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

![Adaptive Neural Controller](image)

### Autonomous Robot: the artificial body


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


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
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
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)
How to do that?

Methodological approach

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

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
External electrical stimulus

Medium IN
CO₂ IN
Heating / Cooling

Local delivery channels
Stimulation / recording electrodes

Optical culture image
Electrophysiological activity

Medium OUT
pH measure
T measure

T and pH regulator
Medium regulator

Incubator
Techniques

- Electrode array
- Delivery channel
- Reservoir (Glass, PS, PMMA)
- MTA (wire-bonded and glued)
- PCB compatible with the multichannel connector

Dimensions: 5 cm x 5 cm
**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
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

Channel 44 - 28 DIV

Channel 51 - 28 DIV

Channel 61 - 28 DIV
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).
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

- s12
- s14
- s16
- s32
- s38
- s43
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 receives 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
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 $U_i(t)$, $i = 1, \ldots, N$
  - 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
Encoding of Sensory Information

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

- **Coding scheme** based on **Gaussian-shaped receptive fields**: for each stimulation site, \( i = 1, \ldots, M \), choice (arbitrary) of a ‘preferred’ stimulus direction \( d_j \).
  - Stimulus intensity, i.e. \( s_i(t), i = 1, \ldots, M \), is computed as:
    \[
    s_i(t) = \sum_{j=1}^{6} G(||d_j - d_i||) \cdot u_i(t) = \sum_{j=1}^{6} G_{ij} \cdot u_i(t)
    \]
    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, \( \delta t \) is sampling time).
  - Generate a spike if \( x_n \leq s_n \cdot \delta t \)
    (this is only appropriate when \( s_n \cdot \delta t \ll 1 \))
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

- Sensory output
- Neural activity
- Robot wheels speeds
- Decoding
- Encoding
- Stimuli
- Spike trains
# Partners of the Neurobit project

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