Prediction of sedative and pro-convulsive side effects of compounds revealed by phenotypic screening using primary neuron cell cultures

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Introduction

Neuronal networks grown on microelectrode arrays (MEAs) exhibit phenotypic fingerprints defined by their distinct spike train patterns. NeuroProof´s spike train analysis includes calculation of >200 parameters describing this spontaneous electrical activity with respect to general activity, burst structure, pattern regularity and network synchronization. Application of test compounds leads to substance specific changes in these spike train patterns in a concentration dependent matter.

Novel compounds often fail during development due to non-tolerable side effects. Binding assays deliver data on off-targets and indicate putative side effects, however do not verify effects in a complex arrangement of cells. Therefore compounds might be withdrawn from further development without examining their real physiological effects. Hence, there is a need for predictive tests uncovering side effects early during preclinical development.

Approach

The aim was to establish an in vitro screening assay to predict either pro-convulsive or sedative effects in a concentration-dependent manner to screen test compound libraries very early in drug discovery. We compared the acute functional fingerprints induced in 28-div-old primary cortex neuronal networks treated with increasing concentrations of
(1) known sedative drugs vs. activity-reducing drugs
(2) known pro-convulsive vs. activity-increasing compounds.

Two artificial neuronal networks were trained with these calibration data from therapeutically-relevant concentrations to build classifiers separating the respective classes for both questions which is based on identifying best-describing parameters.
We validated the two classifiers using fingerprints from compounds known to exhibit or lacking the side effects.

Conclusion

The presented phenotypic screening approach using the MEA and NeuroProof classification technology allows predicting, both, sedative and pro-convulsive side effects of test compounds.
Validation was performed by testing multiple compounds and sorting into classes.
The analysis is flexible to user requirements by using reference compounds with known positive or negative effects for calibration. Importantly, increasing the number of classifier compounds increases the predictability as more phenotypic modes of action are included into the analysis.

Thus, we present a novel scalable, functional high content in vitro screening method to predict sedative or pro-convulsive side effect properties of test compounds.

Predicting sedative properties using known sedative and inhibitory drugs

For the classifier calibration the 5 inhibitory (on MEA in vitro) but clinically not sedating drugs and 5 clinically sedating drugs were selected. All compounds show inhibition of spike rate at clinically-relevant concentrations (boxes). The aim was to establish a classifier based on these clinically-relevant data which is able to sort the respective drugs into their correct category.

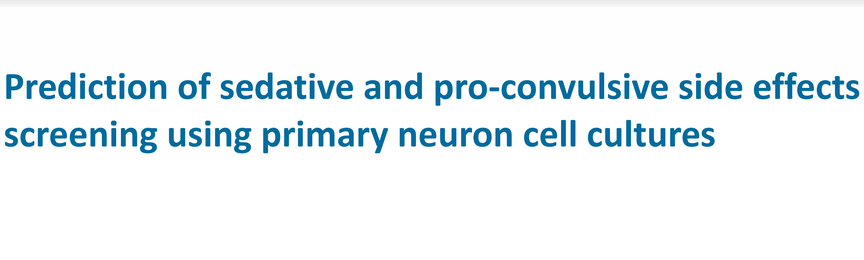


Figure 1: Selection of compounds: Functional concentration-response data for 3 state-the-art pro-convulsive compounds and three excitatory compounds. Boxes indicate selected concentrations used for classification.

Assay Calibration

We aimed to select diverse drugs with different modes of action in order to reduce the bias for one specific mode. Propofol exhibits a completely distinct mode compared to the other four drugs as it completely separated from the combined fingerprint of the other 4 sedative drugs.

		Inhibitory	Sedative	correct	Estimated clinical concentration used for classification
Inhibitory	Amisulpride	100 %	0 %	Yes	100 nM – 10 µM
	Aripiprazole	58 %	42 %	Yes	10 nM – 1 µM
	Flumazenil	54 %	46 %	Yes	10 µM – 100 µM
	Fluoxetine	88 %	12 %	Yes	3 µM – 10 µM
	Fluvoxamine	100 %	0 %	Yes	2 µM – 10 µM
Sedative	Memantine	12 %	88 %	Yes	10 nM – 1 µM
	Diazepam	0 %	100 %	Yes	1 µM – 10 µM
	Flunitrazepam	13 %	87 %	Yes	10 nM – 1 µM
	Methaqualon	33 %	67 %	Yes	3 µM – 30 µM
	Propofol	100 %	0 %	No	5 µM – 50 µM

	Inhibitory	Sedative	Datasets	Chi^2 *** (1.5E-15)
Inhibitory	78 %	22 %	141	
Sedative	33 %	67 %	150	

Assay Validation

Drugs with known sedation

Amitriptyline [1E-07-1E-06]

Mort	Inhib	Sedat
1.E-12	100	0
1.E-11	100	0
1.E-10	100	0
3.E-10	100	0
1.E-09	100	0
1.E-08	100	0
1.E-07	100	0
3.E-07	100	0
1.E-06	57	43
3.E-06	11	89

Levetiracetam [1E-05-5E-04]

Mort	Inhib	Sedat
1.E-07	100	0
1.E-06	100	0
1.E-05	100	0
1.E-04	70	30
5.E-04	22	78
1.E-03	10	90
2.E-03	0	100
5.E-03	13	88
1.E-02	0	100

Clonazepam [1E-08-1E-07]

Mort	Inhib	Sedat
1.E-11	92	8
1.E-10	93	7
1.E-09	86	14
1.E-08	36	64
1.E-07	33	67
3.E-07	0	100
1.E-06	0	100
3.E-06	0	100
3.E-05	0	100
1.E-04	0	100

Chlorpromazine [1E-07-2E-06]

Mort	Inhib	Sedat
1.E-09	100	0
1.E-08	100	0
1.E-07	100	0
7.E-07	90	10
2.E-06	90	10
4.E-06	48	52
7.E-06	0	100
1.E-05	0	100
3.E-05	0	100
1.E-04	0	100

Zolpidem [1E-08-1E-06]

Mort	Inhib	Sedat
1.E-11	100	0
1.E-10	100	0
1.E-09	33	67
1.E-08	22	78
1.E-07	0	100
1.E-06	0	100
1.E-05	0	100
1.E-04	0	100

Drugs with no or low sedation

Risperidone [1E-09-1E-07]

Mort	Inhib	Sedat
1.E-12	100	0
1.E-11	100	0
1.E-10	100	0
1.E-09	100	0
1.E-08	100	0
1.E-07	100	0
1.E-06	100	0
3.E-06	82	18
1.E-05	77	23
2.E-05	64	36
5.E-05	0	100
1.E-04	0	100

D-Cycloserine [1E-07-1E-05]

Mort	Inhib	Sedat
1.E-12	100	0
1.E-10	100	0
1.E-09	100	0
1.E-08	100	0
1.E-07	100	0
1.E-06	100	0
5.E-06	100	0
1.E-05	100	0
3.E-05	89	11
1.E-03	64	36
1.E-03	43	57

Galanthamine [1E-06-1E-04]

Mort	Inhib	Sedat
1.E-10	100	0
1.E-09	100	0
1.E-08	100	0
1.E-07	100	0
1.E-06	93	7
1.E-05	87	13
1.E-05	100	0
1.E-04	93	7
1.E-04	60	40
1.E-03	8	92

Mefloquine [1E-06-8E-06]

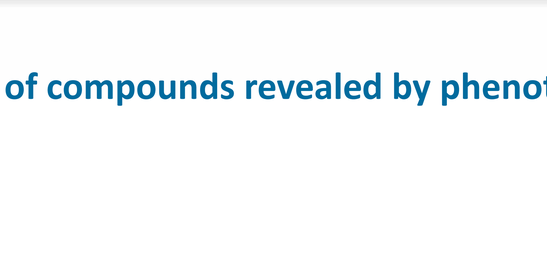
Mort	Inhib	Sedat
1.E-10	100	0
1.E-09	100	0
1.E-08	86	14
1.E-07	86	14
1.E-06	100	0
2.E-06	100	0
4.E-06	100	0
8.E-06	50	50
1.E-05	0	100
3.E-05	0	100

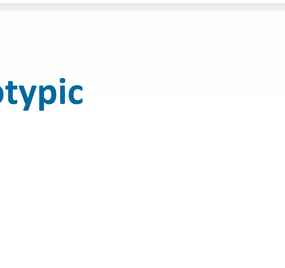
Flovoxamine [1E-07-2E-06]

Mort	Inhib	Sedat
1.E-11	100	0
1.E-10	100	0
1.E-09	100	0
1.E-08	100	0
1.E-07	100	0
1.E-06	100	0
5.E-06	100	0
1.E-05	100	0
2.E-05	44	56

Predicting pro-convulsive properties using pro-convulsants and excitatory

For the classifier calibration 3 excitatory (on MEA in vitro) and 3 state-of-the-art pro-convulsive compounds were selected. All compounds show increase of spike rate. Selected concentrations (boxes) were used for further analysis. The aim was to establish a classifier based on excitatory data points which is able to sort the respective compounds into their correct category.





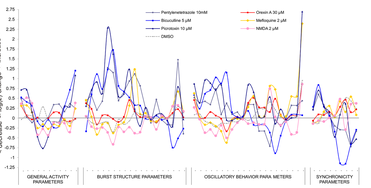


Figure 3: TOP LEFT: Functional concentration-response data for 3 state-the-art pro-convulsive compounds and three excitatory compounds. Boxes indicate selected concentrations used for classification. **TOP RIGHT:** native activity of cortical neuronal networks are affected by compounds. At 5 uM, picrotoxin induces ictal-like events and increases regularity and synchronicity. In contrast, 2 uM NMDA increase activity but not synchronicity. **Left:** Feature chart for the 6 compounds at selected excitatory concentrations (and DMSO): all compounds increase general activity (spike rate = parameter 1, arrow) but the two classes show opposite effects at multiple parameters from the different activity-describing categories. Especially bursting strength, regularity and synchronicity is increased by pro-convulsants (arrows).

Assay Calibration

% classified as	excitatory	pro-convulsive	correct	excitatory concentrations used for classification
excitatory: Mefloquine	94%	6%	yes	1 µM - 4 µM
excitatory: NMDA	92%	8%	yes	5 µM - 10 µM
excitatory: Orexin A	79%	21%	yes	3 µM - 30 µM
pro-convulsive: Bicuculline	18%	82%	yes	5 µM -20 µM
pro-convulsive: Pentylenetetrazole	35%	65%	yes	0.3 mM - 1 mM
pro-convulsive: Picrotoxin	30%	70%	yes	10 µM - 40 µM

% classified as	excitatory	pro-convulsive	Datasets
excitatory	90%	10%	60
proconvulsive	25%	75%	107

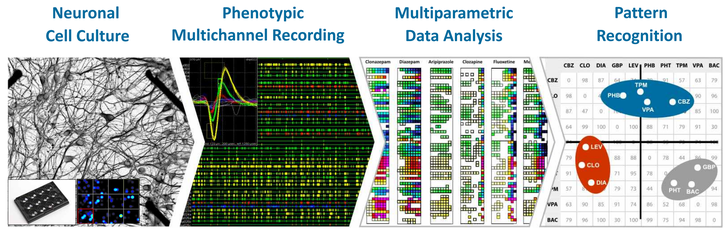
Assay Validation

compounds	excitatory	pro-convulsive	pro-convulsive?	concentrations increasing spike rate >10 %
Acetylcholine	85	15	no	5 mM - 10 mM
AMPA	71	29	no	1 µM - 2 µM
Apomorphine	58	42	no	10 µM - 30 µM
Enkephaline	25	75	yes*	10 nM - 100 nM
Glycine	100	0	no	1 mM - 3 mM
NeuropeptideY	77	23	no	1 pM - 100 pM
Oxotremorine	65	35	no	3 µM - 20 µM
SCH50911	70	30	no	1 µM - 10 µM
Sufentanil	59	41	no	1 nM - 100 nM
Wortmannin	92	8	no	10 µM - 20 µM

Figure 4: Calibration: Compound-specific classification of into either pro-convulsive or excitatory category. All 6 compounds were classified correctly using data from spike rate-increasing concentrations (see Figure 3 TOP Left). Values correspond to '% datasets classified into either group'. **Validation:** We used the classifier to analyze compounds which increase spike rate by >10%. All compounds but Enkephaline were classified as excitatory which coresponds to literature (pubmed search compound name + pro-convulsive). However, Enkephaline is indeed a pro-conculsive neuropeptide (see recent article by Clynen et al. 2014, Mol. Neurobio. 50(2)*).

NeuroProof Technology

Phenotypic Screening with MEA-Neurochips



Primary murine cell culture:
- Frontal Cortex
- Hippocampus
- Midbrain
- Spinal Cord/DRG
Neuronal human Stem Cells

Network spike trains and single neuron action potential

Over 200 descriptors at baseline and compound treatment
- General activity
- Synchronization
- Oscillation
- Burst structure

Data base with functional fingerprints of over 100 basic and clinically compounds

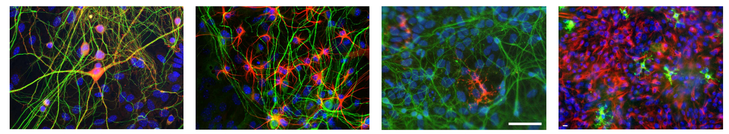
Disease related brain tissue culture
Acute and chronic compound induced disease rescue models

High resolution of spatio-temporal network activity patterns

Specific in vitro assays with optimized compound or disease related parameter selection

Similarity and differentiation from known compound effects
Combination therapy

Primary Cortical Neuron/Glia Coculture



GABAA receptor (alpha1, red), Neurons (Tuj, green), nuclei (blue).

Neurons (Tuj, green), neuronal somata (HuCD, red, nuclei (blue).

Oligodendrocytes (O4, red), Neurons (Tuj, green), nuclei (blue).

Astrocytes (GFAP, red), microglia (Lectin, green), nuclei (blue).

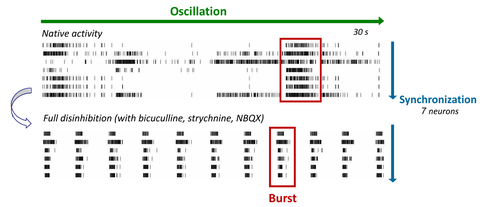
Co-cultures of neurons and glial cells in frontal cortex cultures on MEAs at 28 div are composed of:
Neurons (~20 %), Astrocytes (~70-80 %), Oligodendrocytes (present, neglectable), Microglia (~1-2 %)

Multiparametric Characterization of Neuronal Network Activity

Read out:
- Extracellular action potentials on a single neuron and network activity level
- Spatio-temporal activity changes as well as synchronicity and oscillation in time scales of spikes and bursts

Each specific spike train is described by 200 parameters in 4 categories:

1 General Activity
e.g. spike rate, burst rate, burst period, percent of spikes in



2 Burst Structure
e.g. number, frequency and ISI of spikes in bursts; burst duration, amplitude, area, plateau position, plateau duration

3 Oscillation
Variation over time as an indicator for the strength of the oscillation; in addition e.g. Gabor function parameters fitted to autocorrelograms

4 Synchronization
Variation within the network as an indicator for the strength of the synchronization; in addition e.g. simplex synchronization, percent of units in synchronized burst