MEA Data Analysis For Substance Screening On Neuronal Networks

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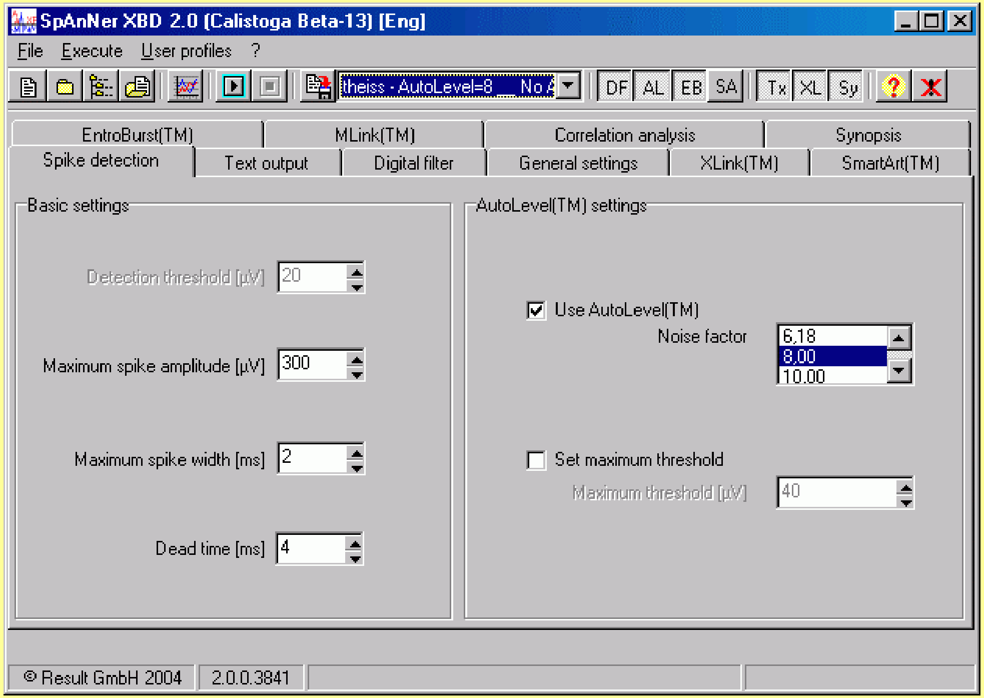
The Challenge:
(How) Can Signal Analysis Contribute To Quality Assurance?

- Task: Automatic and simple standard analysis of spontaneous electrophysiological activity
- Ensuring and controlling primary signal quality
  - Noise-level adjustment & data filtering
- Stable algorithms for network firing pattern analysis
  - Temporal structure: bursts
  - Spatio-temporal structure:
    - Correlation: Pearson's r, Cohen's kappa
    - Synchrony: fraction of empty bins
- Aim: Reproducible quantification of drug effects

The Software Tool

RESULT MEDIZINISCHE ANALYSEVERFAHREN GMBH

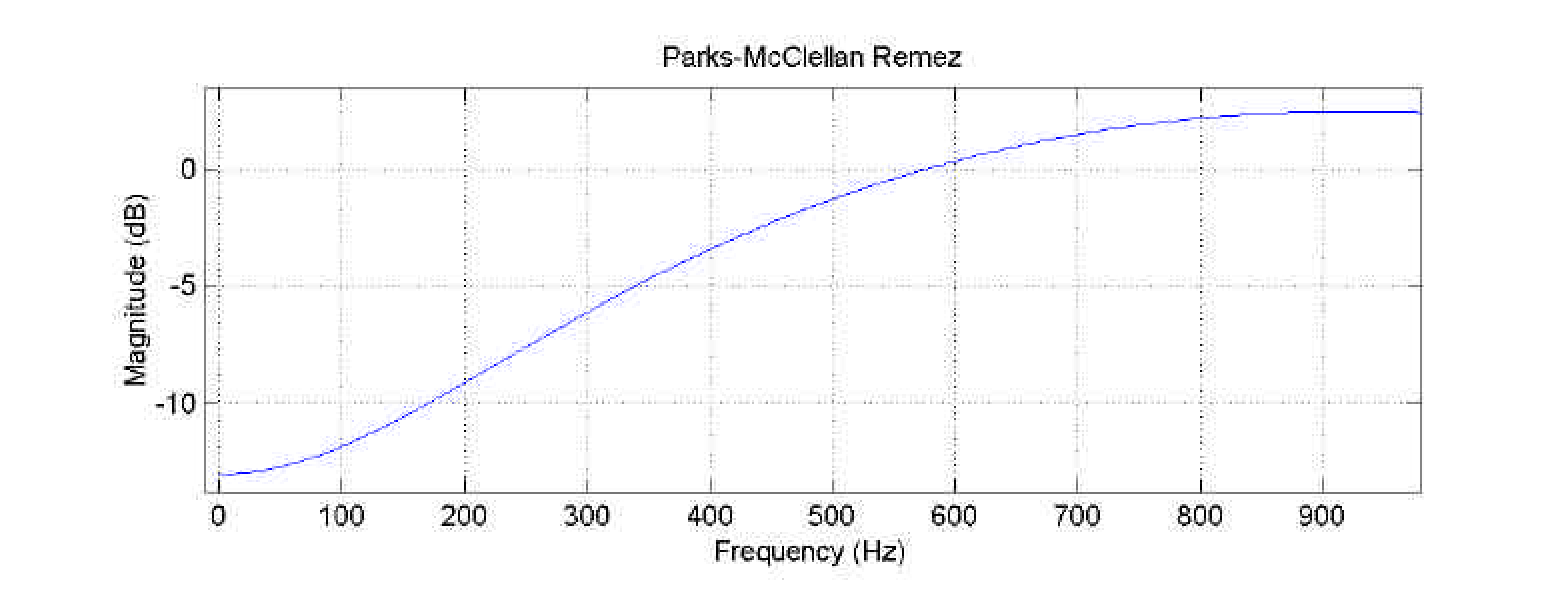


Batch Mode: Raw data files in → Analysis out

Digital High Pass Filtering

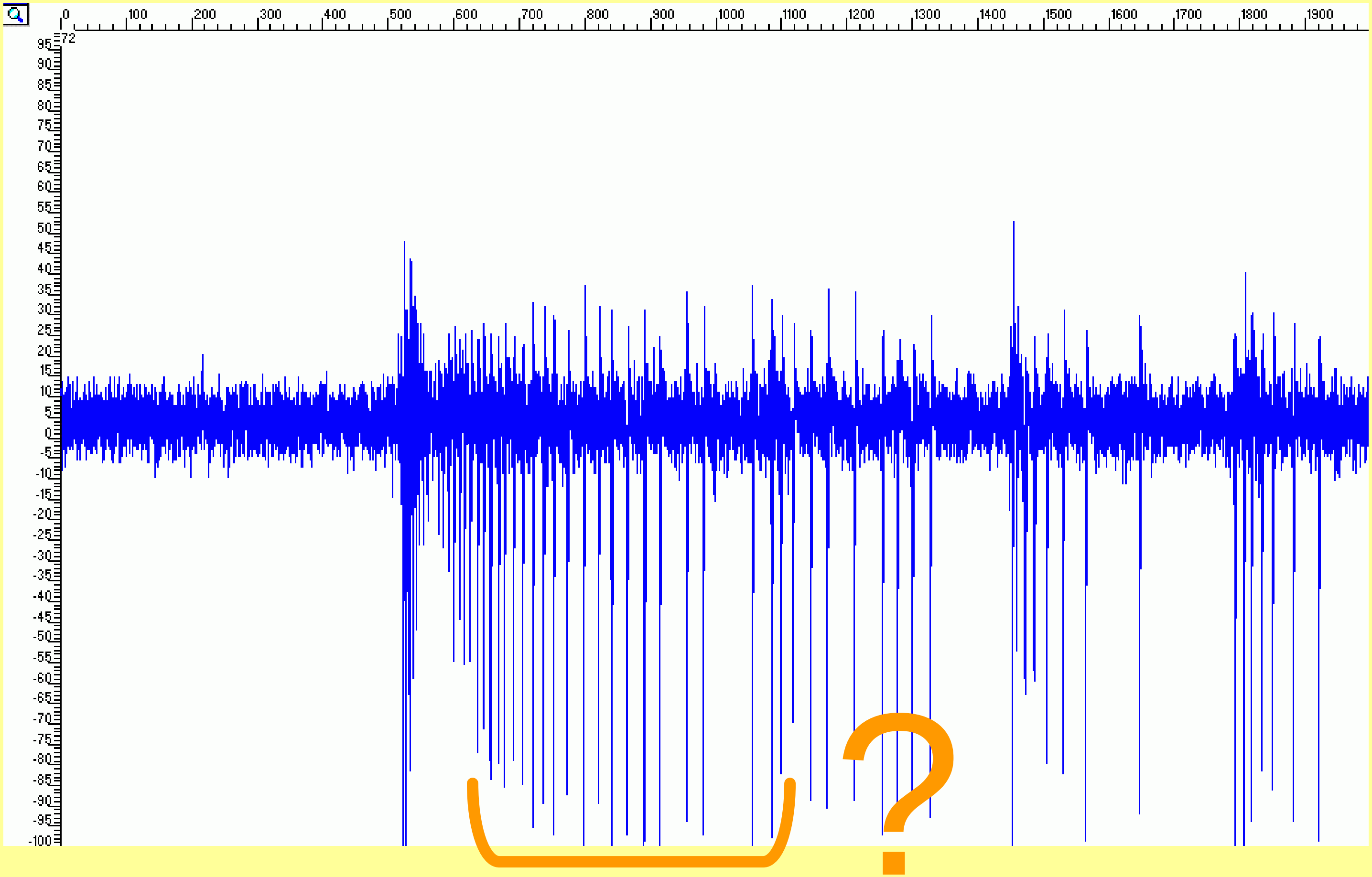
- Aim: remove low-frequency artifacts
  but retain true spike shape
- Problem: cut-off frequency close to zero: instability
- Design: linear phase high pass FIR-filter
  Parks-McClellan: weighted Chebychev approx.

passband	400 Hz	-4 dB
stopband	100 Hz	-12 dB



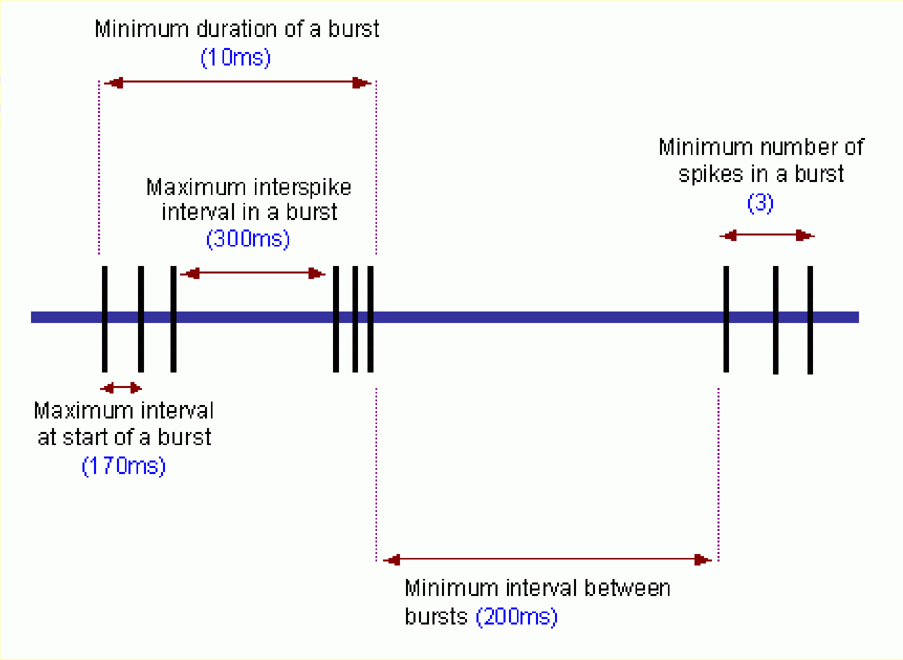
High Pass Filter: Example

Burst Detection



Burst Detection

- Heuristical Method ?
- Pseudo-Integration ?
- String Method ?
- Statistical Method?
- .....



Burst Detection

- Heuristical Method ?
- Pseudo-Integration ?
- String Method ?
- Statistical Method: Surprise ?
- .....

RC-pseudo-integration:

$$
\begin{aligned}
A_n &= A + A_{n-1} e^{-k(t_n - t_{n-1})} \\
A &= A_1 = 25 \\
k &= 0.0253 \text{ sec}
\end{aligned}
$$

Burst Detection

- Heuristical Method ?
- Pseudo-Integration ?
- String Method ?
- Statistical Method: Surprise ?
- .....

String method:

$$burst = \text{vertical string of spikes}$$
$$N_s = \text{min. \# spikes in burst}$$
$$t_s = \text{max. } ISI_{in\ burst}$$

Burst Detection

- Heuristical Method ?
- Pseudo-Integration ?
- String Method ?
- Statistical Method: Surprise ?
- .....

$$
\begin{aligned}
S(n, \Delta t) &= -\ln P_{Poisson}(\#\ \text{spikes during}\ \Delta t \geq n) \\
&= -\ln \left\{ 1 - \sum_{k=0}^{n-1} \frac{(\Delta t \,/\, \overline{ISI}\,)^k}{k!}\, e^{-\Delta t \,/\, \overline{ISI}} \right\}
\end{aligned}
$$

Actual spikes are closer than uncorrelated (Poisson) train.

Burst Detection

- Heuristical Method ?
- Pseudo-Integration ?
- String Method ?
- Statistical Method: Surprise ?
- ...

- ➔ There is NO Gold Standard !

  (*Beauty is in the eye of the beholder* – we cannot *ask the neuron!*)

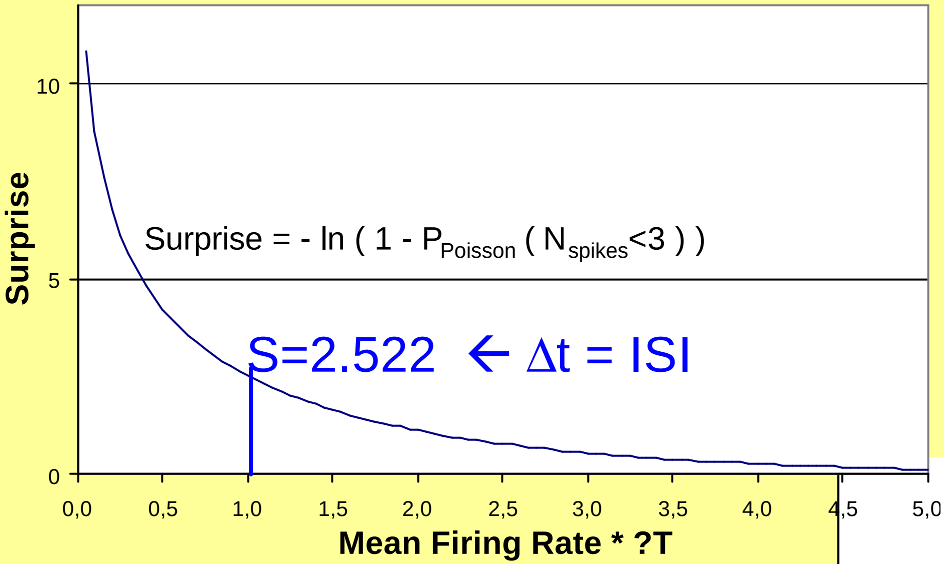
Bursts By Surprise...

RESULT
MEDIZINISCHE ANALYSEVERFAHREN GMBH

- Find 3 spikes closer than ISI, with
- Surprise $S_3 > S_{min}$ compares with Poisson process
- Add spikes as long as S_n increases (within tolerance *tol*)
- Remove spikes at start of burst as long as S_n increases

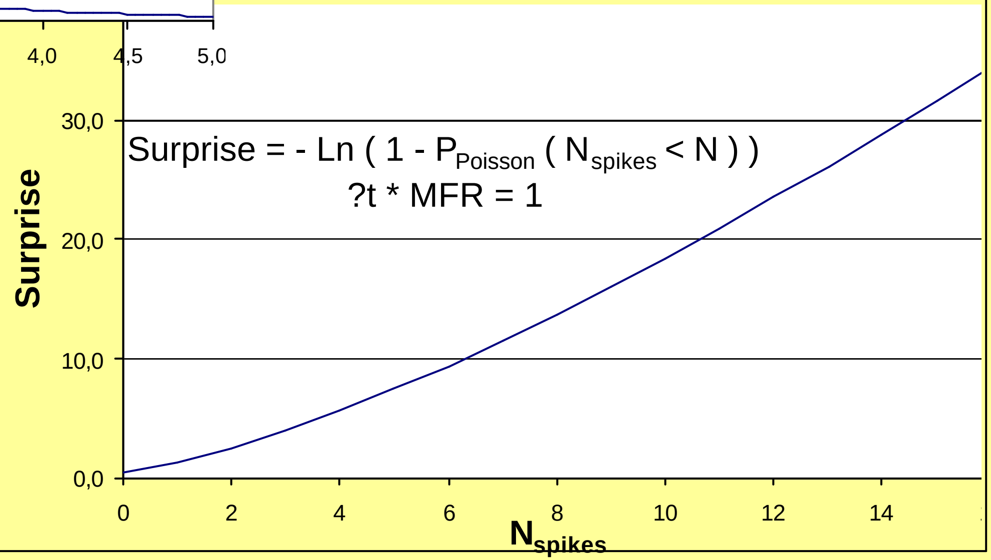
$$
\begin{aligned}
\mathcal{P}(\tau = t_3 - t_1, n \geq 3 \,|\, r_S) &= \sum_{n=3}^{\infty} \frac{1}{n!}((t_3 - t_1) \cdot r_S)^n \exp(-(t_3 - t_1) \cdot r_S) \\
\mathcal{S}_n &= -\ln\left[\sum_{k=n}^{\infty} p(\tau = t_n - t_1, k \,|\, r_S)\right] \\
\mathcal{S}_n &> tol \cdot \mathcal{S}_{n-1} \\
\mathcal{S}_n &> \mathcal{S}_{min}
\end{aligned}
$$

Burst Definition „By Surprise“

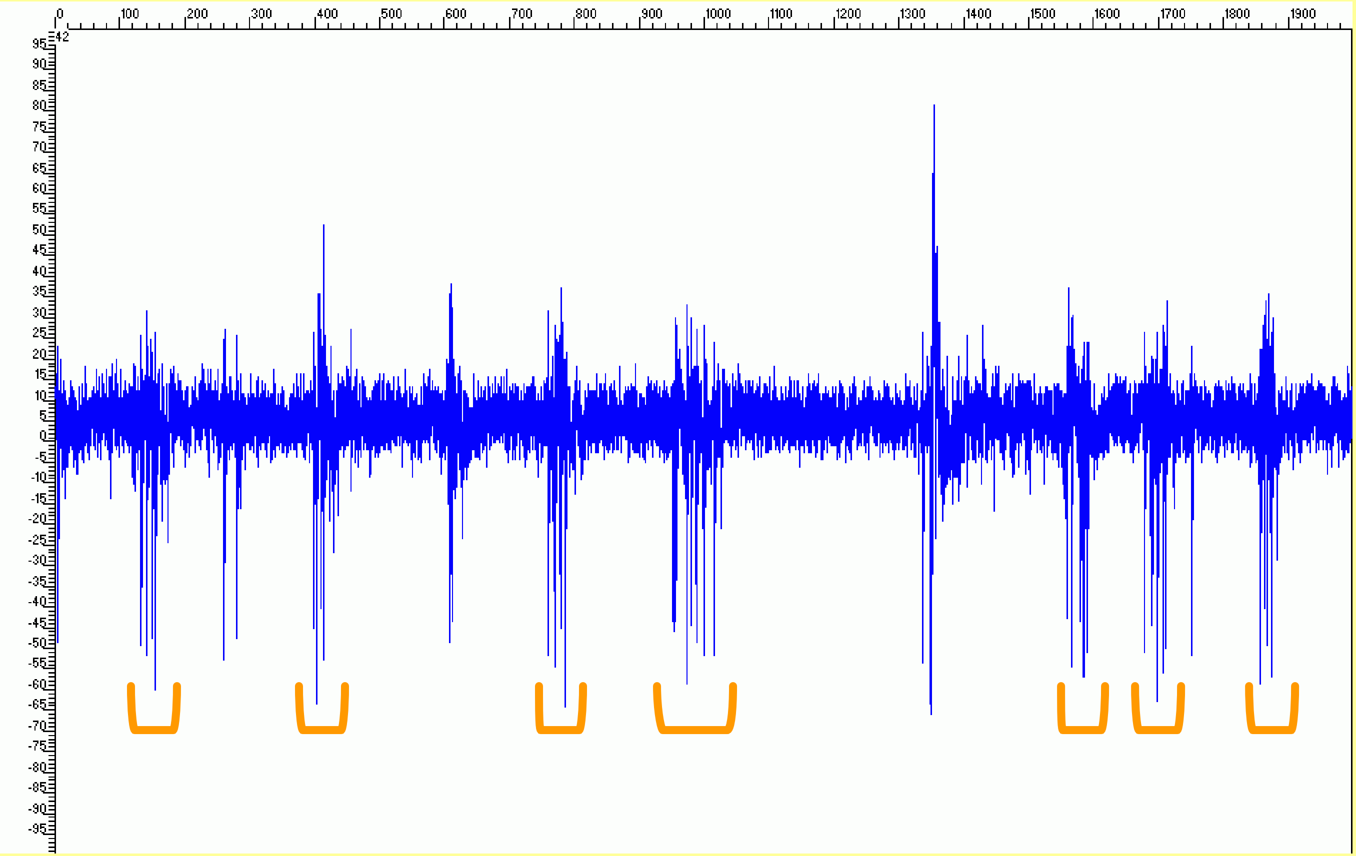


S(n=3, Dt • MFR)

S(N, Dt • MFR=1)



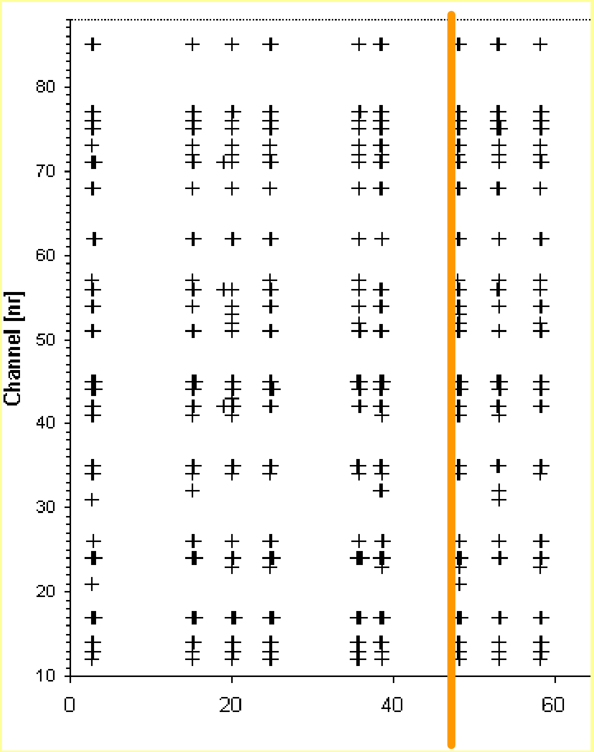
Surprise - Example

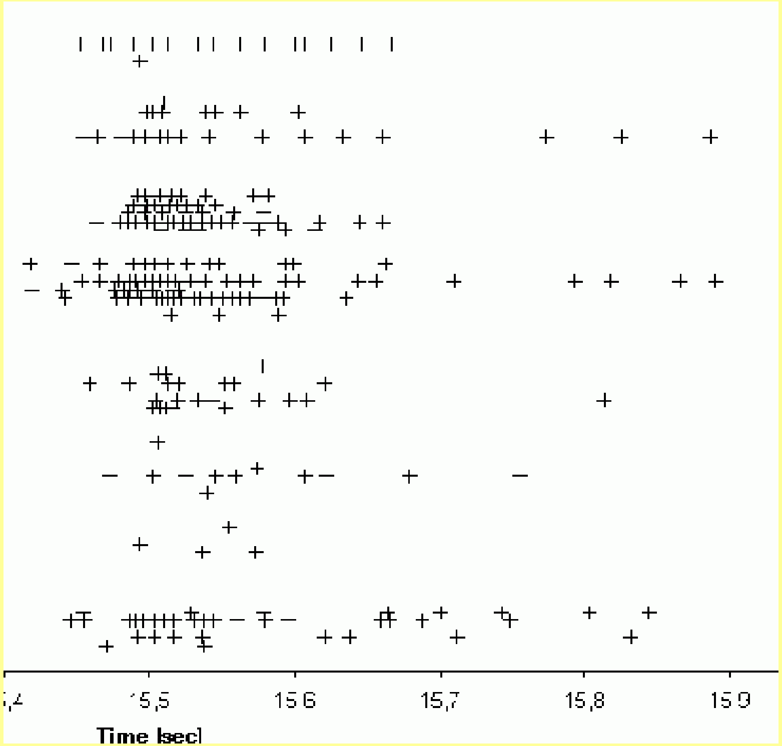


... does what the human observer supposes it to do!

The Synchrony Problem

Blow-Up: 0.5 sec





Obvious synchrony! – Quantification?

The Synchrony Problem

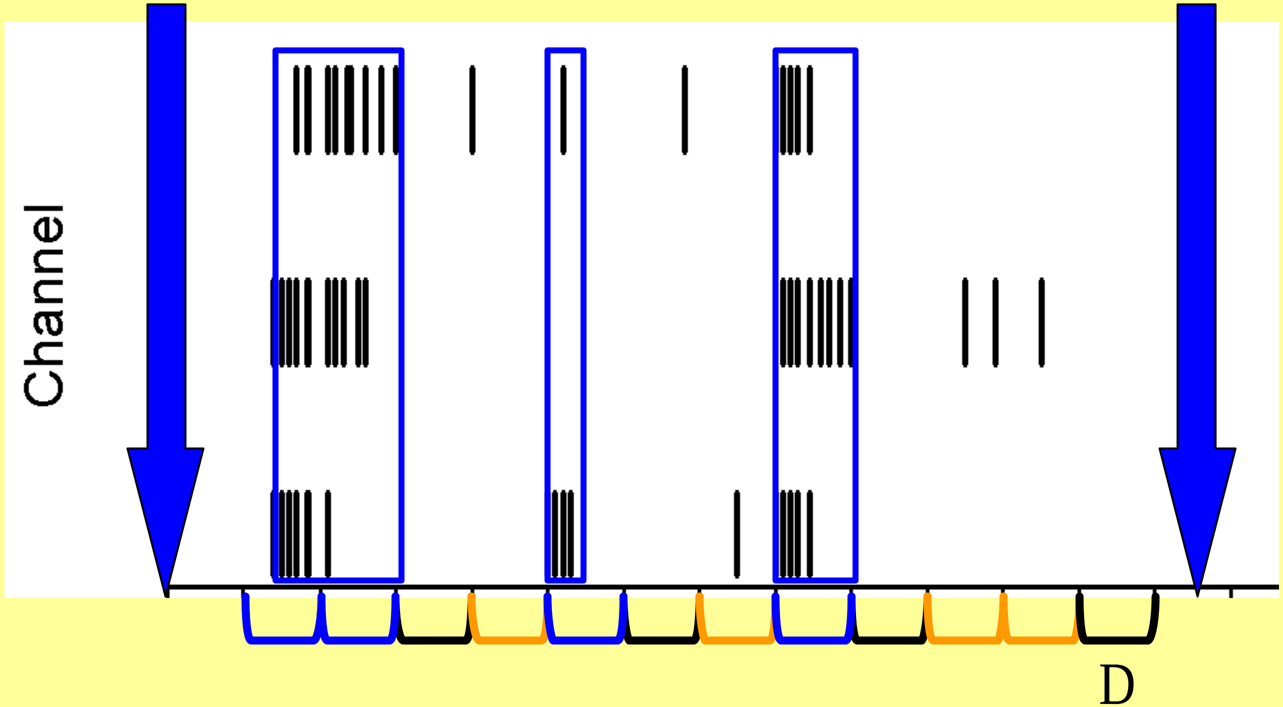
Synchrony of activity

- on which time scale?
  - $O(ISI_{in\ burst})$ 10 ms
  - $O(D_{burst})$ 100 ms
  - O(ß • IBI) 100 – 500 ms
  - O(IBI) 1 – 5 – 20 sec
- across an entire MEA?
- between 2 channels?
- bin discretization necessary
- time scale separation D << IBI

Synchrony parameter ?

Synchrony: Fraction Of Empty Bins

Project spikes down onto virtual channel



B₊ = 4 bins w/ bursts + 4 bins w/ single spikes

... Fraction of Empty Bins

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Calculate ratio **$B_- / (B_+ + B_-)$** „per burst“

IBI-adapted bin-width : D **= ß • IBI** ß fixed

$$Syn = \frac{\textit{spike-free time}}{\textit{length of recording}}$$

$$\frac{B_-}{B} = 1 - \beta \cdot \left[(1.5 + D \div \Delta) - \frac{N_{ssp}}{N_b} \right]$$

D > D ; no single spikes ; ß = 0.1: Syn = 0.85

ß = 0.02 Syn = 0.97

Correlation: Dichotomy

Distinguish only empty and full bins in T:

Dichotomy $N_{spikes}(D_i) = 0$ **or 1**

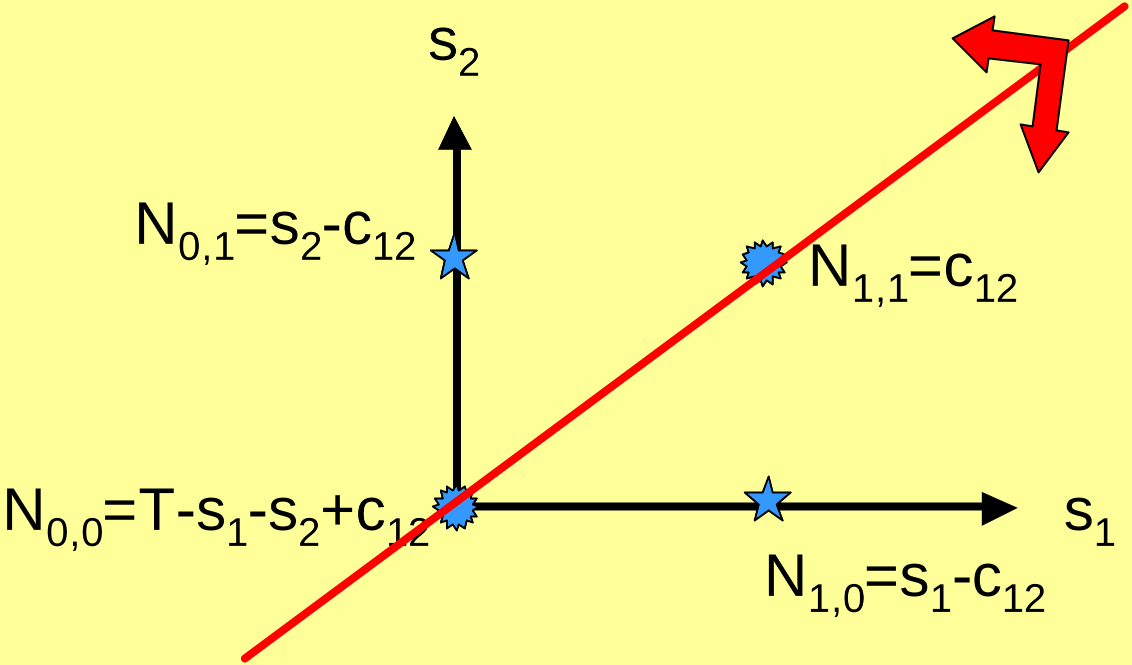
Analysis window represented by binary sequence

bin #	123456...............i.............T
s_1 =13	001101000010010111010000011101000
s_2 =11	001100001010010111000000001101000
c_{12}=10	001100000010010111000000001101000

Þ number s_1, s_2 of bins Δ_i with $N_{spikes}(\Delta_i) > 0$
coincidence count c_{12} of bins Δ_i with spikes on both channels

Regression

4 types of bins: (0,1) (1,1)
(0,0) (1,0)



Minimize mean square deviation of data from regression line.

Pearson‘s correlation coefficient **r** measures goodness of fit.

$$r := \frac{\sum_{i=1}^{n}(x_i-\bar{x})(y_i-\bar{y})}{\sqrt{\sum_{i=1}^{n}(x_i-\bar{x})^2 \sum_{i=1}^{n}(y_i-\bar{y})^2}} = \frac{S_{xy}}{\sqrt{S_{xx}S_{yy}}}$$

Correlation: Pearson‘s R

$$r = \frac{\sum_{k=1}^{T}(x_k - \bar{x})(y_k - \bar{y})}{\sqrt{\left[\sum_{k=1}^{T}(x_k - \bar{x})^2\right]\left[\sum_{l=1}^{T}(y_l - \bar{y})^2\right]}}$$

$$= \frac{c_{12} - s_1 \cdot s_2/T}{\sqrt{s_1\,(1 - s_1/T) \cdot s_2\,(1 - s_2/T)}}$$

Dichotomy only ...

$$r_\infty = \lim_{T\to\infty} r = \frac{c_{12}}{\sqrt{s_1 \cdot s_2}}$$

coincident spike fraction

Fourfold Table:
Chi-Square Statistics
Cramer‘s Phi

$$\chi^2 = \frac{(N_{0,0} \cdot N_{1,1} - N_{0,1} \cdot N_{1,0})^2}{N_{0,.} \cdot N_{1,.} \cdot N_{.,0} \cdot N_{.,1}}$$

$$\Phi = \sqrt{\frac{\chi^2}{N_{.,.}}}$$ **equal to Pearson‘s r**

$$= \frac{c_{12} - s_1 \cdot s_2 / T}{\sqrt{s_1 (1 - s_1/T) \cdot s_2 (1 - s_2/T)}}$$

$F_{max} = r_{max} < 1$ except for $s_1 = s_2$!

Fourfold Table: Cohen's Kappa

$$p_0 = p_e + \kappa \cdot (1 - p_e)$$

Coincidence proportion p_0 =

chance expected coincidence p_e +

excess coincidence $k\,(1-p_e)$

Cohen's Kappa

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proportion in excess of chance

$$p_0 = p_e + \kappa \cdot (1 - p_e)$$

$$p_0 = \frac{N_{0,0} + N_{1,1}}{T_W} \qquad p_e = \frac{N_{.,1}N_{1,.} + N_{.,2}N_{2,.}}{T}$$

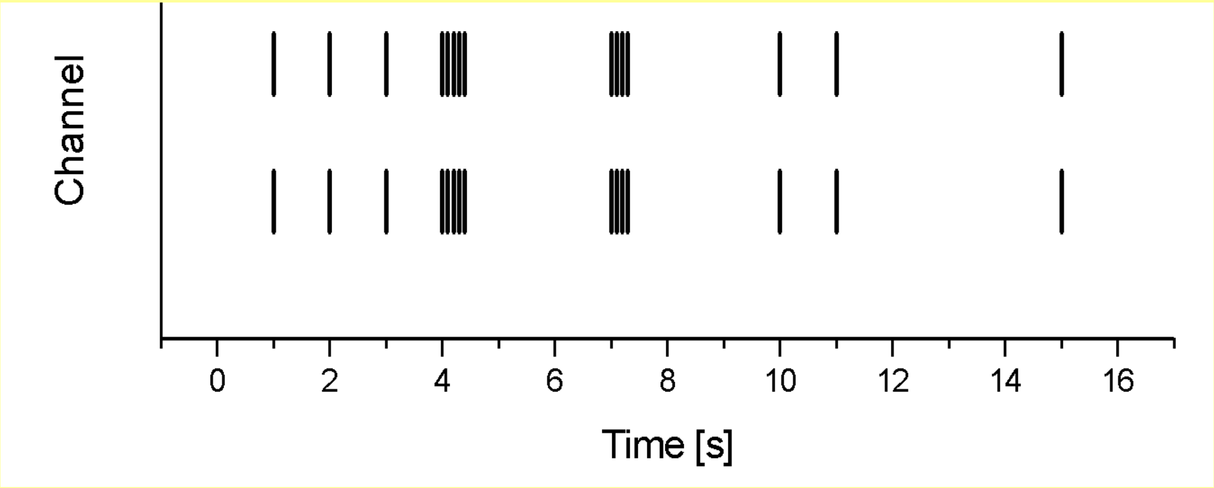
$$\kappa = \frac{p_0 - p_e}{1 - p_e} = 2 \cdot \frac{T \cdot c_{12} - s_1 \cdot s_2}{T \cdot (s_1 + s_2) - 2 \cdot s_1 \cdot s_2}$$

$$\kappa_\infty = \lim_{T \to \infty} \kappa = \frac{2 \cdot N_{1,1}}{2 \cdot N_{1,1} + N_{1,0} + N_{0,1}}$$

$$= \boxed{\frac{2 \cdot c_{12}}{s_1 + s_2}}.$$

coincident spike fraction

Complete Synchrony

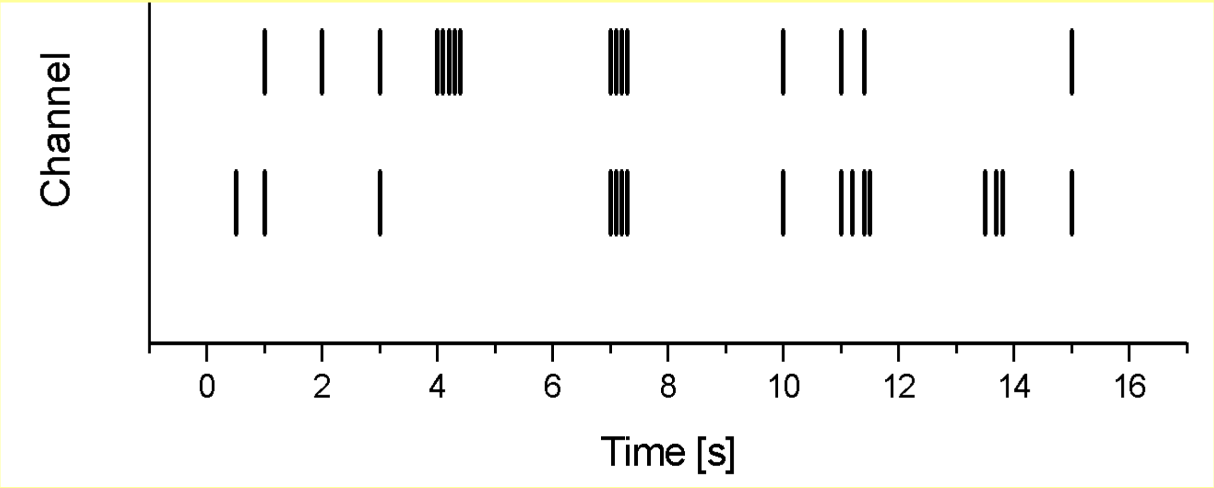


$$s_1 = s_2 = s$$

$$c_{12} = s$$

$$\mathbf{r = k = 1}$$

Partial Synchrony
Equal Firing Rates



$$s_1 = s_2 = s$$

$$c_{12} = \alpha\, s$$

$$\mathbf{r} = k = a$$

Partial Synchrony
Different Firing Rates

RESULT
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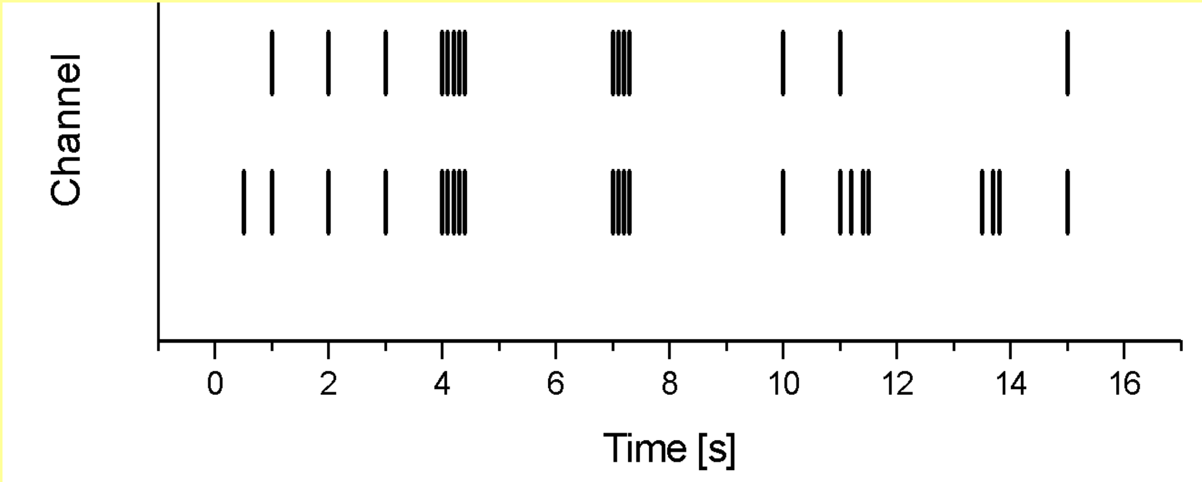


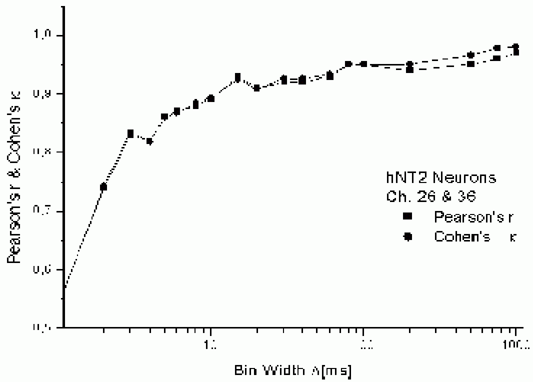
$s_1 = \gamma\, s$ $\qquad$ $s_2 = s \quad (\gamma > 1)$

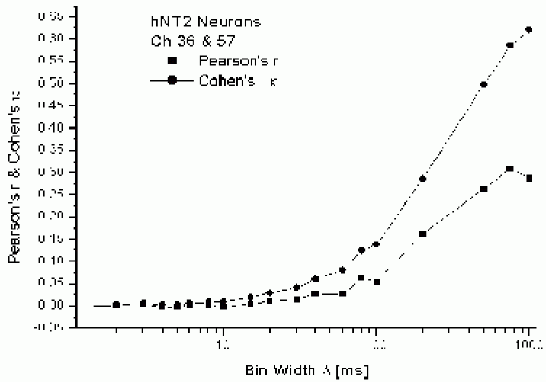
$c_{12} = s$

$r = g^{-1/2}$ $\qquad$ **$k = 2 / (1 + g)$**

hNT2 Neurons: r and κ

hNT2 Neurons: Pearson's r & Cohen's Kappa

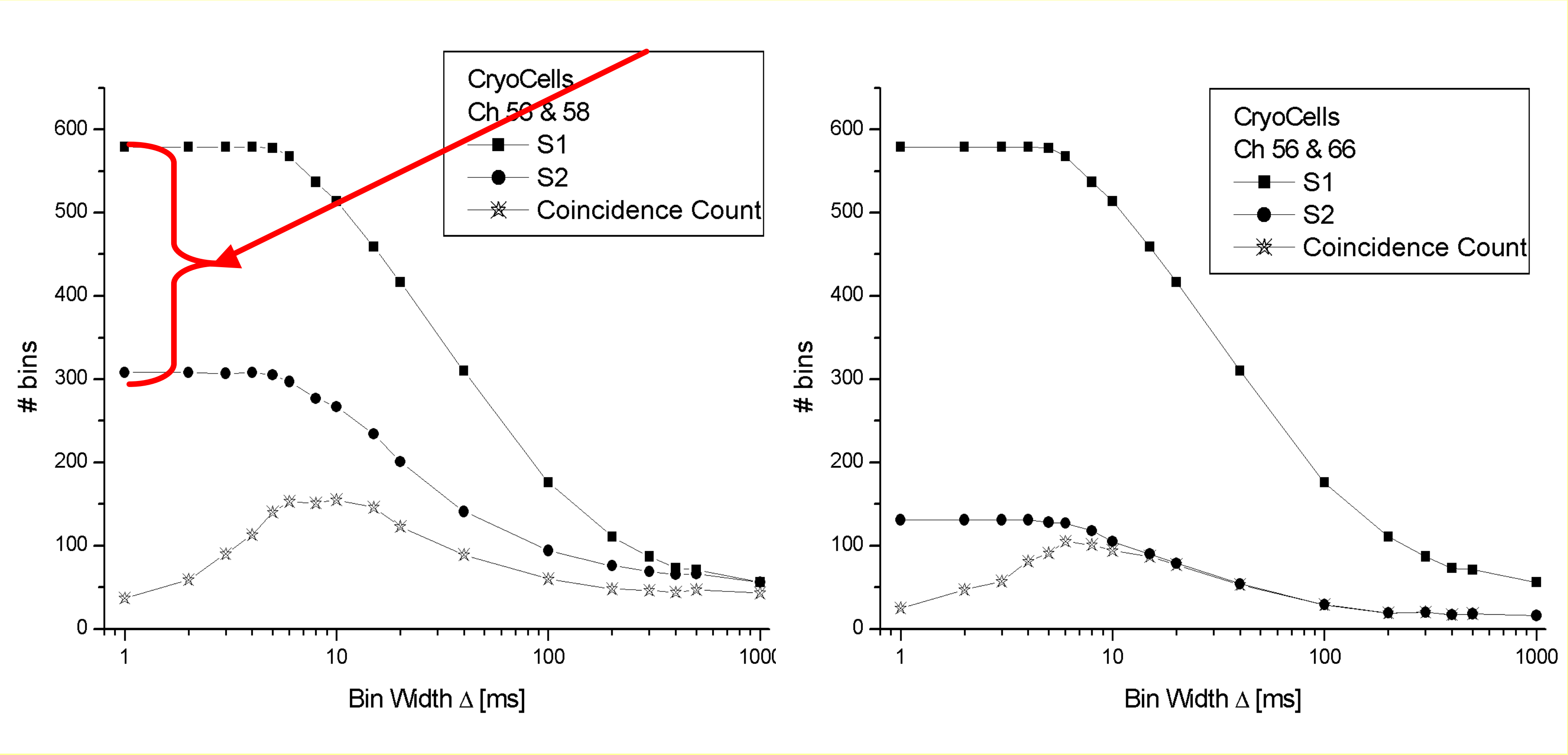




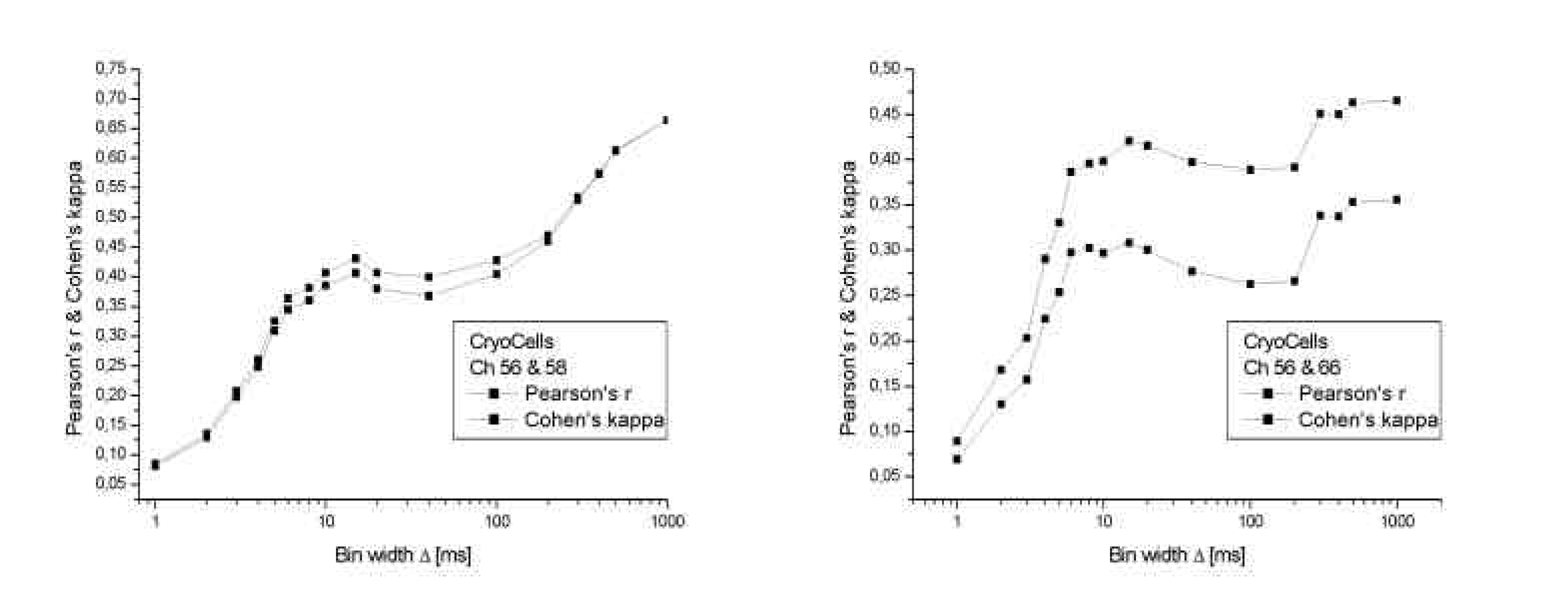
Dependency of correlation parameters Pearson's r and Cohen's κ on bin-width Δ [ms] for hNT2-neurons(chip #4733).

Left panel: 2 strongly correlated, spatially neighboring channels (26 and 36). Right panel: 2 weakly correlated channels (36 and 57).

CryoCells: S_1, S_2, C_{12}



CryoCells: r and κ



ch	IBI [s]	# sp / burst	# spikes	ISI [ms]	Burst duration [ms]
56	5,8	17 ± 11	579	22,4	361 ± 185
58	6,6	10 ± 5	308	13,4	118 ± 59
66	11	8 ± 2	131	13,6	97 ± 25

Correlation Caveats I

For fixed ratio of coincident spikes c_{12}/s
r-values between c_{12}/s and $c_{12}/s - 1$ are possible.
Correlation r falls with increasing s/N .

$$r = \frac{c_{12} - s_1 \cdot s_2/T}{\sqrt{s_1\,(1 - s_1/T) \cdot s_2\,(1 - s_2/T)}}$$

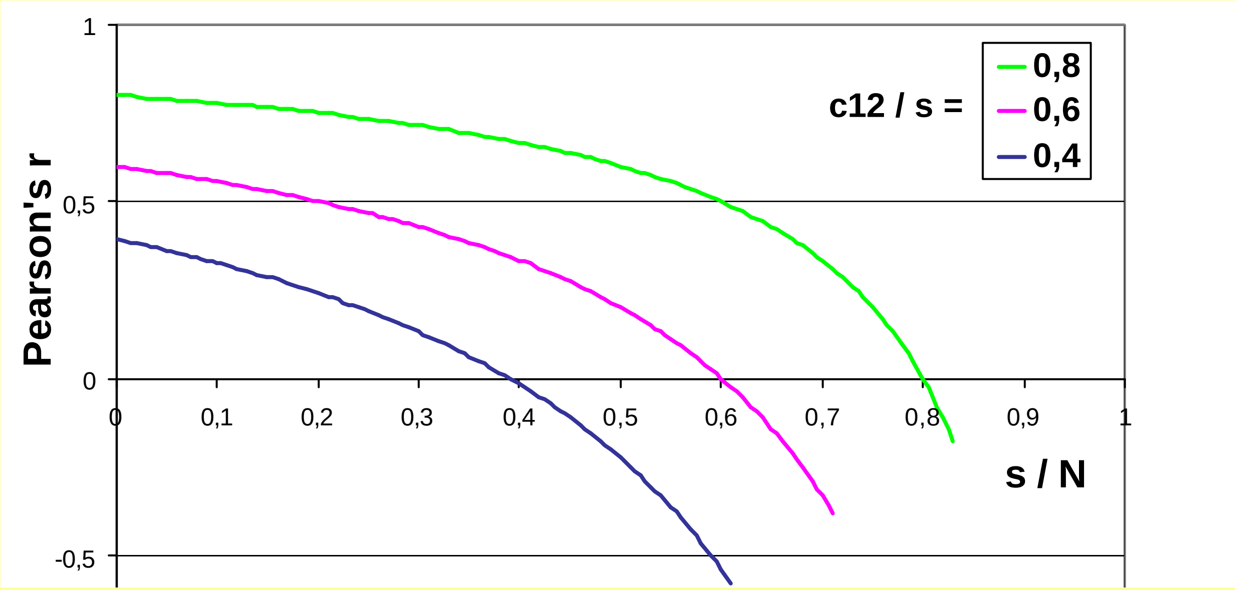
$$= \frac{r_\infty - \sqrt{s_1 s_2}\,/\,T}{\sqrt{(1 - s_1/T) \cdot (1 - s_2/T)}}$$

$$= \boxed{\frac{r_\infty - s/T}{1 - s/T}} \quad \text{for equal firing rates}$$

Correlation Caveats I

For fixed ratio of coincident spikes c_{12}/s
r-values between c_{12}/s and $c_{12}/s - 1$ are possible.
Correlation r falls with increasing s/N .

Counter-intuitive?



Correlation Caveats II

Average correlations of N (N+1) / 2 channel pairs:

Simple mean value of different r-values?

Pooling of s_1, s_2 and c_{12} data?

Fisher's z transform prior to averaging?

Average correlations:

$$\bar{r} = \frac{2}{N(N+1)} \sum_{i<j}^{N(N+1)/2} r_{ij}$$

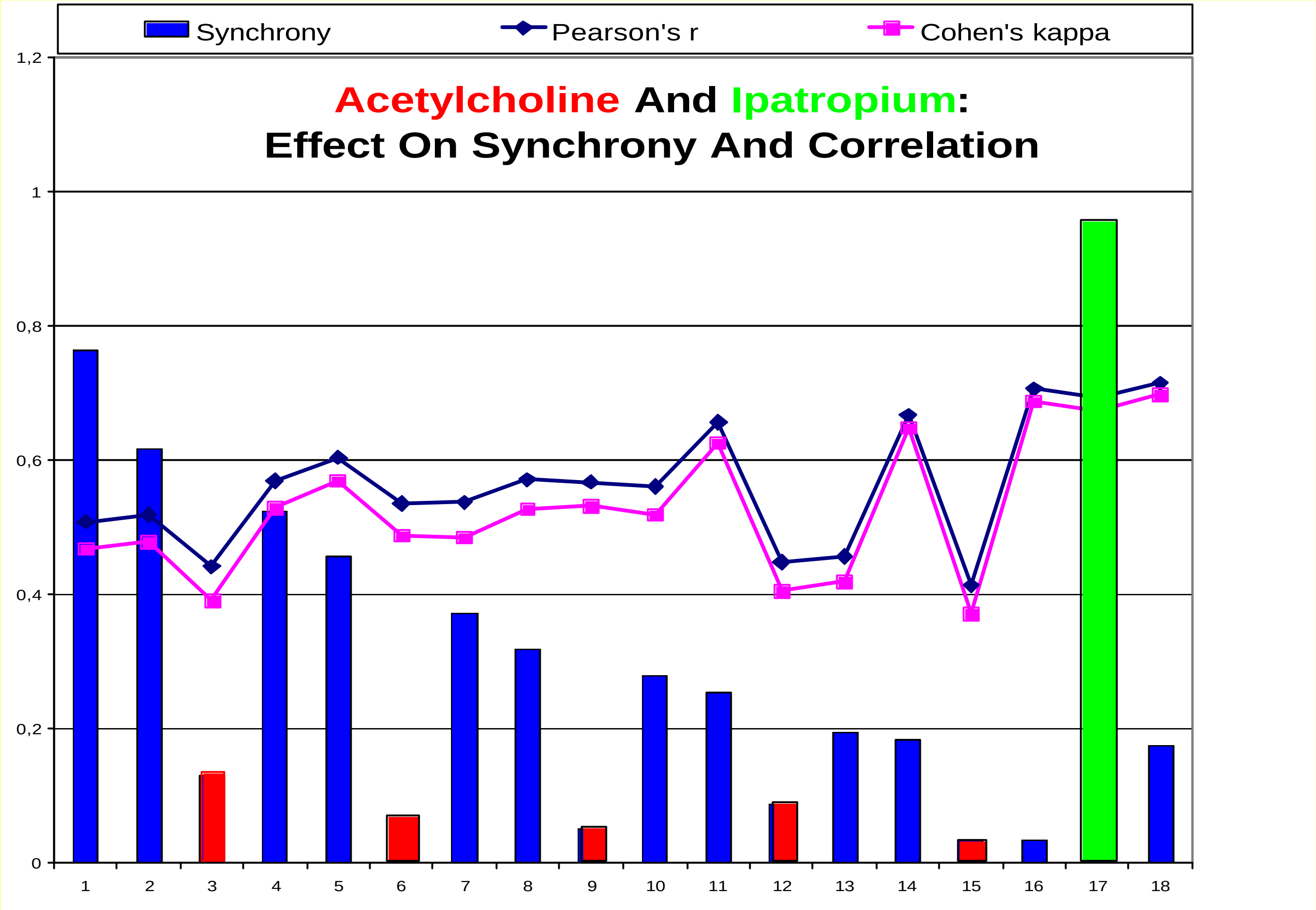
Fisher's z transform

$$z = \sqrt{N_{bins} - 3} \cdot \ln\left(\frac{1+r}{1-r}\right)/2$$

Synchrony vs. Correlation

RESULT MEDIZINISCHE ANALYSEVERFAHREN GMBH

Cryopreserved cortex neurons: 2 x reference & 1 x Ach, alternating



(How) Can Signal Analysis Contribute To Quality Assurance?

- Parameters for quantification of drug effects on spatio-temporal structure of neuronal firing are *in principle* available.
- (Semi-) automatic analysis can be implemented.
- Need for positive and negative control: multi-well MEAs !

- **Real-life tests of pharmacological substances?**

The Team

- RESULT GmbH:
  - Dipl.-Phys. Martin Konieczny
  - Dipl.-Phys. Christian Mayer
  - Cand. Inf. Simon Ofner

- Department of Neurology, University of Düsseldorf
  - Prof. Dr. Mario Siebler
  - Dr. Philipp Görtz
  - Dr. Frauke Otto
  - Dipl.-Biol. Wiebke Fleischer
  - Max Bernardi
  - Brigida Ziegler