

# Comparison of orthosteric and allosteric GABA<sub>B</sub> modulators by their electrophysiological activity patterns on *in vitro* microcircuits from frontal cortex tissue

## Introduction

GABA<sub>B</sub> receptors are inhibitory receptors that are linked via G-proteins to Ca<sup>2+</sup> and K<sup>+</sup> channels. GABA<sub>B</sub>R are discussed to be involved in the etiology of depression and the regulation of behaviour and reward processes.

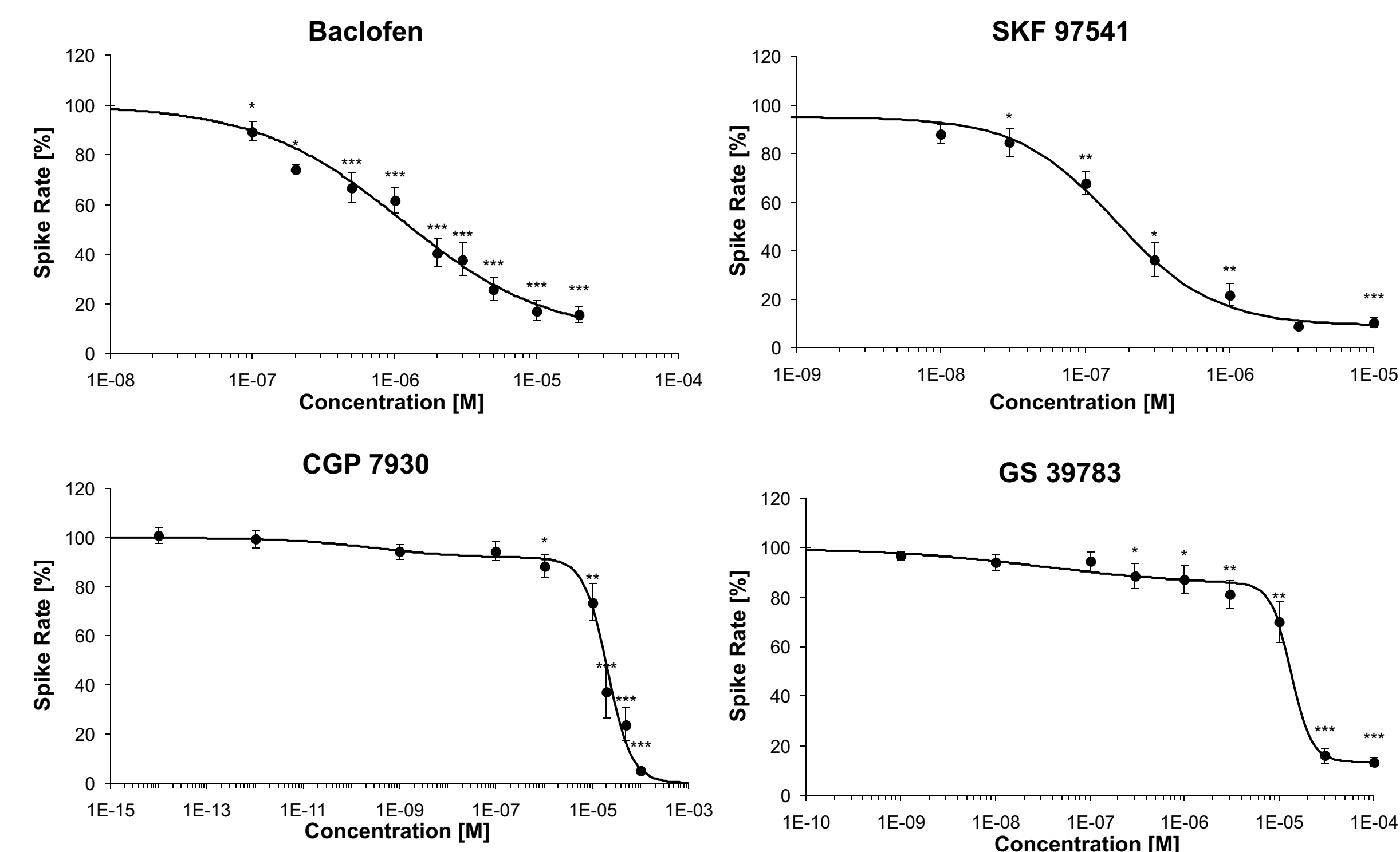
In this study, we investigate the functional effects of the two GABA<sub>B</sub>R agonists baclofen and SKF97541, and the two positive allosteric modulators CGP7930 and GS39783. We used neuronal networks of primary cell cultures from murine frontal cortex tissue grown on microelectrode arrays (MEAs) as a functional *in vitro* model with complex micro-circuitry, which present a powerful tool to characterize pharmacological compounds.

## Results

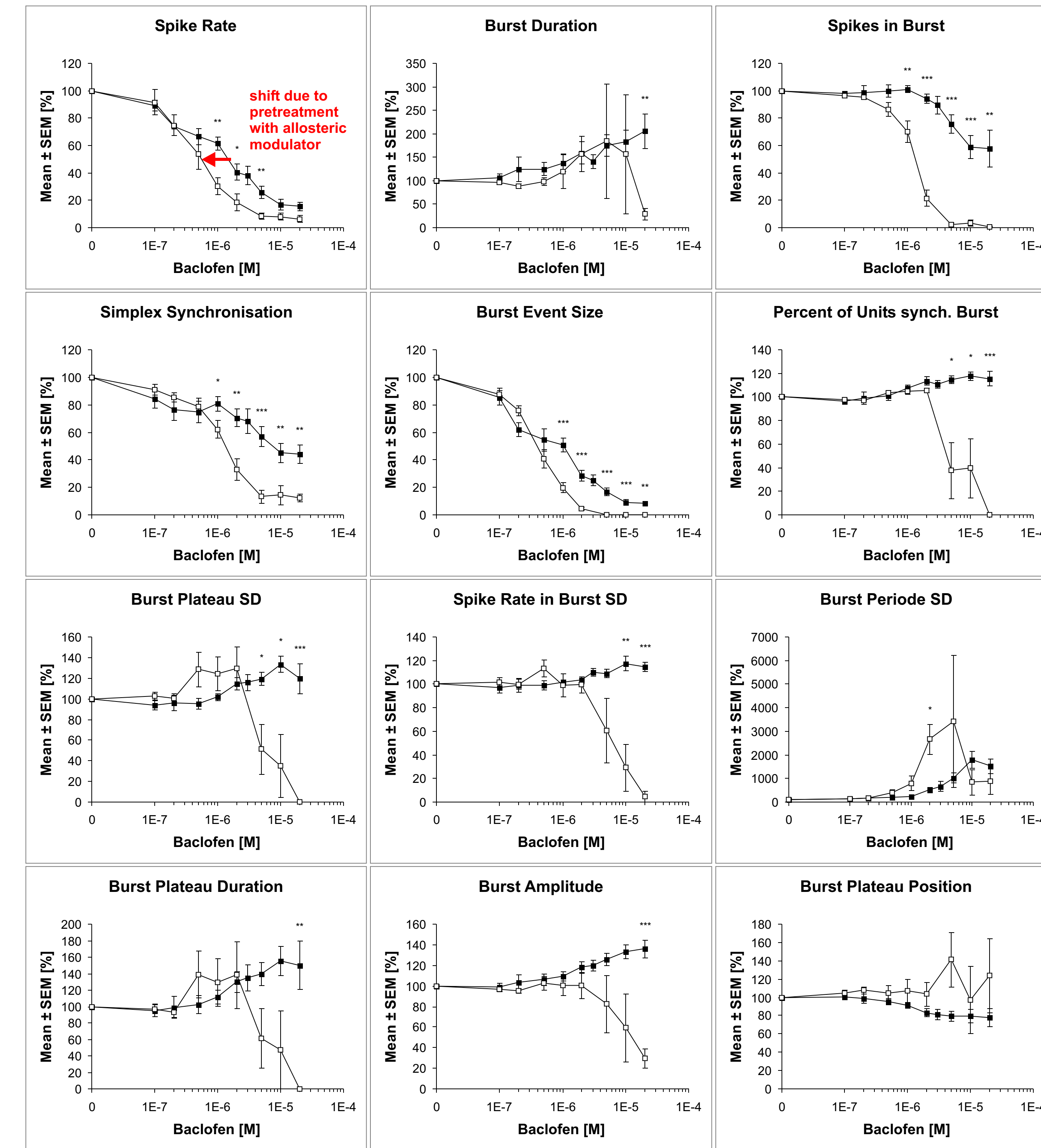
### Multiparametric characterization of the electrical activity patterns

Both allosteric modulators showed without pre-treatment at its alone only slight efficacy. Prestimulation with GS39783 induced a clear shift of the dose-response curve of Baclofen, which showed clearly that GS39783 is an allosteric modulator. On the other hand, prestimulation with the orthosteric compound Baclofen didn't show any effect of the dose-response curve for GS39783 (data not shown). GS39783 induced a significant decrease of the Na<sup>+</sup> peak amplitude, which showed that GS39783 has also ionotropic side effects. A high similarity of the acute effects of GS39783 to Quetiapine and Ketamine confirms antidepressant effects of these compounds, although it should be questioned if these effects can be caused by its GABA<sub>B</sub> receptor modulatory effect or more by its side effects.

Another conclusions from the classification approach is the high similarity for CGP7930 to Chlorpyrifos Oxon which should be taken as a hint for possible negative side effects.



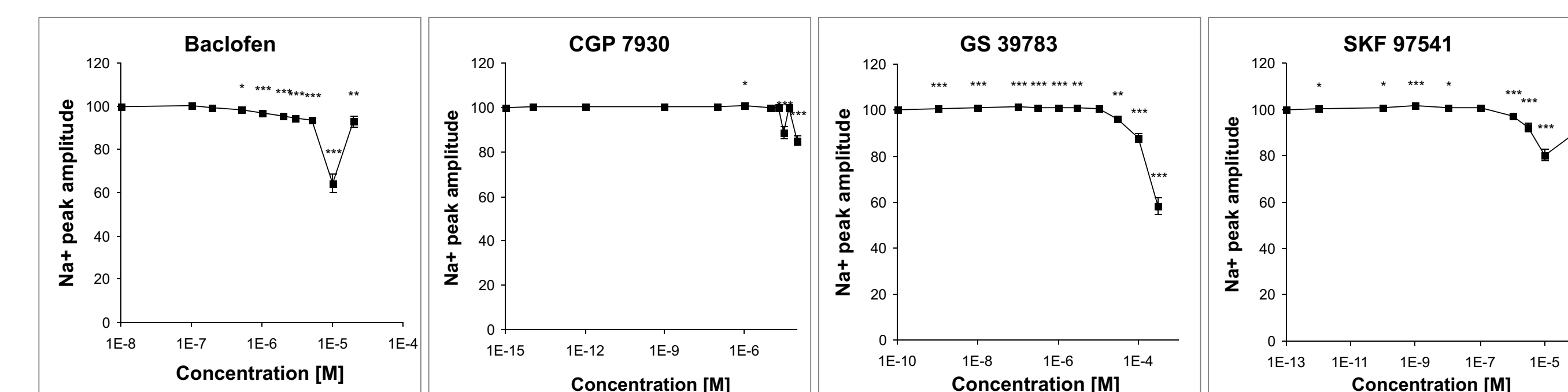
**Acute dose-response effects** of the GABA<sub>B</sub>R allosteric agonists Baclofen and SKF 97541, and the orthosteric modulators CGP 7930 and GS 39783. Displayed are 9 activity describing parameters for treatment of accumulating concentrations. (mean ± standard error, Baclofen: n=13, EC<sub>50</sub>=1.1 μM; SKF 97541: n=14, EC<sub>50</sub>=163 nM; CGP 7930: n=11, EC<sub>50</sub>=21.3 μM; GS 39783: n=9, EC<sub>50</sub>=13.5 μM; . Student's paired t-test: \* p = 0.05; \*\* p = 0.01; \*\*\* p = 0.001).



■ Baclofen only □ pretreatment with 10 μM GS39783

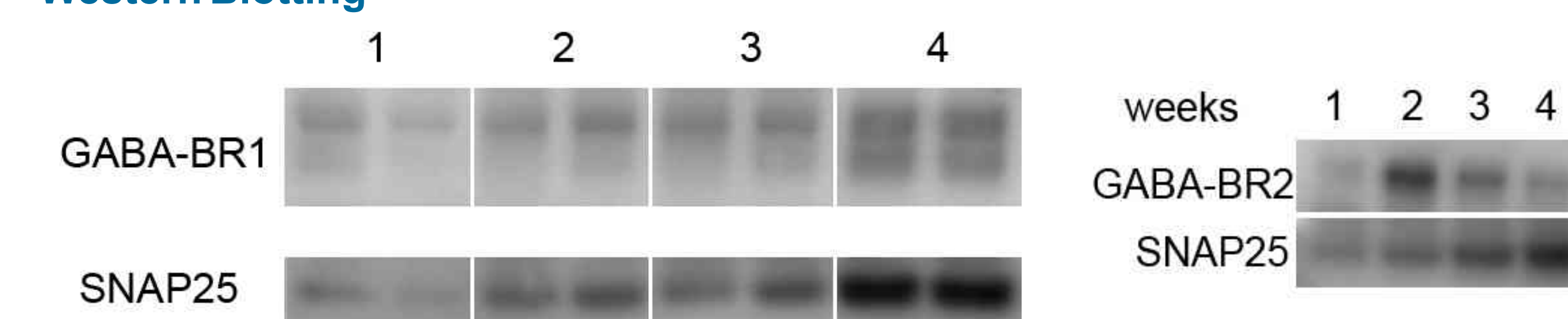
Comparison of acute dose-response effects of Baclofen with and without pretreatment with 10 μM GS39783. Displayed are 12 activity describing parameters for treatment of 8 accumulating concentrations of Baclofen in the range of 100 nM to 20 μM (mean ± standard error, Baclofen: n=13, Baclofen-GS39783: n=9; Student's unpaired t-test: \* p = 0.05; \*\* p = 0.01; \*\*\* p = 0.001).

### Extracellular Action Potentials



Comparison of the changes on the Na<sup>+</sup> peak amplitude (see Methods) reveals a significant decrease of this amplitude for GS 39783, which can be interpreted as a side effect of this compound, which is not metabotropic.

### Western Blotting



Western Blotting showed the development and presence of both GABA<sub>B</sub> receptor subunits, 1 and 2, over the time until 4 weeks when cultures were used for electrophysiological experiments.

### Pattern Recognition and Classification

Classification revealed specific activity patterns for the four GABA<sub>B</sub>R modulators. The electrophysiological activity pattern changes induced by the agonists baclofen and SKF-97541 revealed a very high similarity. Previously, we showed that Baclofen has also a high similarity to Gabapentin. All three substances show a typical increase of the higher frequency pattern within bursts in contrast to the allosteric modulators. Comparison to NeuroProof substance database showed for CGP7930 a high similarity to Chlorpyrifos Oxon, Cortisol, Ethanol and L655,708 (in descending order), and that the GS39783 response is similar to Quetiapine, MK801, Ketamine and Acetaminophen (in descending order).



95 substances were analyzed by 200 parameters, and their similarities determined by our classification algorithm. The distances are projected using the software 'Splitstree' to create a phylogenetic network (Huson and Bryant, Application of Phylogenetic Networks in Evolutionary Studies, Mol. Biol. Evol., 23(2):254-267, 2006).

## Summary

We investigated the modulatory effects of GS39783 and CGP7930 in comparison to the orthosteric compounds SKF97541 and Baclofen with our MEA-neurochip technology. We could confirm the known beneficial and negative effects of these compounds. Since our approach allows a more complex assessment, we could figure out additional views of possible mode of action of these compounds which questions their proof of concept as beneficial GABA<sub>B</sub> modulators.

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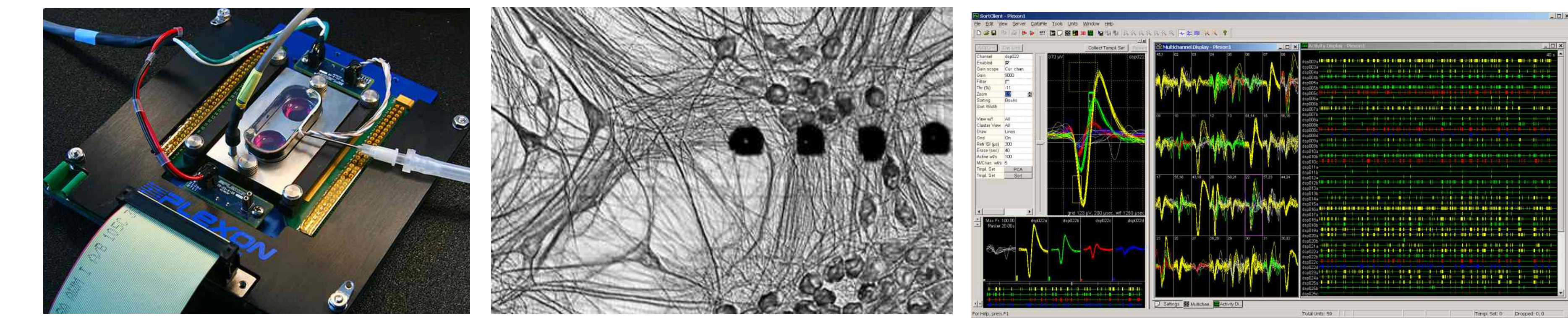
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## Material and Methods

### Primary neuronal embryonic co-cultures

Primary neuronal cells from frontal cortex tissues of embryonic day 16 NMR1 mice were cultured on MEA neurochips. After mechanical and enzymatic (accutase) dissociation, cells were grown in DMEM/10% horse serum without antibiotics. The networks were incubated on PDL and laminin coated MEA's (CNNS, Denton,

Texas) at 37°C, with constant pH at 7.4 in a 10% CO<sub>2</sub> atmosphere until ready for use (generally after 4 weeks *in vitro*). Electrophysiological activity can be monitored starting from a few days *in vitro* (DIV) on for up to several months. Neurons encode information by firing action potential spikes and bursts that can be easily extracted with MEA recordings. Neuronal electrophysiology responds to transmitters, their blockers, agonists and many other pharmaceuticals in a histotypic manner similar to the *in vivo* situation.



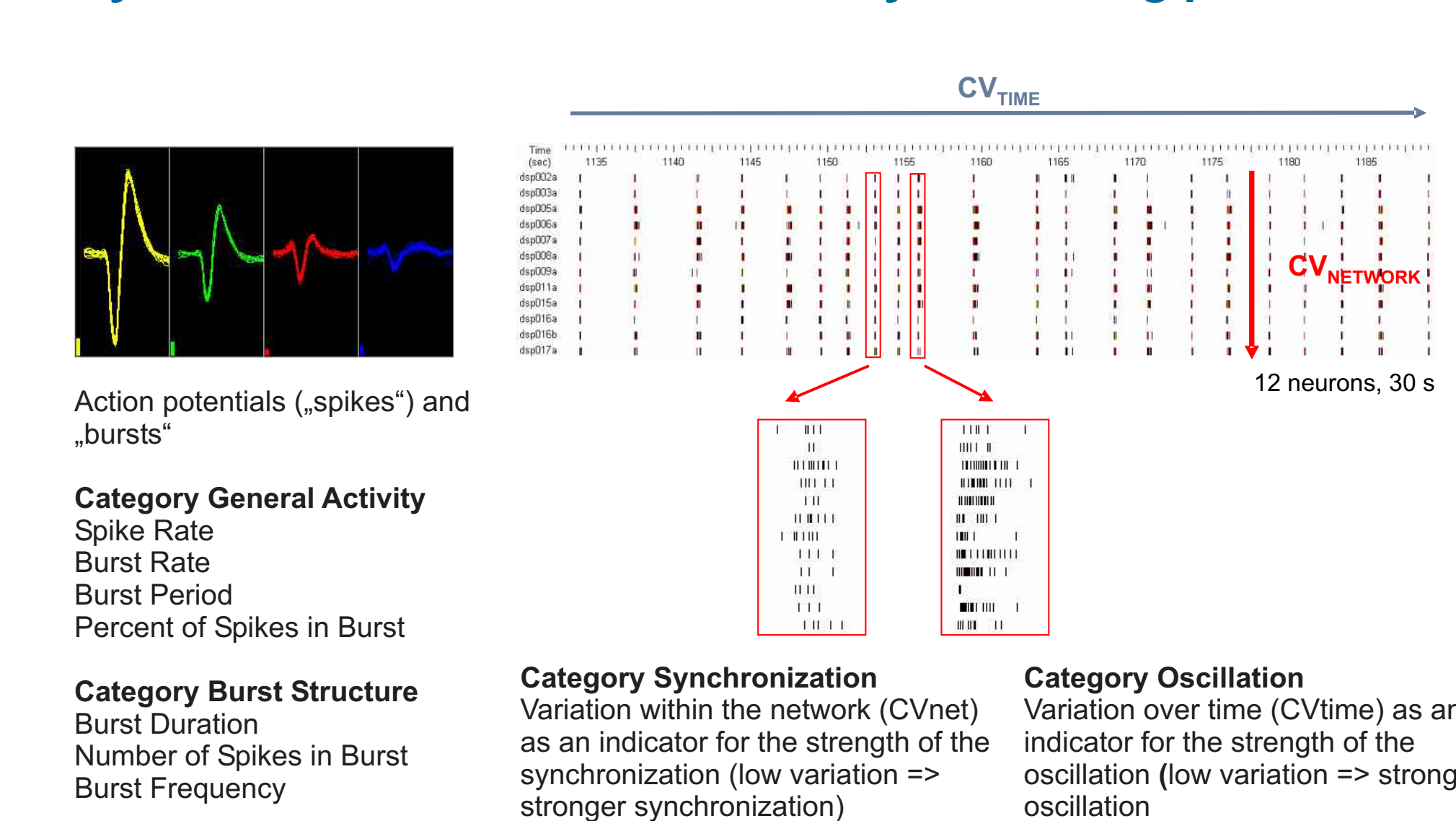
Left: recording set up with heater and CO<sub>2</sub> supply. Right: Electrical activity patterns of the simultaneously recorded spikes (action potentials) of a multitude of neurons on one cortical network.

### Data Analysis

Spike train data were recorded with a microelectrode array neurochip (MEA) setup (Gramowski et al. 2004) consisting of MEA neurochips (CNNS, Denton, Texas, USA), MEA workstations (Plexon Inc., Dallas, TX, USA), and analysis software (Plexon, Inc., and NeuroProof GmbH, Rostock, Germany). Experiments were carried out with increasing concentrations, covering the full dynamic range of action. A stable activity phase of at least 30 minutes was analysed for every application phase. Chemically stimulated networks require a specific data analysis approach due to their spontaneous and complex network behaviour. To characterize the complex influence of a substance on the various network properties we calculated for each stable activity phase of a substance application 200 spike train parameters and normalized those by the native activity. The computed spike train features characterize effects on the general activity, burst structure, regularity of oscillation and synchronicity. These data records were computed for the each substance, creating a "finger print" of its effects. All parameter values are normalized relative to the native level as an internal reference of the activity baseline.

**Extracellular waveform data** were recorded along the time stamps. For each neuron and concentration, waveforms of 5 min activity were averaged (~300 to 1800 spikes) and then analyzed using our in-house software NPWaveX. Nine parameters characterize three possible peaks and the derivatives (slopes) around those peaks, reflecting the dynamics within the extracellularly recorded action potentials (adapted and modified from Gold *et al.*, J Comput Neurosci. 23(1):39-58, 2007).

### Dynamic neuronal networks: activity-describing parameters

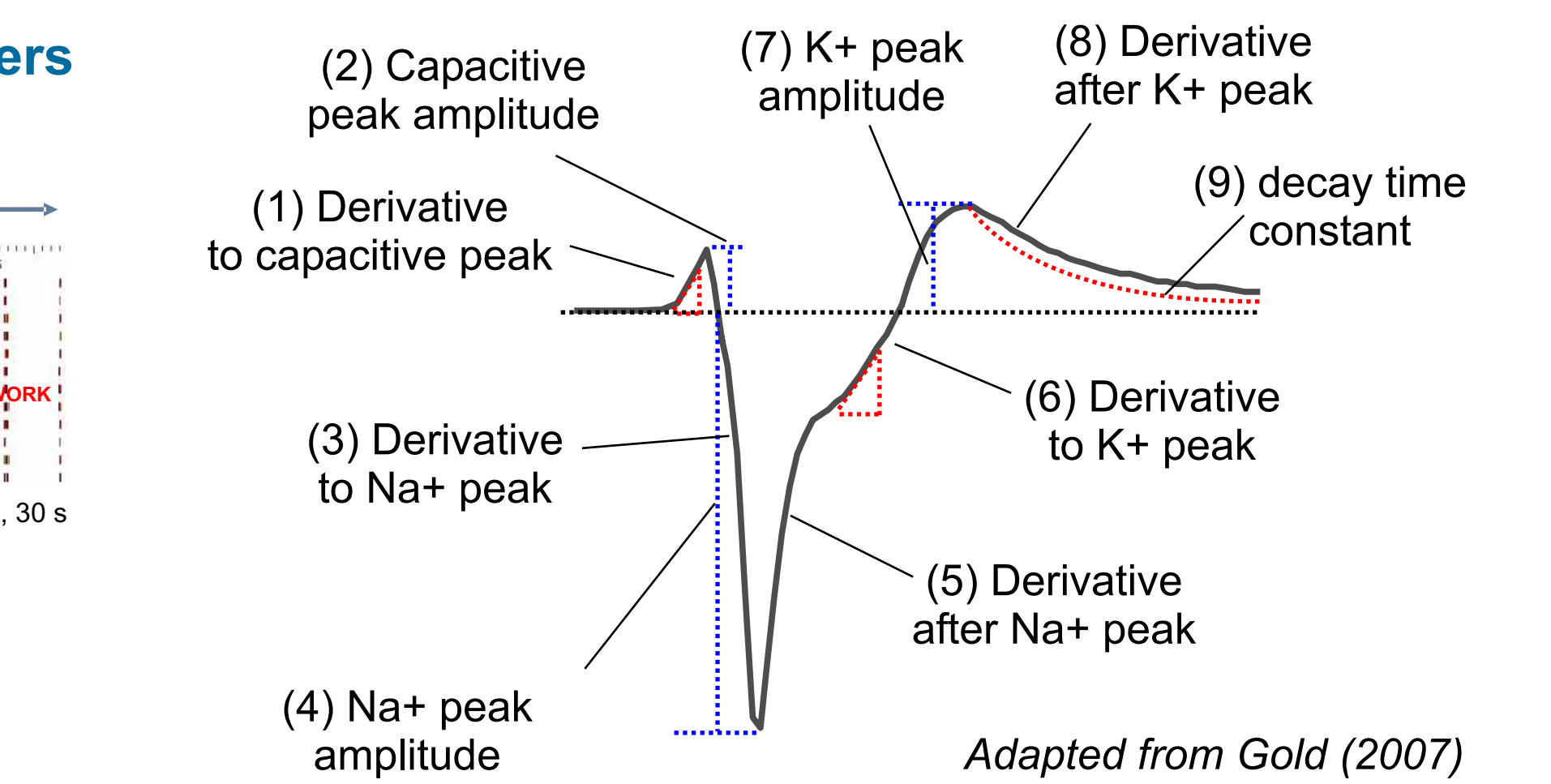


### Pattern Recognition and Classification

To clarify the mode of action of a substance on the activity of frontal cortex networks, we further processed these experiments using methods of pattern recognition. Using a feature selection algorithm, we selected the 40 most descriptive parameters of all 200 spike train parameters based on the reference substances. Feature selection is a widely accepted method in bioinformatics, and a wide range of different approaches have been proposed. We calculated the feature ranking using a MDL (minimal description length) modified algorithm that compares total correct predictions. An artificial neuronal network (multi layer feed forward network and back propagation algorithm without hidden units) was trained with the data sets of all reference substances. The respective data records of each substance was subsequently classified against the reference database. On the basis of classification experiments we performed a similarity analysis and present the relations in sophisticated cluster diagrams.

### Western Blotting

Unboiled cell lysates from different tissues and time points were run on NuPage gradient SDS-gels (Invitrogen) and transferred on PVDF-membranes (iBlot, Invitrogen). The following rabbit antibodies were used: alpha1 (1:750, Synaptic Systems), alpha2. AP-conjugated anti-rabbit secondary antibodies (Invitrogen) were incubated with AP-substrate (Immobilon, Millipore) and chemiluminescence was recorded with a BioRad detection unit and quantified using Imagelab (Biorad).



Characterization of extracellular action potentials (adapted and modified from Gold *et al.*, 2007) with peak amplitudes and derivatives (slopes)