innBW

NMI is alliance partner of
Baden-Württemberg Innovation Alliance

NMI
achieving results

Dr. Udo Kraushaar
Head Electrophysiology

Bringing human neuronal biology to HTS: Functional
Drug Screening with iCell GlutaNeurons and Astrocyte
on the Hamamatsu FDSS/µCELL

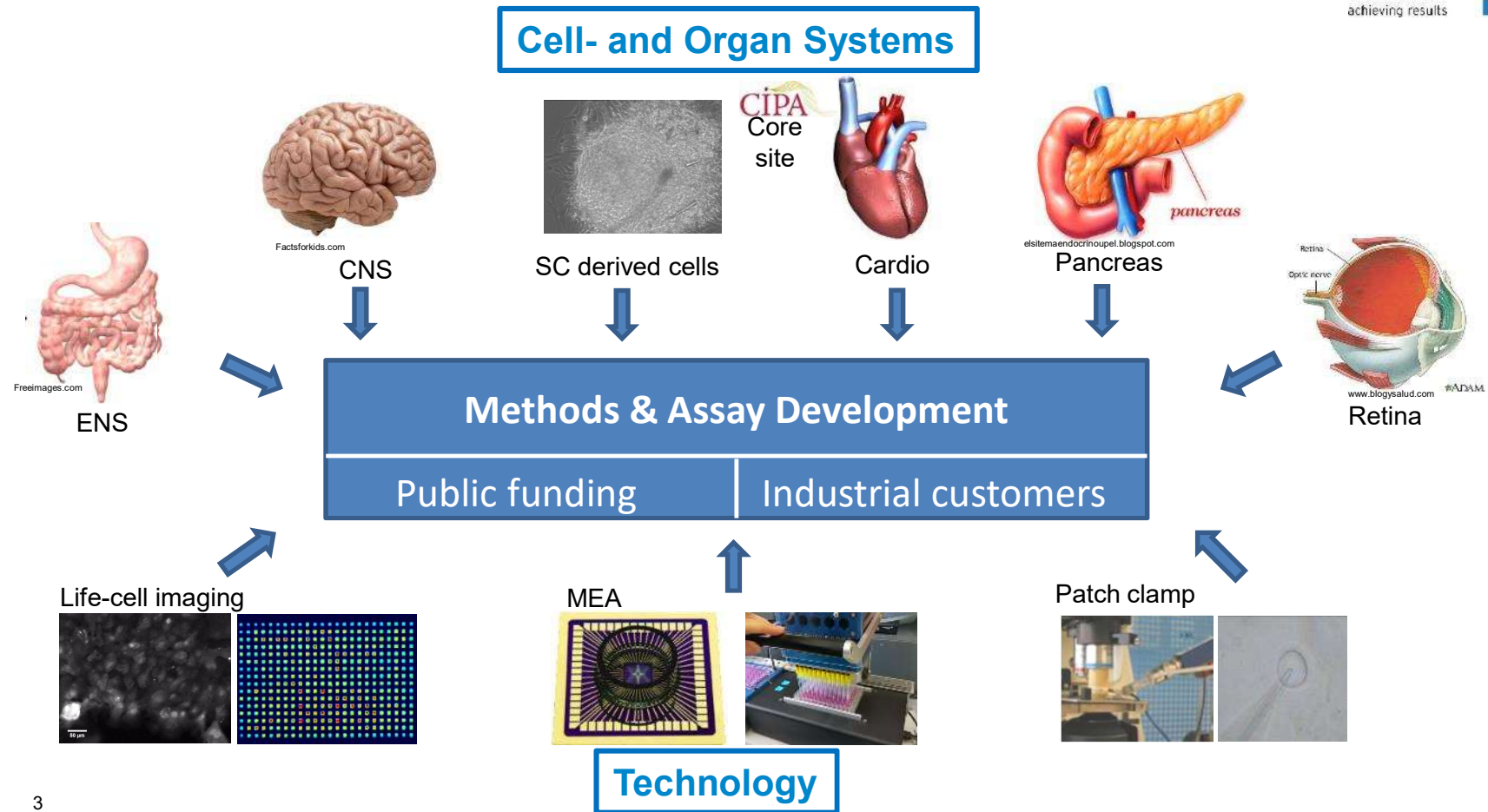Natural and Medical Sciences
Institut at the University of Tübingen

NMI Natural and Medical Sciences Institute
at the University Tübingen

- Established as a foundation under public law (non-profit organization) in 1985

- 200 employees

- Highly interdisciplinary (Physics, Biophysics, Molecular Biology, Biochemistry, Cell Biology)

- Applied R&D, and services for industrial clients

- Joint projects with academic and industrial partners

- Incubator site >17 spin-off companies founded

NMI TT **Pharmaservices** CRO offering pharmaservice

Cell Models / Technology At EPhys Lab

Cell- and Organ Systems

CNS

SC derived cells

Core site

Cardio

Pancreas

Retina

ENS

Methods & Assay Development

Public funding | Industrial customers

Life-cell imaging

MEA

Patch clamp

Technology

NMI
achieving results

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CDI GlutaNeurons on Hamamatsu µCell

1) Establishment of stable assay, 384 well format
   - Cell density
   - Cultivation procedure & time window
   - Buffers & dyes
   - Getting to know the expected activity patterns

2) iCell GlutaNeurons vs. Cocultivation with Astrocytes

3) Functional applications
   - Seizurogenic compound tests (HESI NeuTox subset)
   - Network dynamics by mGluR agonists
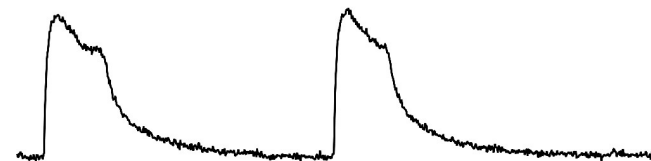   - (simple) disease model
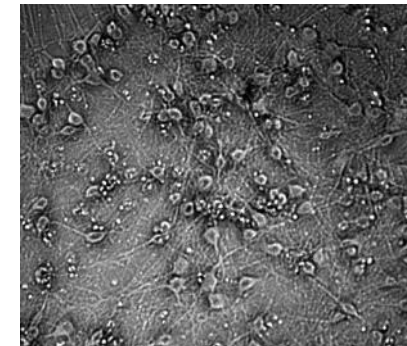
Morphology
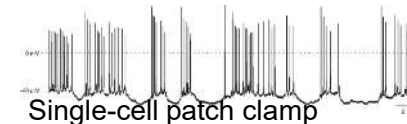
Single-cell patch clamp
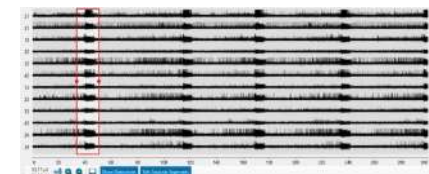
MEA network activity

Basics: Buffer, Dye, Cell density, Coating

NMI
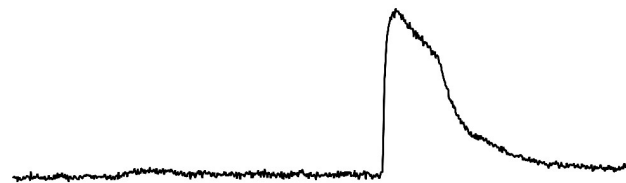
Recording buffer
$2.5Ca^{2+}/0Mg^{2+}$

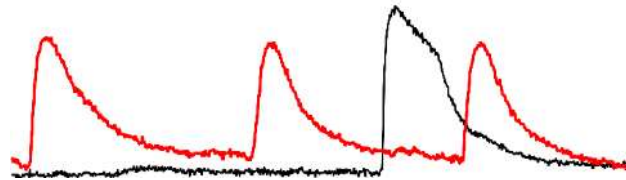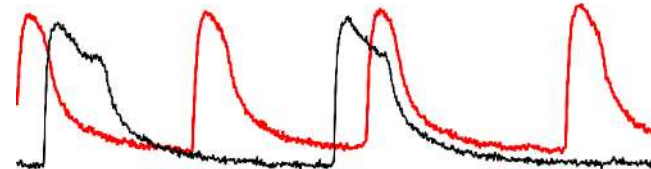Ca^{2+} indicator
Fluo4

30 sec
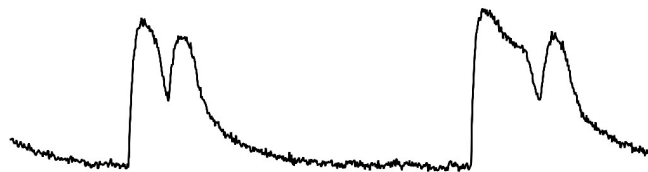
Cell density
20k/well
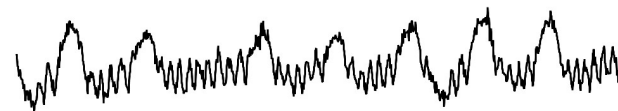
Coating (21 DIV)
0.1%PEI

At time of first experiments lack of ready-to-use protocol
→ First suggestions
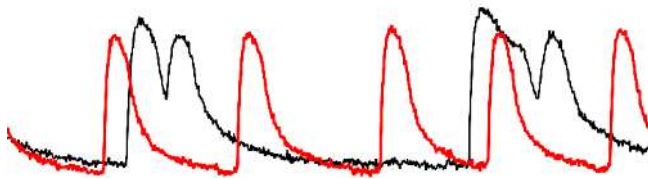
Basics: Buffer, Dye, Cell density, Coating
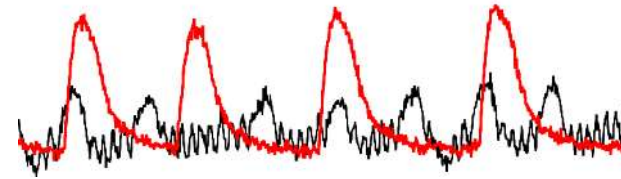
NMI

Recording buffer
$2.5Ca^{2+}/0.2Mg^{2+}$ vs $2.5Ca^{2+}/0Mg^{2+}$

Ca^{2+} indicator
Cal520 vs Fluo4
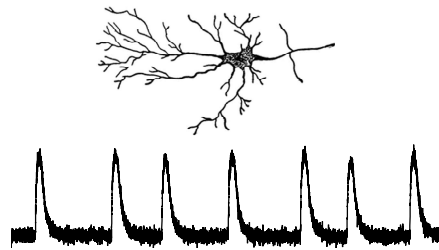
30 sec

Cell density
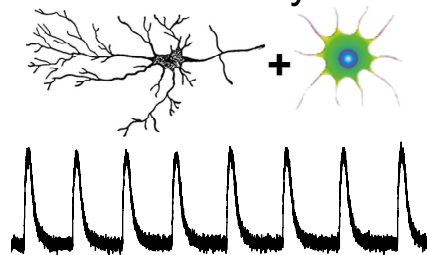30k/well vs 20k/well

Coating (21 DIV)
PLO/Matrigel vs 0.1%PEI

- Coating: PLO/Matrigel
- 30k cells/well (384 well format)
- Cal520-AM (45 min, 2 µM, 37°C, Pluronic)
- Recording Buffer 2.5 mM Ca^{2+}/0.2 mM Mg^{2+}, HEPES
- Stable recording time window DIV14-21

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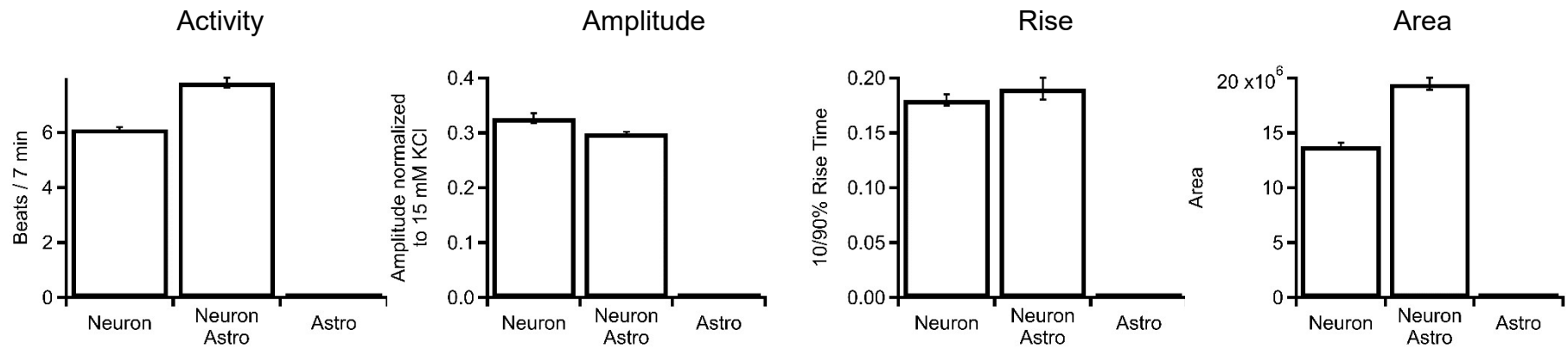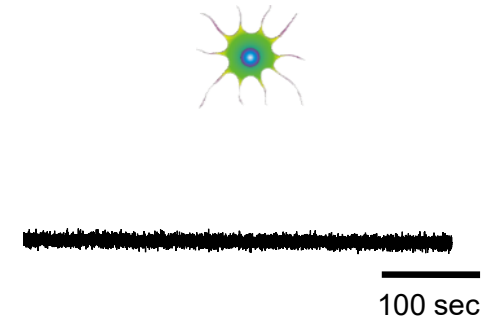# Network activity is +/- independent on cocultivation

Seizurogenic compounds
in vitro surrogate for epilepsy

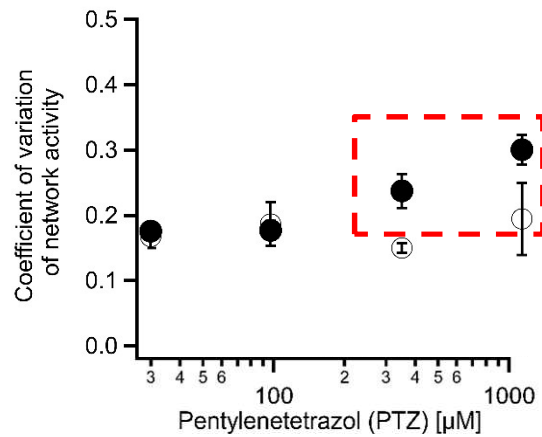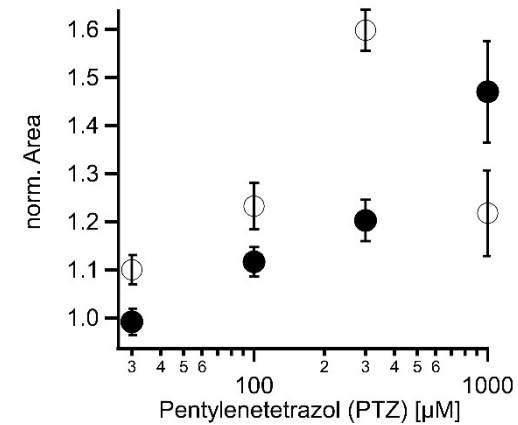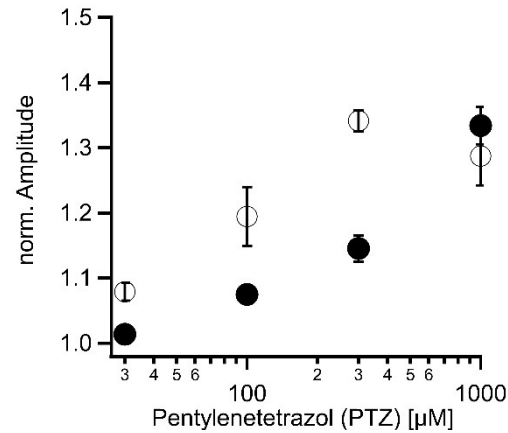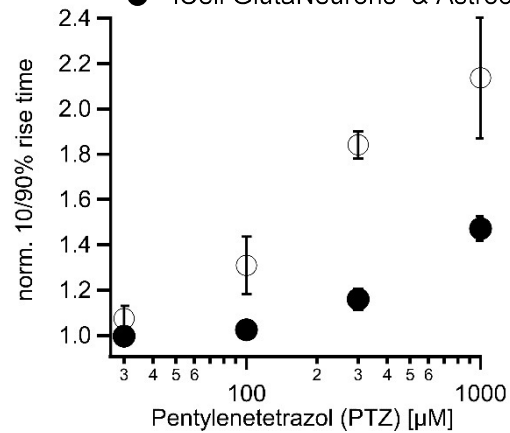| Compound	MoA	Concentration
4-Aminopyridine (4-AP)	K$^+$ channel blocker (IK$_A$)	1 -30 µM
Picrotoxin	GABA$_A$ channel blocker	0.3-10 µM
Pentylenetetrazole (PTZ)	GABA$_A$ / K$^+$ channel blocker (?)	30-1000 µM
Strychnine	Glycin/ACh channel blocker	1-30 µM
Pilocarpine	Muscarinergic ACh agonist	1-30 µM

5 out of 12 HESI NeuTox compounds
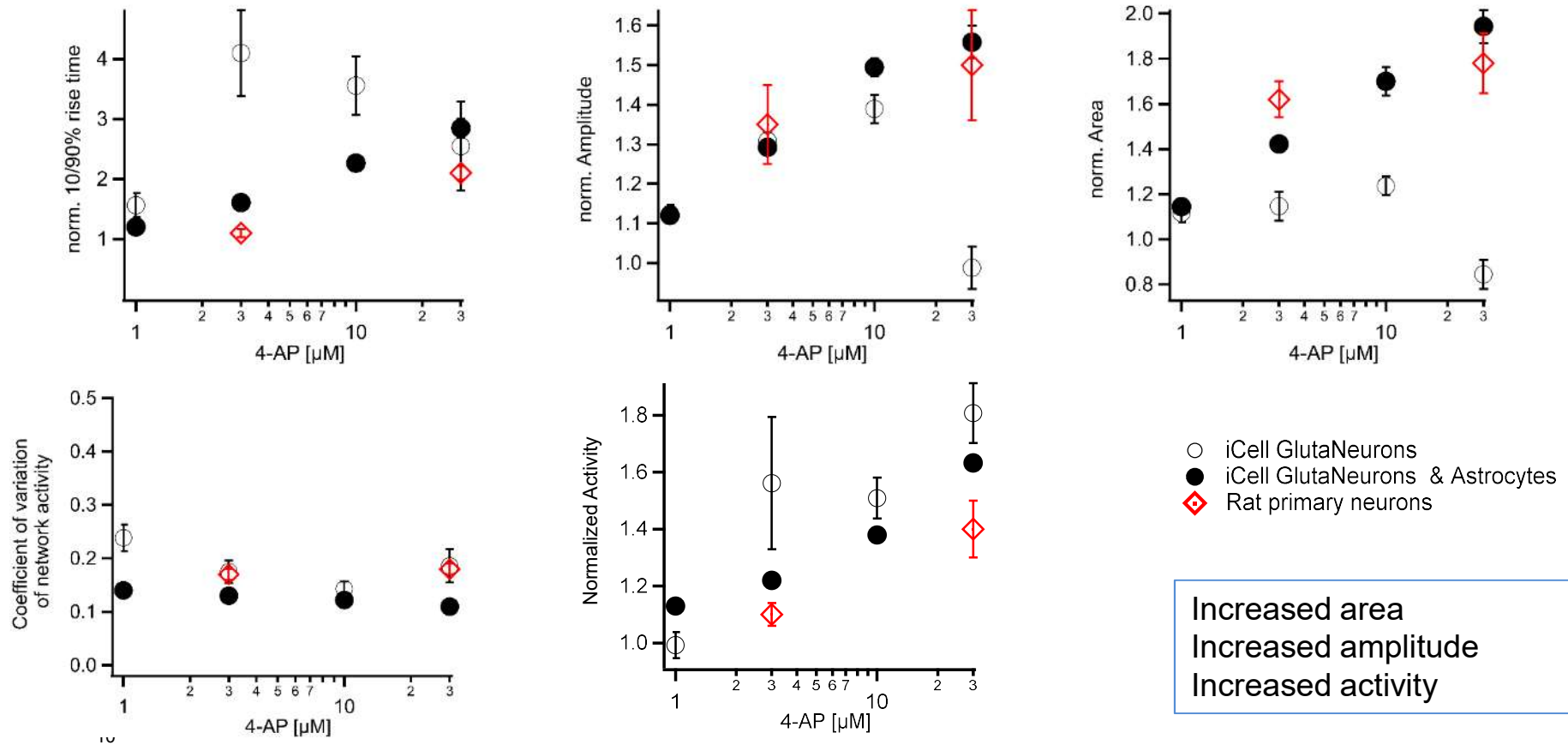
Investigated parameters:

- 10/90% rise time
- Peak amplitude
- Area under the curve

- Activity rate
- CV of signal regularity

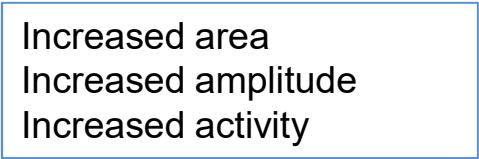# Pentylenetetrazol (PTZ) (GABA$_A$, K$^+$ blocker)

○ iCell GlutaNeurons
● iCell GlutaNeurons & Astrocytes

Increased area
Increased amplitude
Increased activity
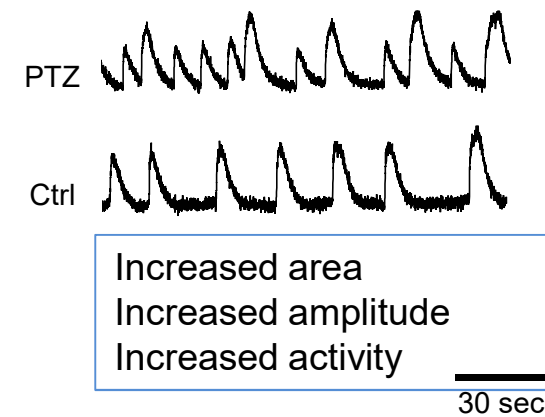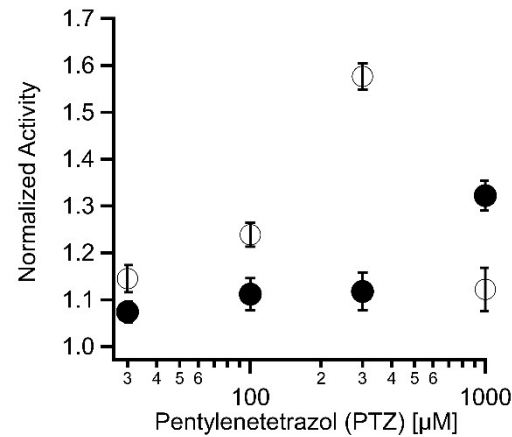
4-Aminopyridine (IK$_A$ blocker)

Picrotoxin (GABA$_A$ blocker)

○ iCell GlutaNeurons
● iCell GlutaNeurons & Astrocytes
◇ Rat primary neurons

Increased area
Increased amplitude
Increased activity

Seizurogenic compounds

| Compound	MoA	Concentration
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Strychnine	Glycin/ACh channel blocker	1-30 µM
Pilocarpine	Muscarinergic ACh agonist	1-30 µM

- **Concentration-dependent seizure-like network activity**
- **Sensitivity equal or higher compared to primary neurons**
- **Cocultivation with Astrocytes → more robustness**

mGluR agonists

mGluRs: G-protein coupled receptors, modulation of synaptic transmission/excitability

| Compound	MoA	Expected action
DHPG	mGluR I/V agonist $GABA_A \downarrow$ $AMPA \uparrow$	Seizurogenic
LY-354740	mGluR II agonist Glu release $\downarrow$	Network activity reduction
L-AP4	mGluR III agonist	Network activity reduction
Picrotoxin + DHPG		Increased seizures, prolonged discharge
Pilocarpin + L-AP4		Partial recovery from seizure

DHPG (mGluR I agonist, seizurogenic)
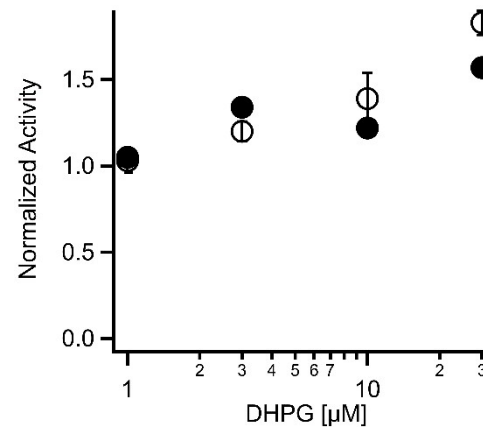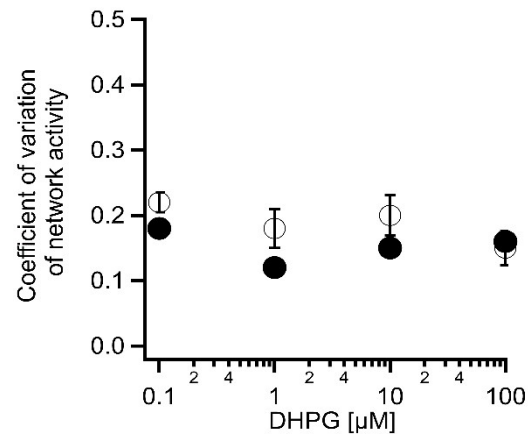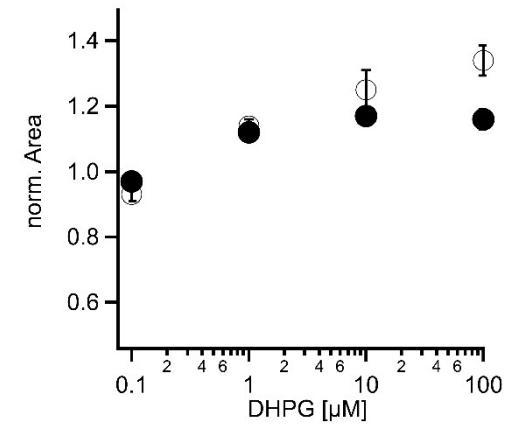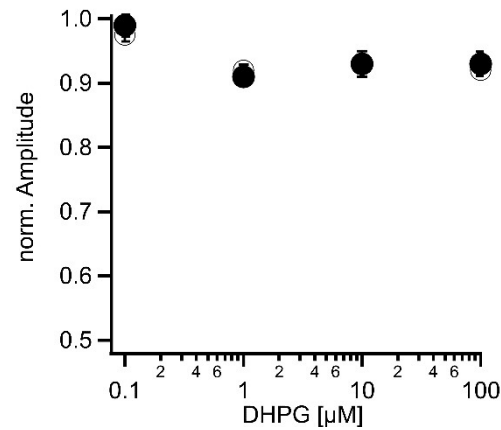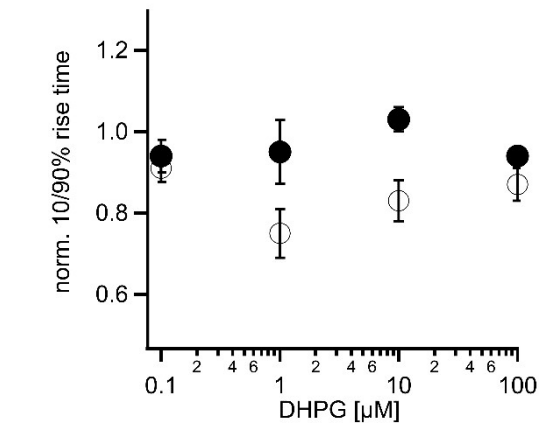
○ iCell GlutaNeurons
● iCell GlutaNeurons & Astrocytes

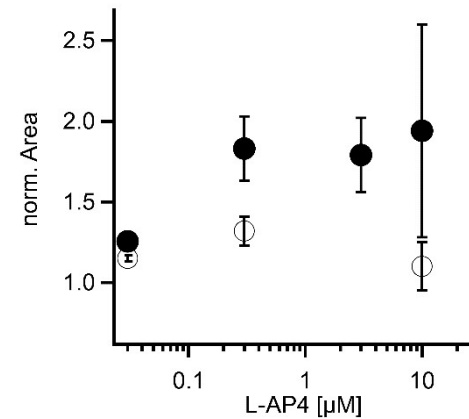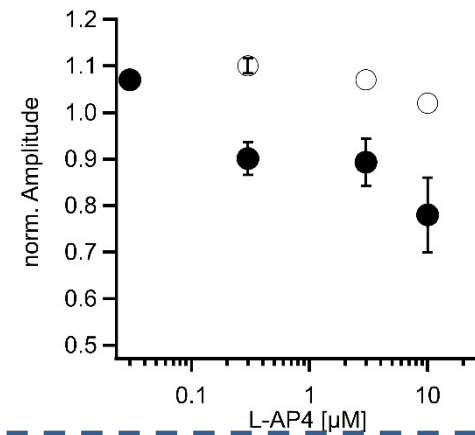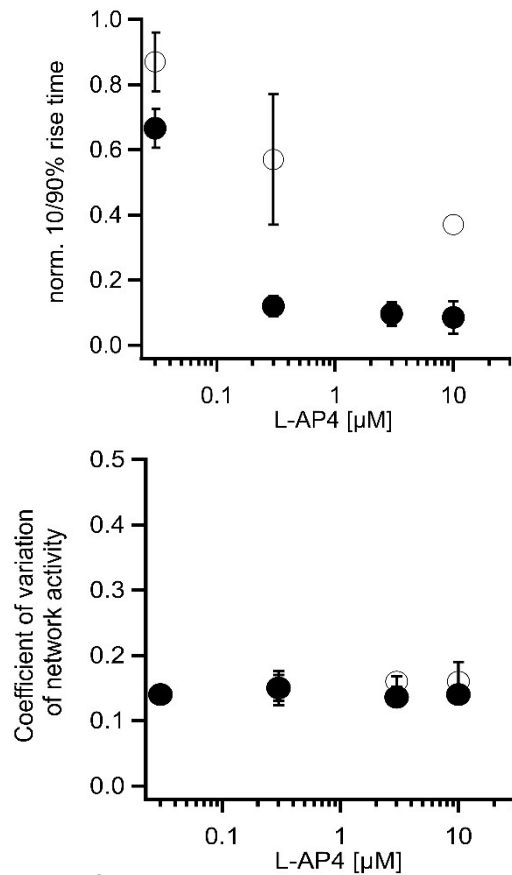Increased area
Increased activity
→ seizure-like activity

LY354740 (mGluR II agonist, network activity reduction)
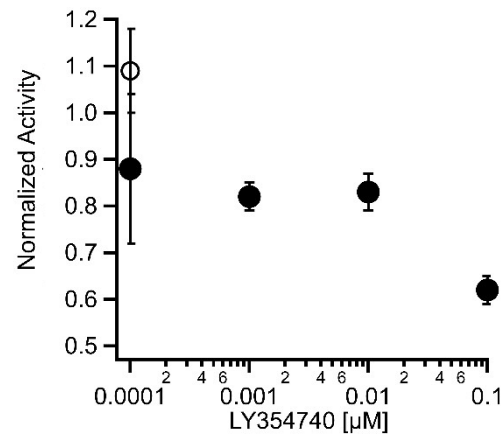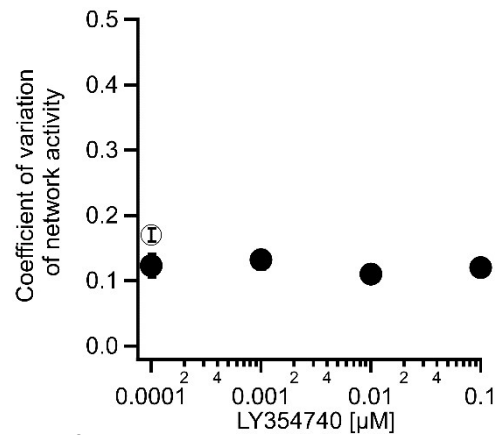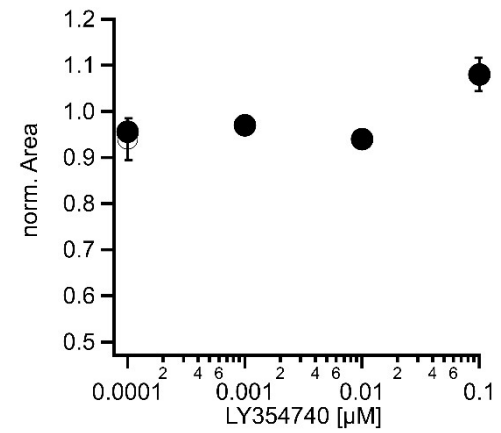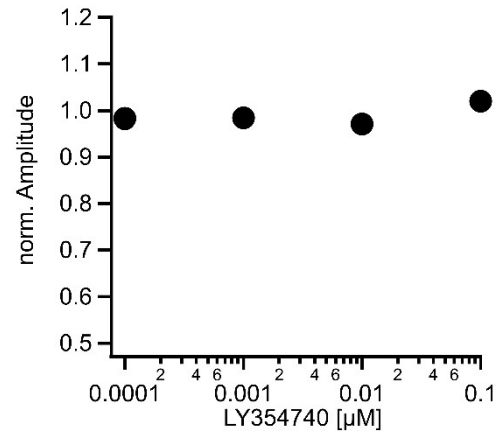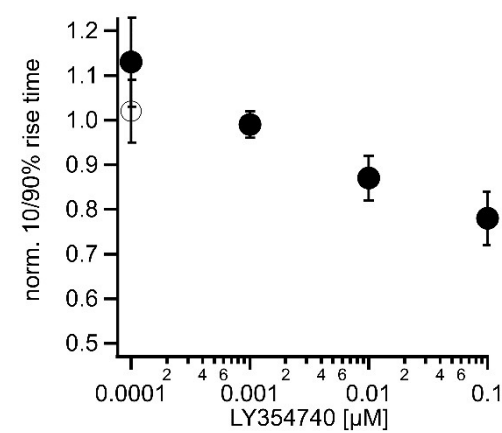
L-AP4 (mGluR III agonist, network activity reduction)

L-AP4 (network activity reduction)
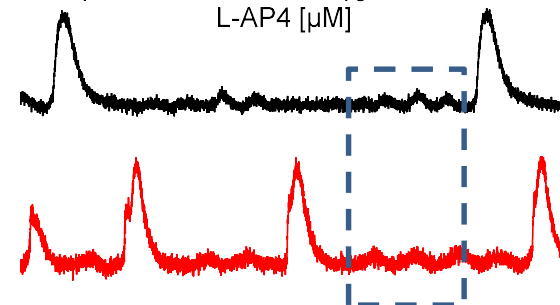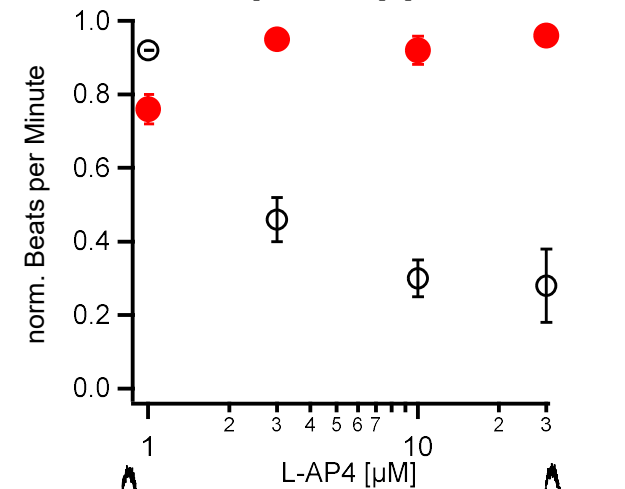
L-AP4 disturbs network synchronicity, not spike rate

control

L-AP4

electrode analysis: L-AP4

24-well MEA

Single well

Electrode layout

100 s
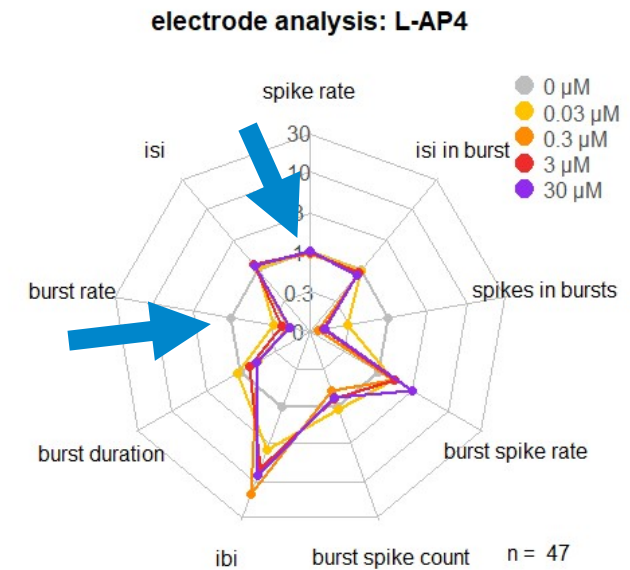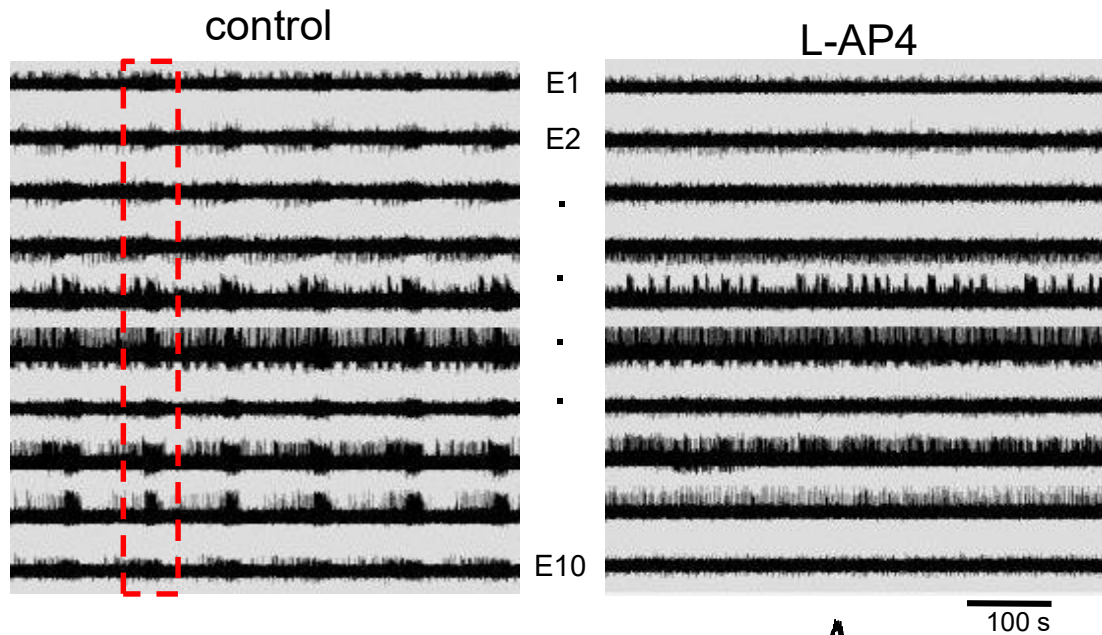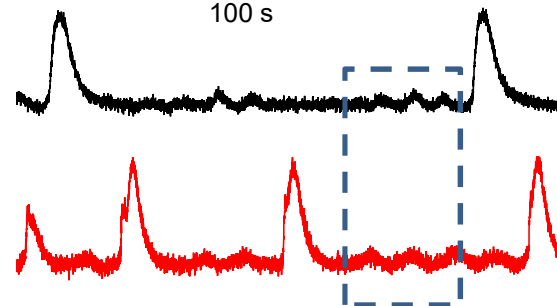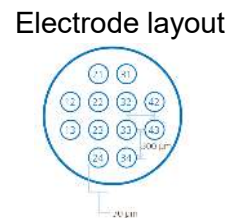
Microelectrode array (MEA),
iCell GlutaNeurons

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DHPG fails to increase seizurogenic activity in the presence of Picrotoxin

NMI
achieving results

Protocol:
- Control recording
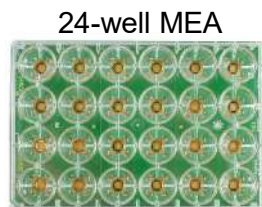- Preincubation 20 min Picrotoxin
- Application of DHPG

Preliminary data: n=3
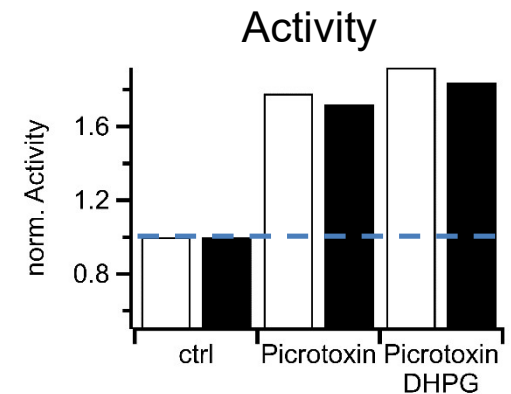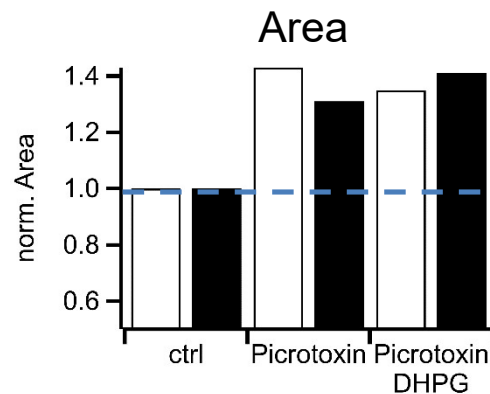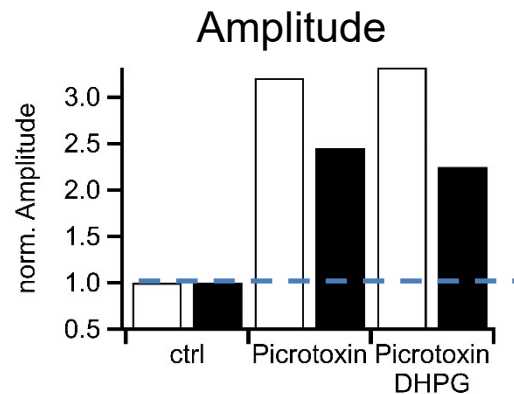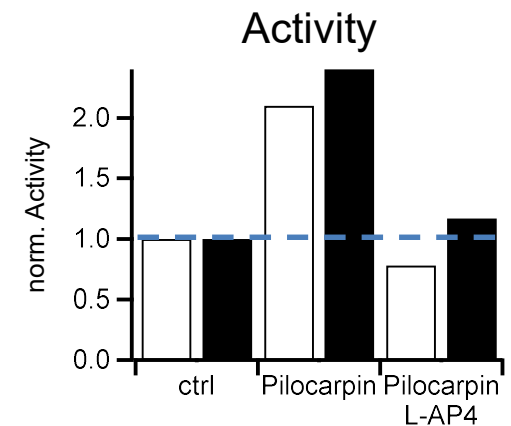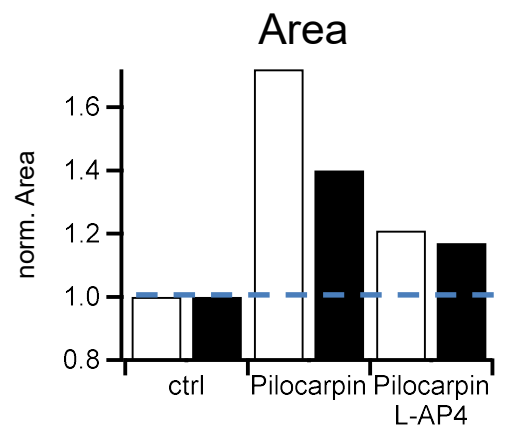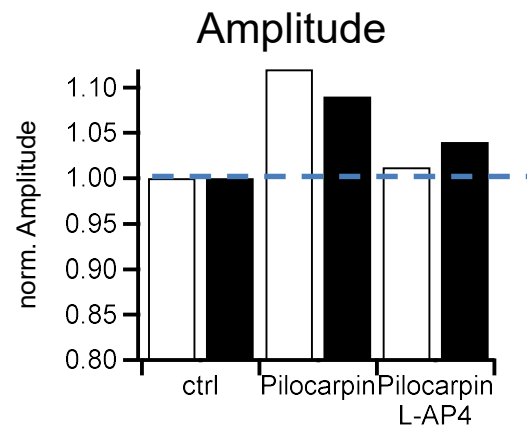
○ iCell GlutaNeurons
● iCell GlutaNeurons & Astrocytes

L-AP4 partially recovers seizurogenic effect of Pilocarpine

Protocol:
- Control recording
- Preincubation 20 min Pilocarpine
- Application of L-AP4

Preliminary data: n=3

○ iCell GlutaNeurons
● iCell GlutaNeurons & Astrocytes

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Amyloid-beta42 application as simple Alzheimer model

- Neurotoxic main component of amyloid plaques

- Main candidate to form misfolded version of tau

- Active form aβ1-42

- Uptake by neurons

- Experiment:
    - treatment 24 h prior to experiment
    - Data normalized to DMSO controls

Wikipedia.de
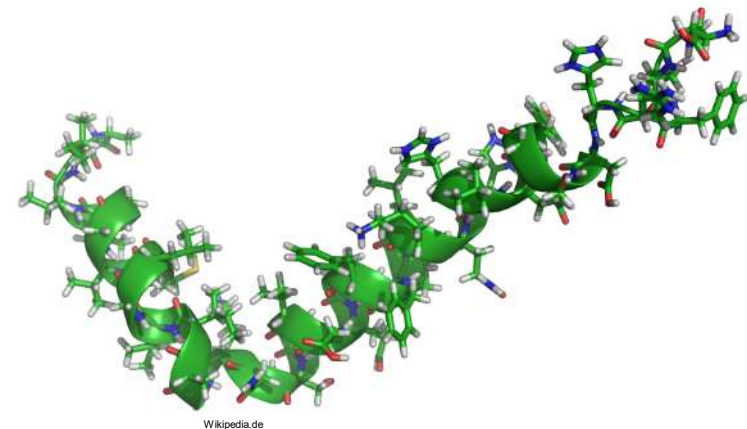
Amyloid-beta42 reduces network activity concentration-dependently
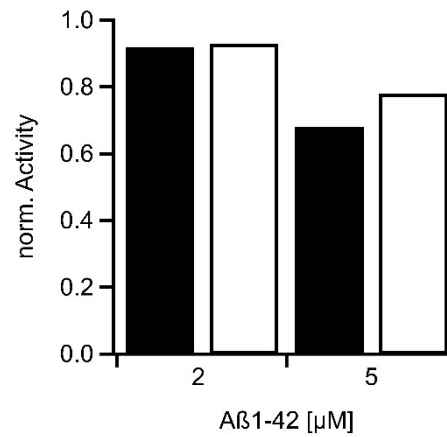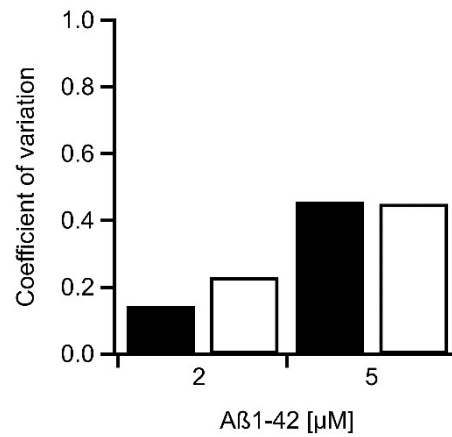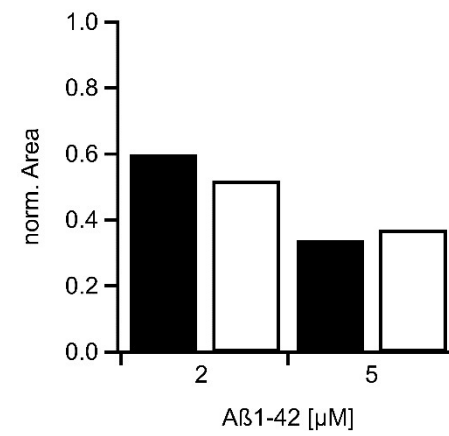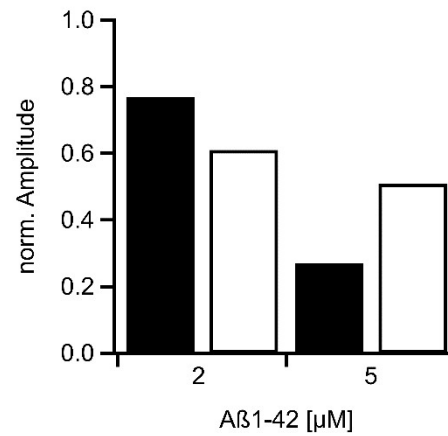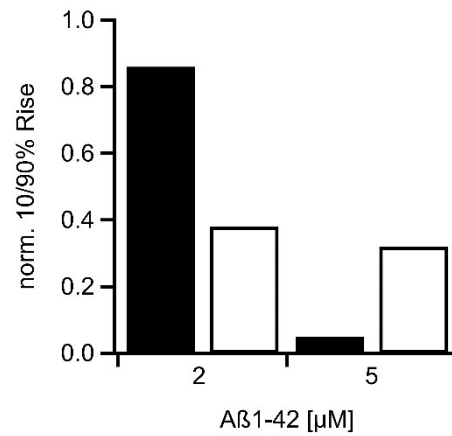
Summary

- Hamamatsu µCell allows to record strong and stable network activity of iCell Glutaneurons
- 384-well format makes experiments fast and cell-saving (=cheap)

- Seizurogenic compounds and mGluR agonists modulate network activity
  of iCell Glutaneurons concentration-dependently
  - Similar or higher sensitivity compared to primary neurons
→ **Neurotox assays**
→ **Neuromodulation research (Alzheimer, Parkinson, Schizophrenia)**

- Cocultivation with iCell Astrocytes
  - Does not alter base network parameters
  - Allow more reproducable recordings compared to iCell GlutaNeurons alone
  - Allow faster recovery from mGluR III activation (L-AP4, to be further investigated)

- Pathological effects by Amyloid-β reproducible
→ **Disease modelling**
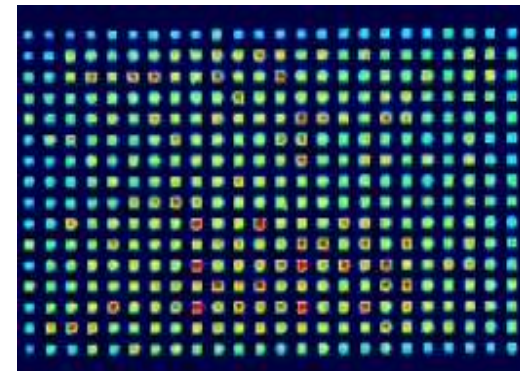
Acknowledgement

NMI:
Electrophysiology:
- Sandra Buckenmaier
- Karin Gebhardt
- Dominik Loser (MEA data)

Molecular Neurobiology:
- Martin Kriebel (primary neurons, A β)

Cellular Dynamics:
Cell sponsoring
- Sabine Lange
- Blake Anson

Hamamatsu:
Organization of Meeting

Novartis Pharma:
Hosting of Meeting

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