Neuro-IT workshop – Alicante 8 July 2003

The NEUROBIT project

A bioartificial brain with an artificial body: training a cultured neural tissue to support the purposive behavior of an artificial body

IST - 2001- 33564 - 1 May 2002 – 30 April 2005

Modulating Neural Networks Dynamics: Electrical Stimulation of *In-Vitro* Cortical Neurons Coupled to MEA Devices and bi-directionally connected to a mobile robot

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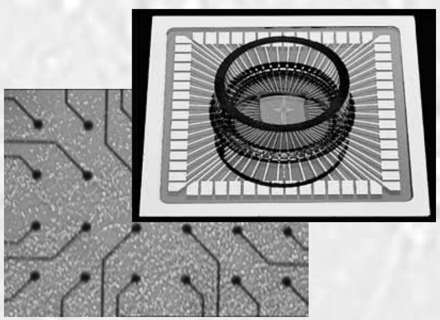
The NEUROBIT project

- The brain is perhaps the most advanced and robust computational system known.
- We are developing a method to study **how information is processed and encoded in living cultured neuronal networks by interfacing them to an artificial body**.

Bioartificial living system

Real environment



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Adaptive Neural Controller

Autonomous Robot: the artificial body

V. Sanguineti, F.A. Mussa-Ivaldi et al. - *Connecting Brains to Robots: An Artificial Body for studying the Computational Properties of Neural Tissues*. Artificial Life, 6(4): 307-324, 2000.

S. Potter - *The neurally controlled Animat: Biological Brains acting with simulated bodies,* Autonomous Robots, *11, 2001*

The rationale of the project

Activity-dependent modification of synaptic efficacy is widely recognized as a cellular basis of learning, memory and developmental plasticity
(Meister et al., Science, 252:939-943, 1991; Katz and Shatz, Science, 274: 1133-1138, 1996)

Stimulation leads to the activation/modulation of a **neuronal ensemble**

The way neurons process information is **distributed** and **redundant**

Main objectives

- To interface in-vitro neurons stably to microelectronic transducers, that allow to monitor and modulate the neuron electrophysiological activity
- To study **learning** and **plasticity** in in-vitro models

Bioartificial neuronal networks → **Bioartificial living systems**

Neurobit

Our goal:

- *to stable interface in-vitro neurons to microelectronic transducers capable to monitor and modify the neuron electrophysiological activity*
- *to study **learning** and **plasticity** in in-vitro-models*
- ***Bioartificial** neuronal networks* → ***bioartificial living systems***

A step forward… **with many possible implications**

to understand and exploit brain plasticity in order to improve brain-computer interfaces, to inspire new computer architectures, and to advance basic neuroscience

Molecules

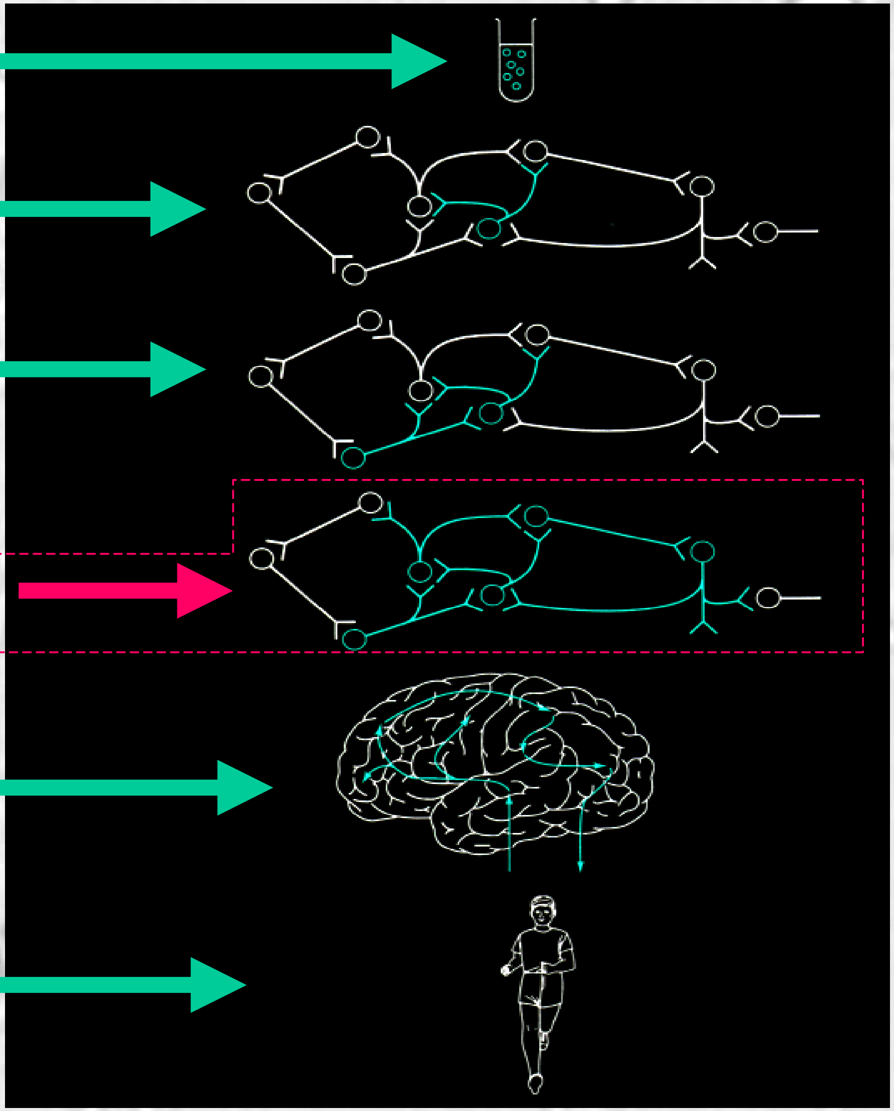
Single neuron

Microcircuit:
Couple of neurons synaptically connected

Neuronal network

Brain mapping and control system

Behaviour



Neurobit

The NEUROBIT project

A bioartificial brain with an artificial body: training a cultured neural tissue to support the purposive behavior of an artificial body (started May 1st, 2002)

Bioartificial living system

Real environment

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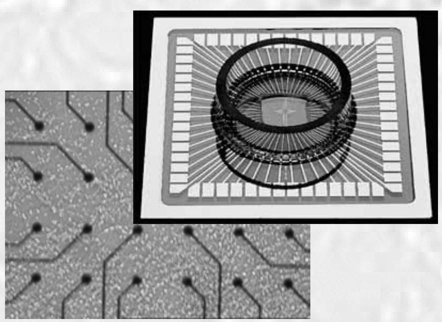

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Adaptive Neural Controller

Autonomous Robot: the artificial body

How to do that?

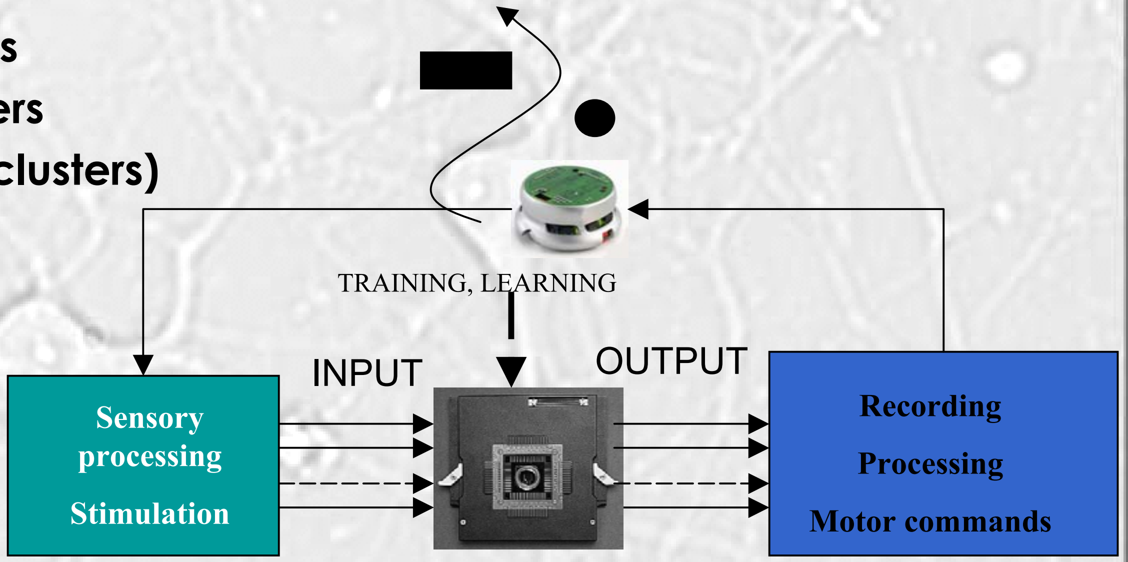
Methodological approach

- NN dynamics characterization
  - Electrical/chemical stimulation
  - Input-output channel selection
- Coding and de-coding strategies

1. *Characterization phase*
2. *Training and conditioning phase*
3. *Application phase (closed loop)*

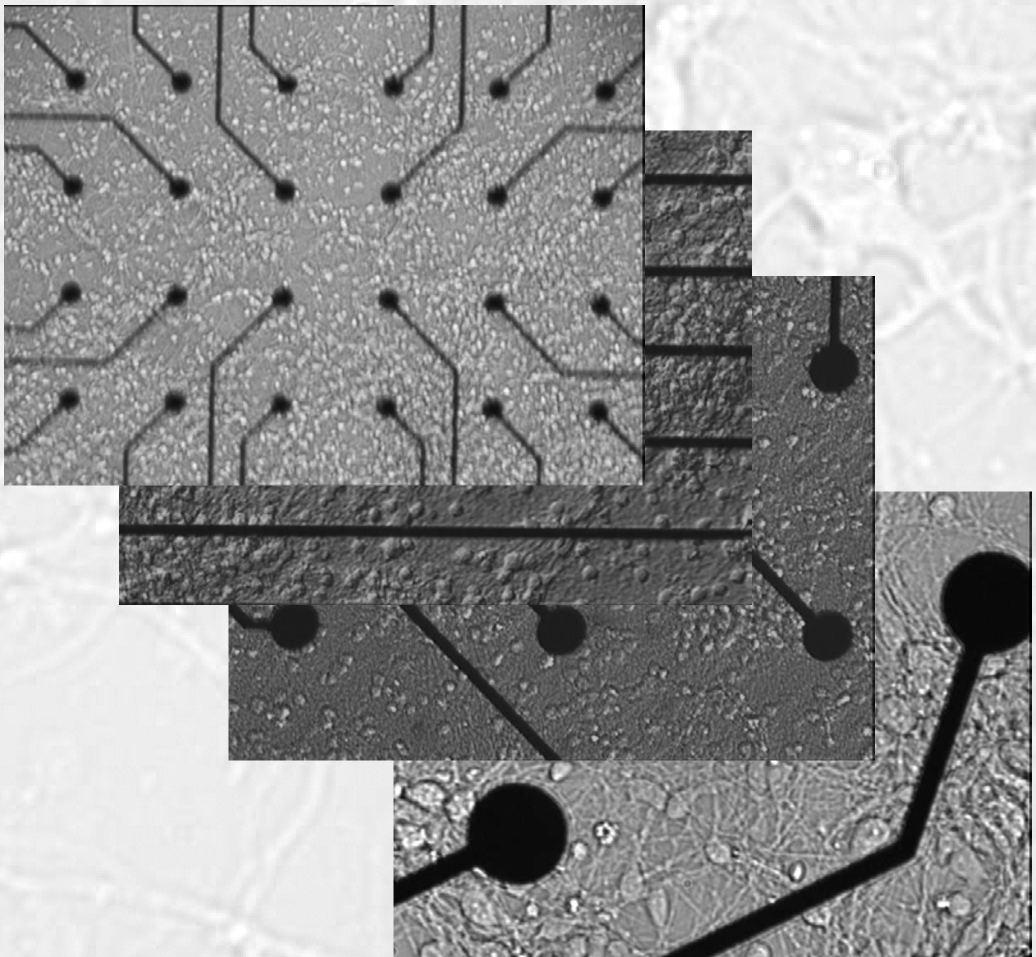
Techniques

- Reliable mini-incubating systems
- Newly designed microtransducers (i.e. Micromachined MEAs with clusters)
- "Real-time" closed loop system



In-vitro neuronal networks

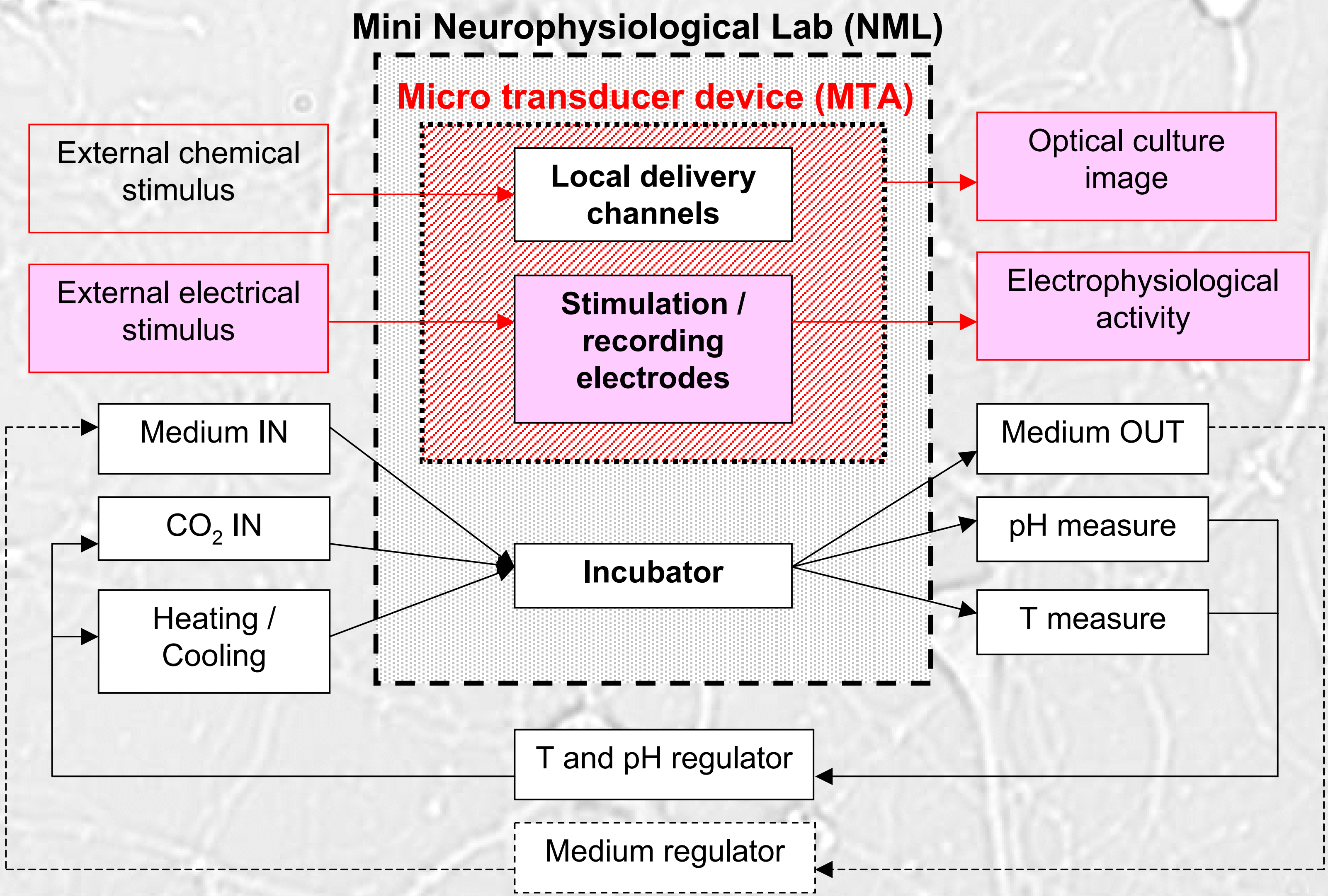
Cortical neurons form rat embryo (E17-18) cultured on MEA substrate (15-30 DIV)



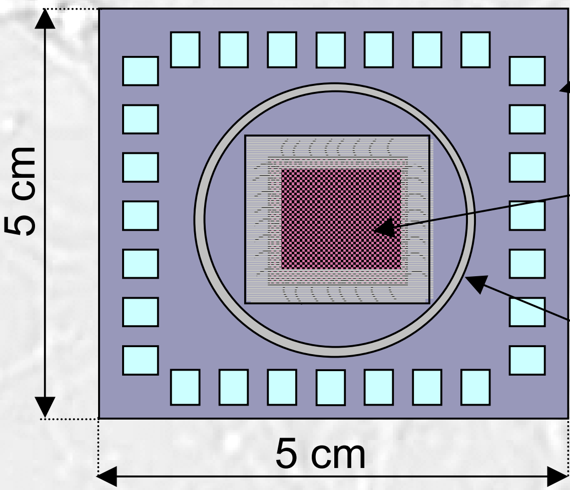
TiN electrodes on glass substrate (30 μm diam., 200 μm spaced)

MultichannelSystems – Reutlingen (Germany)

Techniques


Mini Neurophysiological Lab (NML)
Micro transducer device (MTA)
External chemical stimulus
Local delivery channels
Optical culture image
External electrical stimulus
Stimulation / recording electrodes
Electrophysiological activity
Medium IN
CO_2 IN
Heating / Cooling
Incubator
Medium OUT
pH measure
T measure
T and pH regulator
Medium regulator

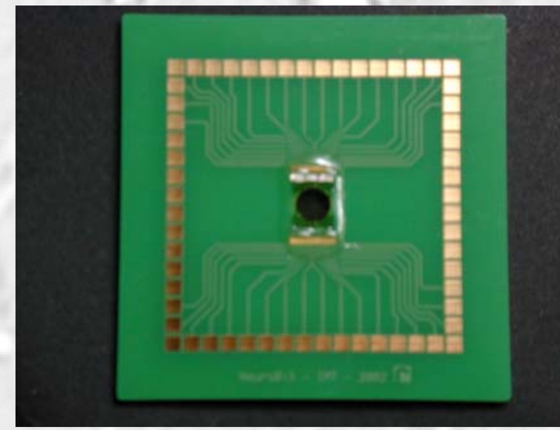
Techniques

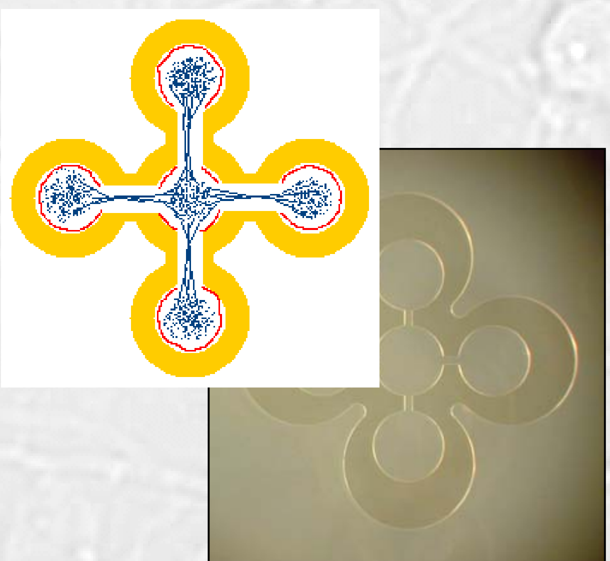


PCB compatible with the multichannel connector

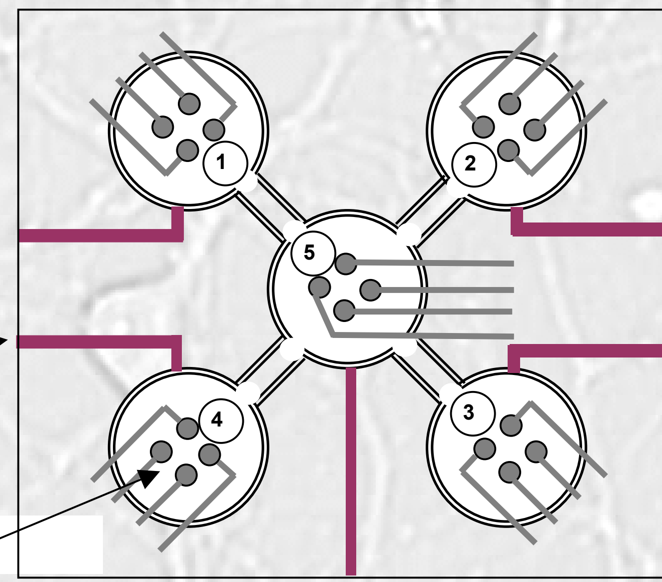
MTA (wire-bonded and glued)

Reservoir (Glass, PS, PMMA)





Delivery channel



Electrode array

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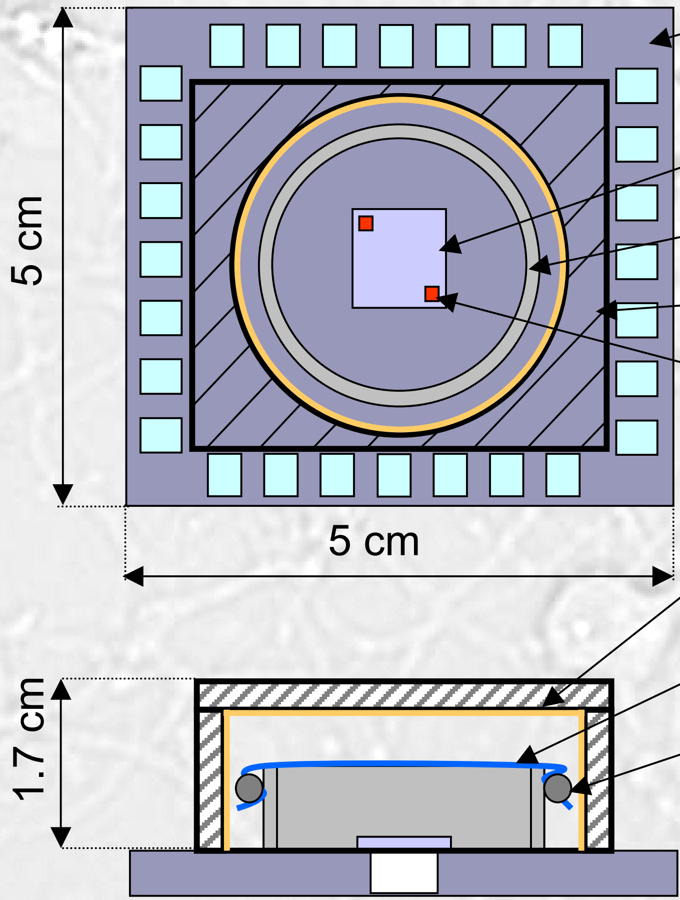
mini-incubator – design



PCB compatible with the Multichannel connector

MTA (wire-bonded and glued)

glass reservoir

chamber (PMMA)

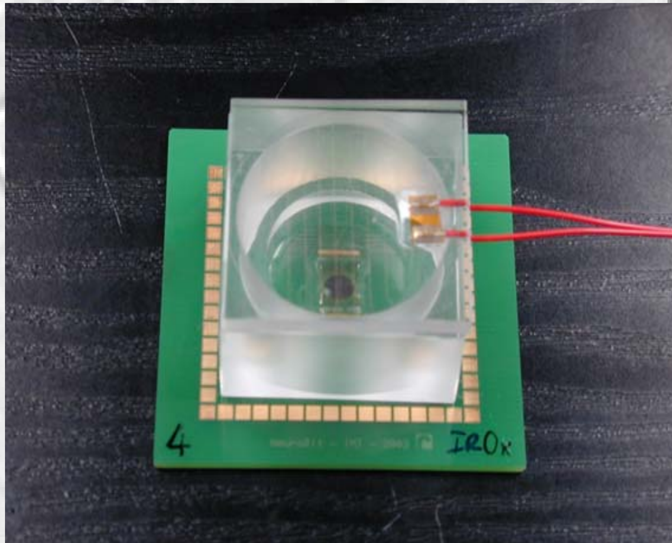
T sensors

heaters

semi-permeable membrane (teflon FEP)

O-ring

mini-incubator - prototype



NN Characterization

Electrical stimulation protocol

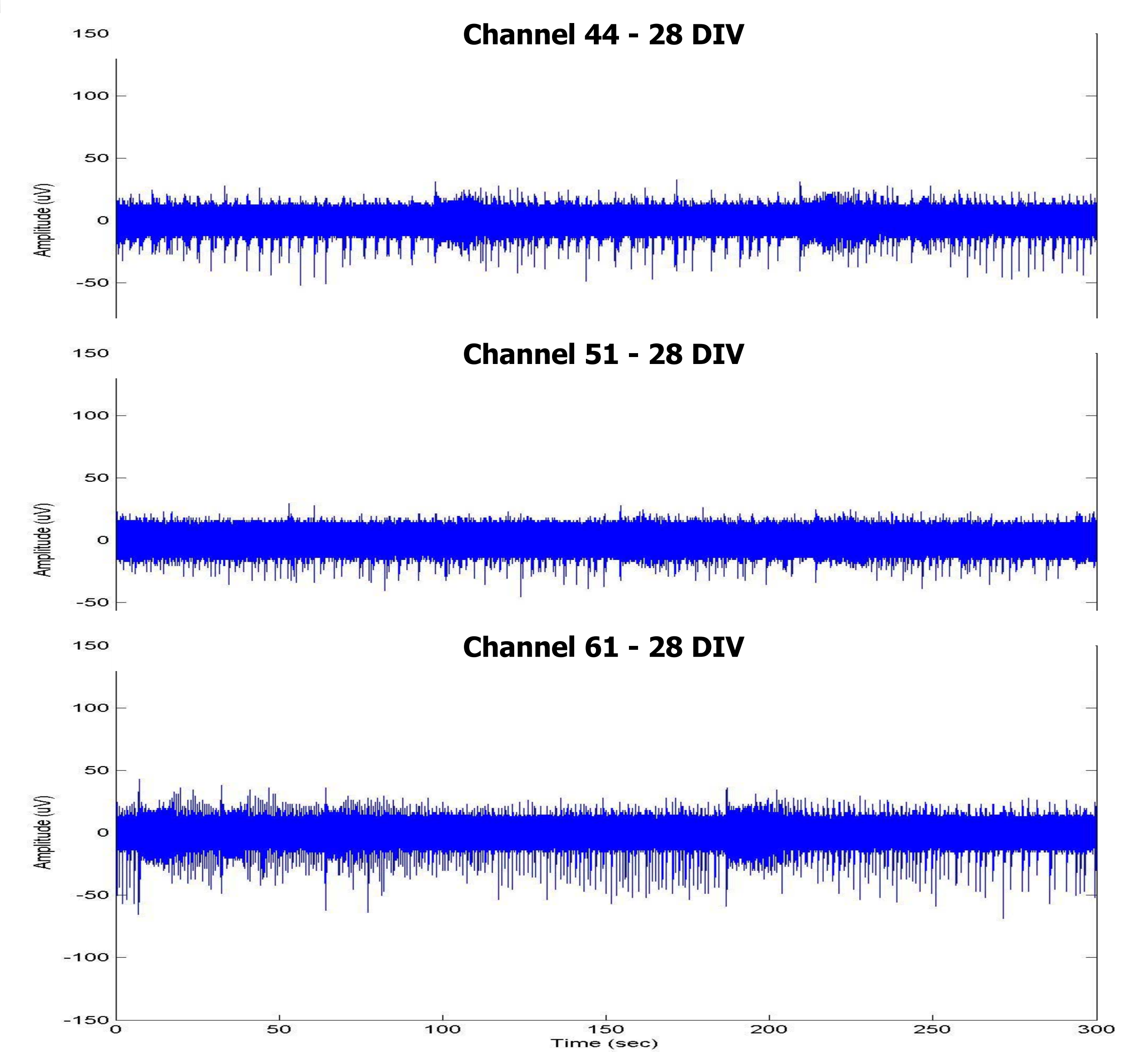
Stimulation parameters have been adapted from literature

Jimbo et al. *Simultaneous Induction of Pathway-Specific Potentiation and Depression in Networks of Cortical Neurons*, **Biophysical Journal 76, 1999.**

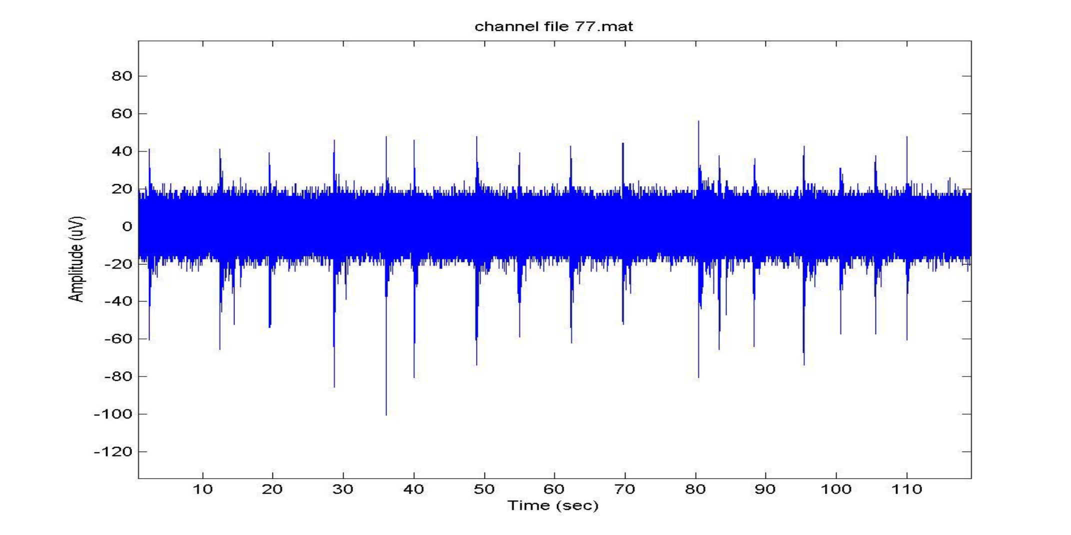
Shahaf and Marom, *Learning in Network of Cortical neurons*, **The Journal of Neuroscience 15, 2001**

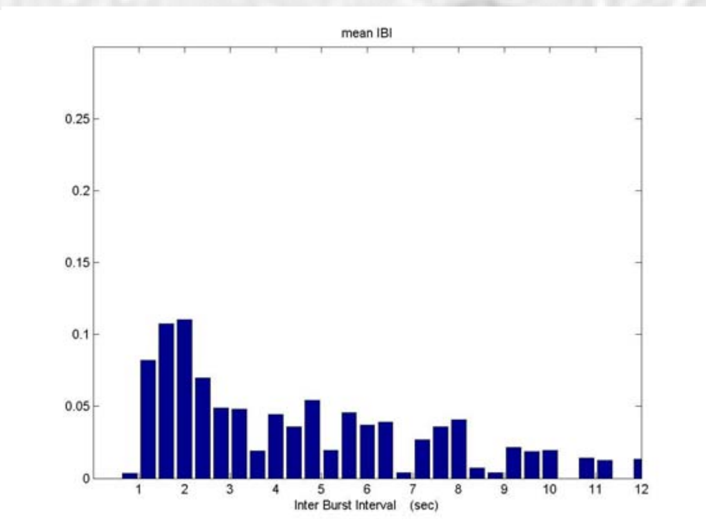
- Spontaneous activity (5 min. recording)
- Train of biphasic pulses, 0.2-0.4 Hz, ± 1-2 V (5 - 7 minutes)
- 10-30 stimulating sites (60 electrodes)
- Experiments performed at different DIV: 15-30

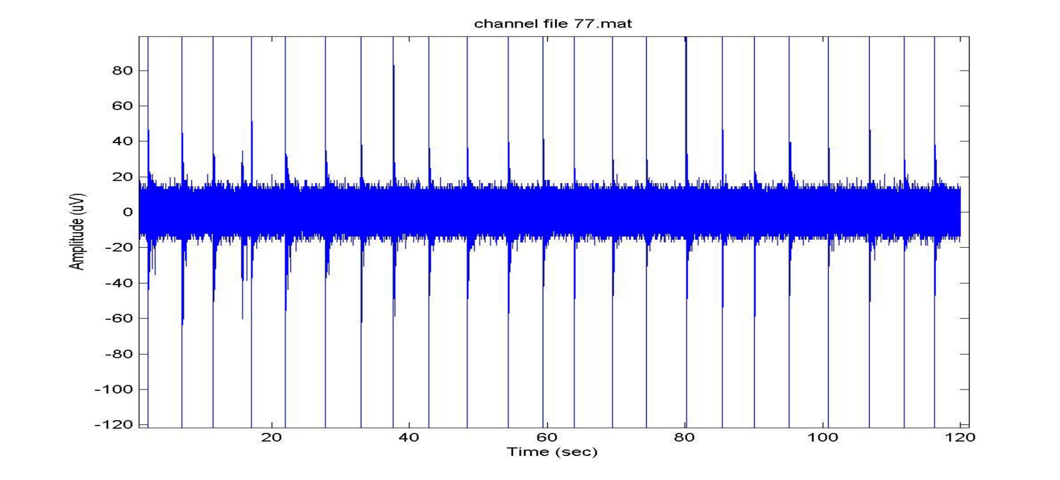
EXP – Spontaneous activity

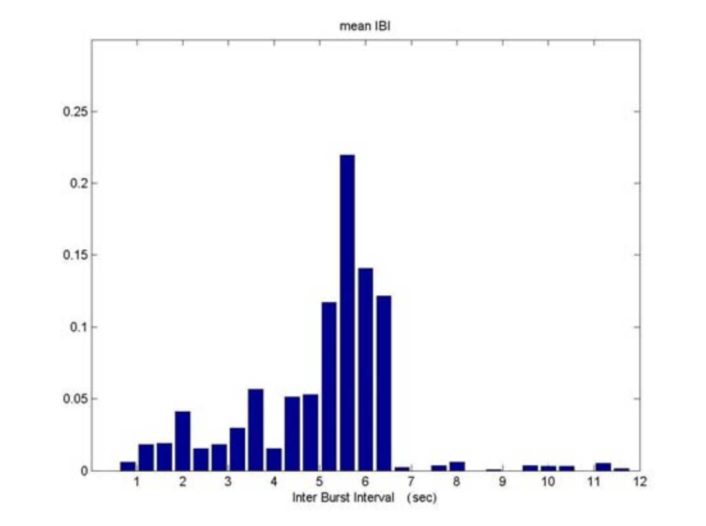


Preliminary results









Average IBI (Inter Burst Interval) in the spontaneous condition and during electrical stimulation: the bursting rate is locked around the stimulation frequency (0.2 Hz = 5 sec).

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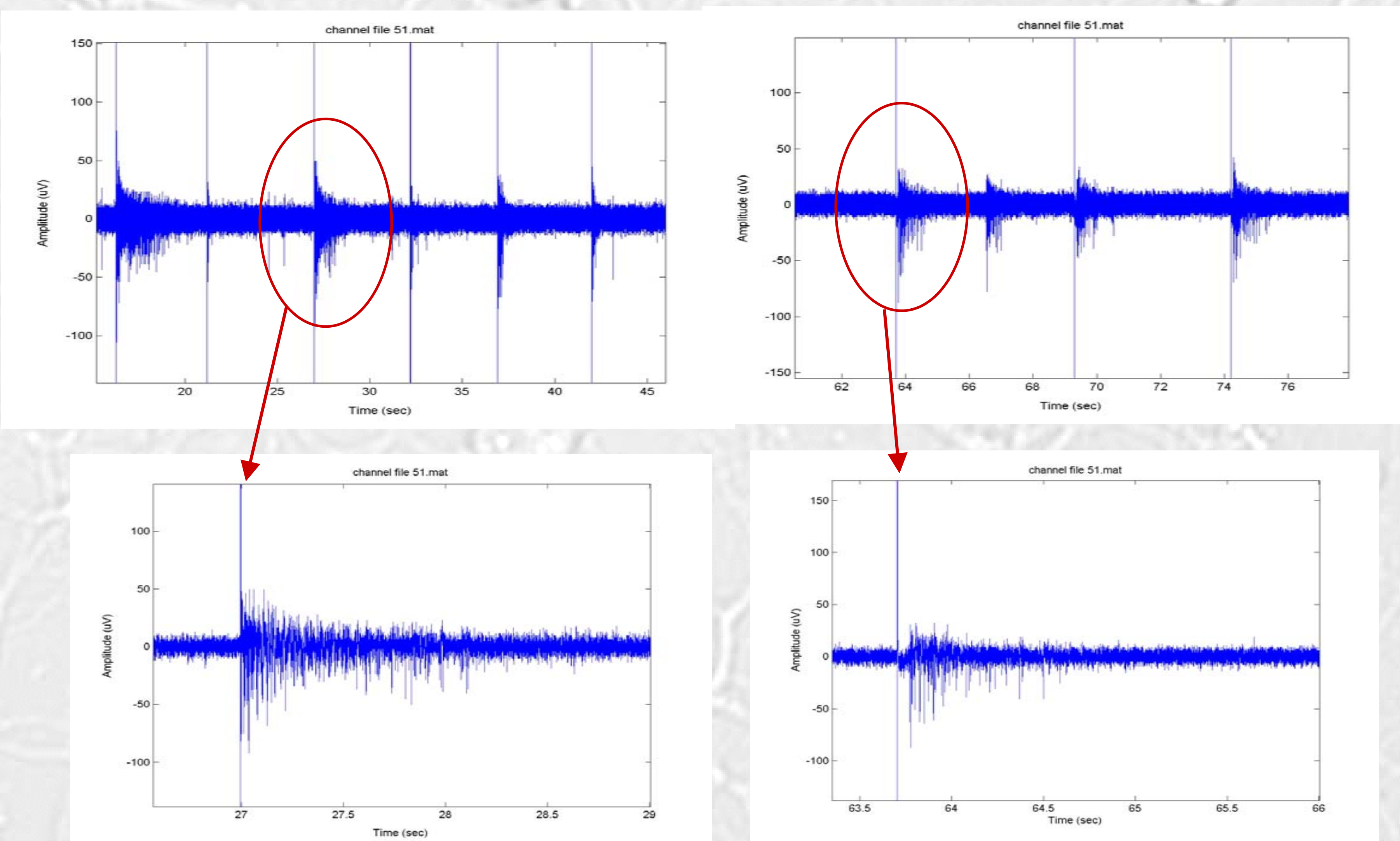
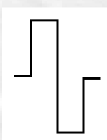
Results

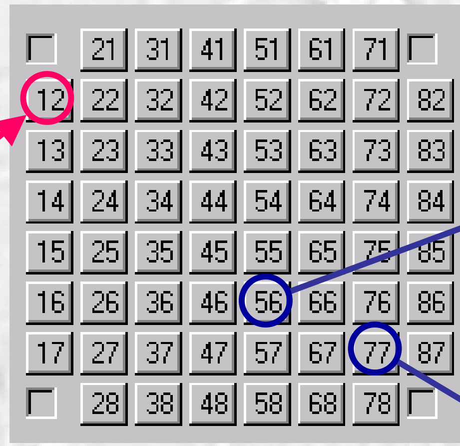
Two visually-identified responses to the stimulus: early and delayed burst

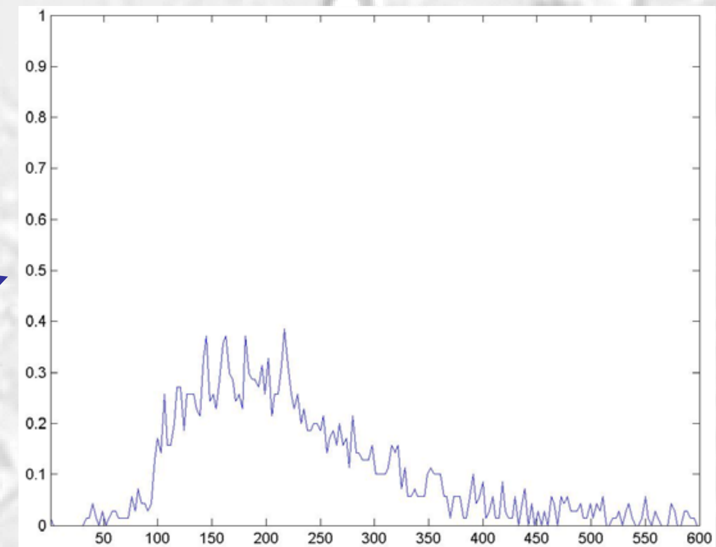


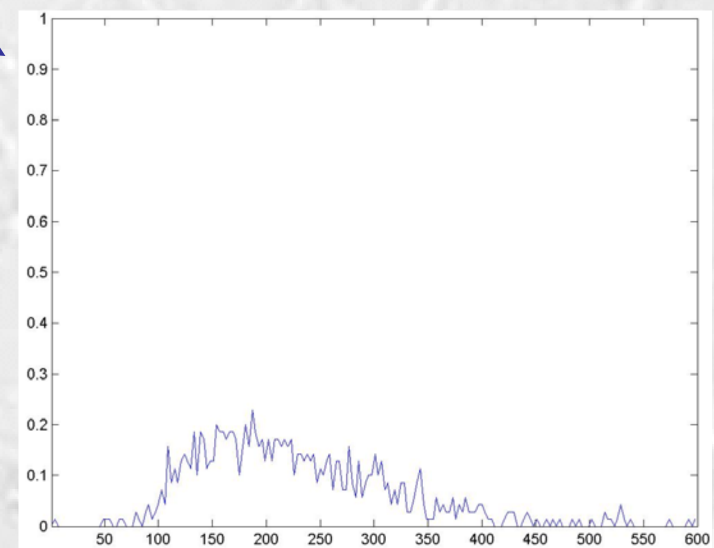
Results



0.2Hz, ±2V

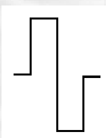




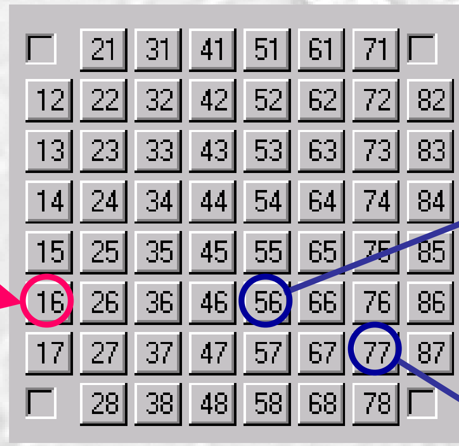


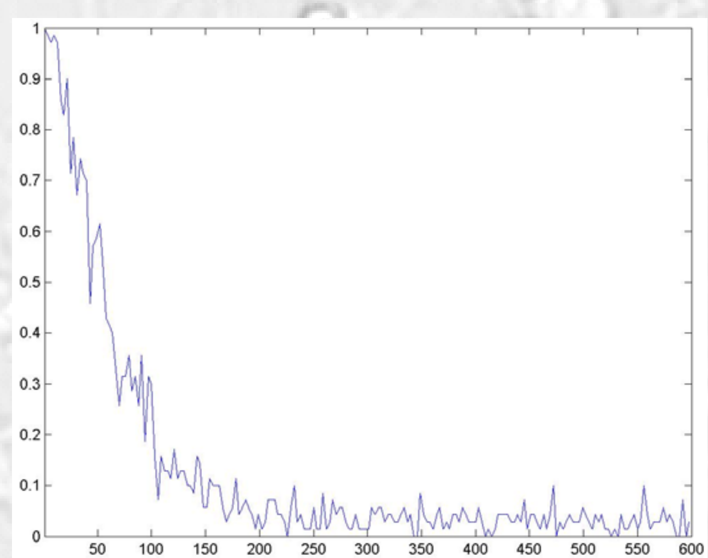
Post Stimulus histograms (PSTH) for 2 recording sites (56 and 77): only the “delayed” response is present

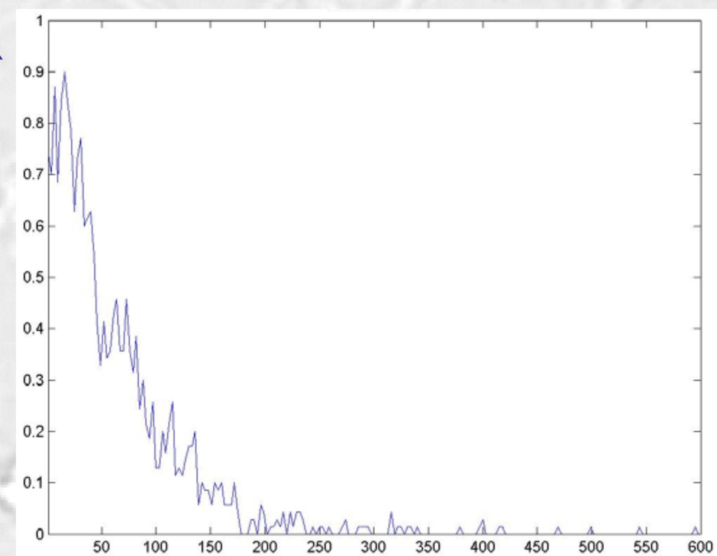
Results



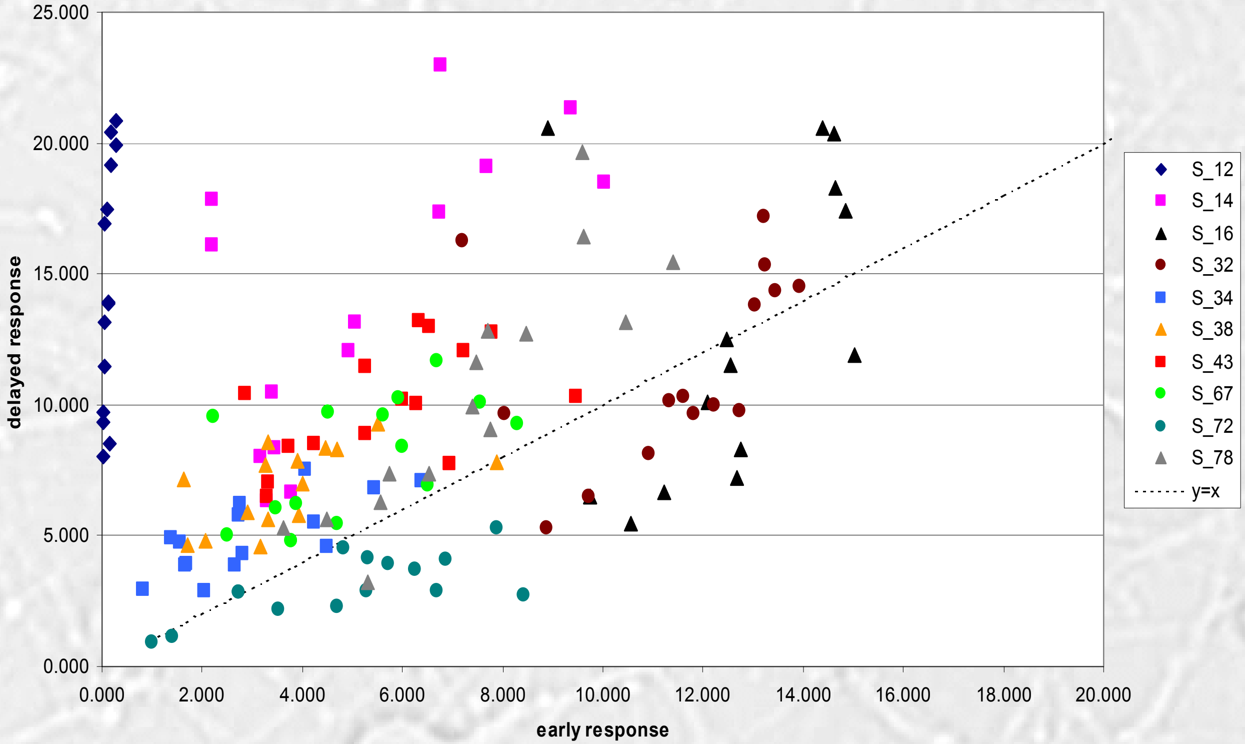
0.2Hz, ±2V

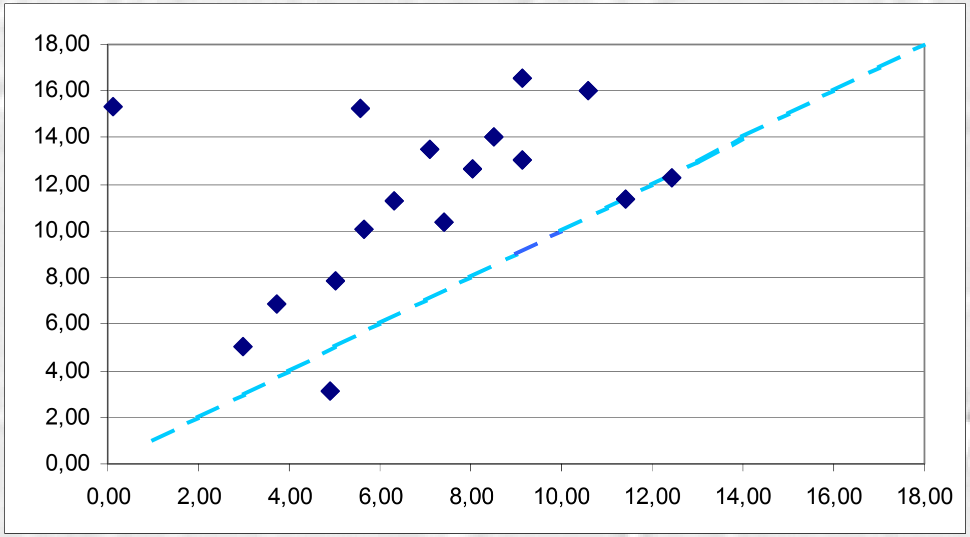






Post Stimulus histograms (PSTH) for the same 2 recording sites (56 and 77): only the "early" response is present



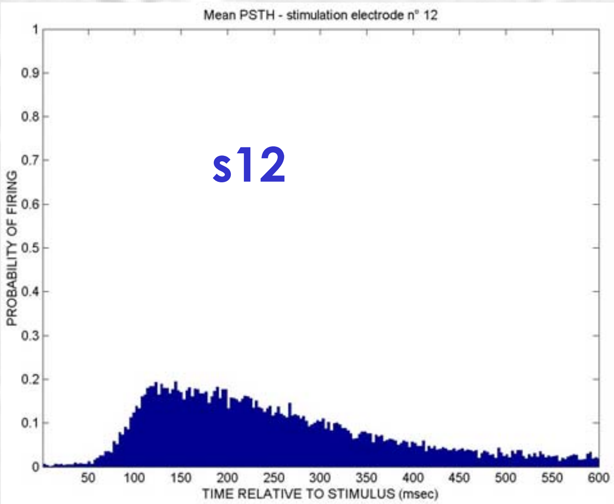


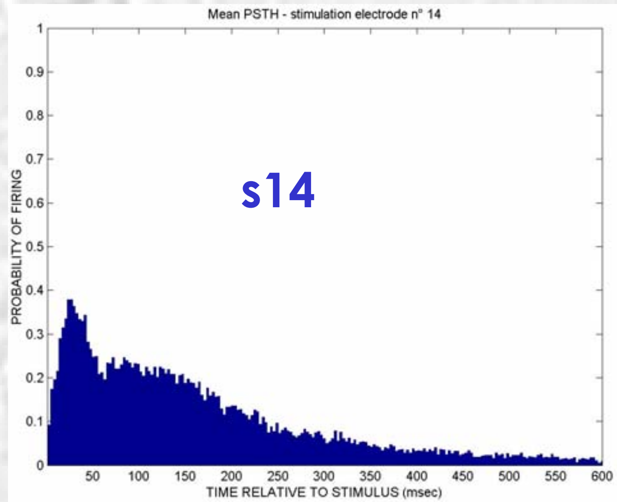
Delayed vs. early evoked spikes

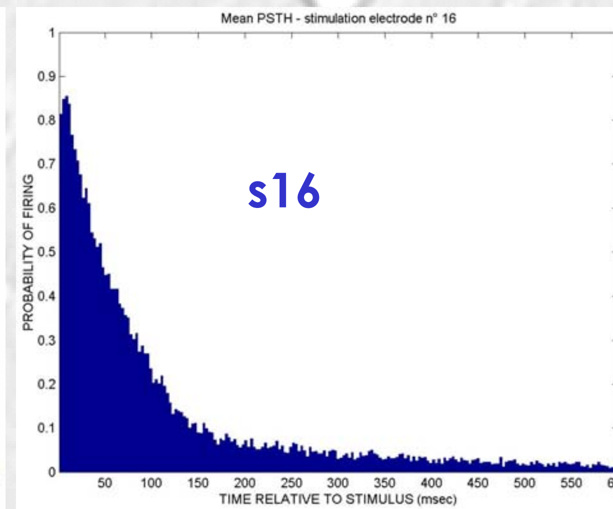
Response averaged with respect to the stimulating sites

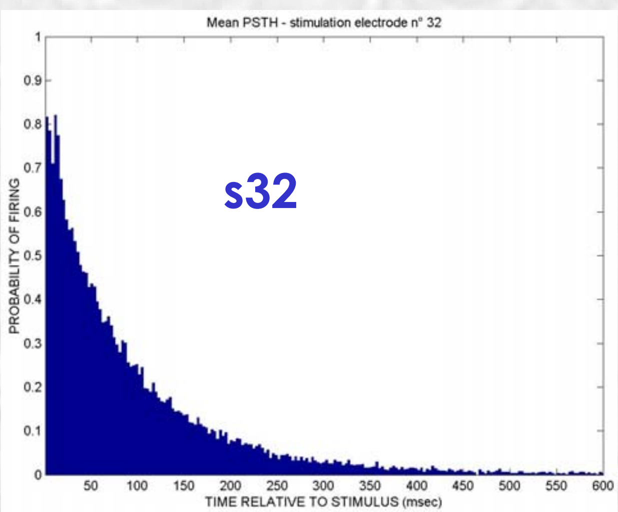
Results

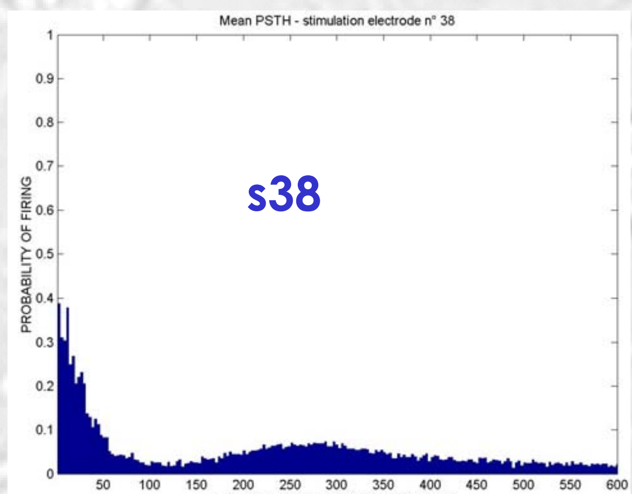
PSTHs averaged on 15 recording electrodes

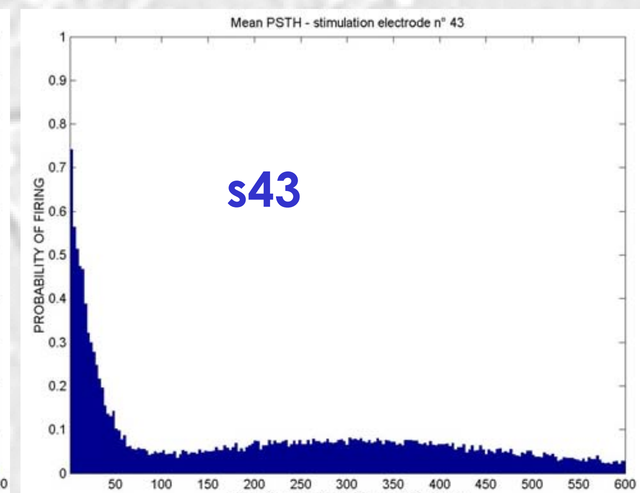


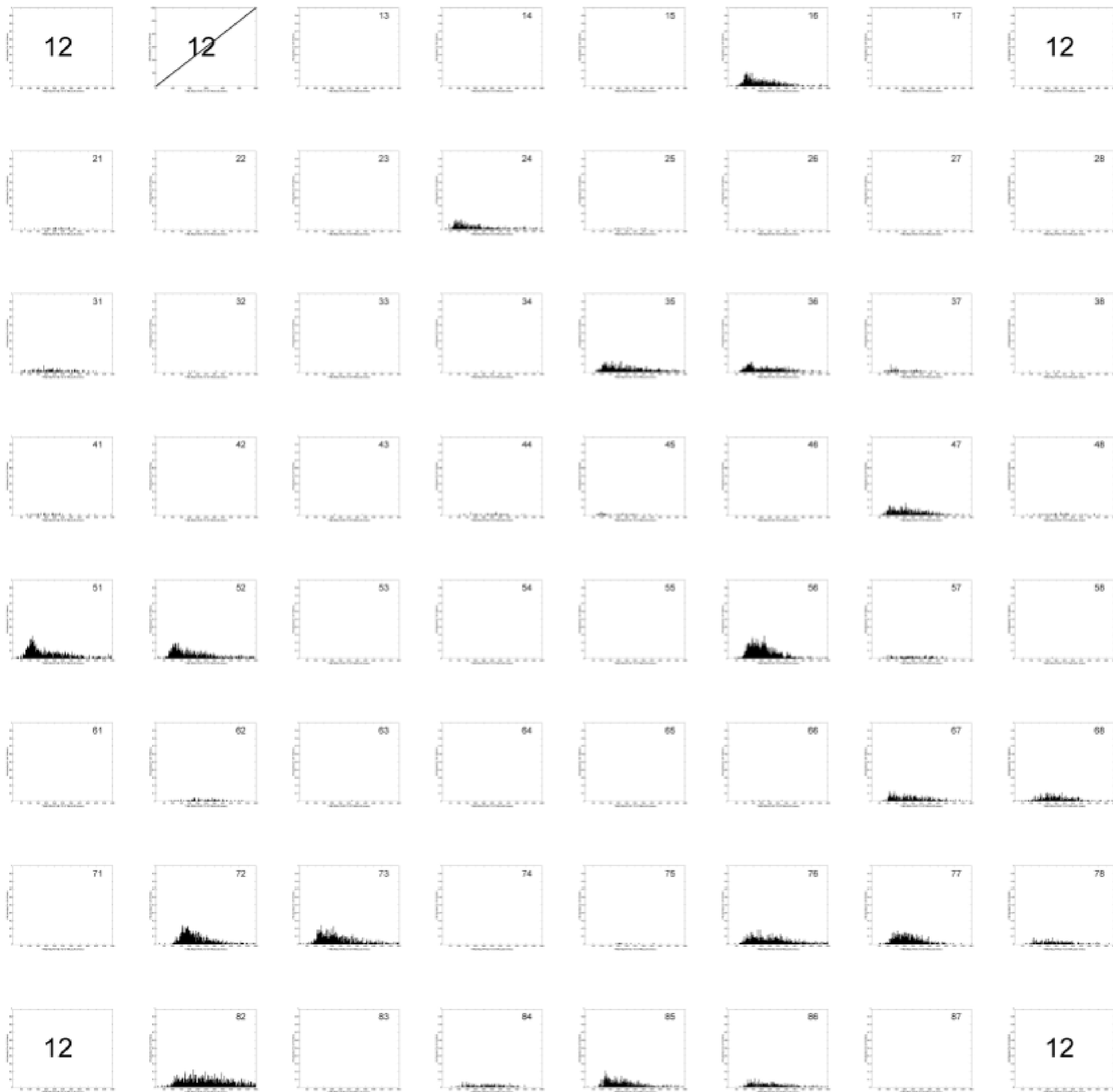








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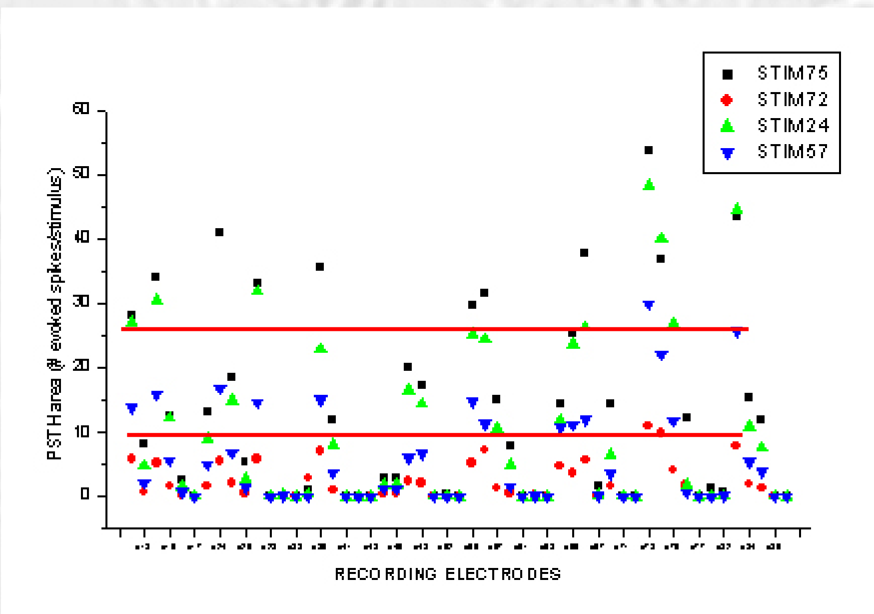
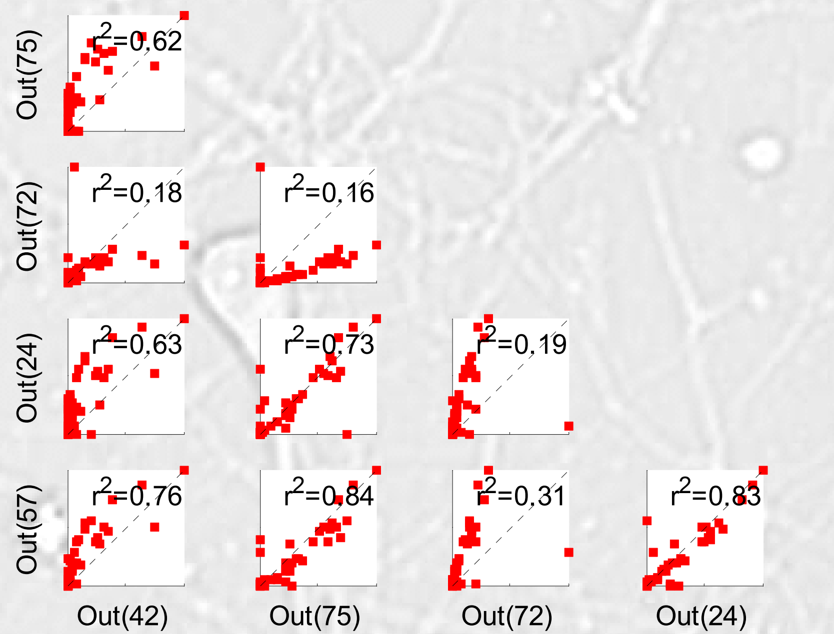
Input-output channels selection

- The network response is **stimulus-dependent**, since different stimulating sites evoke different responses ("distinct patterns" or "states") on the same recording electrodes.

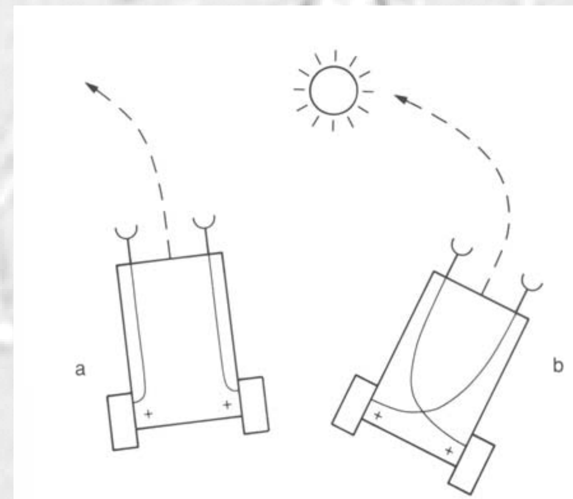
- The network characterization algorithms (IBIH, PSTH) can provide a tool for identifying the recording and stimulating sites candidates to become the "input" sensory channels and the "output" motor channels of our bioartificial neuronal system.

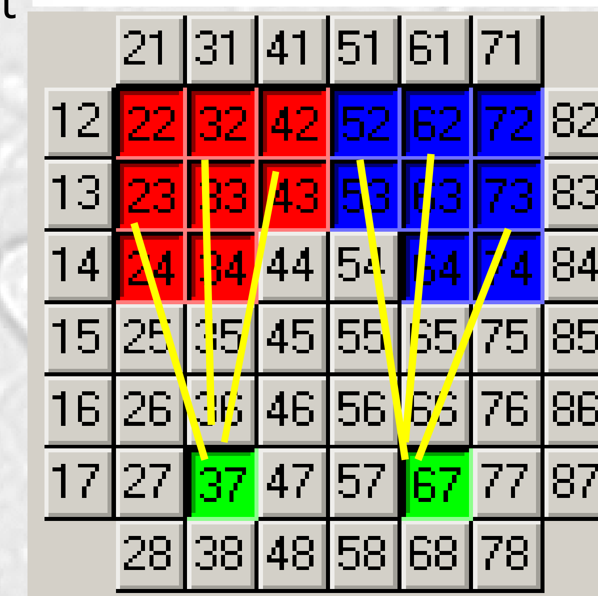


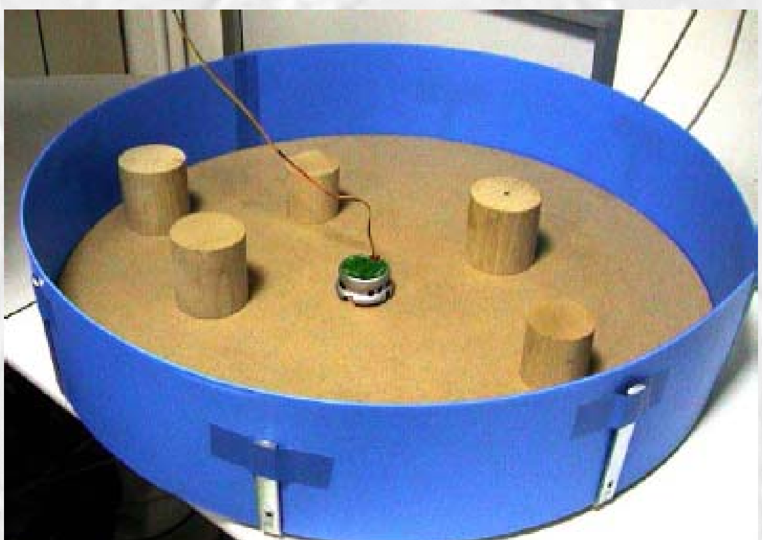


Bi-directional connection and closed-loop experiments

- As a closed-loop experiment, we focus on a simple 'Braitenberg vehicle' that (learns to) avoid obstacles. The robotic body is a Khepera II, with two wheels and eight infra-red (IR) proximity sensors, which moves inside a circular playground, containing a number of obstacles.
- Selectivity of population activity to the site of stimulation points to spatial coding of information. Therefore, we defined separate 'motor' and 'sensory' areas. We used two separate sets of recording sites to control the left and right wheels of the robot

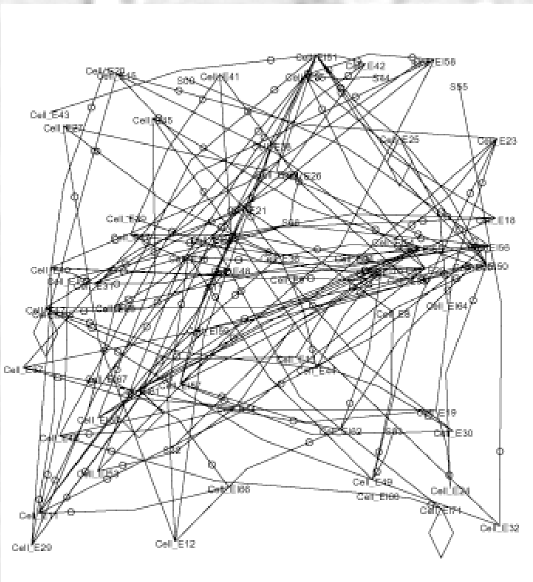


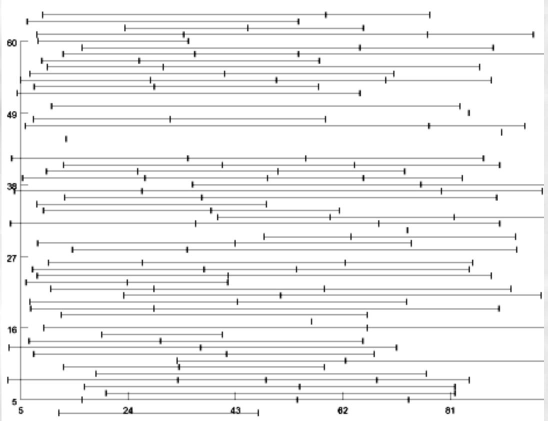


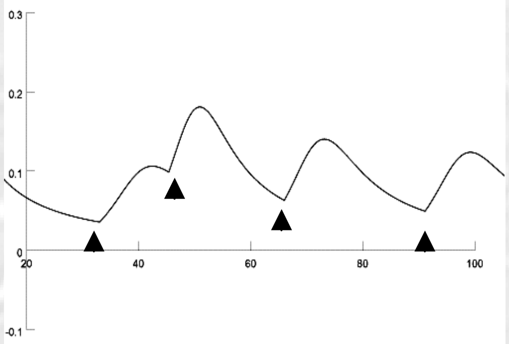


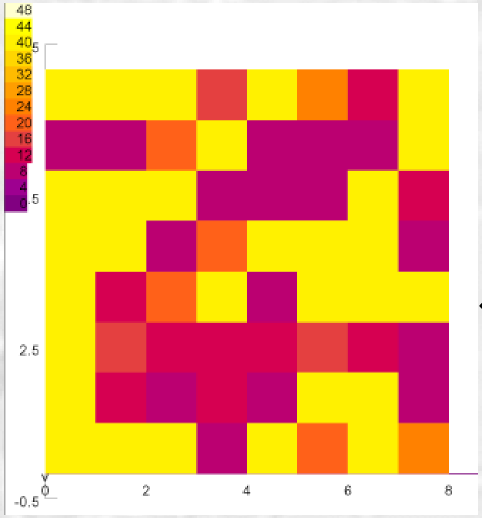
A possible model

- Neuron package environment
  - 64 Neurons. HH neurons, noisy leaky
  - Spontaneous activity
  - 35% of inhibitory synapses.
  - 3,5 connections for each neuron.



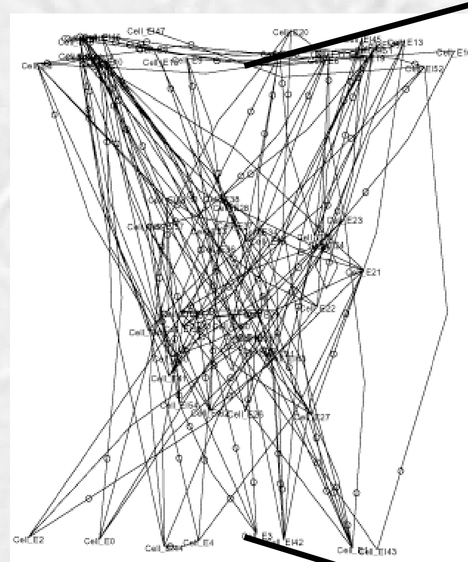


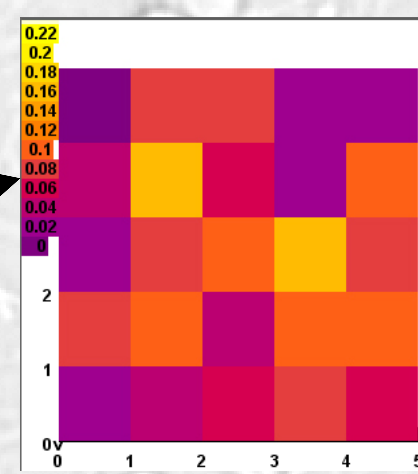


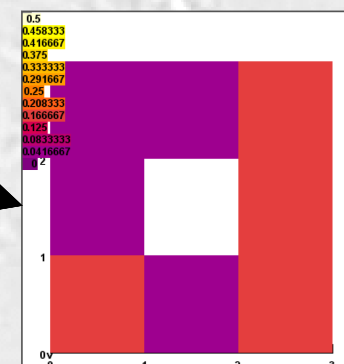


Defining sensor and motor areas

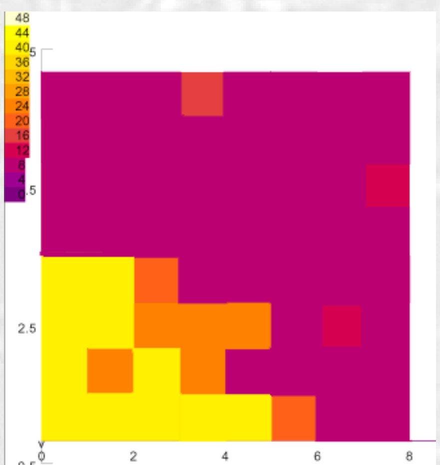
- Motor layer is used to generate the robot movement
- Hidden layers randomly connected.
- Sensory layer receive information from the robot sensor. A sensory vector is generated.

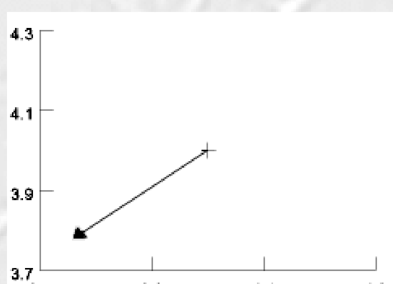






•Population vector coding



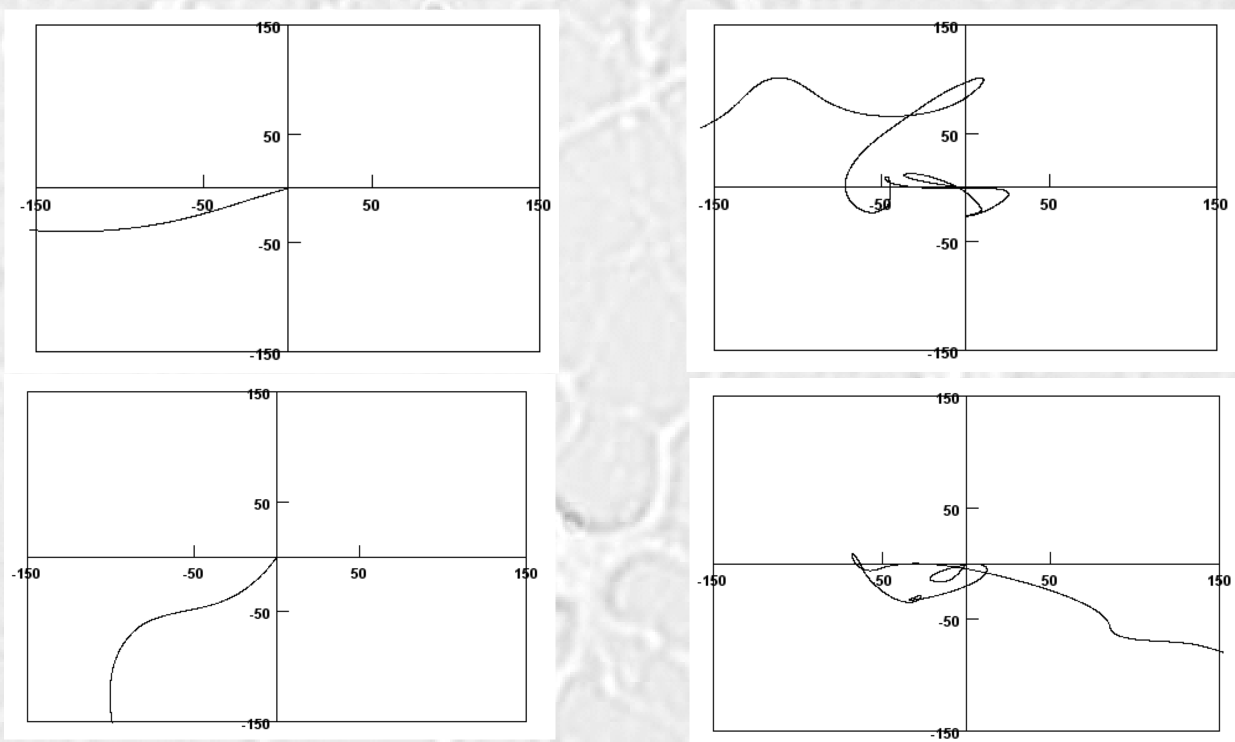


Associative (delta) learning algorithm

- Sensory vector and motor vector are not within the same quadrant : REDUCTION of synaptic weights
- Sensory vector and motor vector are within the same quadrant : INCREASE of synaptic weights

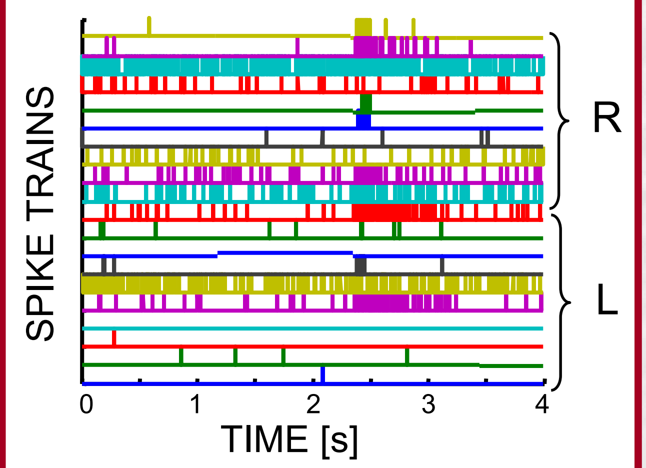
Before learning

After learning

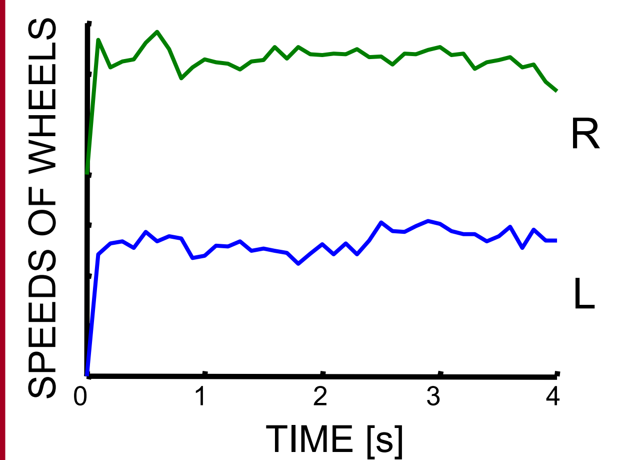


Decoding of Neuronal Signals

- Pre-processing (spike detection)
- Selection of a *N*-dimensional subset of the 60 channels that will be used to generate motor commands
- Estimation of an index of neural activity intensity $U_i(t)$, i = 1,…,N
  - Array of leaky integrators (first-order low-pass filters with a 100 ms time constant)
- Decoding strategy based on ***population coding***
  - Two separate subsets of the recording sites control left and right wheels of the robot
  - Each recording electrode is assigned a 'preferred' motor command (e.g., angular speed, direction of motion) chosen according to a *topographic* rule
  - The control command is computed as a normalized and weighted sum
  - Advantages: the weighted sum prevents each control signal from getting too big in case of prominent bursting activity recorded by the electrodes coding for one of the two sides



DECODING



Encoding of Sensory Information

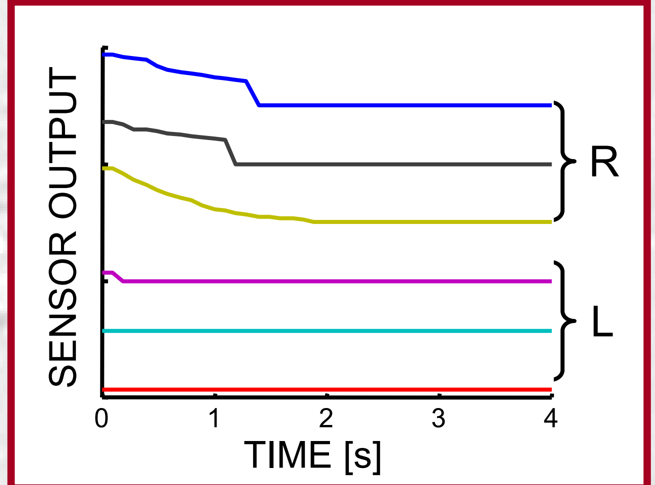
- **Sensory system**: six IR proximity sensors (the two on the back are not used); Let $u_i(t)$, i = 1,…,6 be sensor activity

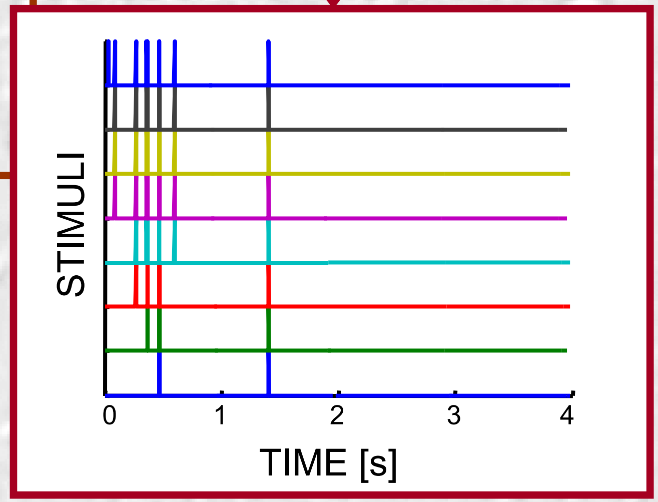
- **Coding scheme** based on **Gaussian-shaped receptive fields**: for each stimulation site, i = 1,…,M, choice (arbitrary) of a 'preferred' stimulus direction d_j
  - Stimulus intensity, i.e. $s_i(t)$, i = 1,…,M, is computed as:

$$s_i(t) = \sum_{j=1}^{6} G\left(\left\|d_j - d_i\right\|\right) \cdot u_j(t) = \sum_{j=1}^{6} G_{ij} \cdot u_j(t)$$

  where d_i are the actual sensor directions; this allows to encode sensory information into an arbitrary number of stimulation sites

- **Generation of spike trains** with Poisson probabilistic distribution:
  - For each stimulation channel, generate a uniformly distributed number x_n between 0 and 1 (n is time step, δt is sampling time)
  - Generate a spike if $x_n \leq s_n \cdot \delta t$ (this is only appropriate when $s_n \cdot \delta t << 1$)





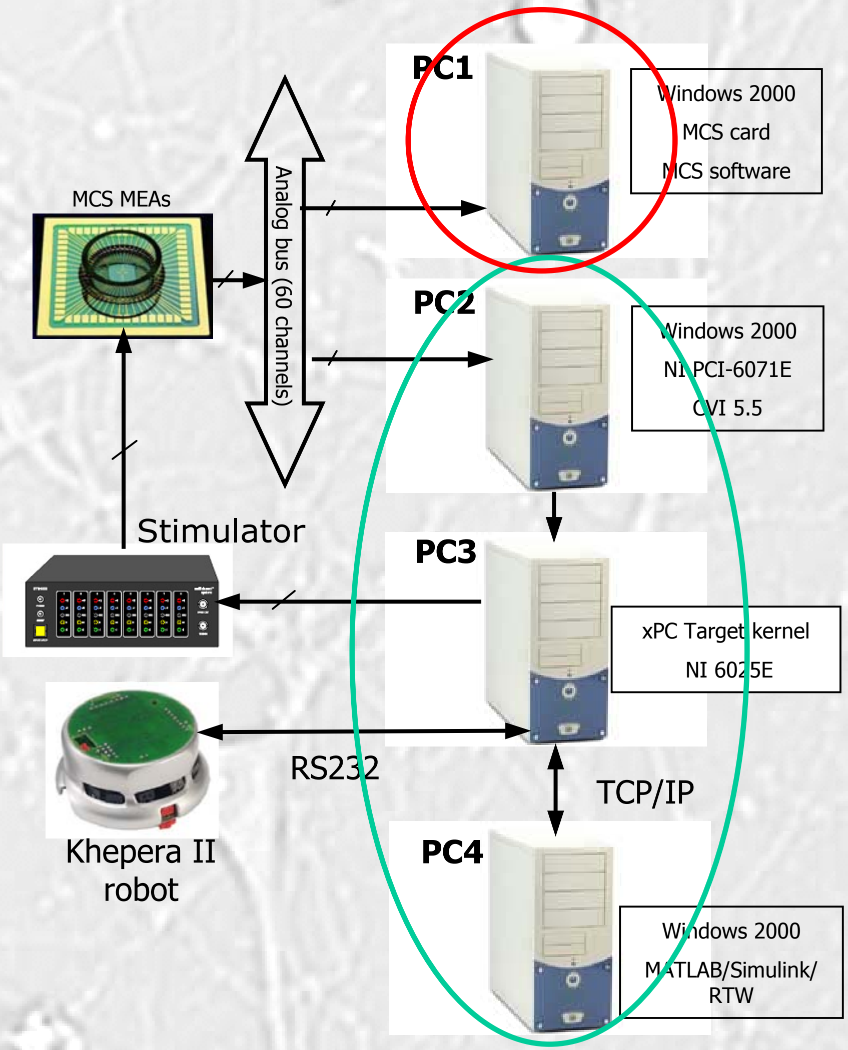
System Architecture: preliminary version

Present architecture

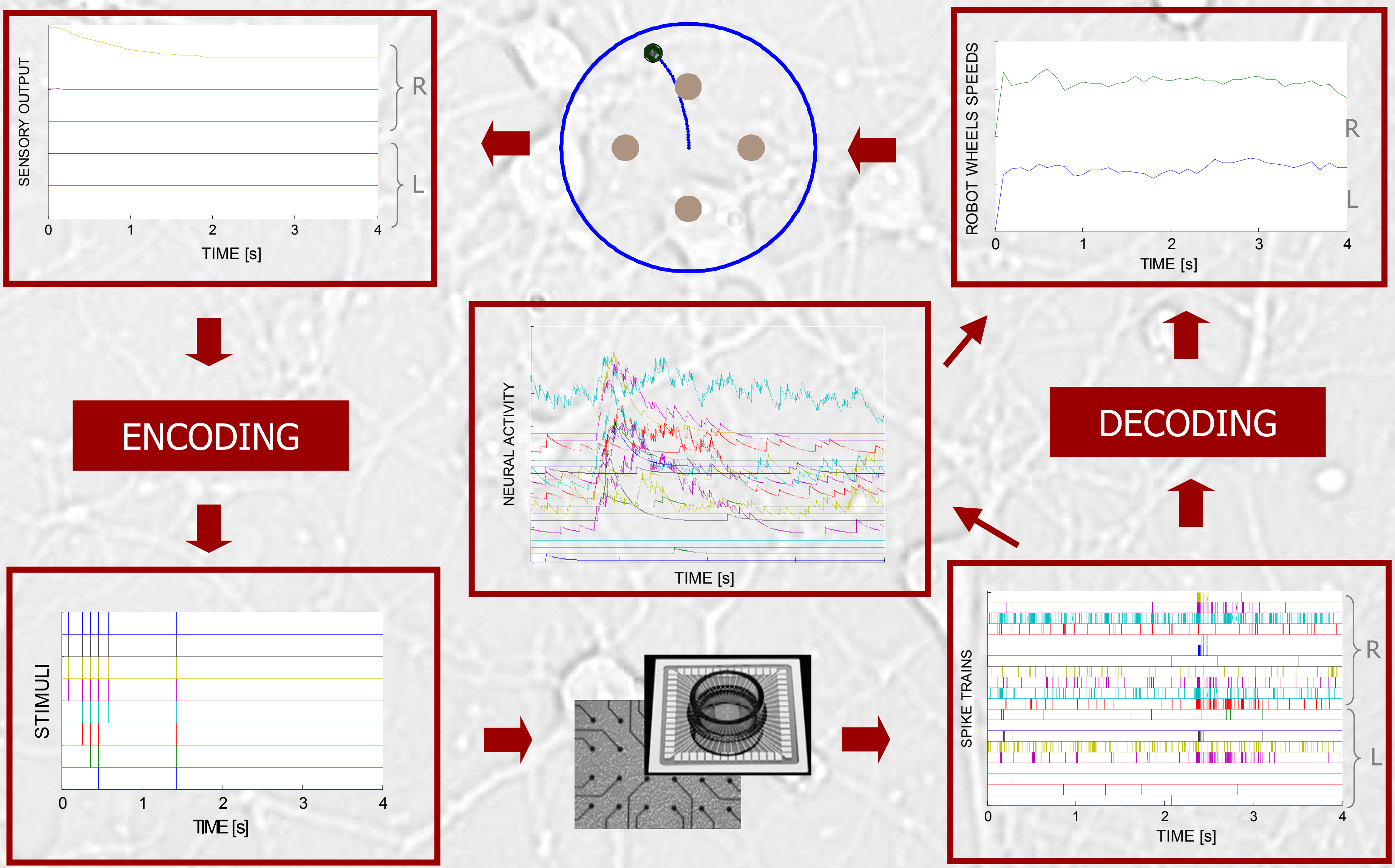
- PC1: Data Logger
  - Acquisition of neural signals from MEAs
  - Recording of Raw Data
- PC2: Spike detection
  - Acquisition of neural signals from MEAs
  - Spike detection
- **PC3: Closed-loop control**
  - **Acquisition of spike trains and generation of control signals (sent to robot via RS232)**
  - **Recording of sensory signals (RS232) and generation of neural stimulation patterns**
- **PC4: Experiment front-end**

Preliminary Tests

1. Open-loop runs with simulated/actual robot, neural data read from file
2. Closed-loop runs with actual robot, and loopback connection (stimulation sites connected to recording sites)
3. Same as 2., with spike detection on PC3



Closed-loop system



Neurobit

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