



# A biosensor for detecting changes in cognitive processing based on nonlinear systems analysis

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## Abstract

A new type of biosensor, based on hippocampal slices cultured on multielectrode arrays, and using nonlinear systems analysis for the detection and classification of agents interfering with cognitive function is described. A new method for calculating first and second order kernel was applied for impulse input–spike output datasets and results are presented to show the reliability of the estimations of this parameter. We further decomposed second order kernels as a sum of nine exponentially decaying Laguerre base functions. The data indicate that the method also reliably estimates these nine parameters. Thus, the state of the system can now be described with a set of ten parameters (first order kernel plus nine coefficients of Laguerre base functions) that can be used for detection and classification purposes. © 2001 Elsevier Science B.V. All rights reserved.

*Keywords:* Biosensor; Hippocampal slice; Nonlinear systems analysis

## 1. Introduction

From antiquity to modern times, there has been a need for detecting threats in the environment. This need is even more acute nowadays as increased environmental pollution as well as activities of groups of terrorists or of various types of fanatics (as illustrated by the attack of the Tokyo subway; Falkenrath et al., 1998; Tucker, 2000) cause serious threats to the general population. Analytical systems have become extremely sensitive and discriminative and are effective sensors as long as the nature of the detected agent is known. This is, in particular, the case of the MM1 (Rostker, 1997), the M22 ACADA, for automatic Chemical Agent Detector (<http://www.gulfink.osd.mil/campmont/index.html>) or a hybrid technology system, the M90-D1-C (Enviro-nics, Milliken, Finland). Even then, such systems do not provide much information on the biological effects of the detected agent. Biological systems, in principle at least, provide ideal detectors and reporters for environ-

mental threats, as they are complex systems, which have evolved to maintain homeostasis despite continuously changing environments. Moreover, they do provide information regarding the biological effects of the perturbing agents. The problem is made more difficult when the agents to be detected affect cognitive processes. In this case, the detection requires either sophisticated behavioral tests not easily implemented in a biosensor, or indirect assays that are strongly indicative of potential cognitive dysfunction. We have developed a biosensor consisting of a multi-electrode array monitoring the functioning of complex neuronal networks contained in a cortical structure involved in cognitive processing. The underlying assumption is that agents that affect hippocampal function will also affect cognitive function in humans.

The evolution of multi-electrode array (MEA) recording from an experimental project to a routine physiological tool makes it possible to study spatially extended populations of interconnected neurons (i.e. networks) in brain slices (Gross, 1979; Pine, 1980; Gross et al., 1982, 1985, 1993; Novak and Wheeler, 1988; Boppart et al., 1992; Meister et al., 1994; Stoppini et al., 1997; Egert et al., 1998; Maher et al., 1999; Oka

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et al., 1999). With a large number of appropriately spaced electrodes, and appropriate support hardware, it is possible to design input patterns with the spatio-temporal richness needed to activate complex network activity. The present data indicate that random train stimulation consisting of as few as 400 impulses delivered over 200 s is sufficient to accurately determine high order kernels, which are a mathematical expression of the nonlinearities of the network. Both theoretical and experimental work indicates that any agent affecting neuronal function will produce a distinct modification of higher order kernels (Scalabassi et al., 1988). Having 64 electrodes in the arrays ensures that there is always a sufficient number of pairs of stimulating/recording electrodes to perform random train stimulation and kernel analysis within each slice (our current experience indicates that as many as four kernels can be obtained from one stimulation site). Furthermore, pairs of stimulating/recording electrodes can be located in different hippocampal subfields, thus providing for an additional spatial parameter that can be used to further characterize the effect of an agent on the state of the system. Thus, not only does random train stimulation offers a rapid way of detecting the presence of a potentially hazardous agent, but it also provides unique information about this agent. By comparison with a known library of molecules, it will be possible to identify the agent itself, or, if not, its site of action. We also present an outline of how our analysis will provide for an efficient classification system for any molecule tested with our system.

## 2. Materials and methods

### 2.1. Hippocampal slice preparation

#### 2.1.1. Acute slices

Adult rats were anesthetized with halothane and decapitated. The brain was quickly removed and bathed in an ice-cold aCSF (NaCl, 128 mM; KCl, 2.5 mM; NaH<sub>2</sub>PO<sub>4</sub>, 1.25 mM; NaHCO<sub>3</sub>, 26 mM; Glucose, 10 mM; MgSO<sub>4</sub>, 2 mM; ascorbic acid, 2 mM; CaCl<sub>2</sub>, 2 mM aerated with 95% O<sub>2</sub>/5% CO<sub>2</sub>). The hippocampus was dissected out and transverse slices (thickness 400–500 μm) were collected using a Leica vibratome (VT 1000S). They were left to equilibrate for at least 1 h in aCSF (containing 2 mM Mg) at room temperature. During the recording session, a slice was transferred to the multielectrode plate (multielectrode array setup (MEA) or multimicroelectrode plate setup (MMEP) and was held down using a metallic ring and a string mesh. The slices were perfused with an aCSF containing a lower concentration of magnesium (1 mM). The slice was positioned carefully over the array with the help of an inverted microscope (4 × magnification,

Leica DML, Leica DM IRB). After documenting the relative position of the slice with respect to the array with an analogue camera (Analogue Hitachi VK-C370, Digital Spot Model 2.0.0), the slice was left for 15 min to equilibrate again. Biphasic current stimulation was achieved using either intrinsic microelectrodes (MEA setup) or an external bipolar Nichrome electrode (MMEP set up).

#### 2.1.2. Cultured slices

A modified version of the roller technique originally described by Gahwiler (1981) was used to culture hippocampal slices on both the MEA and MMEP set-ups. Briefly, hippocampal slices (400 μm thick) were prepared from 7-day-old rats with a McIlwain tissue slicer. Slices were glued over multielectrode arrays using a combination of thrombin (10 μl, 50 units/ml, Sigma) and plasma (10 μl, Sigma). The slices were left for 5 min in order to form a rigid clot. A volume of 3 ml of culture medium was added to the MEA arrays and slices were covered with their respective caps (ALA scientific). The MMEP array, on the other hand, was positioned in a large covered 7 cm diameter Petri dish. Fourteen milliliters of culture medium was added. One liter of culture medium contained Basal Medium Eagle (Sigma B-9638, 4.6 g), Earle Balanced Salts (Sigma E-6132, 2.17 g), and 33% Horse Serum (Gemini-Bio-Products # 100-105). The medium was supplemented by adding: NaCl, 15 mM; ascorbic acid, 0.4 mM; glucose, 36 mM; HEPES, 20 mM; CaCl<sub>2</sub>, 150 μM; MgSO<sub>4</sub>, 1.2 mM; glutamine, 2 mM; insulin (1 mg/100 ml); and 3 ml penicillin/streptomycin (Gibco BRL # 15240-062). The arrays were then left to rotate at 12 revolutions per h with a 15° inclination angle to allow air/CO<sub>2</sub> and medium to alternatively cover the slice. The medium was changed every 2–3 days. Using this technique, we have been able to maintain cultures for over a month with good morphology.

### 2.2. Hardware materials

In order to sample different subhippocampal fields, we used several multielectrode arrays, including the 8 × 8 multielectrode array design (MMEP-4, Gross et al., 1993), and the MEA system (Egert et al., 1998). The signals were amplified, recorded and analyzed using two different hardware and software setups. Multielectrode arrays permitted a spatio-temporal assessment of the neuronal activity in slices. They facilitated the search for the maximum output for a given input. The recorded responses were in the range of 10–100 μV/ms for the slope of evoked postsynaptic potentials (EPSPs) and 200–1500 μV for evoked population spike (PS) amplitude.

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