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Development of artificial neuronal networks for molecular communication

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ABSTRACT

Communication at the nanoscale can enhance capabilities for nanodevices, and at the same time open new opportunities for numerous healthcare applications. One approach toward enabling communication between nanodevices is through molecular communications. While a number of solutions have been proposed for molecular communication (e.g. calcium signaling, molecular motors, bacteria communication), in this paper, we propose the use of neuronal networks for molecular communication network. In particular, we provide two design aspects of neuron networks, which includes, (i) the design of an interface between nanodevice and neurons that can initiate signaling, and (ii) the design of transmission scheduling to ensure that signals initiated by multiple devices will successfully reach the receiver with minimum interference. The solution for (i) is developed through wet lab experiments, while the solution for (ii) is developed through genetic algorithm optimization technique, and is validated through simulations.

1. Introduction

The field of nano/molecular communication is a new area of communication research paradigm, aiming to provide communication capabilities between nanoscale devices [2,23]. Increasing the communication capabilities of nanoscale devices can increase their capabilities and application base, in particular, in the healthcare and pharmaceutical industry. The current research of communication at nano and molecular scale include both molecular communication as well as electromagnetic based nanoscale communication [2,1]. Molecular communication enables communication to be performed between nanoscale devices by utilizing biomolecules as a communication medium, while electromagnetic based nano communication allows communication between nanodevices using wireless technology.

In this paper, we will focus on molecular communication, particularly investigating the use of neurons as a networking component. We will discuss a number of development aspects of neurons that can be implemented as an underlying network to support molecular communication, which includes the following (i) the ability to artificially invoke and suppress signaling in neurons, and (ii) a scheduling design in a neuron topology that could minimize signaling interference. In the case of (i), the solution can be used to allow external devices to interface to neurons and switch the neurons to signal transmission.

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Once devices have switched and signaled the neuron, then the case of (ii) can be used to ensure the signaling transmitted through the neuron network will minimize interference to ensure that signals propagated will reach the destination. We discuss a number of characteristics of neuronal transmission as signaling of Ca^{2+} ions trying to highlight the strict relation between these ions and the transmission of the action potential from a pre-synaptic neuron to the post-synaptic neuron. The signaling behavior of the neurons will be considered in the design process for the scheduling protocol for the neuron networks. Our approach used for designing the scheduling algorithm is based on optimization techniques. Optimization is a common approach used in various network design problems, such access scheduling [19,18], routing and resource management [14] as examples.

The objective of our paper is to present design solutions that could enable nano/molecular communication researchers to use neurons as a communication network component, to transfer and re-use common design approaches, and apply best practices found in conventional communication network to nanoscale communication networks. The paper is organized as follows: Section 2 presents the related work on molecular communication and neuronal networks. Section 3 presents background information on Neurons. Section 4 presents the design of neuron to nanomachine interface, while Section 5 presents the design of the scheduling transmission over the neuronal network. Lastly, Section 6 presents the conclusion.

2. Related works

The related work is separated into two sections, which includes molecular communication as well as neuron networks.

2.1. Molecular communications

A number of solutions have been proposed for molecular communication in recent years. Example of these solutions includes the use of propagation based on molecular diffusion (e.g. calcium signaling [25]), walkway based molecular propagation [10,9], or bacteria networks [5]). Current research activities are investigating the mathematical theory of molecular communication channels, highlighting the challenges of molecular communication based nanonetworks with much addressing the physical mechanisms of molecular communication and molecular communication based nanonetworks.

A key challenge in molecular communication research is noise characterization in volatile aqueous molecular communication channels. For example, in [28,29], Pierobon and Akyildiz presented physical and stochastic noise analysis models for diffusion based molecular communication in nanonetworks. The authors develop a mathematical expression for physical processes underlying noise sources while their stochastic approach characterizes noise sources as random processes. Another challenge in molecular communication is data encoding. Typically, two mechanisms are proposed, which includes concentration encoding and molecular particle encoding. In [21], Mahfuz et al. explore solutions to concentration encoding in diffusion based molecular communication systems. The authors explores both sample and energy based decoding schemes whereby the former samples at a single instant and the latter accumulates samples over a defined period.

Accurate computational and energy models are also a key aspect in the development and understanding of communicating nanodevice. While many energy management models exist for larger scale networks, they are generally not applicable to nanonetworks where nanodevices would be more inaccessible and expected to be more energy self-sufficient. In [16] Kuran et al. propose an energy model for molecular communication via diffusion. Work is also being conducted at the data link and network layers in nanonetworks. In [24], Nakano et al. present a model for in-sequence molecule delivery inspired by out-of-order delivery techniques in computer networks. Simulations using several molecular propagation mechanisms reveal motor driven random walks result in higher probability of in-order reception. As expected, increased symbol transmission periods and receiver buffering time significantly increase probability of successful in-order reception.

While numerous works have investigated communication network theory for diffusion based molecular communications, the area of active transport for molecular communication is still in its infancy. In particular, the investigation into the use of neuron networks for active transport, which is what we aim to investigate in this paper.

2.2. Neuron networks

Neurons form highly complex network, in which they are responsible for processing information in the brain. Kotsavasiloglu et al. [12,15] developed computational models to study the behavior of biological neural networks and also discussed the connection between computational and biological models. The authors performed simulations on the neuronal network of healthy neurons, and varied the synapse failure rate, refractory periods, excitation synapse ratio, as well as synapse delay. Firstly, they focus on the signal transmission and analysis, and investigated the existing critical crossover value regarding the loss of connections by studying the robustness and degradation of dynamics on a network by varying the number of connections which corresponds to the synapses of the biological neural networks. The authors later developed a model to discover the results of synapse loss which can occur in biological systems under certain diseases, such as Alzheimer's and Parkinson's [15].

Breskin et al. [3] set up an experimental design to determine statistical properties of living neural network. They separate the initially connected network to the fully disconnected smaller clusters and use a graph-theoretic approach to study the connectivity. It is observed that if the network's connectivity increases, a percolation transition occurs at a critical synaptic strength. Their study also indicates that connectivity of neural networks is based on Gaussian distribution rather than scale free network. Gabay et al. [6] developed a new approach of pre-defined geometry of neuronal network clusters

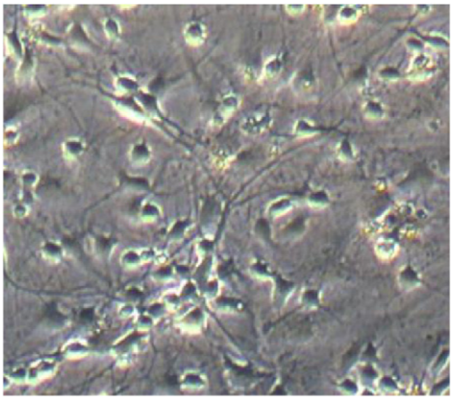

Fig. 1. Examples of pattern of connections in a self-organized network of neurons; please note cell bodies (or soma), axons (larger filaments) and dendrites (smaller filaments). (magnification ×20).

using carbon nanotube clusters. In the proposed approach, neurons migrate on low affinity substrate to high affinity substrate on a lithographically defined carbon nanotube template. Upon reaching the high affinity substrates, the neurons will form interconnected networks by sending neurite messages. A number of works have also looked at mechanism to stimulate neurons, such as the use of LED matrix [8].

Numerous works have studied network properties of neurons, such as connectivity and topology formation network. However, we take a number of these studies further by utilizing the understanding of these networks, and the ability to use them to support molecular communications.

3. Properties of neuron signaling

This section will describe the properties of neurons, where these properties will be used for the design process described in the later sections. Neurons are a basic unit of a neuronal network, where its structure is composed of the cell body, dendrites, the axon and its terminals [4]. Neurons have tremendous abilities to self-organize and form networks through transmission of neurites, as discussed earlier in the works of Gabay et al. [6]. Fig. 1 shows an example of neurons that have self-organized into a network.

As a component of the neural network, neurons are able to process information in two forms, which are electrical and chemical signals. The signaling process is created from an action potential depolarization in the pre-synaptic membrane that opens the Voltage Operated Channels (VOCs), which in turn potentiates the influx of extracellular calcium ions (Ca^{2+}) [31]. Therefore, increases in intracellular calcium concentration initiate exocytosis of synaptic vesicles containing neurotransmitters. The neurotransmitters are transmitted through the synapse between the axon terminal of the pre-synaptic neuron and the post-synaptic neuron. Therefore, the action potential can be seen as a traveling gradient of ions concentration (Na^+/K^+, Ca^{2+}) along the whole length of the cell structure. Based on this property, the information that is transferred from one neuron to the next can be considered as the action potential that is generated by a cascade

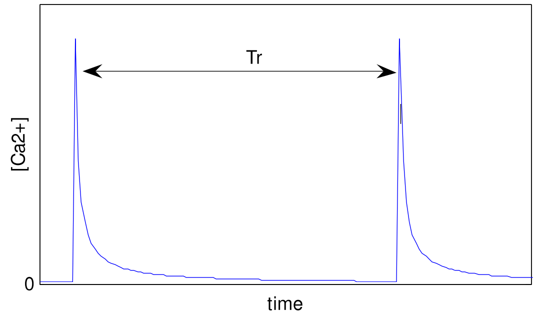

Fig. 2. Intracellular Ca^{2+} concentration in a neuron. Ca^{2+} release events must be separated by at least the refractory time T_r, the time required to replenish internal Ca^{2+} stores.

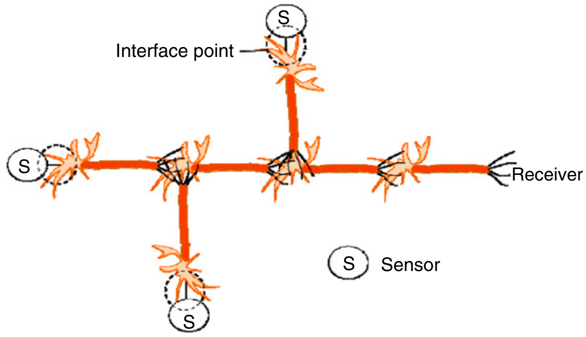

Fig. 3. Interface point between sensor devices to neuron.

of chemical events occurring on the surface of the cell membrane.

Calcium signaling has an inherent property, which is illustrated in Fig. 2. Once calcium within a neuron is activated, there is a refractory period known as T_r. During this refractory period, the neuron will not be able to process any other incoming signals from other neurons, until the T_r period is complete.

4. Design of neuron activation interface

In this section we will present the design of interface to activate neuron signaling. Our scenario application is illustrated in Fig. 3. In our scenario we have sensors that are interfaced to neurons, and activates signaling, where the signaling is propagated to the receiver. Therefore, a requirement is the sensor to be able to emit an agent that can activate the signaling. It is most ideal if this requirement could be achieved through a non-invasive approach (e.g. the firing of the neuron can be controlled externally). Our main objective is to invoke trans-membrane calcium chemical signaling which in turn will induce signaling between the neurons. Therefore, our aim is to also model the calcium signaling that is artificially induced, and to measure this at two different neurons to demonstrate how signals have traveled through the network, as it induces the calcium signaling of the neurons along the path.

We performed experiments to demonstrate this activation process, using primary cortical neuronal cultures obtained from 1-day old rats and plated on customized

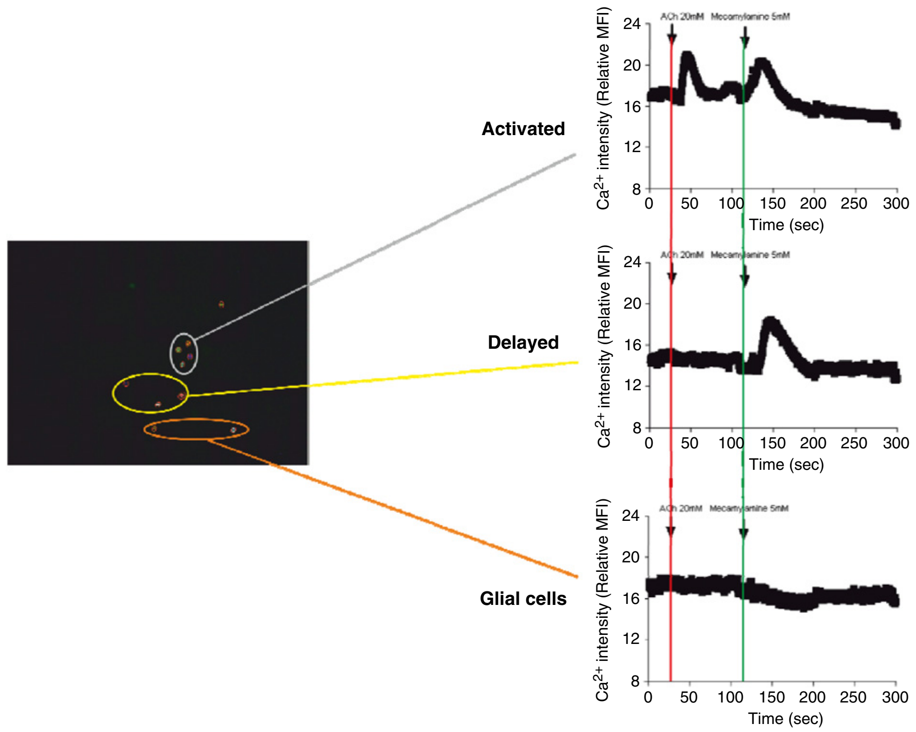

Fig. 4. Fluorescence image of neuronal cells recorded over an interval of 300 s. In this experiment the microinjection and diffusive gradient of Acetylcholine (within the first 30 s of recording) and respective injection and inhibition of Mecamylamine (120 s) is illustrated. Plot of Ca^{2+} flow over the 300 s recording showing different response times of clustered neurons according to their relative position. The red vertical line represents the time flag at which the ACh was microinjected, while the green line represents injection of Mecamylamine as inhibitor. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Microelectrode Arrays (MEAs). In this experiment, Acetylcholine (ACh) is the agonist used to stimulate firing of neuronal action potentials while Mecamylamine is the antagonist which suppresses neuronal firing, thus exhibiting a switch-like function. Neural communication can be demonstrated by Ca^{2+} signaling using *in vitro* cultures. Increasing intracellular Ca^{2+} signifies neuronal activation by enhancing neurotransmitter release and thus potentiating action potentials between neurons. Fig. 4 demonstrates the results from the experiment to show the activation of neurons. Relative mean fluorescent intensity as a measure of basal Ca^{2+} activity was recorded. For application of ACh (20 mM) at 40 s, a steady increase of Ca^{2+} ions was detected while addition of Mecamylamine (5 mM) indicated that Ca^{2+} ion flow was suppressed since fluorescence was reduced below basal levels. Therefore, demonstrating the ability for external sensor devices to use these agents to switch on/off signaling onto the neuron network. At the same time, the experiment also strengthens the idea that Ca^{2+} is a valid marker to track signals transmitted between two neurons.

Fig. 5 demonstrates the results of the experiments on the MEA, where measurements are taken at different points of the network. As we can see in Fig. 5(a), the majority of neurons were in a dormant state during basal measurement. However, following ACh application (Fig. 5(b)), potentiates neuronal firing, thus increasing Ca^{2+} fluorescence intensity. Conversely, Mecamylamine (Fig. 5(c)) suppresses neuronal firing and decreases Ca^{2+} intensity. In this particular experimental example, the white arrow is where the ACh is applied, and shows the neuron firing, and another measurement point is taken at the black arrow, showing the signal propagation. The application of ACh could represent a digital 1 bit transmission through the neuronal network.

5. Design of scheduling protocol for neuronal network

The previous section presented our solution for initiating Ca^{2+} signaling on a neuron from an external sensor device. However, if the sensors emit ACh randomly to initiate signaling, this could lead to large number of interferences in the neuron network, which in turn can lead to corruption of information in the receiver. Therefore, a next requirement in our design is a scheduling protocol to ensure that minimum interference will be encountered during transmission to ensure that signals received are not corrupted. We return back to our scenario presented in the previous section, which is illustrated in Fig. 6. As illustrated in the figure, our aim is to ensure that initiated signals will not result in any collisions during the transmission along the network to a single receiver.

The main aspect of this study is the interaction between the normal activity of the neuron network and the packages of information "injected" simultaneously on the very same "line". As discussed above, the neurons possess a refractory period in which no signals can be transmitted. This results in a sort of bus timing for the signals to pass

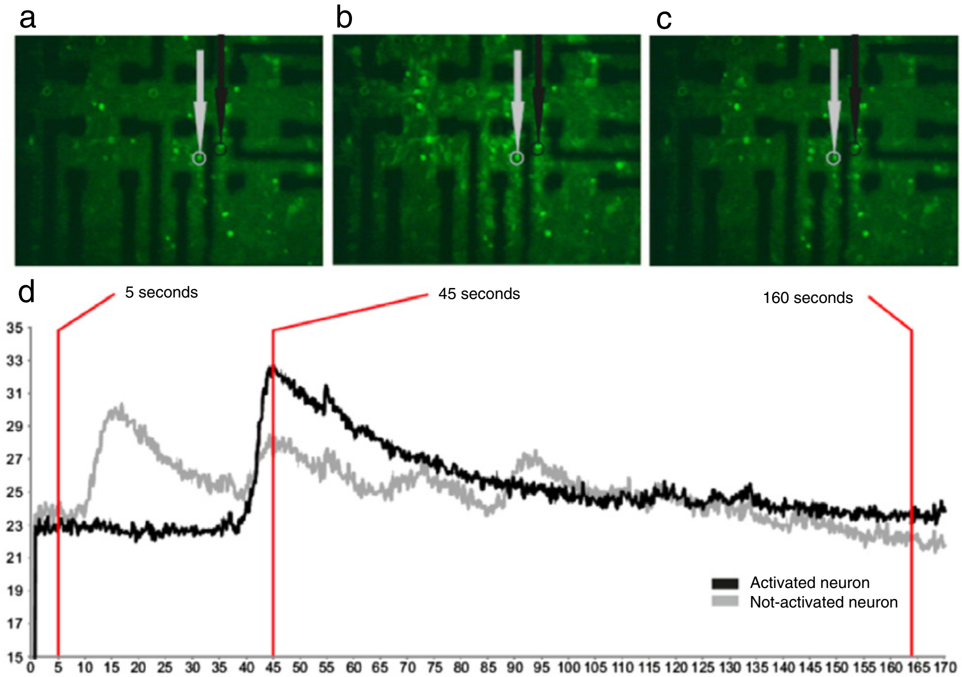

Fig. 5. Fluorescent intensities of intracellular Ca^{2+} in primary cortical neurons cultured on customized microelectrode arrays (MEAs) stained with Fluo-4 AM. The red vertical lines represent the overall course of Ca^{2+} fluorescent intensities for the sample of neurons at the three distinct time points of the experiments. (a) Measurement of Ca^{2+} fluorescent intensities in two sample neurons (gray and black circles) at baseline after 5 s; (b) Fluorescent intensity in the two neurons at 45 s following ACh (20 mM) application demonstrates an increase in Ca^{2+} ion flow as indicated by a brighter intensity of the cell body in the black circle; (c) Fluorescent intensity of the two sample neurons following Mecamylamine (5 mM) addition at 160 s from beginning of recording. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

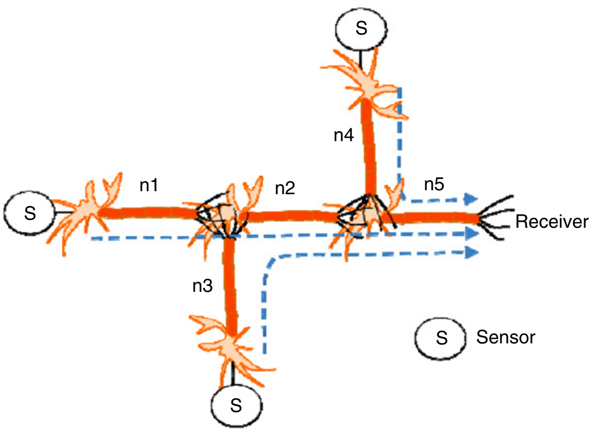

Fig. 6. Sensor transmission along neuronal networks to single receiver.

through the network. Matching the electric signal carried by the action potential with the Ca^{2+} it may be possible to create a parallel communication system that will not compete with the natural one. Fig. 7 illustrates our single bit—Time Division Multiplex Access (TDMA) scheduling, where we aim to schedule the firing of specific neuron with respect to time. Fig. 7(a)–(d) shows the neurons that are fired with respect to time, while Fig. 7(e) shows this from a time division perspective (each color represents a single bit of information transmitted from a specific sensor). Fig. 7(e) also shows the single bit transmission for each time slot. The reason that only a single bit is transmitted per slot is based on two assumptions—(1) there are only two amplitudes that can be produced for bit 1 and 0, and (2) after transmitted, the neuron has a waiting time of T_R during the refractory period, where this waiting time can be used by another sensor to transmit to maximize parallel transmission.

Before we explain our TDMA scheduling algorithm, we will first describe some inherent differences between a neuron link and a wireline communication link. In most communication networks, each link will usually have different bandwidth values. Therefore, the routing process between a source to destination will usually be able to

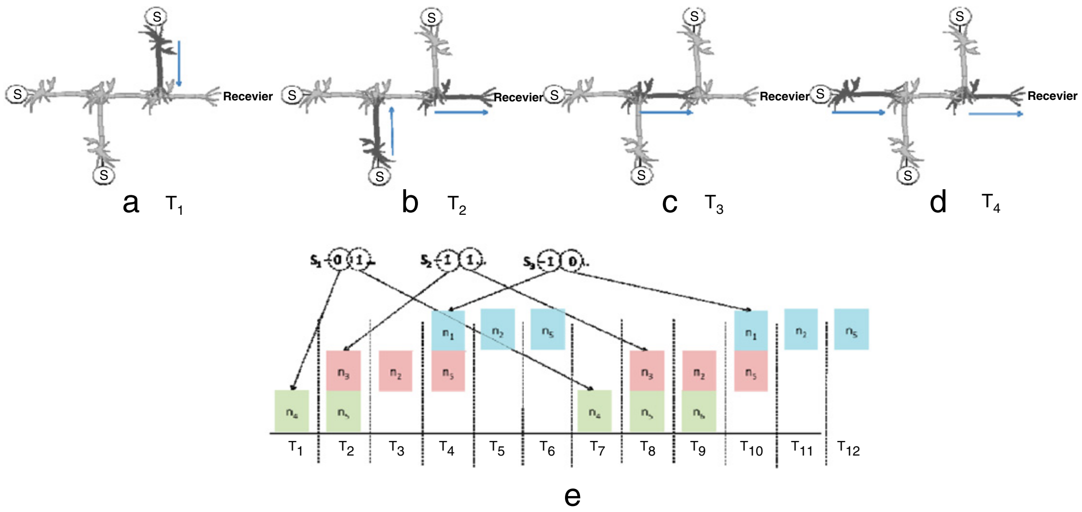

Fig. 7. Single bit—TDMA scheduled transmission from different sensors along a neuron network (each color represents a single bit information from a sensor). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

accommodate a number of flows. However, this is different in the case of a neuron link, where each link of the neuron can only accommodate finite number capacity (this capacity may only represent a single bit). At the very same time, once a neuron is fired, as described in Section 3 (Fig. 2), there is a refractory period where the Ca^{2+} returns to the intracellular stores. During this refractory period, no signal can be transmitted through the neuron. However, this is different from a conventional wireline link, where flows that are terminated can accommodate new flows immediately. While there are differences, there are also similarities between the two. Firstly, as signals are propagated from neuron to neuron, this could be compared to a burst-like traffic behavior found in conventional communication links. Secondly, delays in intermediate nodes of a communication network (due to queuing delays) are very similar to synaptic delays found between the junctions of the neurons. We will consider a number of these properties when we are designing our TDMA scheduler for the neuronal network.

The scheduling design for the neuron network is based on an optimization problem, and the specific technique that we have applied is based on genetic algorithm. The following sections will describe background information on genetic algorithm and some of their applications, the problem formulation for the TDMA scheduling protocol, and we will also present the simulation results of our proposed design algorithm.

5.1. Genetic algorithm

Genetic Algorithm is an optimization search heuristic [7]. The search process is through a guided search that is inspired from the natural evolution. The first step is by creating a random initial population of solutions. This initial population will then go through a series of evolutionary generations, where an optimum solution will slowly emerge based on certain genetic operators. These operators include crossover, mutation, and selection. Each solution of the population is called a chromosome, and has an associated fitness function. Therefore, the optimal solution will be achieved, once the fitness function of the population starts to converge and stabilize.

Genetic algorithm has been used in a number of different communication network problems. Example of these problems includes communication network routing [30,13], as well as network services [32,17,26]. In these various applications, genetic algorithms have produced improved performance compared to numerous approaches, both in design and run-time applications. Therefore, in the same way that genetic algorithm has been successfully applied to communication network problems, we aim to re-use this approach for design of scheduling in neuronal networks. At the same time, since our problem is defined as an optimization problem, we believe that genetic algorithm is an appropriate approach.

5.2. Problem formulation

The objective of our design problem is to maximize the number of signaling messages (x_{si}) as well as minimize the time difference between the sensors that release the ACh ($t_{a,si}$) to activate the signaling, over a period of time T_p. Information that is provided for the optimization problem includes the number of sensors $s_i = (s_1, s_2, \ldots, s_i, \ldots, s_M)$, where M is the total number of sensors; location of the sensors as to which neuron this is connected to; total number of N neurons, where $n_j = (n_1, n_2, \ldots, n_j, \ldots, n_N)$; as well as the neuron topology. Therefore, the objectives can be represented as:

$$maximize \sum_{i}^{M} \sum_{y}^{T_p} \sum_{j}^{N} x_{s_i,j,t} \tag{1}$$

$$\sum_{k.l}^{s_i} \frac{1}{|t_{a,k} - t_{a,l}|}, \quad l \neq k, \tag{2}$$

subject to:

$$n_{i \neq j,t} \quad (i,j) \in N \tag{3}$$

$$t_{a,j} \leq T_p \quad j \in s_i, \tag{4}$$

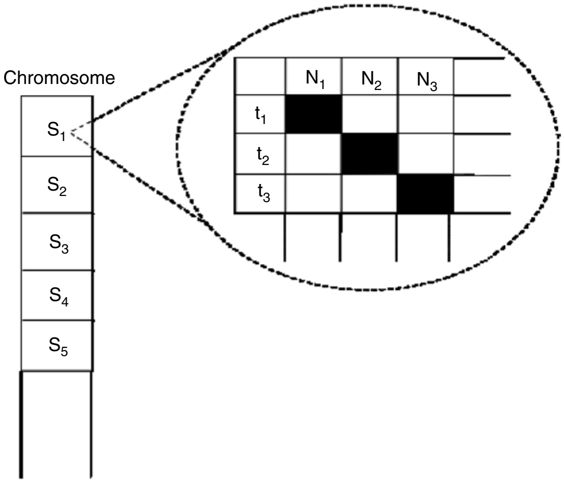

Fig. 8. Chromosome structure which is composed of an array of sensor, which contains a two-dimensional array composed of time steps and neurons in the topology.

where x is the message passing through a neuron. Objective (1) is to maximize the total number of parallel number of neurons transmitting messages in the topology, where $x_{s_i,j,t}$ is the message from sensor s_i passing through neuron j at time t. Objective (2) is to minimize the difference in time ($t_{a,si}$) that sensor s_i fires the neuron through the release of ACh (the aim here is to pack the firing time between the sensor to be as close as possible). Eq. (3) specifies that at a specific time t, the neurons that are fired in the topology must be unique, while Eq. (4) specifies that all initial timing of a sensor t_a must be less that the T_p.

5.3. Genetic operators

Chromosome: As described earlier, the genetic algorithm operates by evolving over a set of solutions, until an optimum solution is reached. Each solution in a genetic algorithm is referred to as a chromosome. For our specific application, the chromosome structure for our solution is illustrated in Fig. 8. The chromosome is composed of a set of sensors, where each sensor is composed of a two-dimensional array, where the rows represent the time steps for the whole time period T_p, while the columns represent the neurons of the topology. During the initial population creation, a random initial time t_a and neuron n_a is selected and set to 1. The next period is set to $t_a + t$ and neighbor neuron n_j of n_a, and this continues until we reach the last neuron of the topology or T_p. This procedure is repeated for all sensors. The time steps and neurons that have been set are recorded, so that when there is a conflict, the solution is eliminated, as this is an infeasible solution. The fitness function of each chromosome is calculated as:

$$f_c = \log\left[\alpha \sum_{k,l}^{s_i} \frac{1}{t_{a,k} - t_{a,l}} + (1-\alpha)\sum_{j}^{N} n\right], \quad k \neq l,\ k,l \in s_i. \tag{5}$$

Selection: A roulette wheel selection process is used for selection of chromosome solution for the next generation.

Table 1
Genetic algorithm configuration.

Population size	200
Number of generations	200
Crossover probability	70%
Mutation probability	5%

Table 2
Configuration for Topology 1.

Number of neurons	43
Number of sensors	11
Total time steps	40
Weight (alpha)	0.5

The roulette wheel selection operates as follows: A total sum of fitness f_T for all chromosome is calculated, after which a probability P_S is calculated per chromosome by the ratio of f_c/f_T. Therefore, this ensures that the fitter chromosomes are selected for the next generation.

Crossover: The crossover probability P_{CO} is randomly assigned to each chromosome. After selection of each generation, each chromosome's P_{CO} is checked and compared to a crossover threshold. If the value is over the threshold, a crossover is performed with another chromosome with a higher value threshold. The crossover performed is a single point crossover, where the crossover point is selected randomly at a specific gene in the chromosome.

Mutation: The mutation is performed by checking if the assigned mutation probability P_M is over a threshold. A chromosome selected for mutation is performed by selecting a random time and neuron point in the two-dimensional array of the gene and changing the bit. This is then checked to make sure that it is a feasible solution.

5.4. Performance

We evaluated our algorithm on two neuronal network topology shown in Fig. 9.

A crucial requirement in our performance evaluation is the development of a suitable topology. A number of studies have investigated neuron network topologies. A common topology to represent tree topology of neuron networks is through using *Diffusion Limited Aggregation* (*DLA*) [20]. Through the branching structure, information are transferred and received. We developed a similar random tree-like topology that mimics a dendritic tree of interneuron [20], where we produced two topologies of size 43 neurons and 151 neurons. We evenly distributed sensors in the topology at a ratio of 1/4 to the number neurons. For each topology we only have one single receiver (denoted as R) in the figures.

The configuration parameters for the Genetic Algorithm are shown in Table 1. The number of neurons, sensors, as well as total time steps for the simulation is presented in Tables 2 and 3 for Topology 1 and 2, respectively.

5.5. Genetic algorithm performance

The performance of our fitness function and its convergence speed is shown in Fig. 10. We can see that the convergence to the fittest solution converges much faster for

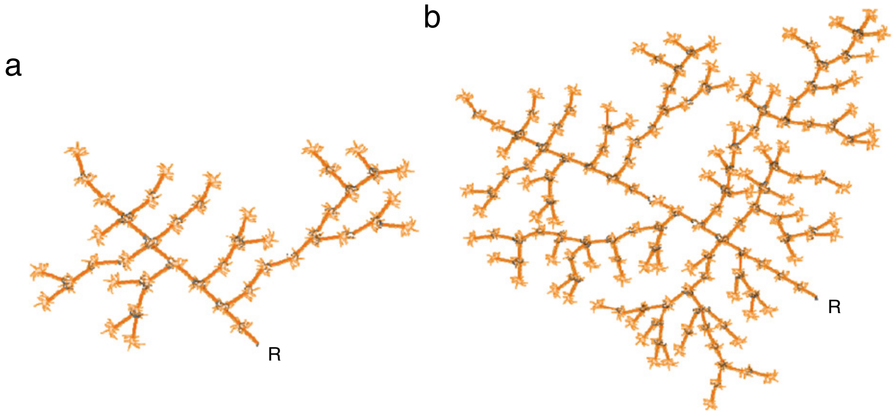

Fig. 9. Topology of neuron network evaluated (a) Topology 1, 43 neurons, (b) Topology 2, 153 neurons.

Table 3
Configuration for Topology 2.

Number of neurons	151
Number of sensors	40
Total time steps	320
Weight (alpha)	0.5

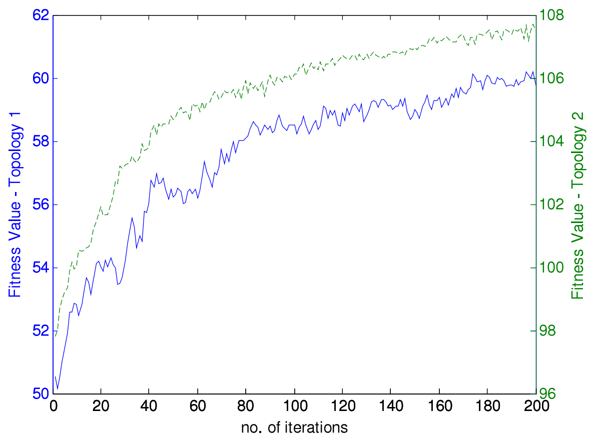

Fig. 10. Convergence performance for Topology 1 and 2 ($\alpha = 0.5$).

Topology 1, compared to Topology 2. For simplicity, we have set the weighting value α of the fitness function to be 0.5. In our future work, we intend to determine the optimal weighting value α.

5.6. Neuron network performance

Simulation results for GA based scheduling designs for both topologies are illustrated in Table 4, where the tests includes the transmission blocking rate, average neuron utilization, average transmission delay. As expected, the GA based scheduling resulted in successful reception of all transmitted messages for both topologies.

Figs. 11 and 12 shows the number of active neurons with respect to the time for the GA based solution and compares this to the random signaling of sensors. The result is aimed to show the number of parallel neurons that are fired in one instance of time. As stated previously,

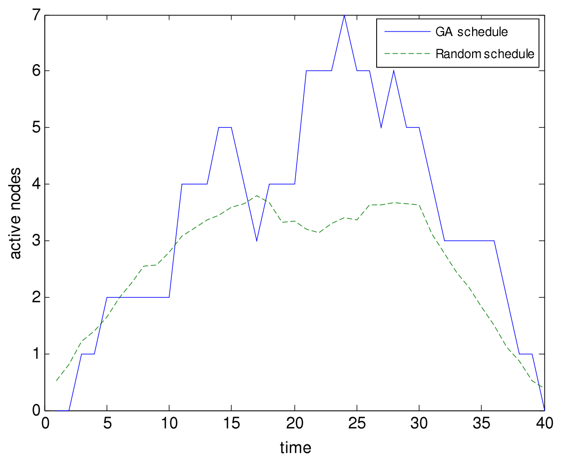

Fig. 11. Comparison of active neurons for Topology 1 between GA and Random ($\alpha = 0.5$).

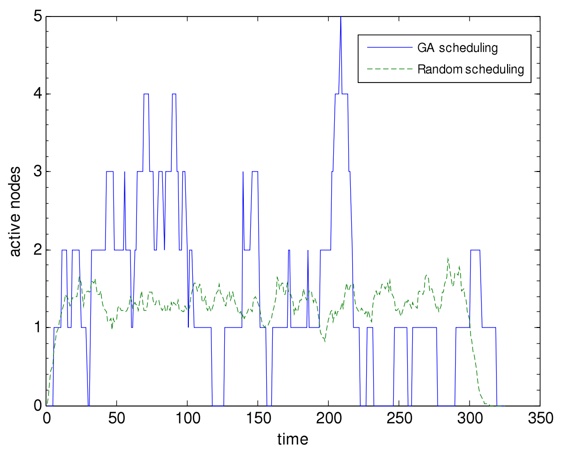

Fig. 12. Comparison of active neurons for Topology 2 between GA and Random ($\alpha = 0.5$).

the goal of the GA fitness function is to maximize neuron utilization and minimize the signaling time between the sensors. For Topology 2, the GA simulation has an average link usage of 1.45 with a minimum of 0 to maximum of 5

Table 4
Simulation results from TDMA scheduling design.

	Blocking rate		Average neuron utilization		Transmission delay (time)		Max. link usage	
	GA	Random	GA	Random	GA	Random	GA	Random
Topology 1	0	0.241	1.36	1.67	5.364	4.711	7	8
Topology 2	0	0.11	1.45	1.24	10.58	9.50	5	6

whereas the random simulation resulted in an average link usage of 1.24 nodes over all simulations with minimum of 0 and maximum of 6. As can be seen in Figs. 11 and 12, the GA simulation exhibits typical characteristics of TDMA scheduling in that the state of the system is fully determinable at any time.

The sensor locations and resulting transmission schedule from the GA is simulated. For random simulation, sensor locations are distributed normally across the neuron network and all transmit events are also normally distributed in the total transmission period (see Table 4). As with GA configuration, each sensor transmits once in the transmission period. We can see that the blocking rate for the random is approximately 0.241 and 0.11 for topology 1 and 2 respectively. The blocking rate is higher in topology one because the transmission events are confined to a much smaller time period and node group. However the average transmission delay is slightly higher than the GA solution. This is expected, since the random signaling does not consider the interference between sensor signaling, and may initiate signaling very close to each other.

The ability to design and construct neuronal networks to specific topology is crucial to the solution that is discussed in this paper. In [11] Jang et al. present a novel method that uses carbon nanotube patterned substrates to direct neuron growth. The authors report highly directional neurite growth along carbon nanotube lines which is attributed to high absorption of neuron adhesion protein by carbon nanotube patterns. This method could be used in our solution to create the neuronal network topologies discussed in this paper.

Similarly, a method to connect bionano sensors to neural networks is essential for our solution. Recent studies have shown that carbon nanofibers can be used to interface between bionano devices and neuron cells. For example, in [27], Nguyen-Vu et al. demonstrated the use of vertically aligned carbon nanofibers as an interface to neuronal networks. The authors predict this technology will have applications in implantable neural devices and the development of neuromodulation based systems. In the context of our solution, it can provide the mechanism by which bionano sensors can interface and communicate via neuronal networks.

6. Conclusion and remarks

As the field of nanotechnology gains momentum through their numerous application base, in particular for healthcare, research in communication capabilities between the devices is still in its infancy. Molecular communication aims to address communication between nanodevices in biological environment. In this paper, we present a development of artificial neuron networks for molecular communications. Inherently, neurons form self-organizing networks that enables information processing. Due to this property, our aim is to design solution that can enable communication between devices connected through a neuronal network. Our scenario is a number of sensors that can transmit information through the neuronal network to a single receiver. Our very first design is to address a mechanism that interfaces between nanodevices to neurons that can initiate neuron signaling. We present our solution through experimental work, where we allow signaling to be initiated through administering Acetylcholine to cultured neuron, and this signaling can be suppressed by administering Mecamylamine. This in turn provides capabilities for nanodevices to create switches as they are interfaced to the neurons. The second stage of our design is to determine optimal scheduled timing of release of Acetylcholine to initiate signaling, in order to minimize any interference in the neuron topology. This is set as an optimization problem, where our aim is to minimize the timing of signaling between the sensors, while maximizing the number of parallel neurons fired. Simulation results have validated our design and comparisons have been made to random signaling of sensors.

The aim of our proposed solution, as described earlier, is to develop molecular communication solutions that can exploit neuronal networks, and at the same time, to design these processes by re-using principles and approaches from communication networks. We believe, that is the first step toward investigating neuronal network as a solution for molecular communication, and can open numerous opportunities for future work.

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Appendix. Experimental setup

Primary cultured cortical neurons and plating

Primary cortical neurons were dissociated and prepared from 1-day old Ham–Wistar rats (BioResources Unit, Trinity College, Dublin 2, Ireland) as described by [22]. The cortices were dissected after humane death and decapitation and meninges gently peeled from neonate brains. Tissue was digested with trypsin from bovine pancreas (Sigma) in sterile PBS and incubated for 25 min at 37 °C. This step was neutralized with trypsin inhibitor type II S: soybean (0.2 μg/ml Sigma) and DNase (0.2 mg/ml). Cells were gently titrated and passed through a sterile mesh cell strainer (40 uM) for single cell suspension. Following centrifugation, cells were re-suspended in pre-heated neurobasal media supplemented with glutamax (2 mM), heat-inactivated horse serum, penicillin & streptomycin (100 units/ml) and B27-supplement.

Cells were seeded onto customized microelectrode arrays (MEAs), fabricated by standard lithographical processes onto borosilicate glass, at a density of 1×10^6 cells/ml coated with laminin (0.05 mg/ml) and incubated in a humidified atmosphere 5% CO_2: 95% air at 37 °C. A sealed gasket made of polydimethylsiloxane (PDMS, Dow Corning, USA) was placed over the cells to contain the neurobasal media to prevent evaporation and housed in a sterile Petri dish.

Calcium signaling

Fluo-4 AM Calcium indicator (Invitrogen, USA) was used as a fluorescent indicator of mitochondrial calcium. The co-culture of neurons and astrocytes on day-in-vitro (DIV) 5–7, were loaded with 4 μM Fluo-4 AM and pluronic F-127 which was dissolved in recording buffer and incubated in the dark for 45 min at 22 °C. Cells were washed and incubated for 30 min at 22 °C. Relative mean fluorescent intensity was measured using optical microscopy.

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