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Screening for Neurotoxicity Adverse Outcome Pathway (AOP) Perturbations in Water Quality Monitoring

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Aim: Propose specific *in vitro* assays to include in Effect Directed Monitoring (EDM) test batteries that cover potential toxic effects not yet screened for by water quality monitoring test facilities

Why *in vitro* bioassays?

- 3R principle compliant
- Non targeted screening → effects of undefined mixtures
- More hazard-oriented
- Amenable to High Throughput Screening (HTS)

Approach

1. Literature review → database of currently applied *in vitro* bioassays
2. Comparison with database of representative chemicals of emerging concern and their related human-relevant AOPs → identify KE's not yet screened for in water quality monitoring
3. Selection of an assay gap → Neurotoxicity
4. Literature review → database of available *in vitro* bioassays measuring perturbations of KE[1] in human neurotoxicity AOPs
5. Selection of an assay to evaluate its potential for use in water quality monitoring

Criteria for the selection of micro-electrode array assay (MEA)

- Endpoint related to a KE commonly found in neurotoxicity AOPs → Network Activity (e.g. Mean Firing Rate)
- High Throughput potential
- Ability to detect wide range of neurotoxicants
- Overlap with chemicals that have already been tested with EMERCHE chemical list

Criteria for the selection of chemicals

- Different neurotoxicity AOPs covered
- Chemicals that have been found negative and positive *in vitro* and are or are not known neurotoxicants (3rd column of **Table 1**)
- Toxicokinetic parameters available in EPA dashboard for IVIVE[2] modelling (unbound fraction in human plasma, *in vitro* intrinsic hepatic clearance etc.)
- Included in the EMERCHE and ToxCast list of chemicals (*except fluoxetine)

Research questions

❖ Can the MEA assay be used in water quality monitoring to cover the gap of human neurotoxicity adverse outcome pathways?
❖ How much do toxicokinetic parameters affect the results of the assay?

Next Steps

- Evaluate the assay by testing it in the lab with neurotoxicants found in water and/or use literature data (concentration-response curves)
- Quantify toxicokinetic parameters (amount of chemical in media and cells) to establish whether or not they affect the result and if it is essential to quantify them when performing an assay

Take home message

There are perspectives to improve neurotoxicity screening in water quality monitoring, be implementing an assay that can detect a wide range of neurotoxic chemicals

Experimental procedure for MEA

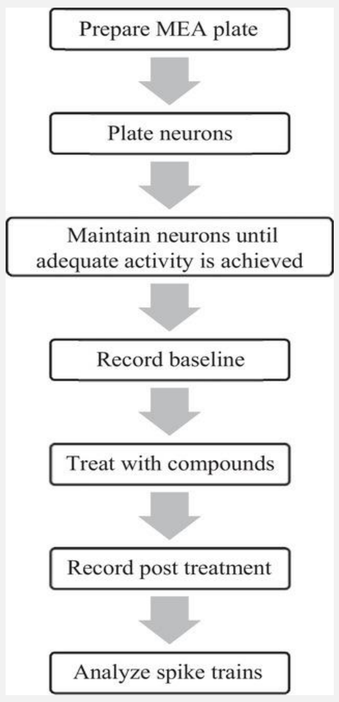


Figure 2
Work flow diagram of experimental procedure for the MEA. After exposure, the network activity is compared to the measured baseline activity (% control) [8]

How different MIEs[3] can end up causing altered mean network firing rates (MFR) and patterns

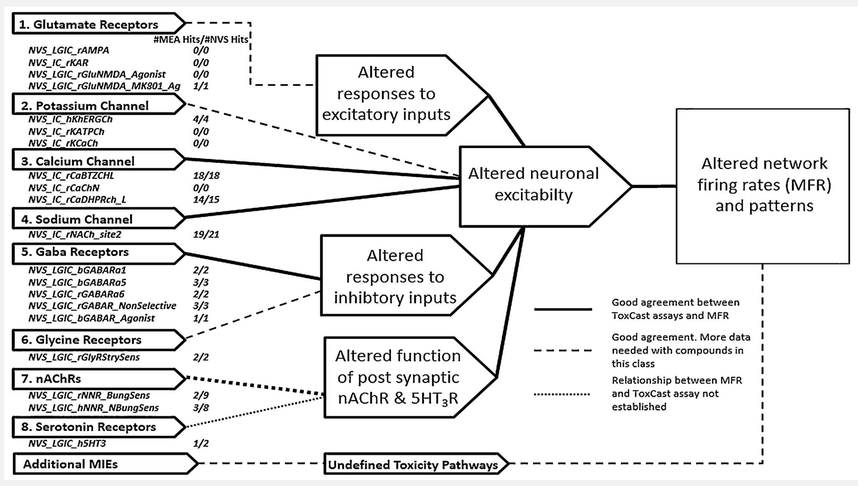
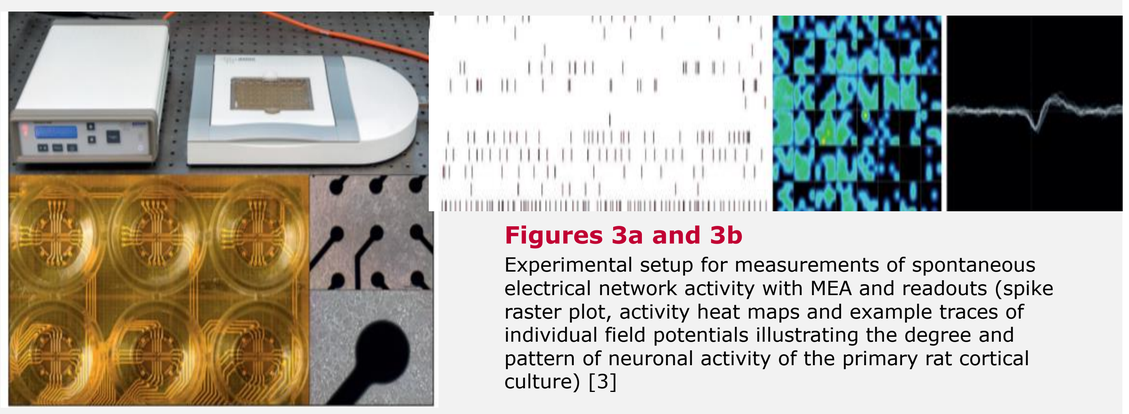


Figure 1
Proposed Toxicity Pathways linking changes in ion channel function to altered network formation function on MEAs. Groups of potential MIEs are listed on the left-side and under each group is the list of ToxCast Novascreen (NVS_IC) assays that assess compound interactions with relevant receptors. The next column has the KE, ultimately leading to altered MFRs and patterns that can be detected by the MEA. [1]

Experimental set-up and readouts from MEA



Figures 3a and 3b
Experimental setup for measurements of spontaneous electrical network activity with MEA and readouts (spike raster plot, activity heat maps and example traces of individual field potentials illustrating the degree and pattern of neuronal activity of the primary rat cortical culture) [3]

Chemical	Mode of Action (MoA)	Covered scenario	$logK_{ow}$	NOAELs from *in vivo* studies	Type of study	Reference
Fluoxetine	Inhibits the serotonin re-uptake transporter protein (SSRI)	Positive in MEA (*in vitro*) and is a known neurotoxicant *in vivo*	4,33	Not available		[2]
Fipronil	Binding to the picrotoxin site of ionotropic GABA receptors	Positive in MEA (*in vitro*) and is a known neurotoxicant *in vivo*; lowest NOAEL value (along with Diazinon)	4,28	0,02 mg/kg bw/day	2-year oral / rat	[1], [4]
				0,9 mg/kg bw	DNT oral / rat	
Cypermethrin	Sodium channel modulator	Positive in MEA, but produced only a small decrease in MFR and is a known neurotoxicant *in vivo*	0,88	0,5 mg/kg bw/day	2-year rat DNT study	[5]
				20 mg/kg bw	Rat acute neurotoxicity study	
Aldicarb	Acetylcholinesterase (AChE) inhibition	Compare sensitivity of MEA with that of AChE inhibition assay	6,04	0,01 mg/kg bw/day	Clinical signs of neurotoxicity from human dietary exposure study	[6]
Diazinon	Acetylcholinesterase (AChE) inhibition	Compare results with those from AChE inhibition assay	1,25	2,5 mg/kg bw	Acute toxicity and neurotoxicity rat studies	[7]
				0,017 mg/kg bw/day	90-day neurotoxicity rat study	
Flusilazole	Ergosterol biosynthesis inhibition	Was found positive in MEA (*in vitro*) but is not considered a neurotoxicant *in vivo*	3,41	Not available		[1]
Spiroxamine	Fungal RNA polymerase inhibition	Was found positive in MEA (*in vitro*) but is not considered a neurotoxicant *in vivo*	3,7	Not available		[1]
Acetaminophen	Not identified	Negative control	2,89	Not available		[1]

Table 1
Chemicals of interest to derive or use from literature concentration-response relationships. The chemicals were selected in order to cover the scenarios mentioned in the 3rd column of the table. The $logK_{ow}$ values as a measure of lipophilicity along with the NOAELs from mammal neurotoxicity studies are also presented.

Abbreviations/Clarifications

1) KE: Key events
2) IVIVE: *In vitro-in vivo extrapolation*
3) MIEs: Molecular Initiating Events

* Oral doses estimated based on self reports of amount of commodities consumed, measured residue levels in commodities, and average body weights for given age and sex

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