

Towards Unconventional Computing through Simulated Evolution: Learning Classifier System Control of Non-Linear Media

Larry Bull¹, Adam Budd,¹ Christopher Stone,¹ Ivan Uroukov,²
Ben De Lacy Costello² and Andrew Adamatzky¹

UWE Learning Classifier Systems Group Technical Report – *UWELCSG07-001* (Jan '07)

¹Faculty of Computing, Engineering & Mathematics, University of the West of England

²Faculty of Applied Sciences, University of the West of England

Coldharbour Lane, Frenchay

Bristol BS16 1QY, U.K.

Abstract. We propose that the behaviour of non-linear media can be controlled automatically through evolutionary learning. By extension, forms of unconventional computing, i.e., massively parallel non-linear computers, can be realised by such an approach. In this initial study a light-sensitive sub-excitable Belousov-Zhabotinski reaction in which a checkerboard image comprising of varying light intensity cells projected onto the surface of a thin silica gel impregnated with a catalyst and indicator is controlled using a Learning Classifier System, as is a form of *in vitro* neuronal network. In the former, pulses of wave fragments are injected into the checkerboard grid resulting in rich spatio-temporal behaviour and a Learning Classifier System is shown able to direct the fragments to an arbitrary position through dynamic control of the light intensity within each cell in both simulated and real chemical systems. Similarly, a Learning Classifier System is shown able to control the electrical stimulation of cultured neuronal networks such that they display elementary learning, i.e., so that they respond to a given input signal in a pre-specified way. Results indicate that the learned stimulation protocols identify seemingly fundamental properties of *in vitro* neuronal networks. Use of another learning scheme presented in the literature confirms these properties.

Keywords: Chemical Computing, Genetic Algorithm, Neuronal Computing, Reinforcement Learning.

1 Introduction

There is growing interest in research into the development of hybrid wetware-silicon devices focused on exploiting their potential for 'non-linear computing'. The aim is to harness the as yet only partially understood intricate dynamics of non-linear media to perform complex 'computations' (potentially) more effectively than with traditional architectures and to further the understanding of how such systems function. The area provides the prospect of radically new forms of machines and is enabled by improving capabilities in wetware-silicon interfacing. We are developing an approach by which networks of non-linear media – reaction-diffusion systems and *in vitro* neuronal networks - can be produced to achieve a user-defined computation in a way that allows control of the media used. Evolutionary algorithms are used to design the appropriate networks by searching a defined behavioural space to create a computing resource capable of satisfying a given objective(s). In this paper we begin by examining a Belousov-Zhabotinsky (BZ) [Zhakin & Zhabotinsky, 1970] reaction-diffusion system in which the networks are created via light and present initial results from the general control/programming methodology. We then apply the same approach to the control of a 3-D form of cultured neuronal network.

Excitable and oscillating chemical systems have been used to solve a number of computational tasks such as implementing logical circuits [Steinbock et al., 1996; Toth et al. 1994], image processing [Kuhnert et al., 1989], shortest path problems [Steinbock et al., 1995] and memory [Motoike et al., 2001]. In addition chemical diodes [Agladze et al., 1996], coincidence detectors [Gorecki et al., 2003] and transformers where a periodic input signal of waves may be modulated by the barrier into a complex output signal depending on the gap width and frequency of the input [Sielewiesiuk & Gorecki, 2002] have all been demonstrated experimentally. However, to some degree the lack of compartmentalisation in these simple chemical systems limits the domain of solvable tasks thus making it difficult to realise general-purpose computing. This proposed methodology of utilising networks of coupled oscillating chemical reactions may provide a solution. The fact that these coupled oscillators can be controlled via the application of external fields such as light provides a possible method for undertaking a number of complex computations provided an effective methodology for realising large scale networks can be found.

A number of experimental and theoretical constructs utilising networks of chemical reactions to implement computation have been described. These chemical systems act as simple models for networks of coupled

oscillators such as neurons, circadian pacemakers and other biological systems [Kawato & Suzuki, 1980]. Over 30 years ago the construction of logic gates in a bistable chemical system was described by Rössler [Rössler 1974]. Ross and co-workers [Hjelmfelt et al., 1991; 1992] produced a theoretical construct suggesting the use of “chemical” reactor systems coupled by mass flow for implementing logic gates neural networks and finite-state machines. In further work Hjelmfelt et al. [Hjelmfelt & Ross, 1993; Hjelmfelt et al., 1993] simulated a pattern recognition device constructed from large networks of mass-coupled chemical reactors containing a bistable iodate-arsenous acid reaction. They encoded arbitrary patterns of low and high iodide concentrations in the network of 36 coupled reactors. When the network is initialized with a pattern similar to the encoded one then errors in the initial pattern are corrected bringing about the regeneration of the stored pattern. However, if the pattern is not similar then the network evolves to a homogenous state signalling non-recognition.

In related experimental work Laplante et al. [1995] used a network of eight bistable mass coupled chemical reactors (via 16 tubes) to implement pattern recognition operations. They demonstrated experimentally that stored patterns of high and low iodine concentrations could be recalled (stable output state) if similar patterns were used as input data to the programmed network. This highlights how a programmable parallel processor could be constructed from coupled chemical reactors. This described chemical system has many properties similar to parallel neural networks. In other work Lebender and Schneider [1994] described methods of constructing logical gates using a series of flow rate coupled continuous flow stirred tank reactors (CSTR) containing a bistable nonlinear chemical reaction. The minimal bromate reaction involves the oxidation of cerium(III) (Ce^{3+}) ions by bromate in the presence of bromide and sulphuric acid. In the reaction the Ce^{4+} concentration state is considered as “0” “false” (“1” “true”) if a given steady state is within 10% of the minimal (maximal) value. The reactors were flow rate coupled according to rules given by a feedforward neural network run using a PC. The experiment is started by feeding in two “true” states to the input reactors and then switching the flow rates to generate “true”-“false”, “false”-“true” and “false”-“false”. In this three coupled reactor system the AND (output “true” if inputs are both high Ce^{4+} , “true”), OR (output “true” if one of the inputs is “true”), NAND (output “true” if one of the inputs is “false”) and NOR gates (output “true” if both of the inputs are “false”) could be realised. However to construct XOR and XNOR gates two additional reactors (a hidden layer) were required. These composite gates are solved by interlinking AND and OR gates and their negations. In their work coupling was implemented by computer but they suggested that true chemical computing of some Boolean

functions may be achieved by using the outflows of reactors as the inflows to other reactors i.e. serial mass coupling.

As yet no large scale experimental network implementations have been undertaken mainly due to the complexity of analysing and controlling so many reactors. That said there have been many experimental studies carried out involving coupled oscillating and bistable systems [Stuchl & Marek, 1982; Crowley & Field, 1986; Bar-Eli & Reuveni, 1985; Bar-Eli, 1985; Crowley & Epstein, 1989; Holz & Schneider, 1993]. The reactions are coupled together either physically by diffusion or an electrical connection or chemically, by having two oscillators that share a common chemical species. The effects observed include multistability, synchronisation, in-phase and out of phase entrainment, amplitude or “oscillator death”, the cessation of oscillation in two coupled oscillating systems, or the converse, “rythmogenesis”, in which coupling two systems at steady state causes them to start oscillating [Dolnik & Epstein, 1996].

In this paper we adapt a system described by Wang et al. [1999] and explore the computational potential based on the movement and control of wave fragments. In the system they describe the application of Gaussian noise (where the mean light level is fixed at the subexcitable threshold of the reaction) in the form of light projected onto a thin layer of the light sensitive analogue of the BZ reaction was observed to induce wave formation and subsequently “avalanche behaviour” whereby a proliferation of open ended excitation wave fragments were formed. Interestingly calcium waves induced in networks of cultured glial cells [Jung et al. 1998] display similar features to the ones identified in this chemical system which the authors postulated may provide a possible mechanism for long-range signalling and memory in neuronal tissues.

The study of *in vitro* neuronal networks has the potential to discover the underlying behaviours of neurons since they are typically created from dissociated cells; the self-organizing characteristics of such cells become identifiable. Such networks have already been reported as being capable of simple learning, memory and other computation-like behaviours.

It is well-established that *in vitro* neuronal networks display a strong disposition to form synapse and sensitivity to electro-chemical stimulation. Shahaf and Marom [2001] have highlighted these latter characteristics in their work with cultured rat neurons in commercially available multi-electrode hardware (Multichannel Systems Ltd.

MEA-60). Some electrodes are designated as input sources and those remaining are monitored for recurring patterns in action potentials; such technology enables network/ensemble level analyses (e.g., [Potter, 2001]). They were able to demonstrate a simple form of supervised stimulus-response learning in the cultured networks such that a required response for a given input was obtained from a pre-determined neuron/electrode through timed stimulus removal. That is, drawing on ideas proposed during the 1940's by behavioural psychologists, they showed that with incremental single-step training, desired discrete output computations could be achieved from essentially randomly connected neuronal networks.

Shahaf and Marom's [2001] work is related to that by DeMarse et al. [2001] who have used the same hardware to randomly control a simulated mobile robot, again with feedback from output to the inputs. That is, they have presented an approach to *in vitro* AI wherein the neuronal network exists within a feedback loop to its environment: the sensors of the simulated mobile robot are fed directly into the network and its responses fed to the robot's actuators. They report the emergence of a number of repeated spiking patterns during the control scenario.

Ruaro et al. [2005] describe the use of neuronal networks for an image processing task. Here two spatial patterns are exposed to the network through appropriate electrode stimulation. They show that the response of the network to one pattern can be trained to be significantly higher than for the other. It has also recently been shown that controlled pair-wise stimulation can be used to alter network response thereby indicating a rudimentary memory mechanism for *in vitro* networks; response to a given stimulus on one electrode alters if another has been stimulated within a time window (e.g., [Takayam & Jimbo, 2006]).

Machine Learning techniques, such as Evolutionary Algorithms (EAs)[e.g., Michalewicz & Fogel, 1999] and Reinforcement Learning (RL) [e.g., Sutton & Barto, 1998], are being increasingly used in the design of complex systems. Example applications include data mining, time series analysis, scheduling, process control, robotics and electronic circuit design. Such techniques can be used for the design of computational resources in a way that offers substantial promise for application in non-linear media computing since the algorithms are almost independent of the medium in which the computation occurs. This is important in order to achieve effective non-linear media computing since they do not need to directly manipulate the material to facilitate learning and the task itself can be defined in a fairly unsupervised manner. In contrast, most traditional learning algorithms use

techniques that require detailed knowledge of and control over the computing substrate involved. In this paper we control the BZ and neuronal networks via a reinforcement learning approach which uses evolutionary computing to create generalizations over the state-action space – Holland’s Learning Classifier System [Holland, 1986], in particular a form known as XCS [Wilson, 1995].

The paper is arranged as follows: The next section describes the sub-excitable BZ system which forms the basis of the chemical computing aspect of our research. The next section describes a computational model of the system and XCS. Initial results from using the evolutionary learner to control the simulated and real chemical system are then presented. The form of neuronal cell culture we are developing is then described, along with the XCS control scenario used. Initial results from controlling the electrical stimulation via XCS are then presented together with confirmation via another learning protocol presented in the literature. Finally, all results are discussed.

2 Chemical Experimental System

2.1 Materials and Equipment

Sodium bromate, sodium bromide, malonic acid, sulphuric acid, tris(bipyridyl) ruthenium (II) chloride, 27 % sodium silicate solution stabilized in 4.9 M sodium hydroxide were purchased from Aldrich and used as received unless stated otherwise.

An InFocus Model Projector was used to illuminate the computer-controlled image. Images were captured using a Panasonic NV-GS11 digital video camera. The microscope slide was immersed in the continuously fed reaction solution contained in a custom-designed Petri dish, designed by Radleys, with a water jacket thermostatted at 22 °C. A Watson Marlow 205U multi-channel peristaltic pump was used to pump the reaction solution into the reactor and remove the effluent.

2.2 Experimental Procedures

2.2.1 Making Gels

A stock solution of the sodium silicate solution was prepared by mixing 222 mL of the purchased sodium silicate solution with 57 mL of 2 M sulphuric acid and 187 mL of deionised water, similar to the procedure used by Wang et al. [1999]. Pre-cured solutions for making gels were prepared by mixing 5 mL of the acidified silicate solution with a solution consisting of 1.3 mL of 1.0 M sulphuric acid and 1.2 mL of 0.025 M tris(bipyridyl) ruthenium (II) chloride. Using capillary action, portions of this solution were transferred onto microscope slides with 100 μm shims and Plexiglas covers. The transferred solutions were left for 3 hours to permit complete gellation after which the covers and shims were removed and the gels washed in deionised water to remove residual tris(bipyridyl) ruthenium (II) chloride and the sodium chloride byproduct. The gels were 26 mm by 26 mm, with a wet thickness of approximately 100 μm . The gels were stored under water and rinsed right before use.

2.2.2 Catalyst-Free Reaction Mixture

The bulk of the catalyst-free reaction mixture was freshly prepared in 300 mL batches, which involved the *in situ* synthesis of stoichiometric bromomalonic acid from malonic acid and bromine generated from the partial reduction of sodium bromate. The catalyst-free reaction solution consisted of the 0.36 M sodium bromate, 0.0825 M malonic acid, 0.18 M sulphuric acid and 0.165 M bromomalonic acid. To minimize the deterioration during the experiment, this solution was kept in an ice bath. This solution was continuously fed into the thermostatted reactor, with a reactor residence time of 30 minutes.

2.2.3 Experimental setup

The spatially distributed excitable field on the surface of the gel was made possible by the projection of a 10-by-10 cell checkerboard grid pattern generated using a computer. After Wang et al. [1999], the checkerboard image comprised the heterogeneous network of two light levels, from a low of 0.394 mW cm^{-2} to a high of 9.97 mW cm^{-2} intensity cells, representing excitable and non-excitable domains respectively. A digital video camera was

used to capture the chemical wave fragments. A diagrammatic representation of the experimental setup is shown in Figure 1.

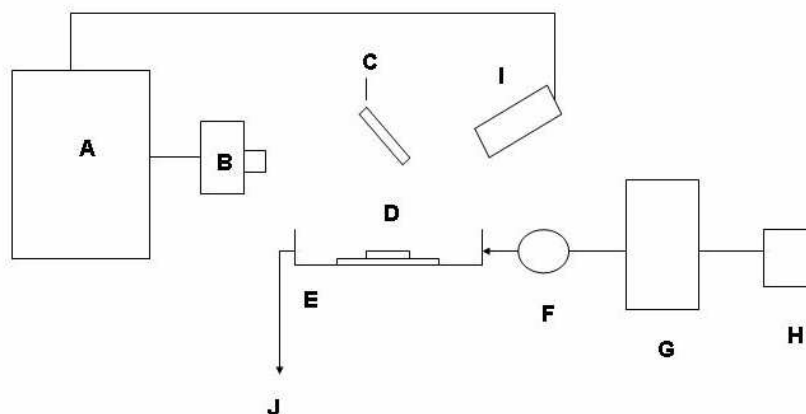


Figure 1: A block diagram of the experimental setup where the computer, projector, mirror, microscope slide with the catalyst-laden gel, thermostatted Petri dish, peristaltic pump, thermostatted water bath, reservoir of catalyst-free reaction solution, digital camcorder and, the effluent flow are designated by A, B, C, D, E, F, G, H, I and J, respectively. The catalyst-free reaction solution reservoir was kept in an ice bath during the experiment.

2.2.4 Data Capturing and Image Processing

A checkerboard grid pattern was projected onto the catalyst-laden gel through a 455nm narrow bandpass interference filter and 100/100mm focal length lens pair and mirror assembly. The size of the projected grid was approximately 20mm square. Every 10 seconds, the checkerboard pattern was replaced with a uniform grey level of 9.97 mW cm^{-2} for 400 ms during which time an image of the BZ waves on the gel was captured. The purpose of removing the grid pattern during this period was to allow activity on the gel to be more visible to the camera and assist in subsequent image processing of chemical activity.

Captured images were processed to identify chemical wave activity. This was done by differencing successive images on a pixel by pixel basis to create a black and white thresholded image. Each pixel in the black and white image was set to white, corresponding to chemical activity; if the intensity of the red or blue channels differed in successive images by more than 1.95%. Pixels at locations not meeting this criterion were set to black. The thresholded images were automatically despeckled and manually edited to remove artefacts of the experiment,

such as glare from the projector and bubbles from the oxidative decarboxylation of malonic acid and bromomalonic acid. The images were cropped to the grid location and the grid superimposed on the thresholded images to aid analysis of the results.

3 Computational System

3.1 Model

The features of this system were simulated using a two-variable Oregonator model modified to account for the photochemistry [Field & Noyes, 1973; Krug, 1990]:

$$\frac{\partial u}{\partial t} = \frac{1}{\varepsilon} \left(u - u^2 - (fv + \Phi) \frac{u - q}{u + q} \right) + D_u \nabla^2 u$$
$$\frac{\partial v}{\partial t} = u - v.$$

The variables u and v represent the instantaneous local concentrations of the bromous acid autocatalyst and the oxidized form of the catalyst, HBrO_2 and tris (bipyridyl) Ru (III), respectively, scaled to dimensionless quantities. The ratio of the time scales of the two variables, u and v , is represented by ε , which depends on the rate constants and reagent concentration; f is a stoichiometric coefficient. The rate of the photo-induced bromide production is designated by Φ , which also denotes the excitability of the system in which low light intensities facilitate excitation while high intensities result in the production of bromide that inhibits the process, experimentally verified by Kádár et al. [1997]. The scaling parameter, q , depends on reaction rates only. The system was integrated using the Euler method with a five-node Laplacian operator, time step $\Delta t=0.001$ and grid point spacing $\Delta x=0.15$. The diffusion coefficient, D_u , of species u was unity, while that of species v was set to zero as the catalyst was immobilized in gel.

3.2 Evolutionary Algorithm

XCS represents a significant development of Holland's Learning Classifier System formalism and has been shown able to tackle many complex tasks effectively (see [Bull, 2004] for examples). It consists of a limited size population [P] of classifiers (rules). Each classifier is in the form of "IF condition THEN action" (*condition*→*action*) and has a number of associated parameters. Conditions traditionally consist of a trinary representation, {0,1,#}, where the wildcard symbol facilitates generalization, and actions are binary strings.

On each time step a match set [M] is created. A system prediction is then formed for each action in [M] according to a fitness-weighted average of the predictions of rules in each action set [A]. The system action is then selected either deterministically or stochastically based on the fitness-weighted predictions (usually 0.5 probability per trial). If [M] is empty a covering heuristic is used which creates a random condition to match the given input and then assigns it to a rule for each possible action.

Fitness reinforcement in XCS consists of updating three parameters, ε , p and F for each appropriate rule; the fitness is updated according to the relative accuracy of the rule within the set in five steps:

- i) Each rule's error is updated: $\varepsilon_j = \varepsilon_j + \beta(|P - p_j| - \varepsilon_j)$ where $0 \leq \beta \leq 1$ is a learning rate constant.
- ii) Rule predictions are then updated: $p_j = p_j + \beta(P - p_j)$
- iii) Each rule's accuracy κ_j is determined: $\kappa_j = \alpha(\varepsilon_0/\varepsilon)^v$ or $\kappa=1$ where $\varepsilon < \varepsilon_0$
v, α and ε_0 are constants controlling the shape of the accuracy function.
- iv) A relative accuracy κ_j' is determined for each rule by dividing its accuracy by the total of the accuracies in the action set.
- v) The relative accuracy is then used to adjust the classifier's fitness F_j using the moyenne adaptive modifée (MAM) procedure: If the fitness has been adjusted $1/\beta$ times, $F_j = F_j + \beta(\kappa_j' - F_j)$. Otherwise F_j is set to the average of the values of κ' seen so far.

In short, in XCS fitness is an inverse function of the error in reward prediction, with errors below ε_0 not reducing fitness. The maximum $P(a_i)$ of the system's prediction array is discounted by a factor γ and used to update rules

from the previous time step and an external reward may be received from the environment. Thus XCS exploits a form of Q-learning [Watkins, 1989] in its reinforcement procedure.

A Genetic Algorithm (GA) [Holland, 1975] acts in action sets [A], i.e., niches. Two rules are selected based on fitness from within the chosen [A]. Two-point crossover is applied at rate χ and point mutations at rate μ . Rule replacement is global and based on the estimated size of each action set a rule participates in with the aim of balancing resources across niches. The GA is triggered within a given action set based on the average time since the members of the niche last participated in a GA (after [Booker, 1989]).

The intention in XCS is to form a complete and accurate mapping of the problem space through efficient generalizations. In reinforcement learning terms, XCS learns a value function over the complete state/action space. In this way, XCS represents a means of using temporal difference learning on complex problems where the number of possible state-action combinations is very large (other approaches have been suggested, such as neural networks – see [Sutton & Barto, 1998] for an overview). The reader is referred to [Butz & Wilson, 2002] for an algorithmic description of XCS and [Bull & Kovacs, 2005] for an overview of current formal understanding of its operations.

3.3 XCS Control: Simulator

The aforementioned model of the BZ system has been interfaced to an implementation of XCS in a way which approximates the envisaged hardware-wetware scenario. A 3x3 grid is initialized with a pulse of excitation in the bottom middle cell as in the wetware experiments described in section 2. Two light levels have been used thus far: one is sufficiently high to inhibit the reaction; and, the other low enough to enable it. The modeled chemical system is then simulated for 10 seconds of real time. A 9-bit binary description of the 3x3 grid is then passed to the XCS. Each bit corresponds to a cell and it is set to true if the average level of activity within the given cell is greater than a pre-determined threshold. The XCS returns a 9-bit action string, each bit of which indicates whether the high ($\Phi=0.197932817$) or low ($\Phi=0.007791641$) intensity light should be projected onto the given cell. Another 10 seconds of real time are then simulated, etc. until either a maximum number of iterations has passed or the emergent spatial-temporal dynamics of the system match a required configuration. In this initial work, a fragment is required to exist in the middle left-hand cell of the grid *only*. At such a time, a numerical

reward of 1000 is given the system and the system reset for another learning trial. To be able to obtain this behavior reliably, it has been found beneficial to use an intermediate reward of 500 in the presence of a fragment in the target cell, regardless of the activity on the rest of the grid (see [Dorigo & Colombetti, 1998] for related discussions). The XCS parameters used for this were (largely based on [Wilson, 1995]): $N=30,000$, $\beta=0.2$, $\mu=0.04$, $\chi=0.8$, $\gamma=0.71$, $\theta_{del}=20$, $\delta=0.1$, $\varepsilon_0=10$, $\alpha=0.1$, $\nu=5.0$, $\theta_{mma}=512$, $\theta_{GA}=25$, $\rho_I = \varepsilon_I = F_I = 10.0$, $p_{\#}=0.33$. Other parameters for the BZ model were $\varepsilon=0.022$, $f=1.4$, $q=0.002$.

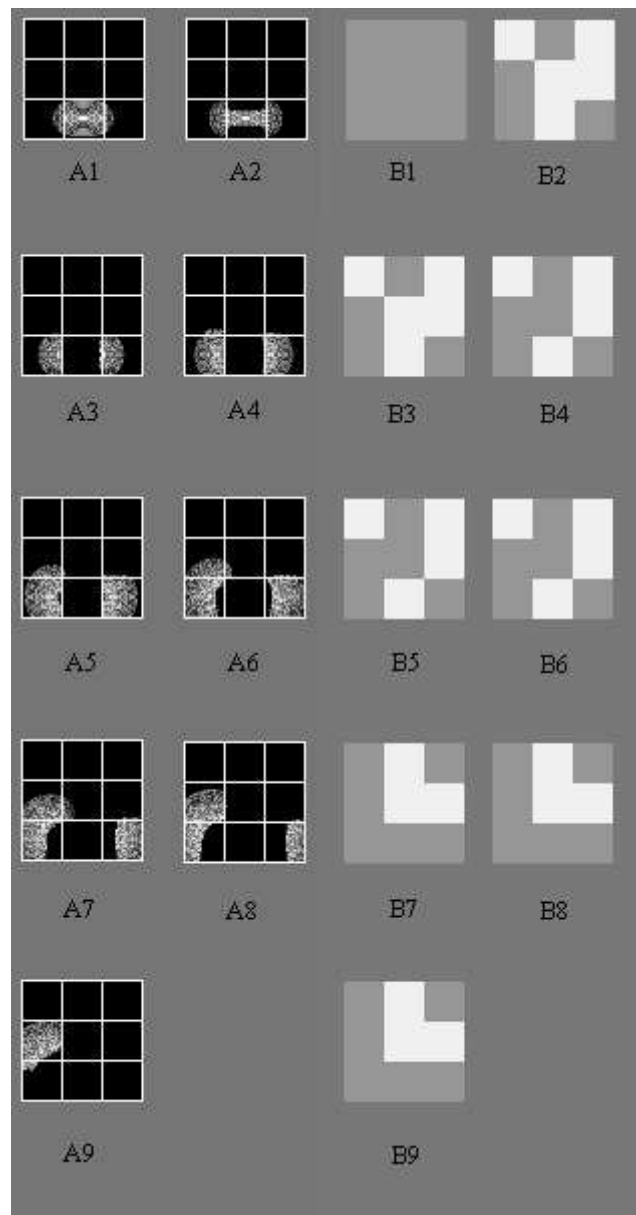


Figure 2: Example control of the simulated chemical system (A) under the learned light programs (B).

Figure 2 shows a typical light program and associated wave fragment behaviour sequence, here taking 9 steps to solve the problem, which appears optimal with the given parameters of the simulator and allowed time between XCS control steps. Figure 3 shows the average reward received by the learner per trial on exploit trials only, as a 50-point moving average (after [Wilson, 1995]). As can be seen, this approaches the maximum of 1000 in the time considered; the XCS controller is reliable in its ability to develop a fragment controller in the given scenario.

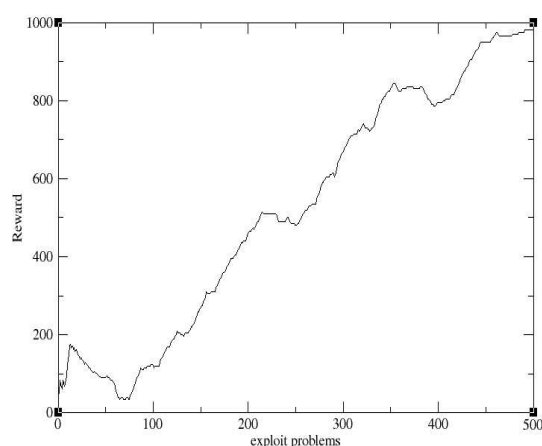


Figure 3: Showing the average reward received by the XCS controllers with increasing number of learning cycles or problems.

3.3 XCS Control: Chemistry

Given the success of the simulation experiments, the LCS was connected to the chemical system described in section 2. The scenario for the chemical experiments was the same as for the simulations, although it must be noted that there is a slightly longer delay before the XCS is able to control the initial light levels once the pulse is added to the grid due to the image processing required. Figure 4 shows an example result using the same parameters as before. This solution was discovered on trial 4 and then refined over the subsequent three trials to

that shown. It can be seen that XCS has learnt to control the fragment in the real chemical system as it did in the simulation.

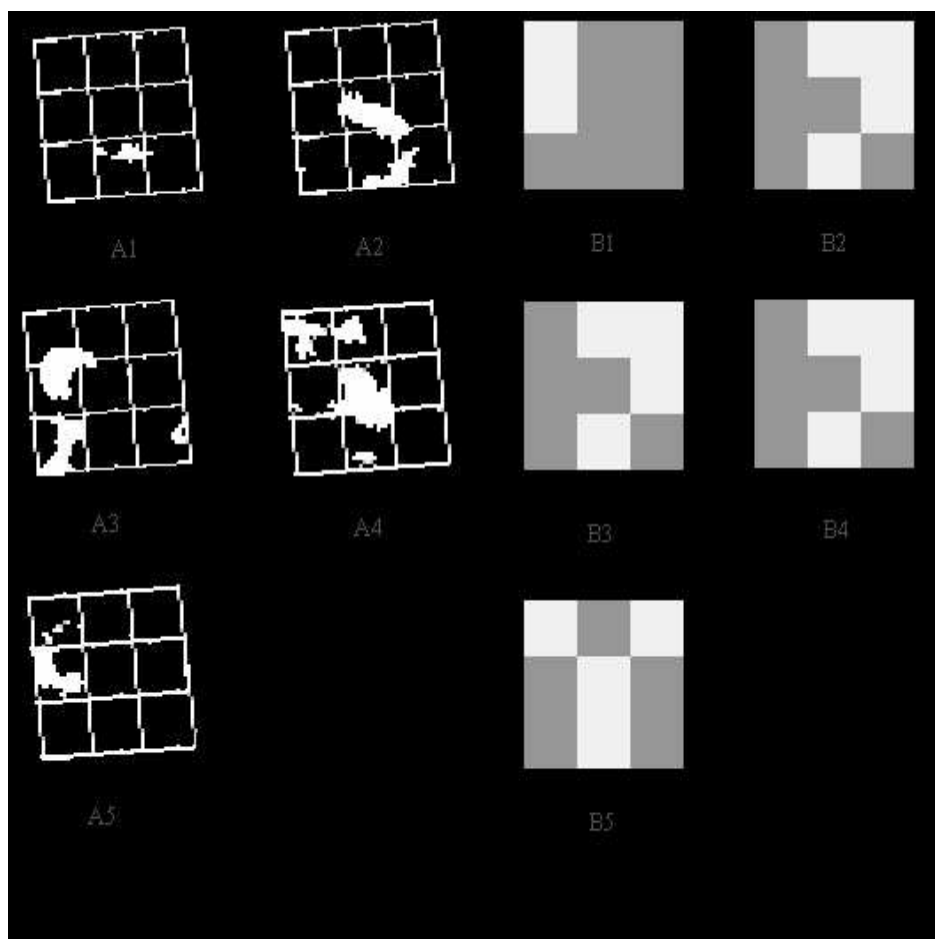


Figure 4: Example control of the real chemical system (A) under the learned light programs (B). Skewing of cell images is due to camera set-up.

4 Neuronal System

The majority of *in vitro* studies of the electrophysiological properties of neuronal networks exploit either tissue slices or monolayer cell cultures. For example, all the research described in the introduction used monolayers, i.e., cells in a network grown across the surface of a multi-electrode array dish. However it has long been known that aggregated, i.e., 3-D, neuronal cell cultures exhibit properties that are remarkably similar to their *in vivo* counterparts. For example, early studies showed structures identical to hippocampal architecture [DeLong, 1970][Sidman, 1970] and Seeds [1971] showed how the temporal biochemical differentiation of brain cell aggregates was very similar to that seen during development in mice, much more so than equivalent monolayer cultures. Indeed, the amount and type of cell differentiation was suggested to be the main difference between monolayer and aggregate cultures (e.g., [Moscana, 1961] [Trapp et al., 1979]).

Advances in cell culturing mean that it is now possible to differentiate neuronal and neuroglial cells obtained from ovoid primary cultures and maintain them for relatively long periods of time, typically several months. These organotypic cultures are derived from hen embryos at day 7 *in ovo*. We have recently described how the maturation of spontaneous spiking behaviour in aggregated cultures of such cells is typically very similar to that reported in monolayers of mammalian cortical cells [Uroukov et al., 2006]. However, response to simple stimulation has been shown able to cause an increase in the relative spiking frequency during maturation, typically up to around two times larger after fourteen days in culture (DIV14). This result indicates strong self-organizing processes within the neuronal networks of such aggregate cultures wherein networks containing mutual inhibition form under steady-state (unstimulated) conditions in such a way that external stimulation causes significant excitation within the structure. It is this feature we aim to explore further using evolutionary computation. Figure 5 shows an example of the aggregates used in this study.

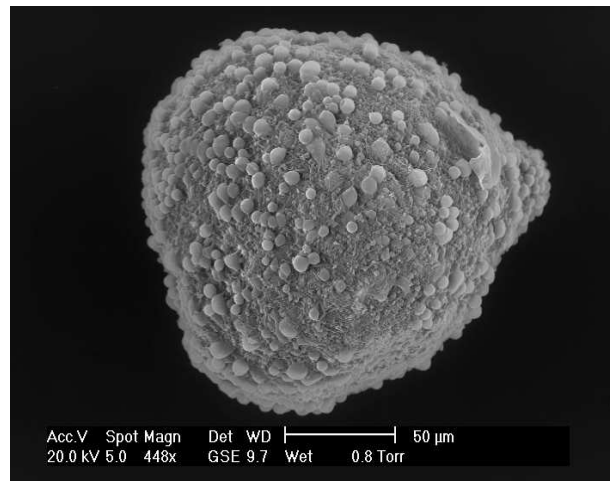


Figure 5. Scanning Electron Microscope image of DIV21 hen embryo aggregate neuronal culture.

4.1 Materials and Equipment

Fertilized eggs type '*Galus Domesticus*' (*Red Island Hens*) were obtained from a local poultry farm and transported to our laboratories; eggs underwent a pre-conditioning phase at room temperature. After marking and recording, the eggs were incubated for 7 days at 37 °C in an egg incubator (Octagon 100, Brinsea Ltd. UK). During the incubation, appropriate levels of humidity were maintained.

On the day of spheroid preparation, eggs were removed from the incubator at a precise time in order that the E7 embryonic stage had been reached [Hamburger & Hamilton, 1951]. The embryo was removed using aseptic techniques and placed in a 100 mm Petri dish with Hanks Balanced Salt Solution (HBSS, Life Technologies). Several embryos were pooled and then washed three times in HBSS. Under sterile conditions, the neuroepithelial tissue was removed under a stereo Leica Zoom 2000 microscope (Leica Co, Germany) with transmission and reflected illumination. The tissue was cleaned of meninges and transferred to a 25 mm Petri dish with culture medium (DMEM, Nutrient Mixture F-12 Ham, Fetal Bovine serum, L- Glutamin Solution, Penicillin-Streptomycin Solution, Progesterone-water soluble, Putrescine dihydrochloride, 3,3,5-Triiod-L-thyronine, Selenium dioxide, Holo-Transferrin Human, Insulin, all from SIGMA). The tissue was then collected and placed in a test tube with 5 ml of culture medium and triturated to prepare a cell suspension that was then filtered through a 35 μm Nybolt membrane. A trypan blue dye (0.4% w/v) exclusion assay was conducted and a cell

count performed under light microscopy using a haemocytometer. The plating cell concentration was 0.5×10^6 viable cells per ml of cell culture medium, 3 ml per well in a six-well culture plate. The six-well plates were placed on a gyrotatory shaker (Innova 2000, New Brunswick Scientific Co., Inc.) and cultured at 37 °C, 5 %CO₂ / 95% air at 75 rpm. Cell culture medium was refreshed every other day by removing some 50% of the old medium and refilling with fresh media warmed to a physiological temperature.

Multi-electrode arrays (MCS-2100, 3-D Multi Electrode Array, Multi Channel Systems MCS GmbH, Aspenhaustrasse 21, 72770 Reutlingen, Germany) with (3D-40 x 40 x 70 µm, spaced on 200 µm) pyramid shaped electrodes were used to record electrical activity of the spheroids. The multi-electrode (MEA) dish surface was modified with 10 µg/ml aqueous solution of Polymer Ethylene Imine (PEI) (Fluka Chemie AG, Buchs, Switzerland) under sterile conditions. The molecular weight of PEI varied between 0.610 and 1.010 according to product specifications. After the modification, two washing steps with demineralized (DEMI) water were undertaken before the plating of the spheroids.

The electrical recordings from the spheroids was performed with a 60 channels data acquisition system, where the sampling frequency of each channel was set to 25 kHz and the single channel amplification kept at 1200 with a digital resolution of 12 bits. At these conditions, data sampling of the input band of spikes within 5 kHz including a high pass 300Hz filter was performed in a way that was similar to other studies (e.g., [Jimbo et al., 2000]). The spikes were detected by threshold depending upon the standard deviation and the offset of noise. A set of data was monitored and raw signal, filtered signal and spikes chosen in order to perform fast and reliable recordings and analysis with the MC Rack software (Multi Channel Systems MCS, GmbH). The recorded data was written in the custom '*.mcd' MC Rack format and stored for further data analysis. The recorded signals were analyzed and the spike parameters extracted using the MC Rack software and analyzed with bespoke software as a post processing step.

The stimulation of the hen embryo brain spheroids was realized with the 8 channel programmed generator STG2008 (Multi Channel Systems MCS, GmbH). The stimulation protocol was created within the MC_Stimulus II software (Multi Channel Systems MCS, GmbH). The elaborated protocol shared features of relevant published studies (e.g., [Wagenaar et al., 2005]), consisting of a single sharp biphasic impulse of 300 µs and voltages between 300 mV and 2000 mV each phase per sweep of 1 sec.

4.2 XCS Control: Scheme

In the current study XCS has been applied to the control of the electrical stimulation of the neuronal networks in the following way. Firstly, the average spontaneous spiking frequency of a chosen aggregate network is ascertained over a 300 second window. Typically, an individual aggregate covers three or four electrodes in a dish as shown in Figure 6, one or two of which will show a suitably good connection into the neuronal network therein, i.e., spikes will be detected of the kind shown in Figure 7. The standard deviation in the spiking frequency is also calculated over the window. The task of the XCS controller is then to cause the chosen neuronal network to reply to the simple stimulus described above with a spiking frequency of the spontaneous mean plus two standard deviations; a significant increase in typical spiking frequency is required under stimulation.

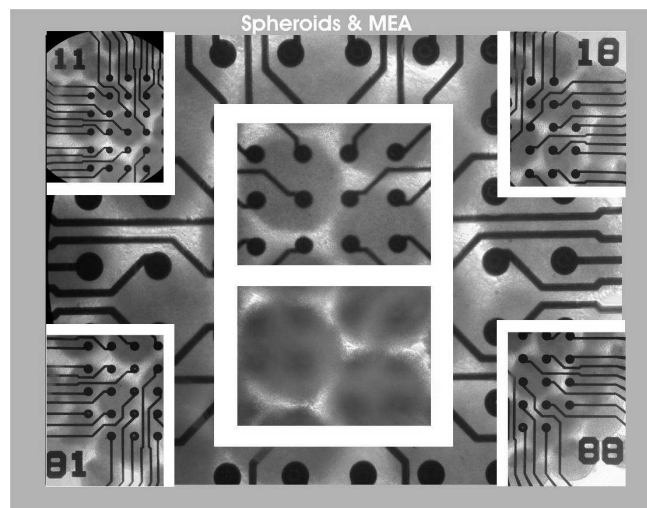


Figure 6. Phase contrast microscopy images taken at various optical magnifications and focal planes of aggregate cell cultures on multi-electrode array dish.

The input to the XCS on each cycle is the spiking frequency of the neuronal network averaged over the last three seconds and the length of time the stimulus was applied. The first number is presented as a fraction of the maximum spiking frequency observed under the 300 seconds of spontaneous behaviour and the second as a fraction of the maximum allowed stimulation time of 600 seconds. The XCS returns one of three actions: to double, halve or maintain the current stimulation time. A reward of 500 is given if the spiking frequency increased over the last stimulation period compared to that immediately prior and a reward of 1000 is given if the target spiking frequency, or one greater, was achieved.

Following [Shahaf & Marom, 2001] we allow a 300 second rest period between applications of the stimulus and truncate the maximum duration of stimulation to be 600 seconds. Thus 300 seconds after the last stimulation period, the XCS controller is given the last recorded spiking frequency of the neuronal network under stimulation, as a three-point running average, and the amount of time for which the stimulus was applied that caused the response. It then adjusts or maintains the stimulus duration for the coming cycle. For the initial cycle a stimulation period of 60 seconds is used.

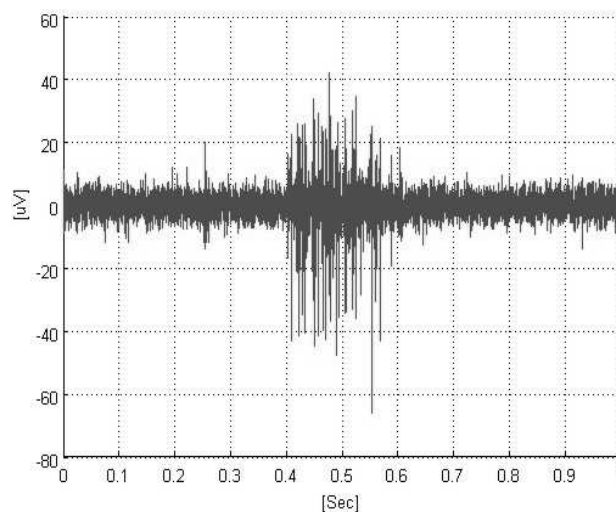


Figure 7. Example spiking behaviour recorded on a single electrode.

Hence the XCS is presented with an environmental input consisting of two real numbers scaled between 0.0 and 1.0; the condition part of the classifiers is encoded as un-ordered pairs of real numbers in the range [0, 1], one pair for each environmental input (after [Stone & Bull, 2003]). A pair is considered to match the corresponding input value if one of the pair is smaller or equal to the target, and the other is larger or equal. The action of the classifier is an integer.

The mutation operator is altered from that in XCS as described above to deal with the new representation. Mutation in the case of the real numbers of the condition is effected either by the addition or subtraction of either a small number drawn from a Gaussian distribution centred on the current value, or a fixed small change (here

0.1). Action mutation is by picking an integer from the set $\{0,1,2\}$ at random, such that the chosen action is different from the current one.

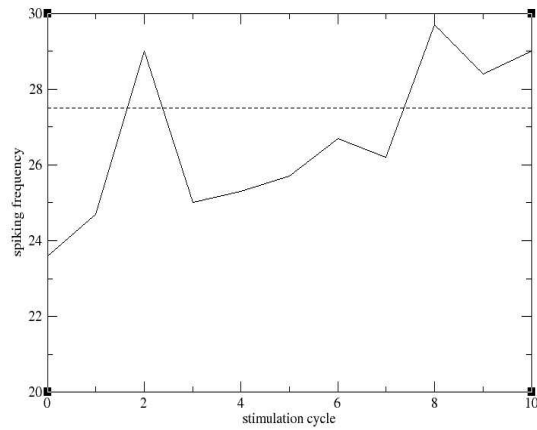
In the initial population, classifier conditions are created randomly in the range $[0,1]$. During cover, the current environmental input e is used as a centre and two values are created in the range $[e-C_{max}, e+C_{max}]$, where C_{max} is 0.1.

The XCS parameters used were again typical for those in the literature: $N=3000$, $\beta=0.2$, $\mu=0.04$, $\chi=0.8$, $\theta_{del}=20$, $\delta=0.1$, $\varepsilon_0=10$, $\alpha=0.1$, $\nu=5.0$, $\theta_{mma}=3$, $\theta_{GA}=2$, $\rho_I = \varepsilon_I = F_I=10.0$. The cell cultures used were all in the range of 20 to 30 days old *in vitro*.

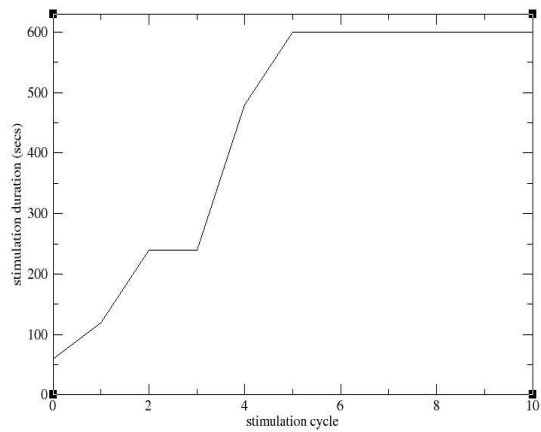
4.3 XCS Control: Results

After a number of experiments it became clear that the XCS was only able to alter a neuronal network's behaviour in roughly a third of cases. Figure 8 shows an example where it was able to cause the required spiking response to the stimulus. As can be seen, and as was typical here, the XCS controller achieves this by increasing the duration for which the stimulation is applied. However Figure 9 shows a case where no significant change in spiking appears to have occurred regardless of how the XCS adjusts the network's stimulation. In other cases the average spiking response decreases during the experiment regardless of stimulation duration (not shown). These figures show both the exploration and exploitation trial behaviour of XCS, i.e., the actual on-line stimulation as experienced by the neuronal networks; unlike the chemical systems above every stimulation has a potential long-term effect on the neuronal network.

Given these findings we implemented the aforementioned behaviour shaping/learning protocol of Shahaf and Marom [2001] for comparison. This scheme, in contrast to the more widespread consideration of neuromodulatory reward mechanisms for learning, is inspired by the work of behaviourists such as Clark Hull (e.g., [1943]). Known as the Stimulus Regulation Principle (SRP) it proposes that reward and hence learned behaviour is achieved through the removal of the driving stimulus. That is, neurons cease a constant alteration to their connectivity when the driving stimulus is removed and hence the behaviour becomes fixed; no other mechanism, i.e., no neuromodulator, is required for such (low-level) learning.



(a)

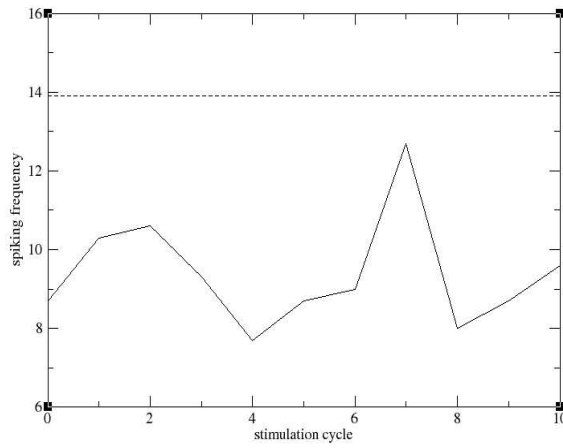


(b)

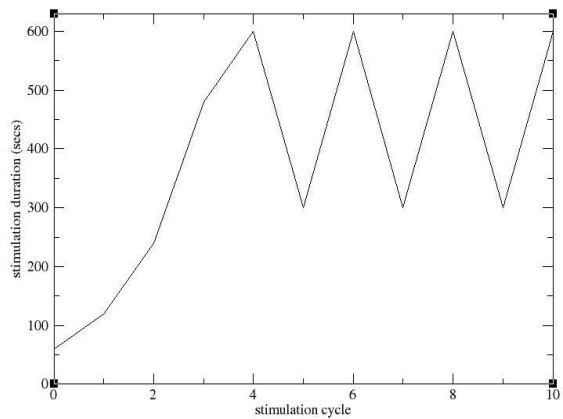
Figure 8. Example learning behaviour under XCS control, showing the spiking frequency response becoming repeatedly higher than the target indicated by the dashed line (a) and how XCS altered the stimulus application time to achieve this (b).

In our implementation the target spiking frequency was again the mean plus two standard deviations recorded under spontaneous behaviour for 300 seconds. The same stimulus was applied as before and removed either

when the required spiking response was obtained (as a running average over the last 3 seconds, as before) or if 600 seconds had elapsed. Again, a 300 second rest period between applications was allowed.



(a)

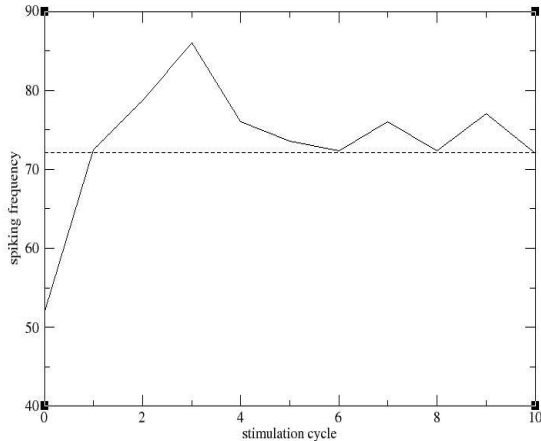


(b)

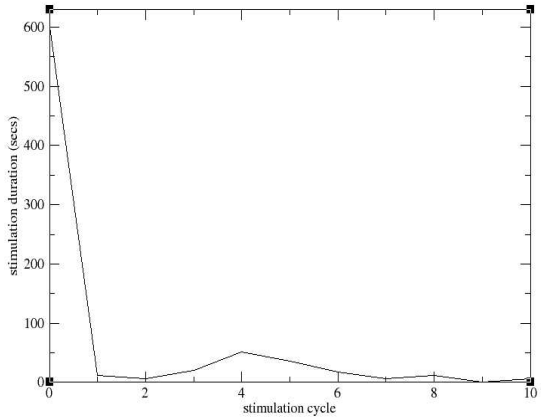
Figure 9. Example unsuccessful learning behaviour under XCS control, showing the spiking frequency response never rising to the target indicated by the dashed line (a) and how XCS altered the stimulus application time (b).

Figure 10 shows a successful experiment akin to those reported by Shahaf and Marom. Here the amount of time the stimulus must be applied before the required spiking frequency is seen rapidly decreases until it is consistently obtained almost immediately with every application. To our knowledge this represents the first reproduction of the work by Shahaf and Marom. However we again found that such results occurred only about a

third of the time. Figure 11 shows an example where the target frequency is never seen, i.e., the stimulation always remains applied for the maximum of 600 seconds, and the spiking frequency drops over time. Examples with no significant change were again also seen (not shown).

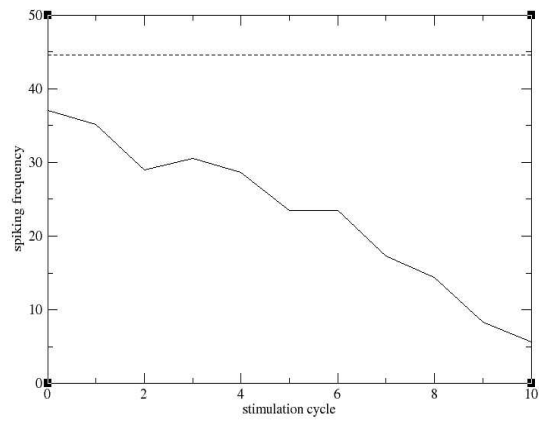


(a)

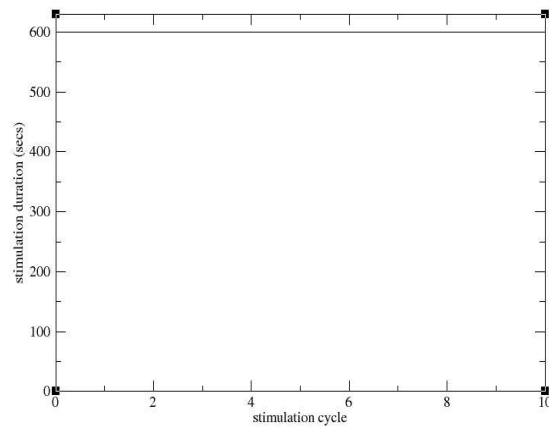


(b)

Figure 10. Example learning behaviour under SRP control, showing the spiking frequency response becoming repeatedly higher than the target indicated by the dashed line (a) and how the stimulus was applied/removed to achieve this (b). The spiking frequency shown is the last recorded on a given cycle.



(a)



(b)

Figure 11. Example unsuccessful learning behaviour under SRP control, showing the spiking frequency response falling away from the target indicated by the dashed line (a) and how the stimulus was therefore constantly applied throughout (b). The spiking frequency shown is the last recorded on a given cycle.

5 Conclusions

Excitable and oscillating chemical systems have previously been used to solve a number of computational tasks. However we suggest that the lack of compartmentalisation in the majority of the previous systems limits the domain of solvable tasks thus making it difficult to realise general-purpose computing. We propose that utilising networks of coupled oscillating chemical reactions will open chemical computing to wider domains and are interested in using light to construct such compartments on the surface of a gel – cells in a regular grid here. In this paper we have presented initial results from a methodology by which to achieve the complex task of designing such systems - through the use of evolutionary learning techniques. We have shown using both simulated and real systems that it is possible control the behaviour of a light-sensitive BZ reaction using XCS.

Neuronal networks represent another form of unconventional system by which to achieve computation. However we suggest that the use of two-dimensional networks is a somewhat impoverished formalism and that organotypic three-dimensional networks represent a potentially fruitful avenue of research. The results from using XCS, and then an SRP-inspired protocol, to induce learning indicate three possible rudimentary responses to simple stimulation from such *in vitro* neuronal networks: *excitation*, giving the potential for significant increases in typical spiking behaviour; *inhibition*, wherein spiking behaviour decreases due to stimulation; and *unchanging*, i.e., no significant shift in spiking behaviour over spontaneous behaviour is seen due to the stimulus. We found that each such behaviour was equally likely and that neither learning protocol could affect the underlying behaviour of a given neuronal network.

Jimbo et al. [199], using monolayer cultures of mammalian cortex, reported simple stimulation to an electrode could induce either an excitatory or inhibitory response at other electrodes - “pathway-dependent plasticity”. Our results therefore suggest the same is also true for aggregate neuronal cell cultures but that a third class of behaviour is possible. It is not clear at this time whether such behaviour is typical in monolayers.

We are currently exploring the use of XCS to elicit more subtle responses to stimulation from neuronal networks and extending the chemical scenario presented to include larger grids containing many more concurrent fragments of excitation. In the longer term, our aim is to study information processing in chemical and neuronal

non-linear media in order to determine fundamental principles for the design of such novel non-linear media computers and to abstract new unconventional computing mechanisms.

Acknowledgements

This work was supported by EPSRC grant no. GR/T11029/01.

References

- Adamatzky, A. (2001) *Computation in Nonlinear Media and Automata Collectives*. IoP Publishing.
- Agladze K, Aliev RR, Yamaguchi T & Yoshikawa K. (1996) Chemical Diode. *J Phys Chem*. 100: 13895-13897.
- Bar-Eli, K. & Reuveni, S. (1985) Stable stationary-states of coupled chemical oscillators. Experimental evidence, *J Phys Chem*. 89: 1329-1330.
- Bar-Eli, K. (1985) On the stability of coupled oscillators. *Physica D* 14: 242-252.
- Booker, L. (1989) Triggered Rule Discovery in Classifier Systems. In Schaffer (ed.) *Proceedings of the International Conference on Genetic Algorithms*. Morgan Kaufmann, pp265-274.
- Bull, L. (2004)(ed) *Applications of Learning Classifier Systems*. Springer.
- Bull, L. & Kovacs, T. (2005)(eds) *Foundations of Learning Classifier Systems*. Springer.
- Butz, M. & Wilson, S.W. (2002) An Algorithmic Description of XCS. *Soft Computing* 6(3-4): 144-153.
- Crowley, M.F. & Field, R.J. (1986) Electrically coupled Belousov-Zhabotinskii oscillators 1. Experiments and simulations, *J Phys Chem*. 90: 1907-1915.
- Crowley, M.F. & Epstein, I.R. (1989) Experimental and theoretical studies of a coupled chemical oscillator: phase death, multistability and in-phase and out of phase entrainment. *J Phys Chem*. 93: 2496-2502.

- DeLong, G.R. (1970) Histogenesis of fetal mouse isocortex and hippo-campus in reaggregating cell cultures. *Dev. Biol.* 22:563-583.
- DeMarse, T.B., Wagenaar, D.A., Blau, A.W. & Potter, S.M. (2001) The Neurally Controlled Animat: Biological Brains acting with Simulated Bodies. *Autonomous Robotics* 11: 305-310.
- Dolnik, M. & Epstein, I.R. (1996) Coupled chaotic oscillators. *Physical Review E* 54: 3361-3368.
- Dorigo, M. & Colombetti, M. (1998) *Robot Shaping*. MIT Press.
- Field, R. J. & Noyes, R. M. (1974) Oscillations in chemical systems. IV. Limit cycle behavior in a model of a real chemical reaction. *J. Chem. Phys.* 60, 1877-1884.
- Gorecki, J., Yoshikawa, K. & Igarashi, Y. (2003) On chemical reactors that can count. *J Phys Chem. A* 107: 1664-1669.
- Hjelmfelt, A. & Ross, J. (1993) Mass-coupled chemical systems with computational properties. *J Phys Chem.* 97: 7988-7992.
- Hjelmfelt, A., Schneider, F.W. & Ross, J. (1993) Pattern-recognition in coupled chemical kinetic systems. *Science* 260: 335-337.
- Hjelmfelt, A., Weinberger, E.D. & Ross, J. (1991) Chemical implementation of neural networks and Turing machines. *PNAS* 88: 10983-10987.
- Hjelmfelt, A., Weinberger, E.D. & Ross, J. (1992) Chemical implementation of finite-state machines. *PNAS* 89: 383-387.
- Holland, J.H. (1975) *Adaptation in Natural and Artificial Systems*. University of Michigan Press.
- Holland, J.H. (1986). Escaping brittleness: the possibilities of general-purpose learning algorithms applied to parallel rule-based systems. In Michalski, Carbonell, & Mitchell (eds) *Machine learning, an artificial intelligence approach*. Morgan Kaufmann, pp48-78.

- Holz, R. & Schneider, F.W. (1993) Control of dynamic states with time-delay between 2 mutually flow-rate coupled reactors *J Phys Chem.* 97: 12239.
- Hull, C. (1943) *Principles of Behaviour*. Appleton-Century-Crofts.
- Jimbo, Y., Tateno, T. & Robinson, H. (1999) Simultaneous Induction of Pathway-Specific Potentiation and Depression in Networks of Cortical Neurons. *Biophysics Journal* 76(2): 670-678.
- Jimbo, Y., Kawana, A., Parodi, P. & Torre, V. (2000) The Dynamics of a Neuronal Culture of Dissociated Cortical Neurons of Neonatal Rats. *Biol. Cybern.* 83: 1-20
- Jung, P., Cornell-Bell, A., Madden, K.S. & Moss, F.J. (1998) Noise-induced spiral waves in astrocyte syncytia show evidence of self-organized criticality. *Neurophysiol.* 79: 1098-1101.
- Kawato, M. & Suzuki, R. (1980) Two coupled neural oscillators as a model of the circadian pacemaker. *J Theor Biol*, 86: 547-575.
- Krug, H.-J., Pohlmann, L. & Kuhnert, L. (1990) Analysis of the modified complete Oregonator accounting for oxygen sensitivity and photosensitivity of Belousov-Zhabotinsky systems, *J. Phys. Chem.* 94, 4862.-4866.
- Kuhnert, L., Agladze, K.I. & Krinsky, V.I. (1989) Image processing using light sensitive chemical waves, *Nature* 337: 244-247.
- Laplante, J.P., Pemberton, M., Hjelmfelt, A. & Ross, J. (1995) Experiments on pattern recognition by chemical kinetics. *J Physical chemistry* 99: 10063-10065.
- Lebender, D. & Schneider, F.W. (1994) Logical gates using a nonlinear chemical reaction, *J. Phys. Chem.* 98: 7533-7537.
- Michalewicz, Z. & Fogel, D. (1999) *How to Solve it*. Springer Verlag.
- Moscona A., (1961) Rotation mediated histogenic aggregation of dissociated cells. *Exp. Cell Res.* 22: 455-475.

- Motoike, I.N., Yoshikawa, K., Iguchi, Y. & Nakata, S. (2001) Real time memory on an excitable field. *Physical Review E* 63 (036220): 1-4.
- Potter, S.M. (2001) Distributed Processing in Cultured Neuronal Networks. In M. Nicolelis (ed) *Progress in Brain Research* vol. 130, pp1-14.
- Rossler, O.E. (1974) In M. Conrad, W. Guttinger & M. Dal Cin (eds) *Lecture notes in Biomathematics*. Springer, pp 399-418 and 546-582.
- Ruaro, M.E., Bonifazi, P. & Torre, V. (2005) Toward the Neurocomputer: Image Processing and Pattern Recognition with Neuronal Cultures. *IEEE Trans. on Biomedical Engineering* 52(3): 371-383.
- Seeds, N.W. (1971) Biochemical Differentiation in Reaggregating Brain Cell Culture. *Proc. Nat. Acad. Sci. USA* 68(8): 1858-1861.
- Shahaf, G. & Marom, S. (2001) Learning in networks of cortical neurons. *Journal of Neuroscience* 21 (22):8782-8788.
- Sidman, R.L. (1970). In F.O. Schmitt (Ed.) *The Neurosciences Second Study Program*. Rockefeller University Press, pp100.
- Sielewiesiuk, J. & Gorecki, J. (2002) Passive barrier as a transformer of chemical frequency. *Journal of physical chemistry A* 106: 4068-4076.
- Steinbock, O., Kettunen, P. & Showalter, K. (1996) Chemical wave logic gates. *J Phys Chem*, 100: 18970-18975.
- Steinbock, O., Toth, A. & Showalter, K. (1995) Navigating Complex Labyrinths: Optimal Paths from chemical waves. *Science* 267: 868-871.
- Stone, C & Bull, L. (2003) For Real! XCS with Continuous-Valued Inputs. *Evolutionary Computation* 11(3): 299-336.
- Stuchl, I. & Marek, M. (1982) Dissipative structures in coupled cells: experiments. *J. Phys Chem*. 77: 2956-63.
- Sutton, R. & Barto, A. (1998) *Reinforcement Learning*. MIT Press.

- Takayam, Y. & Jimbo, Y. (2006) Modification of Evoked Responses Induced by Correlated Stimuli in Cultured Cortical Networks. In *Proceedings of the 5th International Meeting on Substrate-Integrated Micro Electrode Arrays*. BIOPRO, pp22-25.
- Trapp B. D., Honneger, P., Richelson, E. & Webster, H. deF. (1979) Morphological Differentiation of Mechanically Dissociated Fetal Rat Brain in Aggregating Cell Cultures. *Brain Research* 160:117-180
- Toth, A., Gaspar, V. & Showlater, K. (1994) Signal transmission in Chemical Systems: Propagation of chemical Waves through capillary tubes. *J. Phys Chem.* 98: 522-531.
- Uroukov, I., Ma, M., Bull, L. & Purcell, W. (2006) Electrophysiological Measurements in 3-Dimensional *In Vivo*-Mimetic Organotypic Cell Cultures: Preliminary Studies with Hen Embryo Brain Spheroids. *Neuroscience Letters*
- Wagenaar, D., Madhavan, R., Pine, J. & Potter, S.M. (2005) Controlling Bursting in Cortical Cultures with Closed-Loop Multi-Electrode Stimulation. *Journal of Neuroscience* 25(3): 680–688.
- Wang, J., Kadar, S., Jung, P. & Showalter, K. (1999) Noise driven Avalanche behaviour in subexcitable media. *Physical Review Letters* 82: 855-858.
- Watkins, C.J. (1989) Learning from Delayed Rewards. Ph.D. Thesis, Cambridge University.
- Wilson, S.W. (1995) Classifier Fitness Based on Accuracy. *Evolutionary Computing* 3: 149-175.