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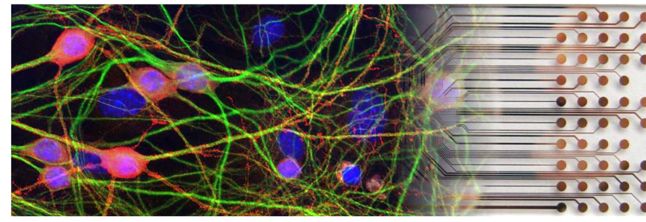

Highly sensitive TTX assay: Cortical network activity responses 100-fold more sensitive to tetrodotoxin than associated action potential waveforms

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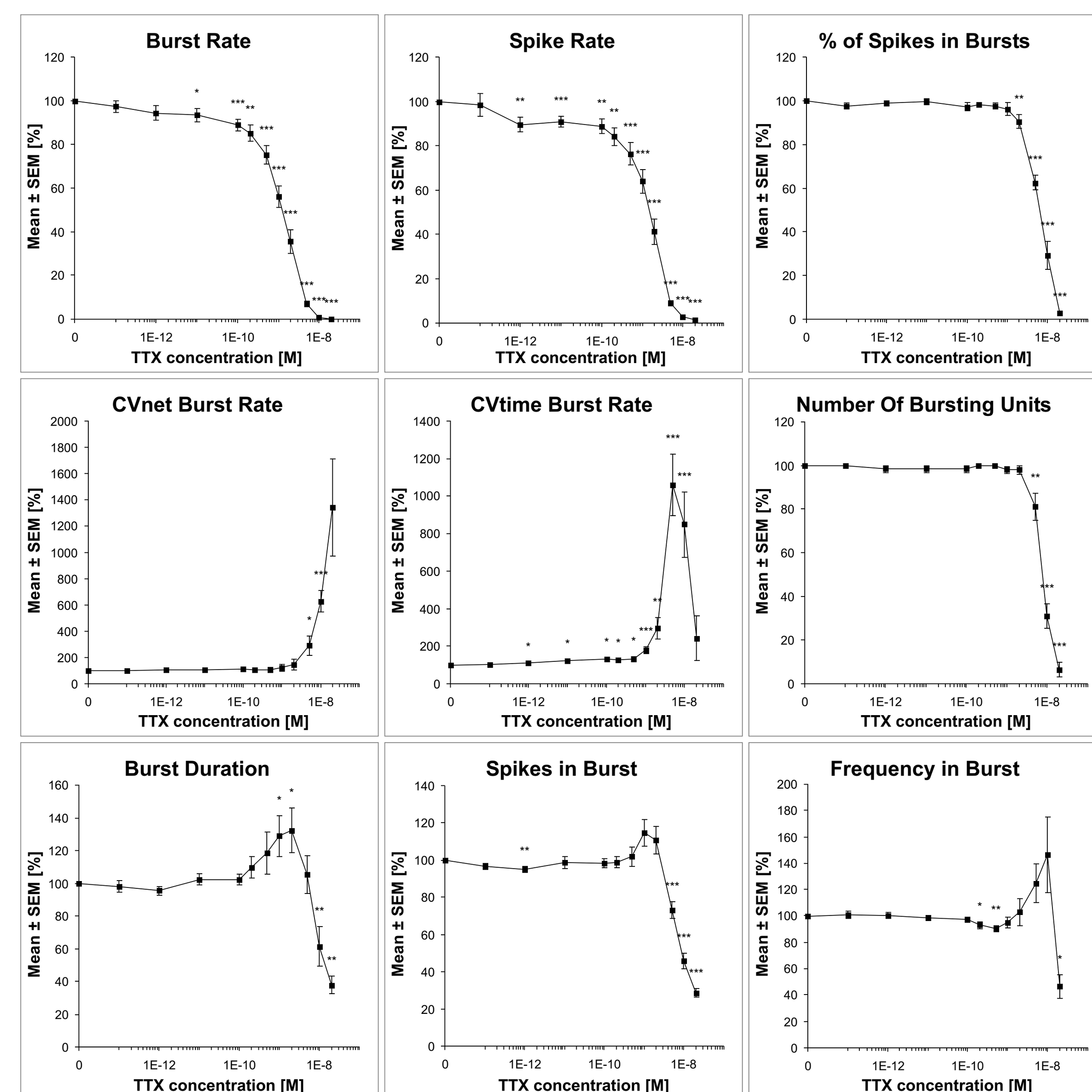
Introduction

Voltage-gated sodium channels (Na_V) play an important role in action potential initiation. So far, nine members of the Na_V channel family have been characterized in mammals, of which 4 are found in neurons in the CNS. These different sodium channels have distinct regulatory and pharmacological properties. Here we studied the electrophysiological activation profile of the Na_V channels sensitive to the specific fast sodium channel blocker tetrodotoxin (TTX). As a sedative, TTX is currently discussed as drug target in pain treatment (e.g. cancer, burns, neuralgia and migraine) as well as an analgesic. We used primary neuronal networks from murine frontal cortex grown on microelectrode array neurochips as a functional *in vitro* model with complex micro-circuitry, a powerful tool to characterize pharmacological compounds. This approach allows us to study changes of extracellular AP's and spike train patterns induced by TTX.

Results

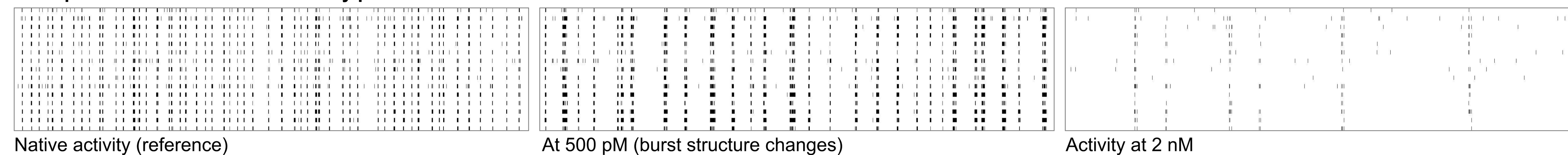
TTX induced biphasic network spike activity changes with EC_{50}s at 1.7 pM and 1.4 nM (spike/burst rate). The activity changes in the second phase were accompanied by changes in burst structure (EC_{50} burst duration=180pM), burstiness, synchronization and oscillation of the network at concentrations higher than 1 nM. Network activity cessation was observed at 10 nM. In contrast, changes in the action potential waveforms induced by TTX started at 1 nM, with an EC_{50} of 18 nM for the decline of the peak waveform amplitude before network activity loss.

Multiparametric characterization of the electrical activity patterns



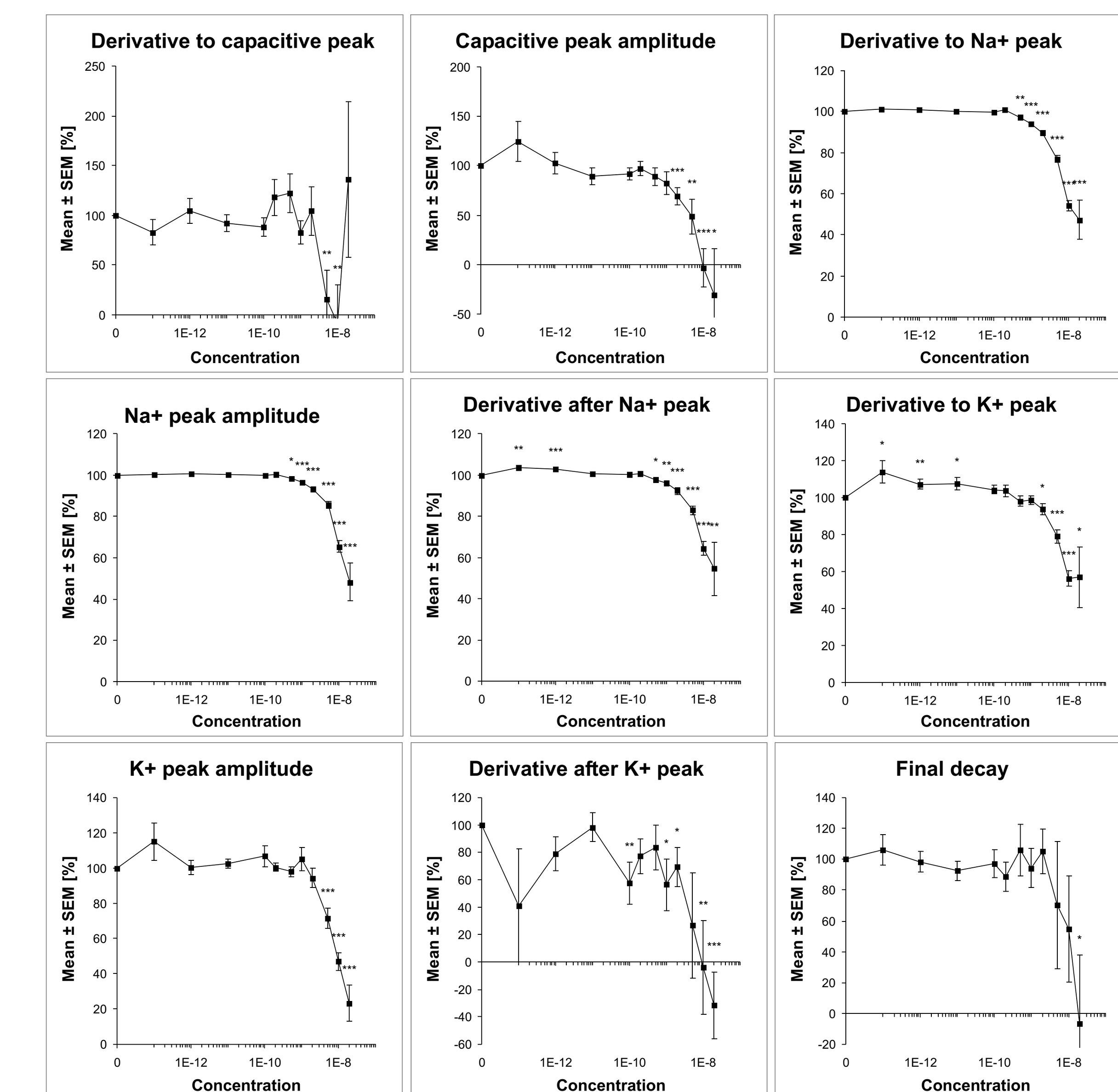
Acute effects of Tetrodotoxin (TTX) on the cortical network activity *in vitro*. 9 network activity parameters for treatment of 11 accumulating concentrations of TTX in the range of 1 fM to 20 nM. (mean ± standard error, n=14; Student's paired t-test: * p = 0.05; ** p = 0.01; *** p = 0.001).

Example of neuronal network activitiy patterns

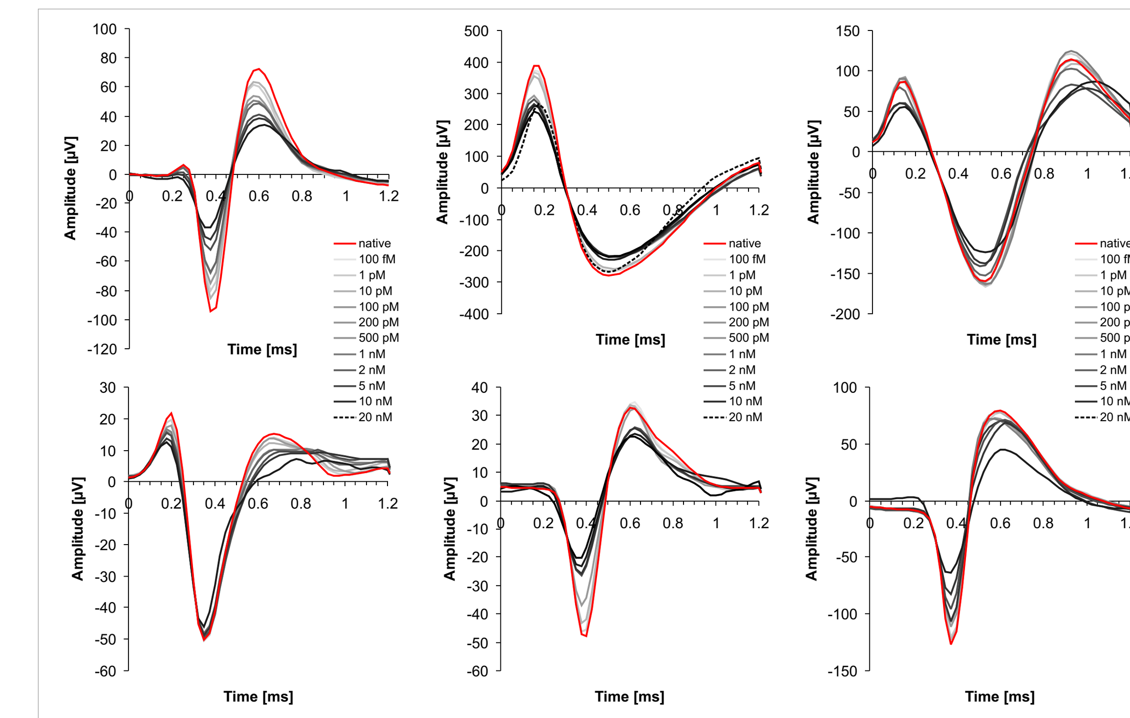


Native activity (reference) | At 500 pM (burst structure changes) | Activity at 2 nM

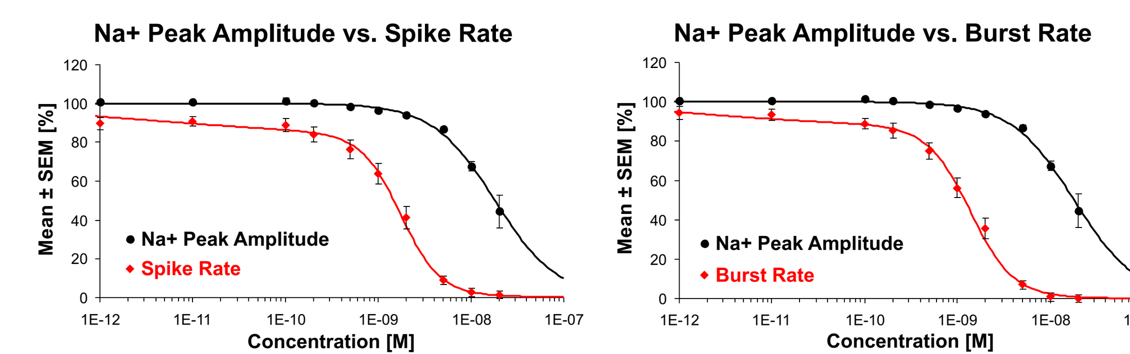
Characterization of the extracellular action potential waveform



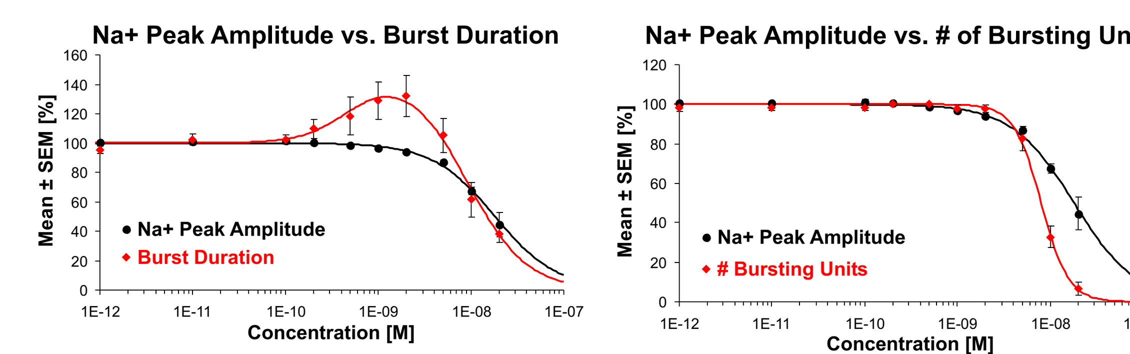
Acute effects of TTX on the waveforms. 9 parameter describing the concentration-dependent changes of the extracellular action potential waveforms. The EC_{50} for the Na+ peak amplitude is 18.1nM which correlates with values from patch clamp recordings (mean ± standard error, n=315; Student's paired t-test: * p = 0.05; ** p = 0.01; *** p = 0.001).



Six examples of extracellular action potential waveforms and their changes after application of increasing concentrations of tetrodotoxin (1 fM to 20 nM).



The cortical network activity shows a 100-fold higher sensitivity to TTX than to spike wave shape changes.



Summary

Neuronal network cultured on MEA neurochips show a high potential for studying both the pharmacological properties of voltage-gated ion channels and their kinetics on complex firing network activity patterns, and their single neuron waveform analyses. An additional advantage is the recording at 37°C temperature.

This is, to our knowledge, the most sensitive and complex TTX assay. It will be valuable to optimize and enhance drug development related to voltage-gated ion channels.

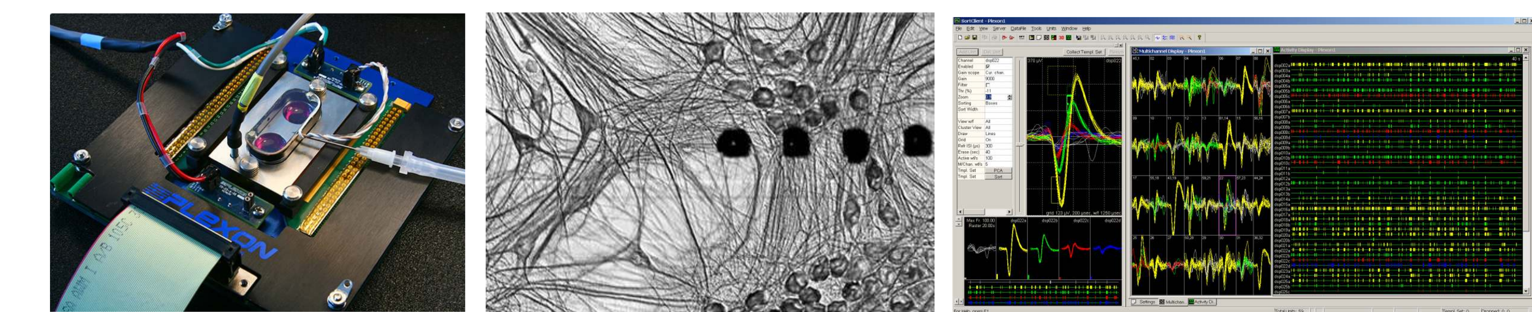
Next Steps

We will further develope the waveform analysis to obtain more parameters and thereby differentiate neuronal cell types by their waveforms, as well as different recording positions at the neuron.

Material and Methods

Primary neuronal embryonic co-cultures

Primary neuronal cells from frontal cortex tissues of embryonic day 16 NMRI 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_2 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 histiotypic manner similar to the *in vivo* situation.



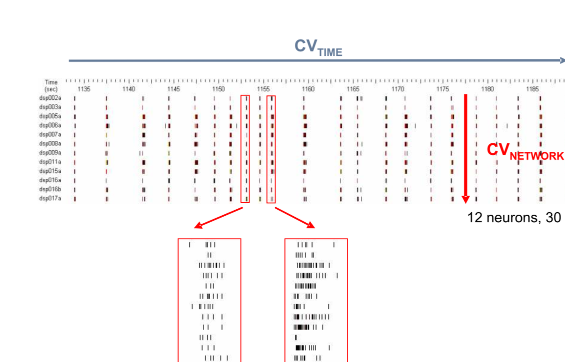
Left: recording set up with heater and CO_2 supply. Right: Electrical activity patterns of the simultaneously recorded spikes (action potentials) of a multitude of neurons of one cortical network.

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 (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, in-house software calculates for each stable activity phase of a substance application 200 spike train parameters, characterizing effects on the general activity, burst structure, regularity of oscillation and synchronicity. 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 timestamps. 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.*, 2007).

Dynamic neuronal networks: activity-describing parameters

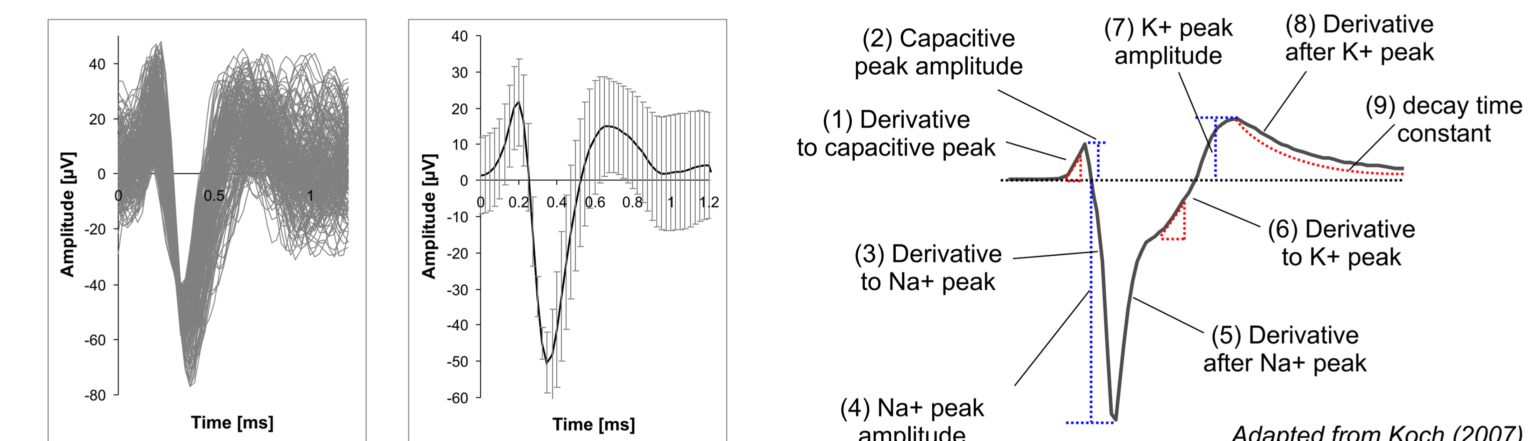


Category General Activity
Spike Rate
Burst Rate
Burst Period
Percent of Spikes in Burst

Category Burst Structure
Burst Duration
Number of Spikes in Burst
Spike Frequency in Burst

Category Synchronization
Variation within the network (CVnet) as an indicator for the strength of the synchronization (lower variation => stronger synchronization)

Category Oscillation
Variation over time (CVtime) as an indicator for the strength of the oscillation (lower variation => stronger oscillation)



Overlay of extracellular action potentials (spike waveforms) of one neuron. Middle: Averaged time course of one neurons (mean ± SD)

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

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Gold C, Henze DA, Koch C. Using extracellular action potential recordings to constrain compartmental models. J Comput Neurosci. 23(1):39-58 (2007)
Gramowski A, Jügelt K, Weiss DG, Gross GW: Substance identification by quantitative characterization of oscillatory activity in murine spinal cord networks on microelectrode arrays. Eur J Neurosci. 19: 2815-2825 (2004)