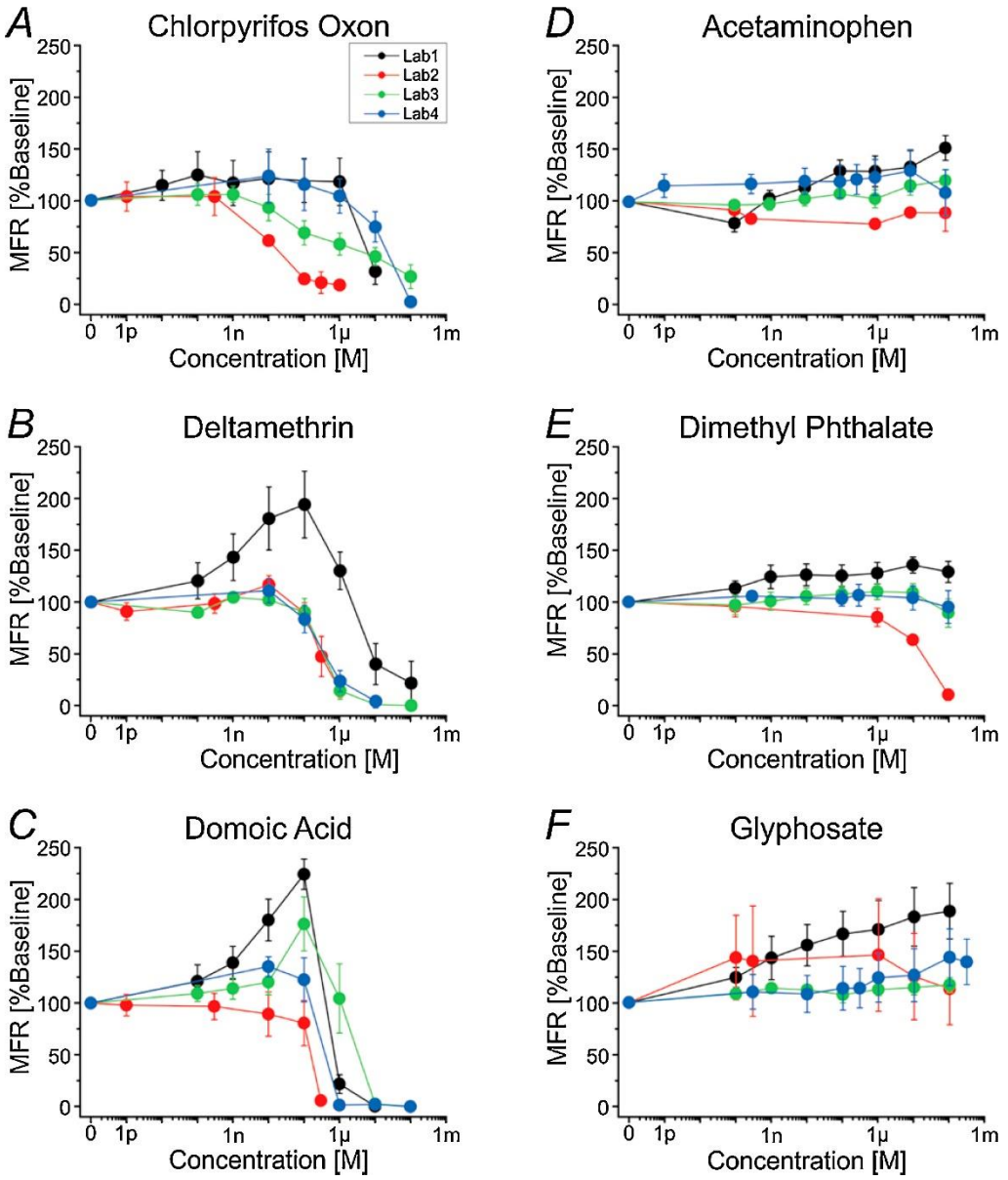
Dingemans et al., NeuroToxicology, 2016

Interlaboratory comparison

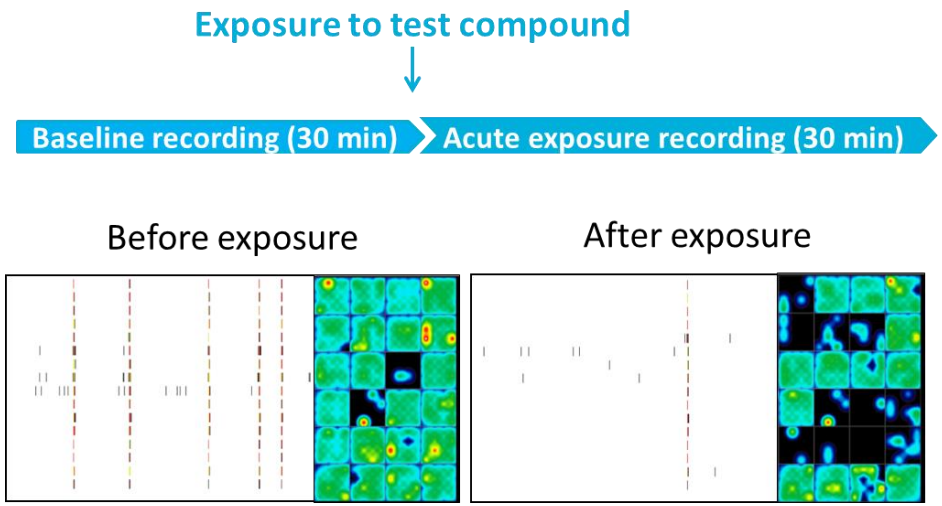


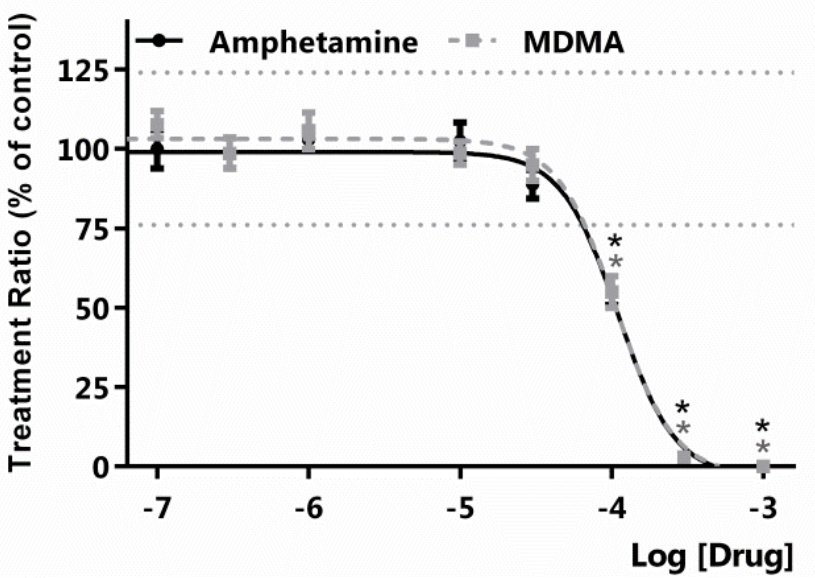
- 4 different labs;
- 4 different culture conditions (E18/PND1, rat/mouse)
- Generally reproducible!

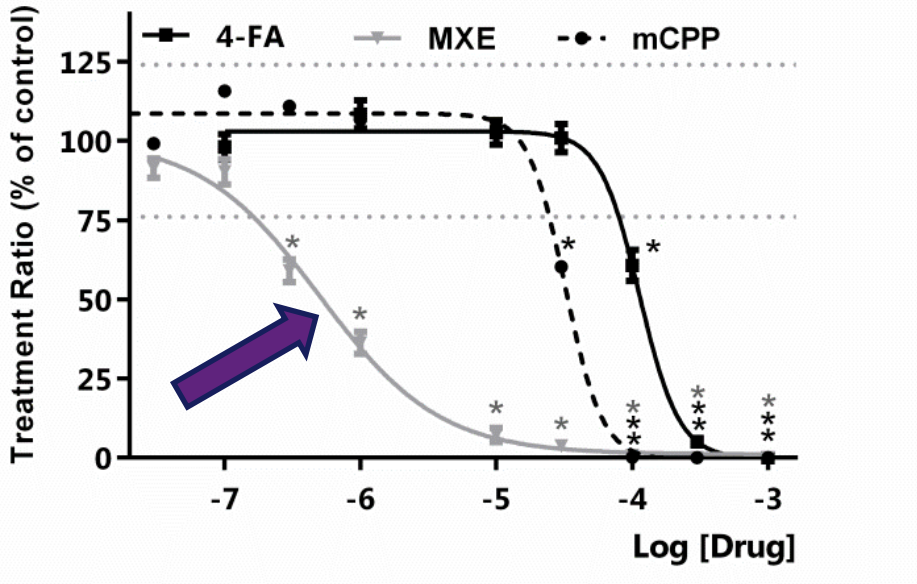


Vassallo et al., NeuroToxicology, 2017

Acute effects of illicit drugs



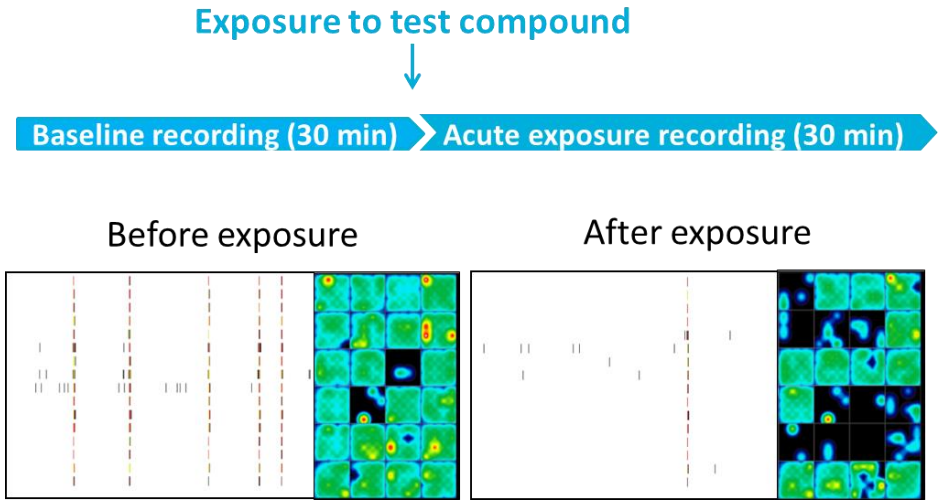




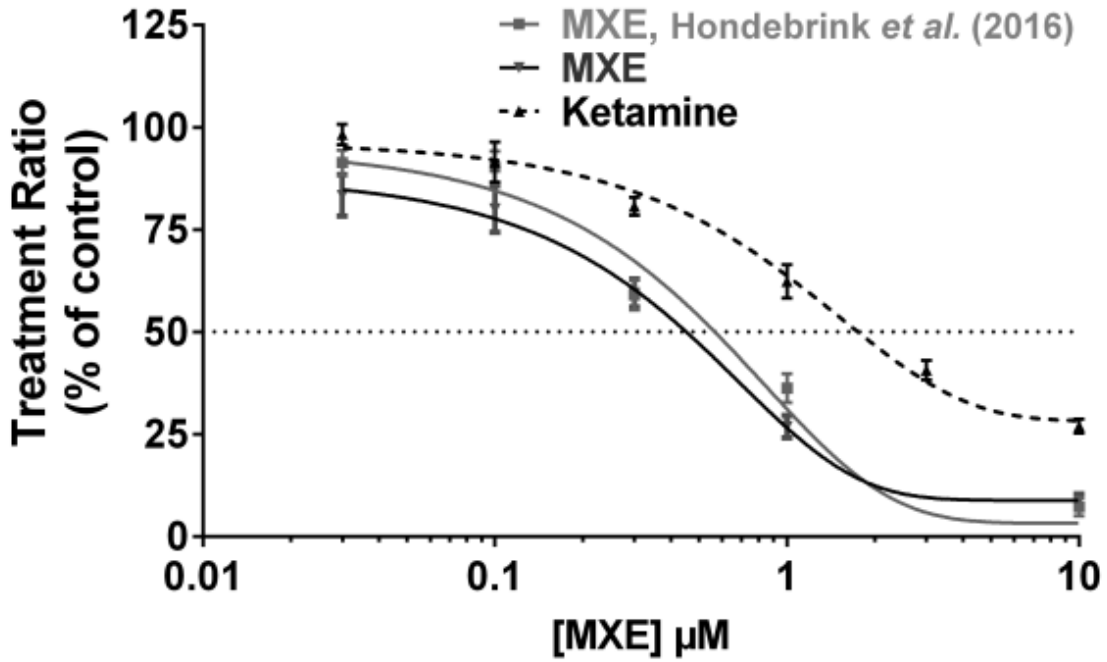
- Acute drug-induced inhibition of neuronal activity
- Comparable potency of amphetamine-like substances
- Remarkable potency of MXE?!

Hondebrink et al., NeuroToxicology, 2016

Acute effects of Methoxetamine



- MXE: Ketamine analogue
- Reproduce & compare to ketamine



❖ IC50 neuronal activity (MXE):

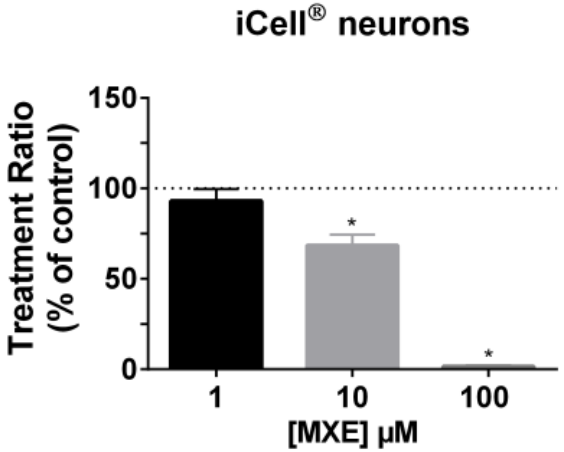
0.5 µM (rat cortex)

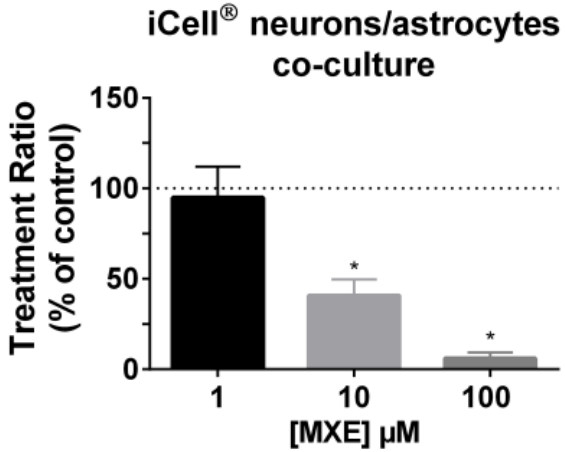
❖ **Human iPSC-derived neuronal cultures?!**

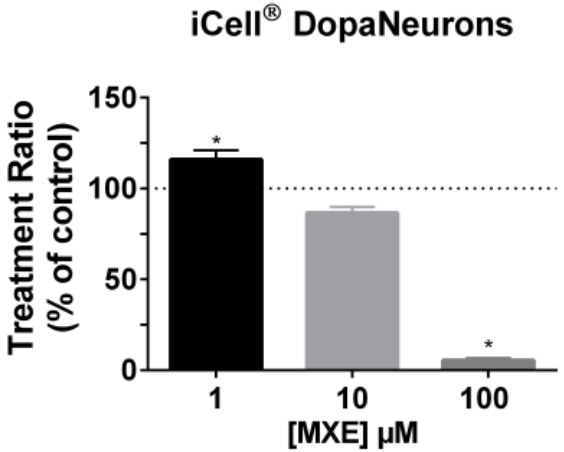
Hondebrink et al., Neuropharmacology, 2017

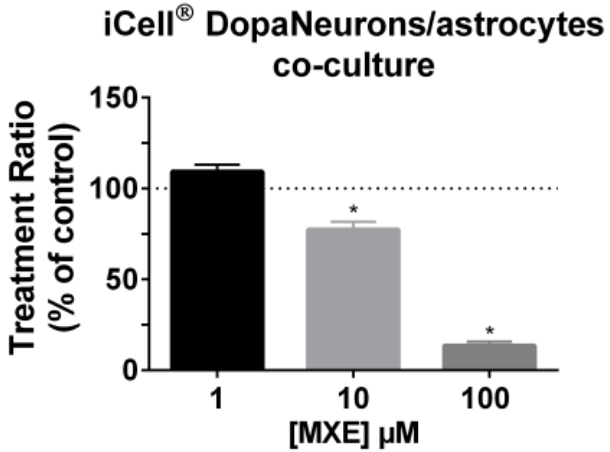
Acute effects of Methoxetamine in human iPSC-derived cultures

- Human iPSC-derived neurons, with/without astrocytes









Neuronal activity:

- IC_{50} (MXE): ~30 µM (without astrocytes)
- IC_{50} (MXE): ~10 µM (with astrocytes)

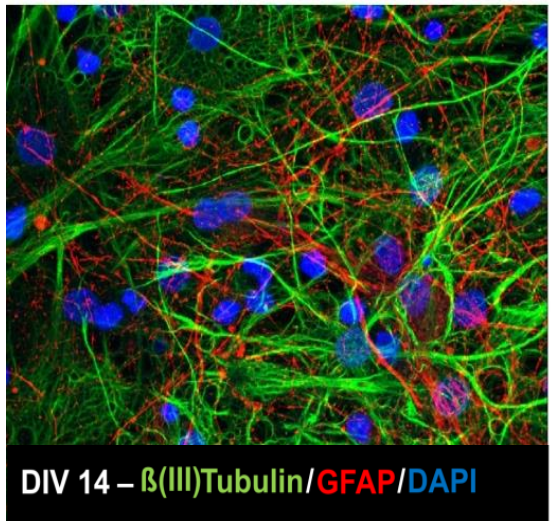
- IC_{50} (MXE) rat: 0.5 µM??

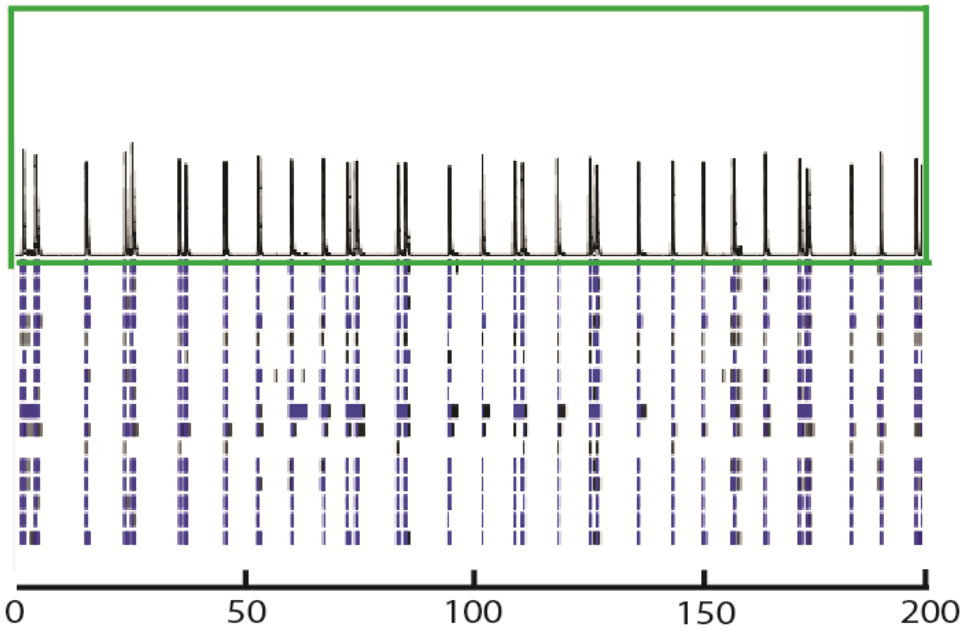
- IC_{50} (MXE): ~50 µM (without astrocytes)
- IC_{50} (MXE): ~30 µM (with astrocytes)

Hondebrink et al., Neuropharmacology, 2017

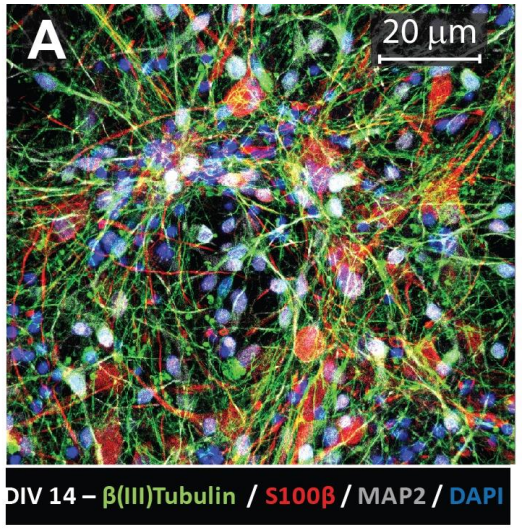
Human vs rat,…

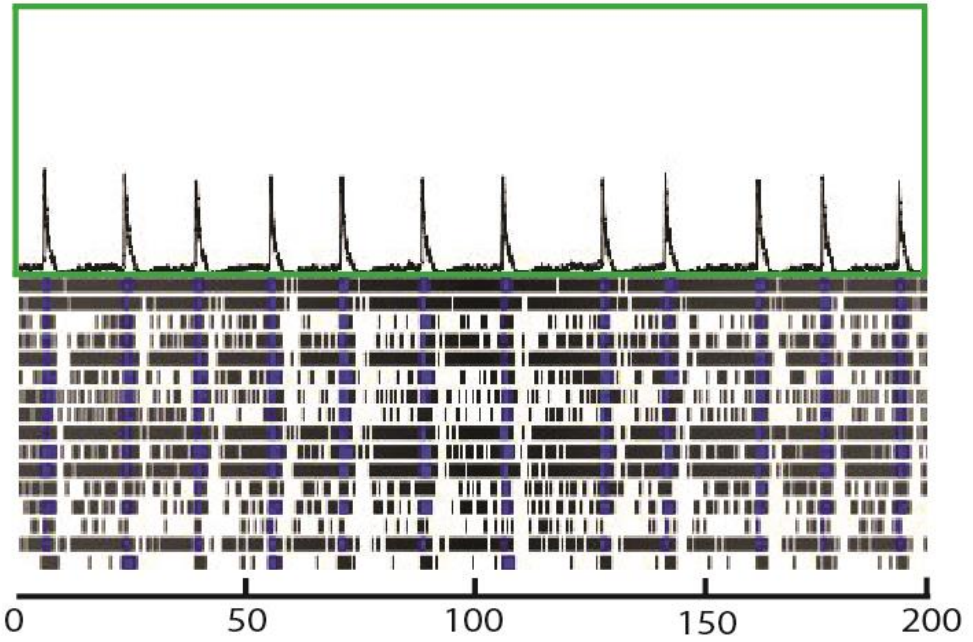
Rodent primary cortical culture





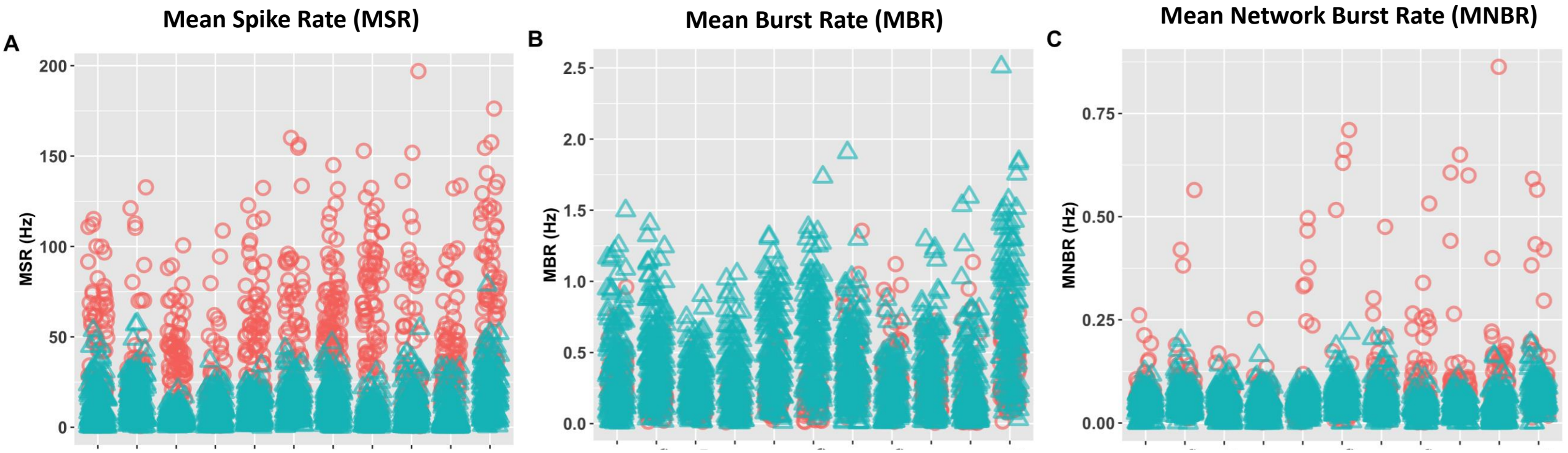
iCell Glutaneurons - Astrocytes





Tukker et al.,

Human vs rat,…



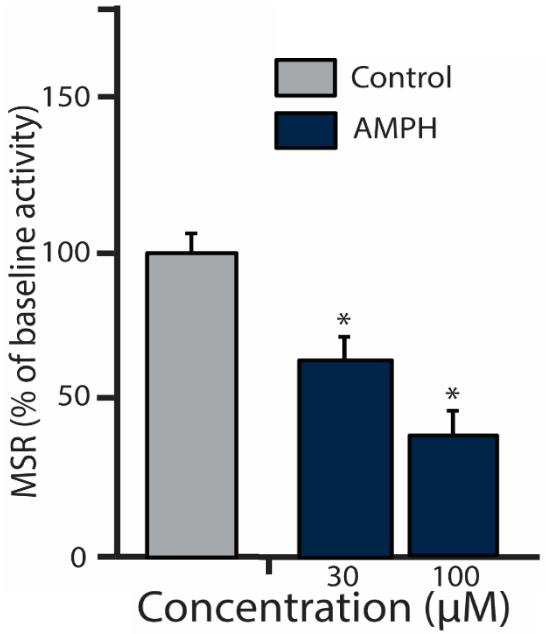
Different experiments, baseline activity

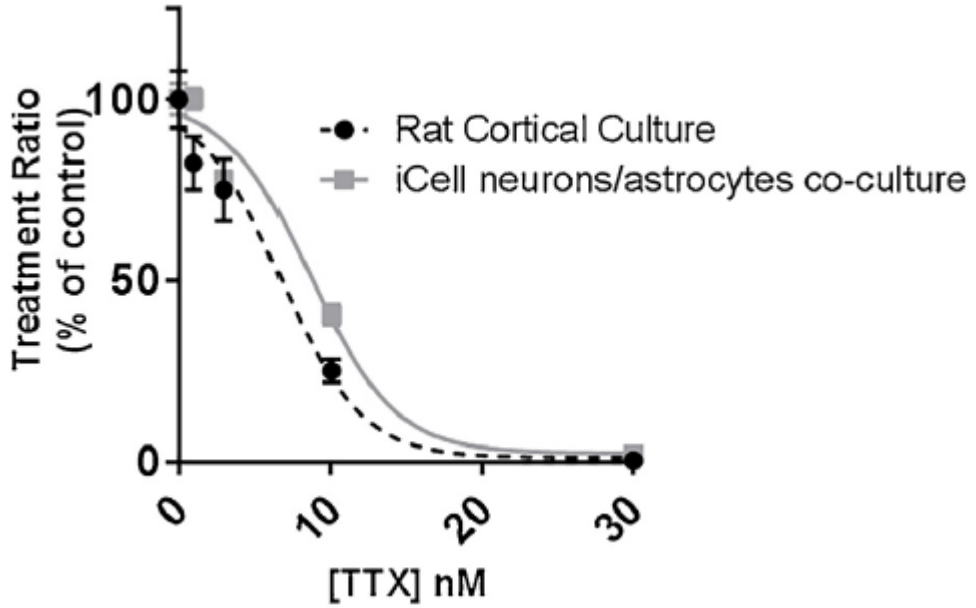
n = 855 wells from N = 25 plates for hiPSC-derived neuronal co-cultures; n = 1927 wells from N = 62 plates for rodent cortical primary cultures

Tukker et al.,

Human vs rat,…

- Many similarities neuronal activity..
- IC_{50} (AMPH) rat cortex ~100 µM
- IC_{50} (AMPH) hiPSC ~100 µM





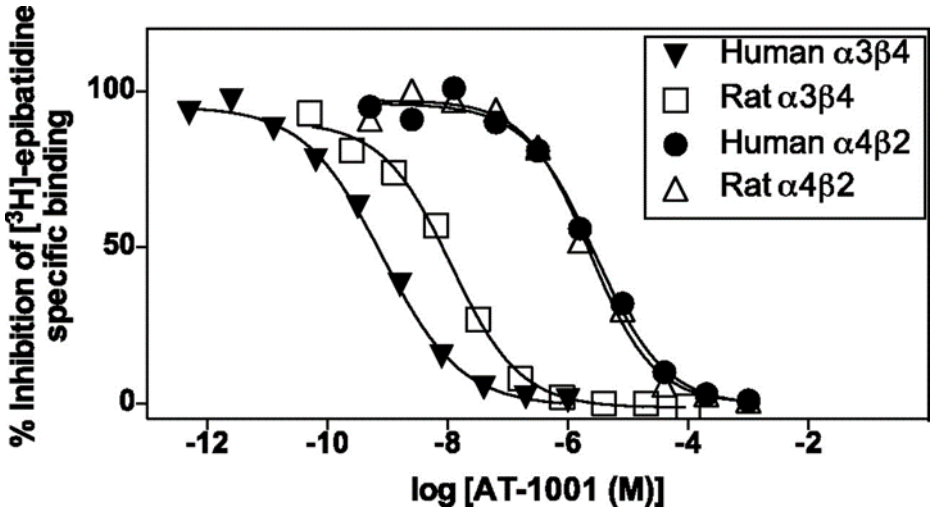
- IC_{50} (TTX) rat cortex ~7 nM
- IC_{50} (TTX) hiPSC ~10 nM

Tukker et al., ALTEX, 2016

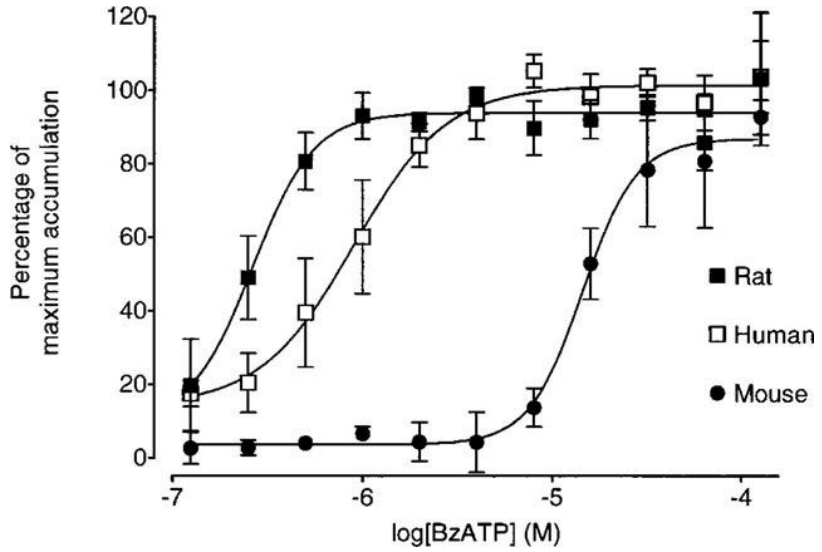
Kasteel & Westerink, Toxicology Letters, 2017

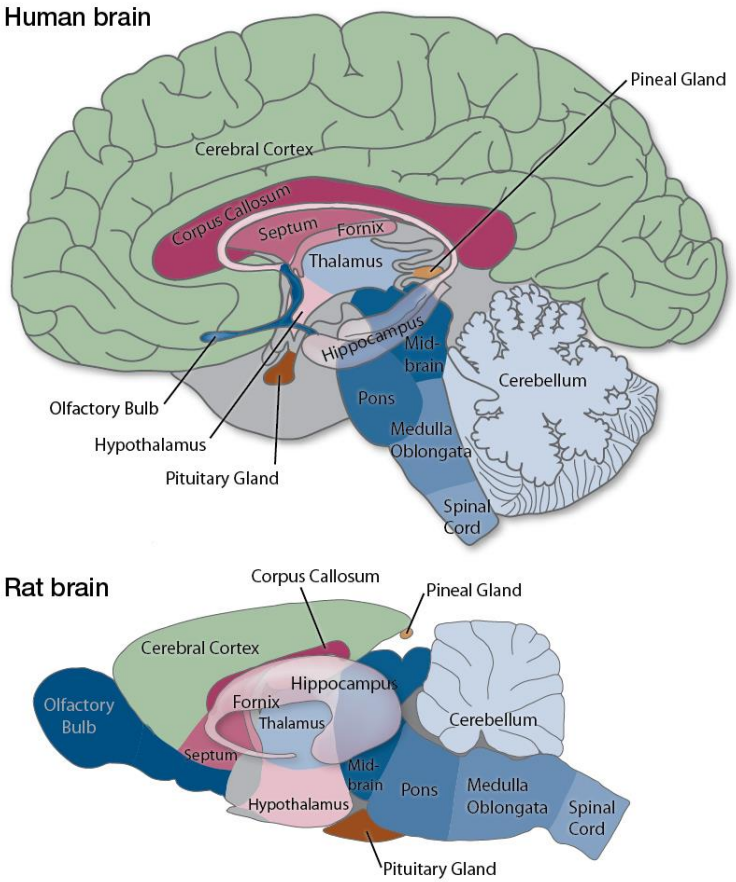
Human vs rat,…

- Also many differences…
- Differences in ACh-R



- Differences in P2X7-R





- And many more…, including MXE sensitivity

Hibell et al. (2000) BrJPharmacol

Tuan et al. (2015) MolPharmacol

Future of *in vitro* neurotoxicity screening?

- Rats are not little humans…
  - Differences in receptors, ion channels and metabolism!

- Human-derived, toxicity models!?
  - Region specific (e.g. dopaminergic) and patient-derived
  - Co-culture models to allow for cell-cell interactions
  - Expensive ☹
  - Model characterization & validation ☹

Take home message

- *MEA recordings offer an integrated, functional screen for neurotoxicity testing of a diverse range of toxicants, including pesticides and illicit drugs*
- *Species differences rat vs human*
- *Suitability of human iPSC-derived neuronal cultures*
- *Composition of culture determines neuronal activity (validation!)*
- *Ultimately replacement of laboratory animal use, while ensuring human safety!*

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