In vitro approaches for neurotoxicity testing

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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!
Drug discovery and safety testing

- Increasing incidence of CNS-related disorders
- Many new drugs do not make it to the market
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  - Safety testing!
- *In vivo* 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!

*In vivo* testing:
  - Expensive,
  - Ethically debated,
  - Not always predictive...
Drug discovery and safety testing

➢ Need for \textit{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

Hondebrink et al. (2012) NeuroToxicology
Extensive *in vitro* battery,..

- Cell viability
- E-phys
- Transporter assays
  - Many (!) different types or receptors/channels!
  - Requires multiple cell types
Integrated *in vitro* assay,..

- Nerve cells form communicating networks that reflect 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
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

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. $n_{wells} = 14-27$, * $p \leq 0.01$

Dotted lines indicate biological variation in control wells (±1x SD)

Hondebrink et al., NeuroToxicology, 2016
Different classes of insecticides induce inhibition of neuronal activity

Known GABA-R antagonist endosulfan evokes neuronal hyperexcitation

Dingemans et al., NeuroToxicology, 2016
4 different labs;
4 different culture conditions (E18/PND1, rat/mouse)
Generally reproducible!
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?!
Acute effects of Methoxetamine in human iPSC-derived cultures

- Human iPSC-derived neurons, with/without astrocytes

Neuronal activity:
- IC$_{50}$ (MXE): $\sim$30 µM (without astrocytes)
- IC$_{50}$ (MXE): $\sim$10 µM (with astrocytes)
- IC$_{50}$ (MXE) rat: 0.5 µM??

- IC$_{50}$ (MXE): $\sim$50 µM (without astrocytes)
- IC$_{50}$ (MXE): $\sim$30 µM (with astrocytes)

Hondebrink et al., Neuropharmacology, 2017
Human vs rat,...

Rodent primary cortical culture

iCell Glutamatergic Neurons - Astrocytes

Tukker et al.,
Human vs rat,...

Mean Spike Rate (MSR)

Mean Burst Rate (MBR)

Mean Network Burst Rate (MNBR)

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
Human vs rat, ...

- Many similarities neuronal activity..
- $\text{IC}_{50}$ (AMPH) rat cortex $\sim 100 \, \mu\text{M}$
- $\text{IC}_{50}$ (AMPH) hiPSC $\sim 100 \, \mu\text{M}$

- $\text{IC}_{50}$ (TTX) rat cortex $\sim 7 \, \text{nM}$
- $\text{IC}_{50}$ (TTX) hiPSC $\sim 10 \, \text{nM}$

Kasteel & Westerink, Toxicology Letters, 2017
Tukker et al., ALTEX, 2016
• 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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