Modeling of bio-nano communication networks for the human body

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MODELING OF BIO-NANO COMMUNICATION NETWORKS FOR THE HUMAN BODY

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Modeling of Bio-Nano Communication Networks for the Human Body

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Abstract

Nanonetwork is a new research focus which presents solutions for agricultural, environmental, medical and security fields. Bio-nano hybrid network, which aims to be applied to the human body, is defined as the cooperation of biological units and possible artificial nanomachines. The physical basis of bio-compatible nanonetwork is the intrinsic signal transmission, encoding and decoding in the human body. By studying the communication mechanism, novel nanonetwork could be suggested and further integrated into the standard computer networks. In the meantime, it is also significant for both researchers of experimental biology and computer science to complete a model in cell level, either to provide chances for signal control and recovery in vivo, or to inspire new methods for artificial intelligence.

Calcium signaling is a ubiquitous phenomenon in living creatures which takes the responsibility of mediating other messengers and inducing cellular activities. Calcium signaling prevails in astrocyte - a kind of non-electrical cell in the nervous system. Therefore, astrocytes undertake information transmission using calcium waves other than electric pulses. The function of astrocyte in the nervous system has been emphasized in recent years, especially its collaboration with the neurons. Besides, the communication between adjacent astrocytes forms a network with nonidentical intra-network channel efficiency. In addition, given the universal existence of calcium signaling (not exclusive in astrocytes), nano controllers are expected to participate and interfere with the original communication procedure thus leading to signal detection and regulation. This will facilitate disease monitoring and treatment.

In this thesis, a conceptual network model is proposed to express the signal transmission from the Peripheral Nervous System to the Central Nervous System. It is divided into four layers: neurons in the Peripheral Nervous System and the spinal cord, neurons in the brain, single astrocyte and groups of astrocytes. The external stimulation is

eventually transformed to the states of the grouped astrocytes. The state combination signifies the current prototypical cognitive pattern induced by the stimulation which is converted into conscious episode through associating with the previous patterns. The parameters of each layer are obtained from specific configurations (e.g. the olfactory sensory system in layer 1 in the model) which could be adapted to other sensory systems. In addition, the model is scalable as the metrics employed such as network range, the number of astrocytes and neurons can be altered to emulate the real-time signal processing in the human body.

In addition, the author builds the nanonetwork of astrocytes and neurons in the cerebral cortex based on their columnar and laminar arrangement. The complete communication procedure from the thalamic input to cortical Layer 5 output is simulated. More importantly, astrocytes are integrated into the neuronal micro-circuits and its function has been analyzed. As an assumed memory unit, astrocytes process the neuronal activities and generate new responses by coupling the past ones in the local domain. Meanwhile, astrocytes also maintain an interconnected network to deliver information to selected neighbors.

The author also envisages a network architecture based on the properties of calcium signaling. Firstly, a detailed explanation of calcium signaling and a more accurate simulation method are provided. Then a two-level network protocol is designed and its performance analyzed. A two-layer protocol stack is established which draws its inspiration from the standard computer network. The physical layer is concerned with the communication channel and entities. The cells are aligned in clusters with dynamic control in certain area. The network layer includes the routing protocols. The layered stack encapsulates the complicated biological process to enable convenient analysis of the message transmission by abstracting the lower layer. Human intervention via nano-controllers at the essential nodes (i.e. central node and gateway nodes) realizes the simple routing according to the routing tables which enhances the message direction control and alleviate the bottleneck effect. Relatively long distance communication is accomplished while the success rate is largely elevated. Given the many source-destination pairs in a typical biological trial, the author is able to model the communication path of a single source-destination pair. The error probability in the proposed ring topology is analyzed

by considering the leakage to the uncontrolled clusters to make the results more complete and accurate.

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Acronyms

ABBREVIATIONS

FULL EXPRESSIONS

3D	Three-dimensional
AMP	Adenosine monophosphate
ATP	Adenosine Triphosphate
ATPase	A class of enzymes that catalyze the decomposition of ATP into ADP and P_i
AP	Action potential
BBB	Blood-Brain-Barrier
BOLD	Blood-Brain-Barrier Blood-oxygen-level dependent
BY	Blue-ON/yellow-OFF ganglion cell
CA1	A small Cornu Ammonis area
Ca^{2+}	Calcium ion
CaMKII	Calmodulin-dependent protein kinase II
CICR	Ca^{2+} -induced-calcium-release
CNS	Central nervous system
CNT	Carbon nanotube
Cx43	Connexin 43
DNA	Deoxyribonucleic acid
EFS	Electrical field stimulation
EPSC	Excitatory post-synaptic current
ER	Endoplasmic reticulum
fMRI	Functional magnetic resonance imaging
GFAP	Glial fibrillary acidic protein
Glu	Glutamate

GJC	Gap junction channel
GPCR	G protein-coupled receptors
GST	Glutathione S-transferase
IC	Integrated Circuit
iGluSnFR	Intensity-based glutamate-sensing fluorescent reporter
IP_3	Inositol 1,4,5-trisphosphate
IP_3R	IP_3 receptor
IPCR	IP_3 -induced-calcium-release
LTP	Long-term potentiation
M1	Primary somatic motor cortex
MAC	Mediam access control
MAMNET	Mobile ad hoc molecular nanonetwork
MEG	Magnetoencephalography
mGluR	Metabotropic glutamate receptor
mOR-EG	A mouse olfactory receptor
NAAMF	Neuronal activity associated magnetic fields
$NF - \kappa B$	Nuclear factor kappa-light-chain-enhancer of activated B cells
NMDAR	N-methyl-D-aspartate receptor
NS2	Network Simulator version 2
$P2Y_2$	One kind of purinergic receptors
PH	Hydrogen ion concentration
PIP2	Phosphatidylinositol 4,5-bisphosphate
PKC	Protein kinase C
$PLC\beta$	Phospholips $C\beta$
$PLC\gamma$	Phospholips $C\gamma$
PMCA	Plasma membrane Ca^{2+} ATPase
PMCC	Plasma membrane Ca^{2+} channel
PNS	Peripheral nervous system
S cone	Short-wavelength-sensitive cone
SERCA	Sarcoendoplasmic reticular Ca^{2+} ATPase
SIC	Slow inward current

STP	Short-term potentiation
TKR	Tyrosine-kinase receptors
V1	Primary visual cortex

Chapter 1

Introduction

In this chapter, the author first presents the background and motivation of nanonetworks. Then different approaches of nanonetworks are introduced and discussed. Based on the intrinsic properties of biological communication, the motivation and objectives of the research are specified. Finally, key contributions of the thesis are listed.

1.1 Background

1.1.1 Nanonetwork - Potential for Research and Industry

In the last two decades, nanotechnology has enabled the fabrication of devices ranging from one to a hundred nanometers. With rapid development in biological and nano areas, new communication methods promise novel solutions for various research issues. Nanonetwork is defined as communication and sharing of information of nanomachines. Nanomachines, which consist of nanoscale components, are considered to be the most fundamental functional units and capable of accomplishing tasks including computing, data storing, sensing and actuation [4].

Nanonetwork will ultimately connect to the classical computer network, not only to expand its coverage, but also to enrich its functionalities. Thus the entire "internet of nano-things" [2] is composed of the nanonetwork, the interfaces and the standard network. In this dissertation, the author focuses on nano-bio communication networks.

Network of nano devices is essential since their interaction increases the capabilities and applications of a single nanomachine in the following aspects [4]:

- The coverage of individual nanomachine is small. Dense deployment of connected nanomachines would better reflect the detail and accurate situation in the given workspace.
- Due to the restricted communication ability, a single nanomachine could not reach remote controller directly. Thus multihop transmission through intermediate nanomachine is necessary.
- To reduce the bottleneck effect of a single nanomachine, as well as the breakdown of key devices, backup nanomachines would be effective for information recovery and reliability.
- Normally a nano device merely possesses a single ability such as sensing, actuation, switching, etc. The command interpretation, handling of surrounding nanomachines and execution requires close cooperation.

Nanonetwork provides plenty of opportunities in a variety of areas. Some of the possible applications of nanonetworks are as follows:

- Food quality control could be facilitated. Nano sensors could be placed in food and drinking water filtering to detect its condition and notify the central control center.
- Biomedical applications could be enhanced. For instance, Fig. 1.1a shows high-resolution ultrasensitive nano-cameras and ultrasonic nano-phones could be implanted into the human body for health monitoring and treatment systems to improve the technique of early cancer detection and diseases treatment [2].

Figure 1.1: Applications of nanonetwork (reprint from [2])

- Multimedia applications could be improved. For example, high-definition holographic videoconferencing is enabled by applying a set of multimedia nano-things with nano-projectors to recreate a high-quality holographic image in the conference room (Fig. 1b), while ultrasonic nano-phones could detect concealed objects [2].
- Wildlife health observation could be realized. Wireless nano sensor networks ensure the safety and comfort of animals by attaching nano sensors to the animals as opposed to macro size sensor/radio collars [5]
- Environmental monitoring is supported. For example, nano actuators and sensors could locate and identify different types of materials and facilitate the biodegradation process [4].
- Advanced military method is suggested. Nanonetworks can be utilized for biological, chemical and nuclear defenses. Nano sensors and actuators could detect the unauthorized chemical, hostile biological agents and radiological materials at the battlefield and other specific areas [4].

1.1.2 Limitation and Challenge of Nanonetwork

As a novel type of network, nanonetwork has unique characterizations which lead to the difficulty in the protocol design and equipment constructions. Therefore, the traditional network fundamentals are not applicable to nanonetwork. The limitations are elaborated as follows:

1.1.2.1 Complexity

The size of nanomachines largely restricts the capabilities of nanonetworks. Hence the structure of a single nanomachine could not be fabricated to be complex. The functions of nanonetwork include data collection, sensing, simple communication, memory, etc. However, complicated computation and large memory are not enabled.

1.1.2.2 Arrangement of Network

Management of nanomachines is also an issue. The motion of certain kinds of nanomachines, e.g. ions and sensors in fluid, affect the control of the network. For example, if a control center is proposed to be aware of the nanomachines in its domain, then accordingly, adjacent control center may have access to nanomachines outside its domain as the boundary of the domains cannot be exactly drawn. Besides, the peer-sensing of nanomachines may be not available due to the high cost.

1.1.2.3 Protocol Stack

If the nanomachine is directly produced using downscaling method from macro network component while the size is in nanoscale, quantum effect would affect the electromagnetic rules. Similarly, the biological nanomachines are conspicuously different from electromagnetic nanomachines. Furthermore, it is challenging to build a common network protocol stack. The corresponding medium access control (MAC) varies from ions and proteins

to carbon nanotubes. Addressing scheme, noise filtering, coding scheme and message recovery should be designed.

- The coding scheme of information should be carefully designed since information comes in various forms, e.g. molecule counts in a time slot, wave burst counts, etc.
- The channel property, especially in biological nanonetwork influences the information transmission. For instance, PH level, calcium wave amplitude/frequency and channel permeability (efficiency) in calcium signaling.
- Under most circumstances, it is impossible for every nanomachine to carry an integer address. The overhead tradeoff would affect the size of artificial nanomachines. Thus a different addressing scheme is needed to locate the sender and the receiver. Data-centric topology is a possible solution whereby only the awareness of a data sink is enough for each data collector.
- In addition to channel noise, the breakdown of the single nanomachine is unpredictable, e.g. the failure to bind to a receiver, the death of a cell, nanomachines running out of energy, etc. Reliability of nanonetwork is also to be resolved.

1.1.2.4 Compatibility

In biomedical applications, the nanomachines should be bio-compatible for the human body. Hence toxic material should not be considered. There is thus a need for bio-compatible nanomachines to be proposed and constructed.

1.2 Types of Bio-compatible Nanonetworks Studied

There are several kinds of nanonetwork such as electromagnetic nanonetwork, bacteria-based nanonetwork, molecular communication etc. In the author's work, three types of bio-compatible nanonetworks are involved.

1.2.1 Cellular Network of Human Nervous System

The nervous system is the most essential structure in animals which coordinates voluntary and involuntary behaviours. Human nervous system, which contains the peripheral nervous system (PNS) and the central nervous system (CNS) - the spinal cord and the brain, is more complicated than animals in the structure and coverage.

Network of human nervous system is defined as the interconnected neurons and astrocytes with the assistance of neurotransmitter and gliotransmitter to exchange information with one another. The network is not the typical nanonetwork since the neuron size in PNS and the spinal cord is beyond the nano scale. However, the study of human nervous system is in the cell level. Besides, cells in the most significant part of the nervous system - the brain are within the nano scale.

1.2.2 Nanonetwork in Human Cerebral Cortex

As an important component of the CNS, the brain is divided into gray matter and white matter according to the anatomical features. The gray matter is also known as cerebral cortex which consists of more cell bodies than white matter. Cerebral cortex is the most essential part of CNS.

More intensively, the author focuses on the nanonetwork in human cerebral cortex. In the traditional viewpoint, neurons rule the brain while the significance of astrocytes is ignored. The modeling of cortical nanonetwork predicts the operation of neurons and integrates the astrocytic function. The clustering and projection of neurons and astrocytes facilitates the entire communication process, including information reinforcement, replication, processing and conversion. Moreover, memory formation has a new explanation.

1.2.3 Calcium Signaling

Calcium ion (Ca^{2+}), which is one of the most important universal second messengers in vivo cells, participates actively in the regulation of cellular activities. Calcium signaling is defined as the signal transduction from other cellular activity to a stable or temporary calcium waves. The frequency and amplitude of calcium waves are affected by cellular activity. In turn, calcium waves could induce diverse cellular behaviour, either local response or distant response. Therefore, calcium signaling could be the fundamental basis of signal transmission, with the help of nano sensor.

Particularly, message encoding and decoding could be performed at designated sender and receiver, respectively. Detection of cellular activities and interfaces to standard network are carried out by nano controllers. Other than reporting anomalies, calcium signaling could also be enhanced for longer distance propagation.

1.3 Research Motivation and Objectives

1.3.1 Network model of Human Nervous System

Nervous system generates thinking, perception, motion, etc. The complexity of nervous system marks the superiority of human. Aiming at uncovering the cause of neurological disorder and dysfunction for more effective therapy against diseases such as Alzheimer's Disease [6], biological experiments and methods have been designed to probe the effect of certain chemical, cell and tissue within the nervous system. On the other side, the nervous system is viewed as a sophisticated information transmission and processing module in computer science to inspire the progress of artificial intelligence. However, owing to the huge amount of information streams, the susceptibility of multiple chemicals and the lack of in vivo experiment technique, the mechanism of this complex system still requires years of research. Conscious processing, memory formation etc. are all the persistent questions in this area.

There are two types of nerves which spread throughout the human body from the CNS: motor nerves are responsible for the control of muscles; sensing nerves undertake the perception of the environment. Reflex is the simplest neural circuit consisting of motor nerve, sensing nerve and CNS. Sensory neuron receives external stimulus and delivers the signal to CNS, thereafter motor nerve from CNS controls corresponding muscle to relax or contract. Other than automatic function such as blood pressure adjustment, the somatic neurons fulfil functions such as touch, olfactory and vision through the cooperation of fibres and the control center (brain).

CNS and PNS (Fig. 2) constitute the nervous system. CNS (in blue) comprises the brain and the spinal cord while PNS (in orange) is primarily bundles of nerves derived from the body of neurons and covered with myelin sheath connecting to the CNS. Generally there exist two directions for signal transmission. The ascending signal pathway begins from the sensing neurons which have receptors to detect the stimulation and traverses the PNS to the spinal cord. The brain is the ultimate receiver where the cerebral cortex [7] is responsible for information synthesis and decision. The descending pathway is the opposite except that the destination is the motor neuron. The ascending and descending pathways are superb rather than reflex in nature since the final receivers are the brain and the spinal cord, respectively. The former involves a series of complicated computations and processing while the latter is an automatic response.

Current research measures and simulates the signal transduction within a field of the nervous system, ie. neural circuits [8], cortex, synapse, etc. However, there lacks a complete model for the entire system providing the relationships of adjacent components and predicting the mechanism of human-specific process.

Since the last decade, astrocytes have been revealed to be involved in the communication and interference with neuronal activity in the human brain. Astrocyte is a kind of star-shaped glial cell located in the brain and the spinal cord [9]. The glia-neuron

Figure 1.2: Nervous system —CNS and PNS

ratio in the cerebral cortex is 3.72 [10] which implies its significant function in the CNS. Other than sustaining the homoeostasis, supplying the energy and penetrating the Blood-Brain-Barrier (BBB), astrocyte interferes with the synaptic activity [11]. Therefore, the communication in the cortex should be reconsidered to incorporate the performance of astrocytes with the neurons.

Given the newly discovered potential of astrocyte in the human brain and the lack of a complete model for the nervous system, the prime motivation in establishing the proposed model is to provide a means to simulate the response of the complex nervous system thus enabling researchers to glean insights into how the entire information processing and transmission takes place upon the presence of a stimulus from the sensory neuron to the astrocyte. With such a model as a starting point, and with future and further validations from biological experiments, a better and clearer understanding of the nervous system can be achieved. This will then pave the way for nano sensors or biological carriers to deliver medication to targeted cell areas for treatment of brain related diseases.

Figure 1.3: Human visual cortex staining (reprint from Fig. 1A in [3])

1.3.2 Nano-Bio Network of Cerebral Cortex

Human cerebral cortex expands 2-5 mm thick and 2500 cm^2 wide [12] which is not identical. Cerebral cortex is differentiated into six cortical layers (tangential to the cerebral cortex plane), each containing a characteristic distribution of neuronal cell types as well as connections. Fig. 1.3 shows the staining of human visual cortex, which indicates obvious difference in the neuron density [3]. The classification method follows the neuron doctrine which has dominated the neurological research for years. This doctrine claims that neurons are the principal cells in the nervous system which work as the primary units for sensing, control, perception and storage.

Apart from intralaminar connections, interlaminar connections exist between different layers. Functional columns which are perpendicular to the cortical surface are aligned in the cerebral cortex. A column is defined as a cluster of neurons sharing identical

receptive field parameters, which traverses from Layers 1 to 6. Recent advances provided evidence of the presence of columnar organizations in the somatic sensory cortex, visual cortex, auditory cortex, and motor cortex [13]. These columns possess direct and indirect connections towards the thalamus. Therefore, a column is an individual and relatively independent functional module in the cerebral cortex.

The essential contact method between astrocytes and neurons is the tripartite synapse formulated by the endfeet of astrocytes and neuron dendrites [14]. Calcium waves and local calcium oscillations prevail in the astrocytic main body and processes, which indicates the possible ability of information carrying. Through gap junction channels (GJCs), astrocytes exchange messenger molecules with one another and calcium waves are synchronized among a group of astrocytes. Similar to neurons, astrocytes have different types and distribution in different cortical layers. The coexistence of astrocytes and neurons in the cerebral cortex underlies their interaction.

According to recent finding, astrocytes in a column are developed from the common origin of neurons [15] which implies their correlation within the same column. In addition, astrocytes in different layers exhibit distinct characteristics. It could be inferred that their responsibilities vary along with the neurons. Therefore, a network with tangential and perpendicular communication of astrocytes and neurons is recognized in the cerebral cortex.

The primary motivation of our work is to provide a simulation model so that biological researchers can gain insight into the communication between the astrocytes and neurons and facilitate the validation and extrapolation of their biological experiments. The model is designed such that it can be readily expanded and adapted with further results from future biological research.

1.3.3 Calcium Signaling Network Layered Stack

Calcium signaling is a ubiquitous phenomenon in many cells such as astrocytes, chondrocytes, hepatocytes and epithelial cells [16]. Ca^{2+} contributes to manage cellular activities such as proliferation, differentiation, ion channel opening, aggregation, secretion, contraction, fertilization and neural signaling [17–20]. According to its significance, calcium signaling is an efficient method for intra-body communication, as well as a common nanomachine for information collection and data delivery to standard computer network.

Other than bio-compatibility, there are mainly four advantages:

- The biological nanomachines have considerable functions including memory, actuation, computing and sensing [4].
- The natural communication in the living body not only inspires artificial bio-engineered network but also provides infrastructures for network design.
- The energy efficiency is guaranteed since chemical energy such as Adenosine Triphosphate (ATP) could drive molecular reactions which consume much less energy than standard networks [21].
- Compared to other biological approaches such as kinesin-motored proteins, bacteria-based DNA coding and massive molecules elicited by sender nanomachine, calcium signaling is not only convenient to be controlled, but is also involved in more intra-body processes.

The most significant distinction between molecular communication and standard communication is that the message is represented as information molecule rather than electromagnetic wave. Owing to the low information capacity of a message molecule, the extremely slow propagation speed and the attenuation within the communication channel, the communication theory of standard network cannot be applied. In this case, a

Figure 1.4: Generation and propagation of calcium waves

unique communication framework and the corresponding network topology should be established.

Numerous research efforts have been made to illustrate calcium signaling while the most acceptable one is the cooperation of ATP and inositol 1,4,5-trisphosphate (IP_3) pathways (Fig. 4) [22]. The plasma membrane Ca^{2+} channel (PMCC) [23] opens under the stimulation and the extracellular Ca^{2+} floods into the cytosol. Then a large amount of IP_3 is generated and IP_3 binds to the IP_3 receptor (IP_3R). Massive Ca^{2+} flows from the intracellular store (e.g. endoplasmic reticulum and sarcoplasmic reticulum). The elevation of Ca^{2+} produces Ca^{2+} blips and puffs [24] thus leading to the calcium waves. IP_3 diffuses through the GJCs and induces the Ca^{2+} release in adjacent cells. Meanwhile, the stimulated cell generates ATP which could diffuse through extracellular space and evoke the calcium waves in the nearby cells.

The behavior of calcium wave is affected by factors such as channel blocker, antagonist, receptor inhibitor [22], etc. These external chemical substances influence the

extracellular environment and the GJC permeability which promote or prevent the generation of calcium waves. The propagation path of calcium waves can be altered through the external focal regulation in certain area. Hence, dynamic control of message spreading becomes possible. This means that transmission in selected directions can be realized as well as broadcasting and it is necessary to design appropriate network topology and protocols.

The motivation and objectives of the author's work are as follows:

- To explain the mechanism of calcium signaling accurately, which leads to the modeling of communication fundamentals such as coding scheme and channel control.
- The simulation of communication basics should be utilized in further network design, thus the differential equations should be simplified to facilitate information inference and interpretation.
- A network model is to be developed to improve the intrinsic message exchange of calcium signaling and provide an interface to classic computer network. Through employment of artificial nanomachines, calcium signaling could be directed and enhanced via intermediate devices.

1.3.4 Contributions of the Thesis

The contributions of the author in this thesis are as follows:

- A conceptual network model is proposed to express the signal transmission from the PNS to the CNS [25]. External stimulation upon PNS neuron is eventually transformed to the states of the grouped astrocytes in CNS. Prototypical cognitive pattern is presented by the current state combination induced by the stimulation. Conscious episode is supposed to be formed through binding of the previous patterns. The parameters of each layer are obtained from specific configurations which

could be adapted to other sensory systems. In addition, the model is scalable as the metrics employed such as network range, the number of astrocytes and neurons can be altered to emulate the real-time signal processing in the human body.

The simulation results from the conceptual network model has been verified against the available biological experiments and mathematical models. A test case of the integrated network is also provided. The proposed model serves as a useful tool to facilitate, complement and verify current and future study in human cognition.

- A communication model of astrocytes and neurons is built based on their columnar and laminar arrangement in the cerebral cortex. Signal transmission from the thalamic inputs, via Layer 4 (L4) and Layer 2/3 (L2/3) to Layer 5 (L5) output is simulated. More importantly, astrocytes are integrated into the neuronal microcircuits and its function has been analyzed. Astrocytes process the neuronal activities and generate new responses by coupling the past ones in the local domain. Meanwhile, astrocytes also maintain an interconnected network to deliver information to selected neighbors.

The proposed network has been simulated with the information flow duly represented and the working mechanism within a column has been studied. The function of astrocyte as a buffering pool for information and a confidential memory unit can thus be envisaged. The proposed model serves as a handy simulation model for biological researchers to gain insight into the communication between the astrocytes and neurons. It also facilitates the validation and extrapolation of their biological experiments. The model is designed such that it can be readily expanded and adapted with further results from future biological research.

- A two-layer protocol stack has been established based on calcium signaling which draws its inspiration from the standard computer network [26,27]. Coding schemes,

communication entities and channel are defined in the physical layer while the network layer includes the routing protocols. The layered stack encapsulates the complicated biological process for analyzing the message transmission conveniently by abstracting the lower layer. Furthermore, a ring topology is introduced for more practical implementation of the protocol stack while the error probability is analyzed by considering the leakage to the uncontrolled clusters to make the results more accurate. Relatively long distance communication is accomplished while the success rate is largely elevated compared to natural calcium signaling. Given the many source-destination pairs in a typical biological trial, communication path of a single source-destination pair could be guaranteed. The feasibility of dynamic control via human factor intervention is demonstrated.

1.4 Organization of the Chapters

The organization and content of the thesis are summarized in Fig. 1.5. The development of the research is from the global view to the detailed. In Chapter 3, the overall nervous system is modeled which includes the body nerves, spinal cord and the brain. The author supposes that astrocytes are the ultimate information handler which gather information through the neuron electric pulses. Through an in-depth study of the brain, especially the cerebral cortex, Chapter 4 discusses the columnar and laminar module of neurons and the relationship with astrocytes. The distribution of astrocytes adjoint to the neuronal structure supports their interaction. More generally, as the astrocyte-astrocyte and astrocyte-neuron connection are related to calcium signaling, Chapter 5 provides a network protocol utilizing the natural communication and artificial nano devices. The calcium signaling is not restricted in astrocytes, but also applied to other cells which employ Ca^{2+} to intermediate cellular activities. In summary, the rest of this thesis is organized as follows:

Figure 1.5: Structure of the Thesis

Chapter 2 introduces related works on the different types of nano-networks covered in the research reported in this thesis, as well as new techniques relevant to nano-device fabrication. Various proposed architectures related to the nervous system and sub-systems are provided. Specifically, the astrocyte-centric doctrine is discussed.

In **Chapter 3**, the author integrates the existing mathematical model and biological experiments in each step of the signal transduction to establish a conceptual network model for the human nervous system. The network is composed of four layers and the communication protocols of each layer could be adapted to entities with different characterizations. The simulation results are verified against the available biological experiments and mathematical models and a test case of the integrated network is provided. As the production of conscious episode in the human nervous system is still under intense research, the model serves as a useful tool to facilitate, complement and verify current and future study in human cognition.

Astrocytes are incorporated into the columnar microcircuit of neurons whereby the memory function of the astrocytes is proposed and modeled in **Chapter 4**. The network is simulated and its performances are observed and estimated. The numerical definitions are acquired from existing biological experiments. The model can be readily adapted and expanded when more and improved results from further biological research become available in the future.

Chapter 5 aims to design a two-layer protocol stack which incorporates nano-controller to realize and enhance the network model for cluster-based calcium signaling. The proposed physical layer specifies coding schemes and communication entities. A three-step communication protocol along with routing mechanisms are explained in the network layer. Furthermore, a ring topology is introduced for more practical implementation of the protocol stack. The proposed network model is aimed at providing insights and facilitating bio researchers in carrying out further experiments on calcium signalling. The comparison of our proposed model against biological experiments shows that the integration of nanotechnology and human intervention is capable of directing the calcium waves and achieving considerable superiority in terms of communication distance and successful transmission rate.

Chapter 6 summarizes the thesis and lists the potential future research directions.

Chapter 2

Literature Review

In this chapter, the author discusses the previous work related to the research. Firstly, the author reviews the literature of related advances in nanotechnology which could be utilized in designing nanonetwork and existing types of nanonetworks. Secondly, the related works of characterization and modeling of calcium signaling are introduced. Finally, the author introduces the studies relevant to the human nervous system, which includes biological experiments and mathematical modeling for the specific portions covered in the research of this thesis.

2.1 Advances in Nanotechnology

The development of nanotechnology has increased the chance of regulating natural biological communication.

2.1.0.1 Signal Stimulation and Chemical Release

Carbon nanotubes (CNTs) which have been discovered in 1991 [28], possess extraordinary mechanical, electrical, thermal and conductivity properties. It has been proposed in [29] that the CNTs could penetrate cells after surface modification such as coating the CNTs with proteins. Thus chemicals could be delivered directly into the cytosol. Additionally,

owing to the electrical conductivity, CNTs could also stimulate cells by sending electrical pulse.

2.1.0.2 Memory Storage

The memory ability of nano unit is proposed in [30] using an atom to store a bit. [31] demonstrates that the hysteresis effect in single-walled CNT could be extensively employed to construct memory device. Furthermore, information encoded in DNA is also a potential memory component for bio-hybrid nanonetwork [32].

2.1.0.3 Detection

The detection technique of cellular activity is essential for the message recovery. Different indicators are responsible for the assay of generated or inverted chemicals. For example, the transcription factor NF- κ B could be influenced by calcium waves. Hence in [33], $(Ig\kappa)_3$ conaluc plasmid in neurons is applied to measure its activity. Another experiment proves that Ca^{2+} facilitates CD_3 phosphorylation in which N-terminal GST tag is used for the unbiased detection [34]. These indicators could be contained in an electrochemical detector which could transform certain chemical release binding to the nanotube. Besides, an optical transducer, voltaic and magnetic detector could also recover messages [30]. Further advancement in detection would enable the identification of calcium wave without disturbing the cellular environment. In such case, the biological method other than chemical or electrical detection can be focused on.

2.1.0.4 Nanocomputing

A nano-chip whose diameter is less than $5\ \mu m$ is designed in [35]. The chip comprised a controller, memory-block and other components which have a bio-compatible coating. The controller unit is made up of IC whose size may be reduced when undertaking customized tasks. Another approach is applying synthesized DNA and chemical reaction

Figure 2.1: Applications of nanonetwork (reprint from [2])

to form a programmable nano controller [36]. In vitro reactions are combined into a network and agents are distributedly controlled via program.

2.2 Types of Nanonetworks

Nanonetworks can be classified into two categories: non-biological approach which employ electromagnetic wave and molecular communication which primarily utilizes biological nanomachines or the method of biological behavior. The comparison of various approaches are listed in Fig. 2.1.

2.2.1 Non-biological Approach

Electromagnetic nanonetwork relates to the information exchange of electromagnetic radiation utilizing downscaling manufacturing technique of silicon-based devices and CNT. CNT can be used to design the communication fundamentals: transmitter and receiver.

- Transmitter: An oscillator generates the carrier signal and sends it to the modulator

along with the information signal. The antenna radiates the combined signal after it is amplified [37].

- Receiver: Radio signal is received by an antenna. The signal is selected by a tunable band-pass filter. After amplification, the demodulator extracts the information signal from the high-frequency signal which would be further processed [38].

Due to the slow speed of electromagnetic wave in CNT (1/100 of that in vacuum), it has been calculated that [39] Terahertz signal is the highest range which could be radiated from 1 μm long CNT. Megahertz range is also enabled for CNT while the efficiency is extremely low. Hence Terahertz signal is favored for CNT.

2.2.2 Molecular Communication

Molecular communication employs molecular motors, protein complexes and genetically engineered cells to act as information carrier and complete expanded applications, rather than electrons or electromagnetic waves [40]. Owing to the biocompatibility, molecular communication could contribute to promising solution to medical treatment. For example, the molecule could be released through external control to facilitate drug delivery, anomaly such as harmful chemical could be detected to report disease, etc. Moreover, molecular communication could be combined with nano devices to enhance its performance and interact with external environment.

Molecular communication is classified into passive communication and active communication. Passive communication is based on the uncontrolled chemical propagation with massive molecules which includes free-diffusion and GJC mediated communication. Active communication requires energy driven process including motor-based and DNA-based communication [40]. It utilizes the bigger size of signal molecules or cellular structure, thus the transport is more reliable.

2.2.2.1 Free-Diffusion

A transmitter and a receiver are the primary components in the free diffusion model. The information is encoded in the concentration of massive molecules. Message is identified by detecting the waveform at the receiver. Channel characterizations are studied in [41, 42]. To increase the signal-to-noise ratio, a large number of information molecule should be released. A drift velocity and routes are proposed to jointly direct a molecule. Free-diffusion model is not a purely biological nanonetwork since the transmitter and receiver are artificial nanomachines while the communication process could not be realized in vivo.

Based on the free-diffusion model, a mobile ad hoc molecular nanonetwork (MAMNET) [43] is designed and analyzed which consists of electrochemical communication. The nanonode is supposed to gather information from outside environment and move in a free-diffusion manner. Inspired by the immune system, MAMNET communication is classified into three phases:

- Collision: the nanomachines walk in Brownian motion and collide randomly.
- Adhesion: collided nanomachines are stuck
- Communication: the information is exchanged by neuro-spike scheme.

Through multiple collision which is similar to multi-hop transmission, the infostation, as a central data sink, would eventually collect the information, make decision and send the message to a micro device standard network.

NanoNS, a simulation tool for molecular communication, is developed to facilitate researchers to study the nanonetwork. The framework is built based on core components of NS2 which integrates the free-diffusion scheme, including entities and channel property. The prototype of the network is a simplified version of the ligand-receptor mechanism.

2.2.2.2 GJC Mediated Communication

Signal molecule diffusion could be mediated through GJC between adjacent cells. The diffusion includes molecule transportation and activation of certain molecule in the neighboring cell. This communication is cell-based which enables message broadcasting within an interconnected cellular network, e.g synchronized heart-beating by cardiomyocytes. Calcium signaling is also an important form of GJC mediated communication while the related study is introduced in the upcoming section of this chapter.

2.2.2.3 Motor-based Communication

Backbone network with molecular motor to impel the information carrier is another solution for directional communication. In [44], chemicals carriers are encapsulated in vesicle driven by ATP along fixed microtubules. The vesicle protects the signal molecules from interference of external environment which would maintain the quality of the message. Thus unicast is realized by consuming chemical energy.

2.2.2.4 DNA-based Communication

A hybrid DNA and enzymatic network is proposed in [45]. The layered network stack is designed by applying DNA and enzyme computing. The network layer is realized by enzymatic computing to identify the routing address. The transport layer and the above layer are assumed to be realized by DNA computation and error correction is included.

The core component of this network stack is the DNA transportation which is fulfilled by contact of flagellated bacteria [4], [46]. The sender bacteria would transfer a DNA chromosome through a pilus - a bridge formed during the transmission.

However, this tentative idea is difficult to achieve due to its complexity and current technological constraints.

2.3 Characterization and Modeling of Molecular Communication

Researchers have been attempting to analyze the mechanism of calcium signaling since the discovery of its existence in 1970s. Plenty of biological experiments have been conducted to observe the possible functions of certain ions or compounds by varying their concentrations in given regions. For example, in [47], a microplatform is designed and realized for demonstrating the propagation of molecular signals through a line of patterned HeLa Cx43 cells expressing gap junction channels. Intercellular communication is allowed over an arbitrary network topology of cells. The propagation of calcium wave is experimented with or without inhibitor of IP_3 receptors in airway epithelial cells [48].

GJC is made up of cell membranes which are formed by homogeneous or heterogeneous connexins [49]. The permeabilities between adjacent cells vary according to the connexin formations [50]. For example, GJC composed of connexin43, has higher conductance than that of connexin32 when permeable tracers are applied [51]. Beyond that, signals including trans-junctional voltage [52], intracellular pH and glucose level [53] could alter the GJC permeability [54]. For instance, the permeability of GJC which consists of connexin43, decreases with the decrease of intracellular PH [55]. Another experiment in [56] reveals that trans-junctional voltage is positively correlated with GJC conductance before reaching the climax and negatively correlated thereafter. Taking advantage of this property, GJC permeability could be influenced manually in the transmission process e.g. changing the local PH conditions leads to the redirection of Ca^{2+} wave (discussed in Chapter 5).

2.3.1 Mathematical Modeling

To investigate the mechanism of calcium signaling and develop further applications, mathematical models have been proposed with regard to the biological results and rea-

sonable hypothesis. Generally, there are three viewpoints on the principle of the second messenger being involved in the origination of calcium waves, i.e. transfer of Ca^{2+} , diffusion of IP_3 and ATP driven.

2.3.1.1 CICR Model

In the calcium-induced-calcium-release (CICR) model [57–60] for non-excitabile cells such as epithelial cells, there exists a large quantity of Ca^{2+} outflow from the stimulated cell to the unstimulated cells. Ca^{2+} and IP_3 could bind to the receptor on the intercellular store to release subsequent calcium flux while the determinant factor is Ca^{2+} from the adjacent cells.

2.3.1.2 IPCR Model

Another possible model is that calcium wave mainly originates from IP_3 diffusion from the stimulated cell although Ca^{2+} leakage conduces to the release of calcium wave in unstimulated cells. The IP_3 -induced calcium wave model is developed on the basis of the Ca^{2+} -induced-calcium-release (CICR) mechanism. The CICR model assumes that the interaction of cytosolic and extracellular Ca^{2+} modulates the Ca^{2+} channel on the surfaces of the internal Ca^{2+} store. However, experiments [61] show that the stimulated cell is capable of producing repetitive calcium spikes in an environment vacuum of extracellular Ca^{2+} and the duration of Ca^{2+} oscillations is longer in experiments than modeling results [62]. Therefore, the function of IP_3 as a Ca^{2+} mobilizing second messenger is envisaged. CICR process collaborating with IP_3 triggers local Ca^{2+} oscillations. The GJC plays an important role in mediating the IP_3 diffusion. Besides, the IP_3 metabolism which regenerates certain amount of IP_3 , assists IP_3 diffusion in initiating the calcium wave in neighboring cells. Different modeling works investigating the characteristics of calcium signaling on account of the above assumptions include deterministic [63], threshold [64] and stochastic [65] approaches.

There are several related works concerning the gap junction model with IP_3 induced calcium waves. In [57], a one-dimensional communication model is presented. Signal switching, filtering and amplification are envisaged. This model claims that apart from the stimulated cell, the resource of calcium wave in a certain cell is from its neighboring cells. A two-dimensional model is presented in [66] and each cell is viewed as a square plot. In [67], the function of cell connectivity and the location of stimulus are researched. Gating probability of gap junction channel is synthesized. In [68], a IP_3 receptor-oriented theoretical model is analyzed under global stimulation. The permeability of gap junction comes in a spacial differential equation. However, the extrusion of cytosolic Ca^{2+} is neglected.

In this thesis, the author adopts this model and combines it with the property of CICR oscillation since calcium wave propagation can be enhanced by CICR oscillation. The author suggests that there does not exist Ca^{2+} transportation between neighboring cells which conforms to the biological experiment observations in glial cells [69] and airway epithelial cells [48, 61].

2.3.1.3 ATP driven

The other model attributes the spreading of calcium waves to the propagation and regeneration of extracellular ATP. This explanation adopts several modules of the IP_3 model. The essential difference from the previous model is that the IP_3 generation in unstimulated cell originates from the extracellular ATP binding to the IP_3R on the plasma membrane instead of IP_3 diffusion from the stimulated cell via GJCs.

Other related work to the calcium signaling communication includes theoretical information model and design of network units. Information coding, network coding and channel capacity are analyzed according to the stochastic process of Ca^{2+} signals in [70].

2.3.2 Characterization of Calcium Waves

The features of calcium waves differ depending on the stimulation method, the cell type and the variation of propagation channels. For example, the stimulation method in rat mesenteric arteries decides the existence of calcium waves. Local phenylephrine stimulation cannot initiate calcium wave unless a small global pheylephrine stimulation is executed [71].

The propagation channel including the GJC and the ATP pathways could be affected by external signals. Firstly, the permeability of GJC can be adjusted leading to the termination of calcium waves in some cells and enhancement in other cells. Secondly, the calcium waves could be prevented by applying the inhibitors (heptanol, octanol, etc.) to block the ATP binding to the purinergic receptors [22].

2.3.2.1 Selective Permeability of Gap Junction Channel

There are numerous types of gap junction channels since they are composed of different connexins. Apart from that, the gap junction may consist of homogeneous or heterogeneous connexins. It has been studied that different connexin formations have selective permeabilities [50] to small molecules including Mn^{2+} , Zn^{2+} , IP_3 , etc. In [72], transfected connexin45 successfully transferred Lucifer Yellow and reduced dye transfer.

Except for the inherent permeability diversity of various connexins, the permeability of certain connexin composition can be regulated by external or internal signals. Research reveals that the gap junction permeability can be altered by protein kinase-catalyzed phosphorylation [73–75], intracellular pH [76], glucose level [53], cyclic AMP [77] and transjunctional voltage [54, 56]. Even Ca^{2+} can regulate the coupling in gap junction channel [78]. For instance, in [53], high glucose level inhibits the intercellular communication in cultured vascular smooth muscle cells through the activation of protein kinase C. In [55], lowering intracellular pH reduces the permeability of gap junction composed of connexin43.

2.3.2.2 Channel Blocker

By utilizing channel blocker to interfere with the propagation of extracellular ATP, calcium wave could also be affected.

In [79], calcium waves are limited in the adjacent cells near the stimulated one after the ATP scavenger cocktail while the range recovers to the normal stage after the washout of ATP scavenging enzymes. It is inferred that ATP change is able to terminate wave propagation in certain cells through IP_3 control.

Hence, dynamic control of message propagation becomes possible. This means that besides broadcast, transmission in selected directions can also be realized and there is thus the need to simulate such communication with an appropriate design of network topology and protocols.

2.4 Cerebral Cortex

From neuron doctrine to the assertion of astrocyte involvement, biological experiments have been conducted to prove the significance of astrocytes. The structure of neurons in cerebral cortex is studied while astrocytes are accompanied with signal exchange. Specifically, modeling works of astrocytes have been proposed.

2.4.1 Neuron Doctrine

Due to the existence of dendrites and axons of neurons, researchers have been confused on whether the tissue in the nervous system is purely made up of cells like plant tissues. As the development of staining technique, reticular theory [80] and electron microscopy, it has been found that the nervous system is composed of discrete interconnected neurons through synapses while there exist interspaces between two neurons.

The neuron doctrine claims that neuron is the core component of the nervous system which has the capability of memory, decision and information acquisition. The constitution of neuron doctrine is as follows:

- Neurons are the fundamental units of the brain while each neuron has sub-structure including cell body, axons and dendrites. These branches vary in the number, form and shape in different area.
- Within a neuron, the section containing nucleus possesses the trophic factors. A neuron could not survive without the nucleus.
- There are two kinds of synapses: electrical and chemical synapses. The electrical synapse is the GJC formed by two adjacent neurons while electric pulses could be directly transmitted. The pre-synaptic cell could arouse voltage variation in the post-synaptic cell. Electrical synapse is more rapid than chemical synapse. Chemical synapse converts the voltage change in the pre-synaptic neuron to the chemical release — neurotransmitter into the GJC, which would bind to the post-synaptic cell membrane receptors. Multiple neurotransmitters are found to either excite or inhibit the post-synaptic neuron.
- The polarization along the axon is through one direction in natural communication although it could propagate in both directions.
- Nerve fiber is the assembly of neuronal extensions which is protected by a sheath.

2.4.2 Astrocyte Involvement in Cerebral Cortex

Albeit astrocytes being discovered in the CNS, they are regarded as merely performing supportive functions because of the inability to convey electric pulses compared to neurons. However, in the last decade [81–83], the astrocyte-centric hypothesis has become

prevailing which challenges the neuron-centric perspective. Experiments reported the existence of calcium waves [22] in the astrocytes suggesting that it is probable for the information to be encoded in a non-electric form. For example, astrocyte of the sensory cortex initiates calcium elevation in response to the peripheral stimulation [83–85]. Moreover, the frequency and amplitude of calcium wave vary according to the types of stimulations which further implies the astrocyte participation.

Furthermore, the discovery of interlaminar astrocyte in layer I of the cerebral cortex as well as the obvious bigger size and more branches of the protoplasmic astrocyte (a kind of astrocyte emerging in all the cortex layers) compared to lower form of animals such as rodent indicate that the development of intelligence may be accompanied with the evolution of astrocyte.

Evidence revealed that astrocyte is involved in the bidirectional communication at the tripartite synapse [83,86]. Recent observation proves such interaction in human brain tissue [87]. It not only influences the firing rate and frequency of post-synaptic neuron AP [88], but also generates calcium wave upon message exchange between the synaptic neurons [83]. Neurotransmitter corresponds to different synaptic pathway. For example, the glutamate (Glu) mediates astrocyte calcium wave in the Schaffer collateral synaptic terminals [89]. In contrast, the astrocytes in the stratum oriens of CA1 (a small Cornu Ammonis area) respond solely to synaptically-released acetylcholine [90].

2.4.3 Neuronal Circuitry

After neuronal microcircuits among laminar cortex have been discovered, various approaches have been envisaged. The ascending and descending pathways including the thalamic input are summarized in [91]. The connection ratio between input and output cortical layers are concluded [92], as well as the connection among the same cortical layer.

The arrangements of a dozen minicolumns are also proposed. A winner-take-all scheme for all the minicolumns within a macrocolumn as part of the sparse distributed

columnar code is introduced in [93]. [91] shows a pattern of connection among large-scale cortical areas that two adjacent areas are linked from the bottom layer of the first one to the top layer of the second one.

2.4.4 Related Modeling Works in Cerebral Cortex

2.4.4.1 Neuronal Model

Other communication method of microcircuits are suggested. For example, the stochastic computational model [94] focuses on the probability distribution of neuronal states and the impact of the background network oscillations. It is demonstrated that the network states of neurons would eventually converge exponentially from the initial states. Intrinsic generic microcircuit dynamics respond quickly with possible solution from learned knowledge and current inputs.

In motor cortex, a top-down laminar organization is established of which Layer 2 (L2) dominates the outflow to the deeper layers while a quantitative framework is provided [95]. The primary somatic motor cortex (M1) converges multiple inputs from the sensory and motor system, which would successively generate corticofugal signals to generate movement. M1 neurons functions in a coordinated way rather than fulfils the task merely by corticospinal neurons. Therefore, the local circuit is significant in discovering the mechanism of M1. After deciding a laminar presynaptic (i.e. postsynaptic connectivity matrix from data collected from mouse motor cortex), it is demonstrated that the ascending signal to the upper layer would induce network-wide events.

2.4.4.2 Astrocytic Model

The involvement of astrocyte in the columnar model is considered in [96]. The wavefronts of two peripheral calcium waves aroused by the neuronal activities in the corresponding minicolumns would interact and lead to a single oval wave. Stellate astrocyte constitute

'panglial syncytium' through chemically and electrically coupling which is specific in mammals, forming a pathway for information communication. In primate order, long process parallel to apical dendrites is proposed to present certain properties. The generation of large-scale astrocytic calcium wave starts from generation of cylindric calcium wave evoked from neuronal release. Thereafter the calcium wave spreads from interlaminar process within the cylinder and propagates in a restricted neighborhood. Eventually, neighboring calcium waves encounter and extend to bigger area.

[97] presents a hypothesis based on neuronal activity associated magnetic fields (NAAMFs) of self-organized magnetic interaction between astrocytes and neurons within the minicolumn to explain neurocomputation, especially memory processing. It is based on two facts: a) Neurons and astrocytes in CNS have magnetic fields which are transient and continuously decreasing, respectively. b) The orthogonal organization of astrocytes and neurons (columnar structure) predicts the three-dimensional (3D) interplay. The information is supposed to be memorized in the astrocytic network. Neuronal minicolumn discharges through repetitive events and would enhance the memory while minicolumn activation would evoke the retrieval process.

Apart from astrocyte-neuron network, [98] studies the astrocyte wave propagation process by varying the topology of the astrocyte network. Astrocytes occupy spatial territories and connect to their neighbors without overlap. The structure of astrocyte networks differs according to the location [99] and the GJC could be adjusted through neuronal activity. The simulation is conducted on more than 1000 cells in a 3D space and the main results show that calcium wave propagation performs better when the mean-shortest path of the network is small. Hence astrocyte favors its nearer neighbors rather than connects to neighbors with identical strength.

The study in [100] shows that the incorporation of artificial astrocytes which present the biologically defined properties involved in astrocyte-neuron communication, improves

artificial neural network performance in solving classification problems. The findings thereby establish the concept of Artificial Neuron-Glia Networks [100].

2.5 Human Nervous System

Owing to the distribution and specification of cells in the nervous system, the function of the human nervous system varies according to the location. Sensory neurons respond to external stimulus; CNS cells collect the converged signals while motor neurons regulate the muscles to complete movements in the body and head. Therefore, the understanding of the human nervous system is developed in a bottom-up manner and the network should be divided in an appropriate way. Generally, there are two kinds of approaches:

- By dividing the connected network into sub-systems with distinct functions such as audition, speech, vision and so on, experiments could be designed to probe and speculate the mechanism within a specific system.
- Generic property of a restricted region is detected, such as the neuronal connection, astrocytic connection and astrocyte-neuron connection. The arrangement of neurons and astrocytes is to be observed.

2.5.1 Sub-system Study

Neural coding [101, 102] focuses on the stimulus-response relationship of a single neuron or neurons in the ensemble. Neural coding is bonded with the type of sensory system. Besides, learning, memory, attention and motor system, it is also concerned with neural coding. The coding scheme includes rate coding, temporal coding, correlation coding and sparse coding. The coding methods within a specific system are introduced as follows.

2.5.1.1 Visual System

It has been suggested from theoretical study that sparse code is employed in the primary visual cortex (V1) to express natural vision. In [103], non-classic receptive field is stimulated, leading to the increase of sparseness in neuron population and decorrelation of neuron pairs. The structure of the stimuli is not crucial for the sparseness while the non-classic receptive field possesses excitatory or inhibitory effects on classic receptive field, which contributes to early vision and higher processing.

In the striate and extrastriate visual cortex of the macaque [104], it has been measured that a code of natural image is not necessary to have correlated lifetime and population sparseness. The neuron firing rate appears to seek the optimization of maximum sparseness and metabolic constraints.

The acceleration of object will generate a response in the visual system [105]. One third of the motion-sensitive neurons in the pigeons pretectal nucleus could detect acceleration of motion. These cells show a plateau-like speed-response curve while the firing rate is not changed over a wide range. They could induce transient responses to motion offset. Acceleration sensitive and insensitive neurons are separated in location.

In [106], the circuitry of cone inputs to blue-ON/yellow-OFF (BY) ganglion cells in the macaque retina is detected. The results indicated that the short (S) wavelength-sensitive cones is correlated with ON responses. One or more S cones contributes to patterned BY cells in a certain region while the received signals are not identical. The multiple inputs of S cones are summed linearly to the BY cells.

Other than the neuron, the astrocyte in the visual cortex exerts a response upon the application of a stimulus [107]. The orientation and spatially distributed frequency could be tuned by astrocytes while the preference is closely related to the neuron map. The sensitivity of hemodynamic mapping signal towards the astrocytes points to the crucial role of the astrocytes in the arrangement of brain imaging.

2.5.1.2 Auditory System

The response to a remarkably wide range of speech frequency is observed in the auditory midbrain of the guinea pig [108]. Since auditory neurons merely show increasing firing rates over a limited hearing range, the self-adjustment of neurons is requested to accurately perceive the extended range. The adjustment is fulfilled by measuring the mean, variance and other complicated statistics of the sound distribution.

Through human non-invasive magnetoencephalography (MEG) recordings in simple and party scenes, monaural and dichotic speeches are examined to uncover the neural coding method [109]. Phase-locked temporal code with difference in two hemispheres is employed. For example, response to speeches towards both ears is weaker and delayed than speech towards a single ear. Furthermore, the top-down attentional signal modulates the auditory coding.

2.5.2 Generic Sonnection Study

AP is the main signal transmitted in neuronal assembly. Nerve conduction has been studied for more than 80 years while the classic HodgkinHuxley model [110] lays the foundation for future improvements from cable-theory perspective. HodgkinHuxley model describes the initiation and propagation of APs in invertebrate giant axon including a series of differential equations, which integrates the electrical variations of axon membrane. Various properties such as conduction velocity of nonmyelinated nerve fiber [111] is derived and the factors affecting the velocity is presented. The vicinity between 'active' and 'resting' regions, which triggers 'local currents', is significant for nerve production.

Synaptic plasticity [112] is the strength or efficiency modification of synaptic transmission. It has been classified into short-term potentiation (STP) and long-term potentiation (LTP). STP lasts for several milliseconds to minutes which is related to sensory

response, short-time memory and transient changes in behavioural status. LTP is assumed to conduce to memory formation. Experiments [113] demonstrate that synaptic plasticity is clustered within a dendric branch, thus the localized potentiation spreads to a global area. Therefore, the entire dendric would be sensitive to the upcoming synaptic input. The function of synaptic plasticity predicts an explanation of perception and memory formation.

In [114], the GJC between astrocytes are supposed to increase the complexity of communication in the cerebral cortex. It has been shown that the astrocyte-astrocyte connection is enlarging the neuroglial interaction loop through intercellular calcium waves. Moreover, the diversity in the neuroglial projection, e.g. an astrocyte could contact multiple neurons, neurons connecting to peer neurons with varying strength, the dynamic property of GJC permeability, etc, would further conduce to the intricate network structure.

2.6 Conclusion

In this chapter, the author first introduces the progress in nanotechnology, leading to the fabrication of nanomachines which would facilitate the construction of nanonetwork. Secondly, different approaches of nanonetwork are described while their frameworks and characterizations are explained. Non-biological network utilizes electromagnetic waves while molecular communication can also employ bio-hybrid nanomachines to facilitate the communication. A number of network architectures and simulation tools have been developed for future study. Thirdly, the fundamentals and property of calcium signaling are discussed. Related mathematical models as well as biological experiments are presented. Fourthly, neuron doctrine and demonstrations of astrocyte involvement in cerebral cortex are provided. Finally, neural coding in sensory systems, synaptic plasticity and neural cable theory are described within the human nervous system.

Chapter 3

Conceptual Network Model from Sensory Neurons to Astrocytes of the Human Nervous System

In this chapter, a conceptual network model is proposed to express the signal transmission from the PNS to the CNS. It is divided into four layers: neurons in the PNS and the spinal cord, neurons in the brain, single astrocyte and groups of astrocytes. The external stimulation is eventually transformed to the states of the grouped astrocytes. The state combination signifies the current prototypical cognitive pattern induced by the stimulation which is converted to conscious episode through associating with the previous patterns. The parameters of each layer are obtained from specific configurations (e.g. the olfactory sensory system in layer 1 in the model) which could be adapted to other sensory systems. In addition, the model is scalable as the metrics employed such as network range, the number of astrocytes and neurons can be altered to emulate the real-time signal processing in the human body.

The author's contributions are as follows:

- The existing observations, biological experimental results and simulation results are studied, consolidated and integrated to build a conceptual network model for the ascending signal pathway in the nervous system.

- The communication fundamentals can be easily changed to model other sensory systems.
- The simulation results from the proposed model have been verified against the available biological experiments and mathematical models.
- The model provides insight as well as facilitates the demonstration of new biological experiments.
- A complete test case for the integrated network is presented and the model can be further validated and expanded from the results of future biological research for better understanding of the nervous system.

The rest of this chapter is organized as follows. The four-layer conceptual network framework is introduced in Section 3.1 and the characterizations of each layer are detailed. Section 3.2 describes the simulation parameters and definitions, the numerical results and analysis. Section 3.3 concludes this chapter.

3.1 Network Design

The ascending signal pathway is separated into different stages according to their anatomy. Fig. 3.1 depicts the network model overview. Layer 1 is defined as the neuron chain outside the brain which is a long distance signal pathway. The neuron chain starts from the sensing neuron and spreads across the spinal nerve/cranial nerve. Layer 2 includes the neurons in the brain which receive APs from layer 1. Surrounded by the neurons in layer 2, the astrocyte in layer 3 will generate calcium waves from its processes to the body. Layer 4 is a large-scale astrocyte network which is made up of groups of small astrocyte networks. Each sub-network includes several astrocytes as presented in layer 3. Compared to the lower layers, information in Layer 4 is the state combination and coupling

Figure 3.1: Four-layer network overview

with layer 3 waves which are not physically loaded in carriers but stored in unknown forms such as background field or expressed genes. These states, in the proposed model, indicate the cognitive pattern produced in a single trial. Definitely, more complicated processing methods upon receiving the message, exist, contributing to the final decision in the brain. Thereafter, the signal would return in the descending pathway resembling the ascending pathway (not discussed in this thesis). The layered network is illustrated as follows:

3.1.1 Layer 1

Layer 1 consists of one peripheral neuron and two intermediate neurons which could be called the sensing pathway. It is classified into two categories according to their locations: the spinal pathway and the cranial pathway. The former begins with the spinal nerve rising from a segment of the spinal cord and travels along the spinal cord to the brain. By comparison, the latter starts at the cranial nerve which directly emerges from the brain

Figure 3.2: Biological entities (A) and network entities (B) in layer 1

or brainstem, eg. the optic nerve which conveys visual information. Fig. 3.2A presents the schematic connection of layer 1 neurons in the spinal pathway and the structure is also applicable to the other pathway. The following anatomy introduction is based on the spinal pathway.

3.1.1.1 Biological Entities

Located in the ganglion of the spinal cord, the peripheral neuron (aka. first-order neuron, afferent) is a bipolar neuron whose longer axon ¹ contains receptors at the terminal and shorter axon makes up a synapse ² with the second-order neuron. The first-order neuron takes the responsibility of sensing the stimulation. Every neuron owns a certain type of receptors such as mechanoreceptors, photoreceptors, thermoreceptors and so on which are specific to the diverse sensory systems including somatosensory system, vision, motion, audition etc [8]. The receptors are implanted in the sensing area i.e. a finger could detect the intensity of stimulation and transform it to an electric pulse. If the stimulation strength exceeds a threshold, the electric pulse would be stable enough to pass through

¹A slender projection of neuron.

²The end of the axon of one neuron forms a cleft with the dendrite of the adjacent neuron.

the nerve fibre hence an AP is triggered. Otherwise, local electric oscillations called graded potentials would be generated [115]. The body of the neuron is housed in the dorsal root ganglion (spinal cord). The sensing neuron has two directions: one goes to the motor area via intermediate neuron constituting a reflex arc; the other links to the dendrite of the second-order neuron in the spinal cord.

Second-order and third-order neurons transmit AP and form an interconnected path from the sensory neuron to layer 2. The axon of the second-order neuron traverses different segments of the spinal cord to the third-order neuron. It is a multipolar cell which forms a number of synapses with different first-order neurons. The second-order neuron in the spinal cord has two kinds of locations: the dorsal column nuclei in the medulla for the lemniscal pathway and the dorsal horn of the spinal cord. The neuron axons of both types get over the midline³ to the contralateral side of the spinal cord. The former crosses midline at the decussation of medial lemniscus and extends to the thalamus. The latter arrives at the contralateral side in the anterolateral quadrant along the lateral spinothalamic tract.

The body of the third-order neuron lies in the thalamus whose axon forms the corticonuclear tract at the ventral posterolateral nucleus and goes up to the cerebral cortex. It is also a multipolar cell with plenty of synapses.

3.1.1.2 Network Entities

The neuron chain is modeled as a signal converter for the receptors of the first-order neuron and two signal amplifiers for the interface between the first-order neuron and the second neuron, as well as the second-order and third-order neurons (Fig. 3.2B). Characterizations of each component are detailed as follows.

Signal converter: Owing to the variety of receptors, the transformation rule upon stimulations differs. Hence, the author models one kind of sensory neuron (i.e. the olfac-

³An imaginary line that divides the body into right and left halves.

tory sensory neurons) for the proposed conceptual model. The feature of the converter could be adjusted to adapt to other sensory systems. For olfactory sensory neurons, it has been observed that the dose-response curve of mOR-EG (a kind of mouse olfactory receptor) obeys the Hill equations (See Appendix A.2.1) if the baseline is subtracted [116]. In Eqn. 3.1, f represents the frequency of AP. C denotes the concentration of the dose. The peak value is f_{max} while the baseline frequency is f_{base} . $k_{1/2}$ indicates the concentration of the stimulation when the frequency is half of f_{max} .

$$f = \frac{f_{max}}{1 + \frac{k_{1/2}^n}{C}} + f_{base} \quad (3.1)$$

Signal amplifier: By means of the interfaces with the previous component in the neuron chain (Fig. 3.2B), the second-order and third-order neurons exhibit signal divergence and convergence. The hierarchical structure of the amplifier is interpreted in Fig. 8-3A of [115] (see Fig. A.1). Each evoked potential in the first-order neuron is split and transmitted to 3 second-order neurons. Subsequently, the signal is aggregated in the second-order neuron and its amplitude and frequency rise. The same applies to the third-order neuron.

3.1.2 Layer 2

3.1.2.1 Entities

Layer 2 entity is defined as the CNS neuron which relays the AP to the cerebral cortex. These intermediate neurons include the first-order and higher-order thalamic relay neurons, as well as the cortical neurons. The signal transmission in this layer is related to the various sections in the brain. An individual intermediate neuron which sustains the frequency of the afferent AP is adopted so as to simplify the proposed model.

3.1.2.2 Communication with Layer 3

The most important function of layer 2 neuron is to interact with the layer 3 entity — astrocyte. The cortical neurons are located next to the processes (branches) of the

astrocytes. Two neurons and an astrocyte constitute a tripartite synapse. Fig. 3.3 shows the bi-directional communication at the tripartite synapse. The pre-synaptic neuron receives an AP and releases neurotransmitters such as Glu, ATP, GABA, etc. to the synapse triggering the generation of inositol (1, 4, 5)-triphosphate (IP_3) in the astrocyte. Then calcium ion (Ca^{2+}) is discharged from the intracellular store in the astrocyte. In return, the astrocyte produces neuroactive molecules such as Glu and D-serine to affect the AP property in the post-synaptic neuron as well as the synaptic plasticity. Through the coordination with neurons, astrocyte summarizes synaptic information and elicits Ca^{2+} spikes. The detailed communication procedure is as follows:

- step a: When an AP arrives at the pre-synaptic neuron, Glu and other neurotransmitters are delivered to the synaptic cleft while some of the Glu binds to the metabotropic glutamate receptor (mGluRs) of the astrocyte.
- step b: IP_3 is synthesized in the astrocyte which binds to the IP_3Rs on the endoplasmic reticulum (ER). The Sarco Endoplasmic Reticulum Ca^{2+} ATPase (SERCA) pumps Ca^{2+} from the ER. When the Ca^{2+} transient exceeds the threshold, Ca^{2+} oscillations are induced.
- step c: Gliotransmitters such as Glu are released to the cleft activating the extrasynaptic Glu receptors (NMDARs) on the post-synaptic neuron. This motivates slow inward current (SIC) in the post-synaptic neuron which is much larger than the excitatory post-synaptic current (EPSC) directly triggered by the pre-synaptic neuron via neurotransmitter binding.

The calcium wave could be encoded by its amplitude or frequency [117]. The coding scheme of the astrocyte is modeled in [118] and the range of AM, FM and AFM are calculated using Li-Rinzel model [119]. [1] couples the neuronal activity while the author concentrates on the relationship of pre-synaptic neuron and the astrocyte in the proposed model.

Figure 3.3: Tripartite synapse: message exchange between astrocyte and neurons

3.1.3 Layer 3

3.1.3.1 Stimuli and Wave Area

The propagation of Ca^{2+} waves within the astrocyte is characterized by the Ca^{2+} waves initiated at different astrocyte process sites. There exist two kinds of Ca^{2+} waves: a) the focal spontaneous Ca^{2+} waves which are not aroused by AP [120]. b) the expanded Ca^{2+} waves which are restricted to the processes under single electric stimulus (AP). However, trains of electrical stimuli evoke propagated Ca^{2+} waves which may cover the soma of astrocyte. In [121], electrical field stimulation (EFS) initiates APs in the mossy fibers⁴ while the number of bursts is related to the propagation area of Ca^{2+} waves. The extracellular Glu is generated at the tripartite synapse which binds to the astrocyte plasma membrane. The Glu concentration near the astrocyte processes is approximately

⁴The unmyelinated axons projected by granule cells in the hippocampus to the CA3

Figure 3.4: Astrocyte with 6 processes

linear to the number of stimuli as well as the area of coverage of Ca^{2+} . Eqn. 3.2 shows the correlation:

$$\# \text{ of stimuli} \propto \text{Glu concentration} \propto Ca^{2+} \text{ area.} \quad (3.2)$$

Consequently, it could be deduced that only sufficient Glu leads to the occurrence of Ca^{2+} wave in the soma due to its free diffusion.

A simplified astrocyte is shown in Fig. 3.4 whose diameter of the soma is $3.5\mu m$ and the diameter of the process is $2\mu m$. Each process has a length of $23.5\mu m$ which marks the territory of the whole astrocyte (estimated from [122]). The diffusion speed of Glu in the extracellular space could be estimated from experiments [121].

3.1.3.2 Assumption of Multiple Inputs from Branches

Each neuron is allocated to only one astrocyte process while the microdomains of different tripartite synapses do not overlap. Hence, APs trigger local Ca^{2+} waves in independent microdomains of an astrocyte. On account of the existence of global Ca^{2+} waves and

the insufficiency of an individual AP to lead an expanded wave, it is assumed that the accumulation of local Ca^{2+} waves from several sites of the same astrocyte would propagate to the soma and other branches hence inducing a global Ca^{2+} wave. Further experiments could be conducted to validate this hypothesis.

3.1.4 Layer 4

Layer 4 includes groups of astrocytes which have a hierarchical structure consisting of a sub-network and a backbone-network (Fig. 3.5). Conscious episode is the data loaded in this layer. We first assess the potential of the astrocyte to aggregate information from the lower layers.

3.1.4.1 Astrocyte and Cognitive Processing

Astrocyte could integrate synchronized synaptic inputs and generate intercellular Ca^{2+} waves among several or hundreds of astrocytes [123]. Intercellular Ca^{2+} waves transmit via GJC — which is made up of plasma membranes of adjacent astrocytes. The extracellular ATP, Glu and other possible gliotransmitters, as well as the IP_3 which diffuse through GJC, cooperate to generate Ca^{2+} release in the unstimulated astrocyte.

The GJC permeability could be modified by external factors, such as pH, transjunctional voltage and intracellular Ca^{2+} [54]. Therefore, the coupling level of two adjacent astrocytes varies depending on intracellular and extracellular conditions. In this regard, the astrocyte network could be divided into plenty of sub-networks while the involvement of a sub-network is flexible.

It is conceived that astrocyte participates in cognitive processing for the following reasons:

- Astrocyte Ca^{2+} waves could propagate to a long distance [124] which have the potential to transmit conscious episodes through layers of cortex or to a distant region of the human brain.

- The discovery of new kinds of astrocytes and the giant size of protoplasmic astrocyte with increasing number of synapses imply the significant function of astrocytes in higher order information processing.
- Astrocyte along with post-synaptic neuronal activity releases pre-synaptic type I mGluRs, thus inducing LTP [83]. Besides, astrocyte could increase its dynamic range.
- Astrocyte extends endfeets on the blood vessel to drive the hemodynamic conditions to supply energy for brain activity which directly regulates the blood-oxygen-level-dependent (BOLD) functional magnetic resonance imaging (fMRI) signals [125]. Since BOLD fMRI is correlated with the prototypical cognitive patterns, astrocyte thus takes part in cognitive processing.

It is proposed in [126] that AP represents the content of external stimulation which is transformed to Ca^{2+} wave as the "feeling" or "awareness" of the information. While the formation of conscious episode is still not well researched yet, the author's assumption is that the state combination of certain astrocyte networks indicates the prototypical conscious pattern such as cold, thirst, hunger and so on. The information of a physical stimulation exerted at a restricted area is encoded in the frequency and amplitude of Ca^{2+} wave while the spatially distributed Ca^{2+} waves encapsulate the cognitive pattern. The conscious episode requires binding of a sequence of prototypical patterns. The phase of Ca^{2+} wave is not discussed here though they may have relevance in the information processing.

3.1.4.2 Sub-Network

The author uses (amplitude, frequency) pair to denote the Ca^{2+} wave in an astrocyte. Each astrocyte has a unique id (order). The state combination is expressed in a one-dimensional matrix while the element is the amplitude-frequency pair. The events are

Figure 3.5: Sub network and backbone network of astrocyte

arranged into several time slots and a stimulus is applied at the beginning of a time slot. For instance, if a 5-astrocyte network with only two activated astrocytes is modeled, its state in a single time slot is:

$$A_s = \begin{pmatrix} (0,0) & (a_2, f_2) & (a_3, f_3) & (0,0) & (0,0) \end{pmatrix} \quad (3.3)$$

Different from utilizing trapped-ion quantum computing [125] to explain the states transition, it is assumed that the entanglement of the conscious episode involves three prototypical cognitive patterns denoted as A_1 , A_2 and A_3 . Matrix $A(t)$ (Eqn. 3.4) stores the conscious episodes formed according to the past stimuli where $A(t)$ will decay as the time passes. The degradation matrix B is described in Eqn. 3.5. β_i denotes the degradation factor. When a new cognitive pattern is received, $A(t)$ would be updated as shown in Eqn. 3.6. α_{ij} represents the impact coefficient of each past cognitive pattern component and current episode to the updated cognitive pattern.

$$A(t) = \begin{pmatrix} A_1 & A_2 & A_3 \end{pmatrix} \quad (3.4)$$

$$B = \begin{pmatrix} \beta_1 & 0 & 0 \\ 0 & \beta_2 & 0 \\ 0 & 0 & \beta_3 \end{pmatrix} \quad (3.5)$$

$$A(t + \Delta t) = \begin{pmatrix} \alpha_{10} & \alpha_{11} & 0 & 0 \\ 0 & \alpha_{21} & \alpha_{22} & 0 \\ 0 & 0 & \alpha_{32} & \alpha_{33} \end{pmatrix} \begin{pmatrix} A_s \\ A_1 \\ A_2 \\ A_3 \end{pmatrix} B \quad (3.6)$$

3.1.4.3 Backbone-network

The backbone-network is the assembly of sub-networks and the transition function remains the same.

3.1.5 Extension

The termination of communication is the conscious episode in the astrocyte network in the proposed network model. In reality, the descending pathway is also indispensable in the generation of movement. Firstly, astrocyte relays and interferes with the post-synaptic neuron AP. Secondly, the AP goes down the neuron chain to the motor neuron and control the movement of muscles.

3.2 Results analysis

The conceptual network model based on the four-layer structure is developed with the continuous time element being simplified to a discrete event list.

The parameters of each layer are first determined and then the simulation results are compared to the biological experiments or mathematical models. After that, a test case of the entire integrated network model is discussed.

Table 3.1: Parameter set

Designation	Value
f_{max}	35.5Hz
$k_{1/2}$	$10^{-5.6}$ M
f_{base}	2.18Hz

3.2.1 Layer 1

3.2.1.1 Signal Converter

The results shown in Fig. 3D in [116] provides a dose-response curve which acts as an experimental proof for the simulation. Data points of the experiment are extracted from the figure. Odorant is conducted to the first-order sensory neuron and AP of specific frequency is generated. The parameters for mOR-EG cell are adapted to fit in the experiment result. The parameter set is listed in Table 3.1. To simulate the actual transmission process, additive white Gaussian noise with $N(0, 0.5)$ is incorporated into the signal converter.

The odor stimulation with changing concentration is carried out in the first-order neuron in layer 1. Stimulus-response result in the simulation and the result in [116] are compared in Fig. 3.6.

3.2.1.2 Signal Amplifier

On the basis of the above theoretical analysis, the author characterizes the signal divergence and convergence into a two-dimensional area where the neurons are patterned in a lattice. 9 first-order neurons receive the stimulus where the strength of the stimulus decreases from the center. As displayed in Fig. 3.7A, AP coverage for first-order neurons is 9 cells (in blue) and expands to 21 neurons when APs are transmitted to the second-order neurons (an additional 12 green nodes). Furthermore, the strength of the 9 centered secondary neurons elevates due to convergence from the surrounding primary

Figure 3.6: The property of signal converter in layer 1: simulation and biological results comparison

neurons. The activated area of third-order neurons enlarges to 37 neurons. Fig. 3.7B presents the frequency map for the 37 third-order neurons under a spatially distributed stimulus whose strength reduces from the center to the edge. The primary receptive field (9 neurons in Fig. 3.7A) extends after two signal amplifiers.

3.2.2 Layer 2

In the proposed network model, there is only one CNS neuron in layer 2 as the intermediate neuron between a single layer 1 neuron and a layer 3 entity (Fig. 3.1). Its frequency is the same as the corresponding third-order neuron in layer 1. Nevertheless, there should be a queue of connected neurons between two layer 1 and layer 3 entities in the actual situation where the signal would experience some attenuation. The single central neuron is representative of the layer 2 entity while the model could be improved when new biological research results are presented in the future.

Figure 3.7: A: first-order neurons send APs to second-order neurons. B: frequency map of third-order neurons

For the communication with layer 3, the author acquires the parameters from the Li-Rinzel model for AFM encoding in the astrocyte combined with the mediation of AP [1]. The frequency of AP and the amplitude/frequency of the Ca^{2+} waves in the astrocyte process are plotted in Fig. 3.8 and Fig. 3.9 respectively. The first bifurcation point is at $f = 15Hz$. When the frequency ranges from 5 Hz to 15 Hz, Ca^{2+} waves follow amplitude modulation while frequency modulation is employed when the frequency is greater than 15 Hz. The second bifurcation point is at $f = 35Hz$ after which the frequency of Ca^{2+} waves rapidly rises to 1Hz. The behaviour of the calcium wave as communication progresses to the next level (shown in Figs. 3.8 and 3.9), are demonstrated by the proposed model which is founded on the published work of [1].

3.2.3 Layer 3

In this layer, the local Ca^{2+} waves propagate from the branches to the soma of a single astrocyte producing the global Ca^{2+} waves. The author analyzes the propagation speed and amplitude degradation of Ca^{2+} wave during the propagation process while its

Figure 3.8: Ca^{2+} amplitude of different action potential frequency

Figure 3.9: Ca^{2+} frequency of different action potential frequency

frequency is proposed to be invariant.

The shape of the astrocyte in the proposed model is depicted in Fig. 3.4 and the

following computation is based on the iGluSnFR concentration in Fig. 6G of [121] (Fig. 6G has been reproduced as Fig A.2B) The wave coverage increases as the stimuli number increases while the speed is not constant (Fig. 3.10B). The propagation speed of Ca^{2+} wave per stimuli is roughly estimated. The speed peaks at 8 stimuli. When the number of stimuli equals to 15, Ca^{2+} wave could reach almost the entire territory of the astrocyte domain (Fig. 3.10A). Glu concentration has similar varying pattern as the Ca^{2+} wave coverage except for the deviation at the two stimuli (Fig. 6F of [121], reproduced as Fig. A.2A). Hence it is inferred that the Glu generated at the stimulation point diffuses to extracellular space and decreases to the minimum concentration required to initiate the Ca^{2+} wave.

The stimulation is applied at a single branch of the astrocyte and it is envisaged that if other branches receive the same AP, the Glu and ATP would transmit over a longer distance⁵. In our simulation, we assume that for every stimulation from the environment, at least 8 spikes are initiated. The number is selected to ensure that the evoked Ca^{2+} wave would propagate across the current astrocyte and it alone, is not strong enough to cover the entire astrocyte territory.

Under this circumstance, the author studies the location of the stimulus which will result in a global Ca^{2+} wave coverage and justifies that if any two branches within an astrocyte get the AP, the astrocyte would induce Ca^{2+} wave. The amplitude of Ca^{2+} wave drops in the propagation as reported in Chapter 5. The decay speed of Ca^{2+} wave follows the diffusion of extracellular neurotransmitters. Therefore, the information embedded in Ca^{2+} wave would have some distortion. The author maintains the frequency

⁵Owing to experimental constraints, currently there are no biological experimental results for scenarios where the stimulation is applied to more than one branch. Research is still ongoing to prove the hypothesis as it is intuitive that with multiple stimuli, the transmission distance should be longer.

⁵Wave propagation speed is expressed as:

$$V = \frac{Area_{current} - Area_{last}}{\#stimuli_{current} - \#stimuli_{last}}$$

Figure 3.10: A: Ca^{2+} wave propagation distance per stimulus. B: Ca^{2+} wave propagation speed⁵ (in terms of area) per stimulus

of Ca^{2+} wave to represent a component of information as there is no published literature so far which indicates that the frequency would change notably.

3.2.4 Layer 4

After the global Ca^{2+} wave floods the astrocyte domain, intercellular Ca^{2+} wave could be formed by the diffusion of extracellular Glu and ATP as well as the IP_3 through the GJC. Permeabilities of GJC are not identical while in every transmission trial they could be varied to form different sub-astrocyte network topology. Fig. 3.11 depicts a lattice-shaped astrocyte network along with the GJC permeabilities between adjacent astrocytes. The red node represents the stimulated astrocyte while the dark blue nodes denote the astrocytes with triggered Ca^{2+} wave. The remaining light blue nodes are the astrocytes failing to generate Ca^{2+} wave as a result of GJC permeability restraints. More detailed explanation on the permeabilities can be found in Chapter 5. Due to the

Figure 3.11: Sub-astrocyte network topology

significance of GJC permeability, the author incorporates its variation to complete the function of the network model.

Other than GJC permeability, the amplitude of Ca^{2+} wave largely restricts the information transmission. In the proposed model, Ca^{2+} wave amplitude ranges from $0.05\mu M$ to $0.53\mu M$ while the sub network diameter is at most $300\mu m$ to ensure the minimum concentration of Ca^{2+} wave.

The parameter set for Eqns. 3.5 and 3.6 is listed in Table 3.2. The author tests an instance that a train of repeated stimuli is applied in a continuous time slot ($7\Delta t$). It is supposed that three astrocytes in a sub-network could be activated and their initial states are resting ($0\mu M$). Ca^{2+} wave amplitude from a single activation is ($0.04\mu M$, $0.15\mu M$, $0.20\mu M$). The author defines the matrix A_1 in Eqn. 3.4 as the "current states" and the following results describe the value of this matrix. The amplitudes of the three astrocytes are plotted in Fig. 3.12. Apparently, repeated stimuli conduce to strong response which lasts for a longer duration. The result conforms with the biological facts that conscious processing is related to memory of past prototypical cognitive patterns.

Figure 3.12: Current states: Ca^{2+} wave amplitude of three astrocytes

Table 3.2: Parameter set

Designation	Value
β_1	0.9
β_2	0.9
β_3	0.9
α_{10}	0.95
α_{11}	0.75
α_{21}	0.7
α_{22}	0.65
α_{32}	0.6
α_{33}	0.55

3.2.5 Integrated Network Model

In this section, a stimulus is projected to a receptive field of first-order neuron as shown in Fig. 3.7A while the central and edge odor concentrations are $C_1 = 10^{-5}M$ and $C_2 = 10^{-5.5}M$, respectively. 49 layer 2 neurons (Fig. 3.7B) are allocated corresponding

Figure 3.13: Instantaneous Ca^{2+} wave amplitudes generated by a single stimulus in all the astrocytes of the backbone-network

to the third-order neurons and they are arranged into 4 groups: the first three groups each consists of two rows and the last one has only one row. Every two adjacent neurons connect to two branches of an astrocyte. Each group refers to a sub astrocyte network. The GJC permeability matrix for the seven astrocytes in a sub network is:

$$P = \begin{pmatrix} \infty & 0.95 & \infty & 0.02 & \infty & \infty & \infty \\ 0.95 & \infty & 0.01 & \infty & 0.9 & \infty & \infty \\ \infty & 0.01 & \infty & \infty & \infty & 0.01 & \infty \\ 0.02 & \infty & \infty & \infty & 0.85 & \infty & 0.88 \\ \infty & 0.9 & \infty & 0.85 & \infty & 0.015 & \infty \\ \infty & \infty & 0.01 & \infty & 0.015 & \infty & \infty \\ \infty & \infty & \infty & 0.88 & \infty & \infty & \infty \end{pmatrix} \quad (3.7)$$

where ∞ denotes connection towards itself or no connection.

Fig. 3.13 provides the amplitudes of Ca^{2+} wave in the 4 sub-networks when a single stimulus is applied triggering a prototypical cognitive pattern.

The scenario that a second stimulus is applied at $t = 4 \triangle t$ is examined. Average

Figure 3.14: Current states: average Ca^{2+} wave amplitude in the backbone-network with 4 sub-networks

amplitude is counted to represent the overall status of each sub-network. The result is shown in Fig. 3.14. The second stimulus "evokes" the fading "memory" of the first stimulus and the response is enhanced.

The proposed conceptual network serves as a fundamental tool to study the performance of the components and will predict their function and interaction. The simulation results presented above are consistent with available biological experiments and mathematics models. If the proposed model is further validated by future biological experiments, the model will facilitate the accurate target application of medication to treat brain related diseases via nano sensors or other biological carriers.

3.3 Conclusion

In this chapter, a conceptual network model based on the cellular characterizations of the nervous system has been developed. This model analyzes the signal processing pathway from the external stimulation on the sensory neurons to the astrocytes, resulting in the

generation of conscious episode in the human brain. The astrocyte-centric hypothesis is adopted in the proposed model as the astrocyte regulates the information transmission. The proposed conceptual network model is divided into four layers according to the anatomy and property of every component within the signal pathway. The simulation results are verified against the available biological experiments and mathematical models and provide a test case of the integrated network. As the production of conscious episode in the human nervous system is still under intense research, the proposed model serves as a useful tool to facilitate, complement and verify current and future study in human cognition.

Chapter 4

Modeling of Communication Between Neurons and Astrocytes in Laminar Cerebral Cortex

In this chapter, a communication model of astrocytes and neurons is built based on their columnar and laminar arrangement. The complete communication procedure from the thalamic input to Layer 5 (L5) output is simulated. More importantly, astrocytes are integrated into the neuronal microcircuits and its function has been analyzed. Astrocytes process the neuronal activities and generate new responses by coupling the past ones in the local domain. Meanwhile, astrocytes also maintain an interconnected network to deliver information to selected neighbors.

The rest of this chapter is organized as follows. The columnar and laminar structures of neurons in the cortex are introduced in Section 4.1. Section 4.2 describes the types of astrocytes and their characteristics. The interconnected network model of astrocyte and neurons are detailed in Section 4.3. Section 4.4 presents the simulation parameters and definitions, the numerical results and analysis. Section 4.5 concludes the chapter.

4.1 Neuron in Cerebral Cortex

A neuron has a cell body, long processes called axon and several small processes called dendrites. Axons are always covered with sheath and bundled together to form nerves. Sensory and motor neurons in peripheral nervous system (PNS) are linked through nerves spreading from CNS. Cerebral cortex receives sensory signal from PNS and sends decision signal to the motor neurons eventually. As neurons make up the majority of the cerebral cortex, the exchange of information in the cortex is mostly fulfilled by the cooperation of the neurons. The laminar and columnar structures in the cerebral cortex are discussed in the following sections.

4.1.1 Laminar Structure

Through scanning of stained cross section, the cerebral cortex could be divided into six layers (at most) [8, 91]. The number of layers in different areas of the cerebral cortex varies. For example, the primary visual cortex has 6 layers while archicortex has only 3 layers. The more ancient layer has fewer layers according to the evolution rule. The 6-layer cortex is exclusive in the mammalian brain which is also called the neocortex. The ratio of the neocortex to the medulla in the brainstem in human overwhelms that of other mammals by 60:1 [127].

From outside (i.e. the pial surface of the cerebral cortex) to inside (i.e. near the white matter), each layer is defined as follows. The first three layers are also called the subgranular layers. Layers 5 and 6 are also called infragranular layers (Fig. 4.1).

Layer 1: Mainly contains profuse dendrites branch of pyramidal neurons as well as horizontally oriented axons.

Layer 2: Contains small pyramidal neurons and numerous stellate neurons.

Layer 3: Contains pyramidal neurons with horizontal projections and non-pyramidal neurons with vertically intracortical axons.

Layer 4: Contains the majority of stellate cells.

Layer 5: Contains pyramidal neurons with axons projected to thalamus and other subcortical structures (e.g basal ganglia).

Layer 6: Contains multipolar neurons, few large pyramidal neurons and many small spindle-like pyramidal.

Synapse is the gap between the dendrite of one neuron and the axon of another neuron. A capsule enwrapping chemicals could be released from the pre-synaptic neuron to the gap. After binding to the post-synaptic neuron, the chemicals enter the cell and an electric pulse may be elicited. Through synapse, neurons pass information to the surrounding neurons.

Neurons are linked by synapses across the layers and within the local layer. The connection entities between the different layers is not identical throughout the 6 cortical layers. The distribution of neuronal types decides the connection pattern. In addition, the responses of the received signal in each layer is dependent on the inherent neuronal dynamics. For instance, the frequency of oscillation in Layers 2/3 is 2 Hz while that in L5 is 10-15 Hz [128].

4.1.2 Columnar Structure

Cortical columns are aggregated by a set of neurons with similar receptive fields. They are perpendicular to the pial surface and have space between each other. The actual column starts from L2 to L6 since there are merely a few scattered neurons in L1. Within

Figure 4.1: Laminar structure of neurons in the cerebral cortex

one cortical column, there are about 50-100 minicolumns, each comprises about 80 neurons. 2×10^8 million minicolumns are estimated in the human cerebral cortex [129]. A minicolumn has an average traverse diameter of 40-50 μm and 80 μm for spacing [130].

It has been proposed that the minicolumns are the elementary functional units of the cortex [131]. Adjacent minicolumns have overlapped receptive fields which may enhance the response of external stimulus by receiving effective redundancy and information are gathered in a spatially distributed way (Fig. 4.2).

4.2 Astrocytes in Cerebral Cortex

From low order mammals such as mouse to primate order mammals such as chimpanzee, the complexity of astrocytes has been progressively identified in the following aspects:

- a) New forms of astrocytes [9] appear different from the traditional stellate form (protoplasmic astrocyte). Two types of new astrocytes - interlaminar astrocyte and varicose

Figure 4.2: Columnar structure of neurons in the cerebral cortex

projection astrocyte are discovered in the primate order. The functions of these novel astrocytes are still under research and it is probable that they have distinct abilities from the protoplasmic astrocytes.

- b) The size of astrocyte increases. For example, protoplasmic astrocyte in human has a 2.6-fold diameter and 10-fold more GFAP¹-defined processes compared to rodents [122].
- c) A single astrocyte encompasses more synapse, from 12000~20000 of rodents to 27000~2 million in human. Thus the more vibrant activity and participation of astrocytes could be inferred through more contact sites.

The function and anatomy of the interlaminar astrocyte and the protoplasmic astrocyte are discussed as follows:

¹Glial Fibrillary Acidic Protein

Figure 4.3: Interlaminar and protoplasmic astrocytes in the cerebral cortex

4.2.1 Interlaminar Astrocyte

Interlaminar astrocytes are primate-specific and abundantly populate L1 of the cerebral cortex. Fig. 4.3 shows the location of interlaminar astrocytes. The cell body and short processes prevail in L1 while the long processes expand farthest to L4. Bundles of interlaminar astrocyte form the palisade shape. The diameter of the cell body of the interlaminar astrocyte is 10 nm which is much smaller than the protoplasmic astrocyte. Long processes of interlaminar astrocytes contain massive mitochondria which indicates that they may undertake energy-consumption task. It has also been found that the long process in Albert Einstein's brain has more bulbous endings and mitochondria [132]. Scientists speculate that the number and property of interlaminar process is relevant to the intelligence and utilization of the brain. The long process terminates in a neuropile, blood vessel or penetrates the domain of the protoplasmic astrocytes. Each interlaminar astrocyte has one or two long processes which also guards the edge of the neuron minicolumns. The tortuous long process could also form GJC with protoplasmic astrocytes.

4.2.2 Protoplasmic Astrocyte

Protoplasmic astrocytes are the most common cells found in the human brain. They are more popular in white matter to support the living and working expenses of the neurons in the gray matter. However, protoplasmic astrocytes are also found in Layers 2 to 6 in the cerebral cortex. The star-shape protoplasmic astrocyte has a round cell body with thousands of spreading short processes or long processes. In [133], researchers revealed that there are similar stellate astrocytes with different length of processes while they have distinct chemical property such as GFAP staining. In this thesis, the author categorizes these astrocytes into protoplasmic astrocytes since their anatomy has mutual characteristics and the features which will be studied in the following sections are similar.

The domains of neighboring astrocytes do not overlap with one another. GJC are the channel formed by massive pores on the membranes of two adjacent astrocytes where ions and certain compounds could diffuse through. An activated astrocyte possesses calcium waves with certain amplitude and frequency. When the calcium wavefront arrives at the GJC, mediating messengers such as Inositol 1,4,5-trisphosphate (IP_3) and GFAP are synthesized and released. The neighbor astrocyte thus generates calcium ion (Ca^{2+}) from the distant process and subsequently propagates to the whole cell. Consequently, calcium wave in the astrocyte network could spread via a trail of GJCs.

4.3 Network of Neurons and Astrocytes in Cerebral Cortex

Astrocyte:neuron ratio in the cerebral cortex is 1.48 [10] and the ratio in the neocortex is 0.266 [134]. Thus the network in cortical layers is composed of interconnected protoplasmic astrocytes and neurons. Neurons receive inputs and generate fast responses while astrocytes, in contrast, have a rather long responding period. Hence astrocytes are

Figure 4.4: Microcircuits of neuronal minicolumns in the cerebral cortex

proposed to function as "cache pool" and storage of perceptual states. The circuits of neurons and astrocytes as well as the interweaving of both are detailed in the following.

4.3.1 Microcircuits of Neurons

Thalamus receives ascending signal from PNS. Thereafter, thalamic afferent projects to certain position of the cortex according to the function of the systems. For example, the parvalbumin-containing nuclei which relays sensory information of a single sensory modality projects to Layers 3, 4 and 6. However, the calbindin-containing cell which receives more diffuse inputs projects to L1 and a small portion of L3 [135]. Generally, spiny neurons in L4 are the main target of excitatory thalamocortical afferents [136] .

It is observed that interneurons in L4 are the parallel target of thalamic inputs.

The interneurons in turn release GABA². Action potentials (APs) generated in spiny neurons of L4 excite the pyramidal neurons in L2/3. Likewise, the interneurons in L2/3 also receive inputs from L4 to inhibit the pyramidal neurons in L2/3. Simultaneously, L2/3 neurons also receive intralaminar excitatory signals [136]. Each L4 spiny neuron innervates 300-400 L3 pyramidal cells while 300-400 L4 cells converge on each L3 target [92]. Connection ratio in L4-L4 is 1/3 of that in L4-L2/3 [137].

Excitatory connection also projects from L2/3 to L5 [92]. L5 is supposed to be the behavior/movement outputs to the corresponding columnar module through the inter-laminar connections [138]. L2/3 carries sensory information and L5 is responsible to carry out the action. After the filtering of sensory information, relevant aggregated signals are exported to L5.

The receptive field of the same column is identical [13]. The thalamic input will be transmitted to the cortical neurons in the same column. Based on the results from rats [15], it could be inferred that inter-column communication exists. Minicolumns are only found in mammals [139] which signifies the more complex brain structure of higher order animals.

4.3.2 Astrocytes and Neurons in a Network

Consciousness episode is related to direct sensory response while thinking and learning require combinations of several conscious episodes - working memory. Hence long-term memory is important in this process [140]. Astrocytes are fundamental to working memory [141] which could also be inferred from the slow peak time.

Local protoplasmic astrocyte enwrap several neurons in their domains and these neurons have 4-5 contacts to the dendrites [142]. Each contact is a tripartite synapse, which is a special synapse made up of two neurons and one astrocyte (Fig. 4.5). The end of

² γ -Aminobutyric acid to inhibit the spiny neurons

Figure 4.5: Protoplasmic astrocyte and the surrounding neurons in the cerebral cortex

an astrocyte process interacts with a traditional synapse. Gliotransmitters such as glutamate are released into the cleft and assist the initiation of calcium oscillations in the astrocyte process. In turn, the neurotransmitter generated by the astrocyte affects the frequency of the electric pulse in the post-synaptic neurons. Calcium wave expands to the entire astrocyte eventually. Protoplasmic astrocyte has a longer attenuation period than neurons [140] and the working memory could be stored after sensory input vanishes. Despite the intrinsic decay of the astrocyte, the original response will be enhanced if several repeated stimulus are exerted.

The communication between protoplasmic astrocytes via GJC enables information handling and filtering. Thus the signal could be shared globally. The calcium fluxes triggered in the local astrocyte is limited and will not flood the whole astrocyte unless the received neuronal input exceeds the threshold. In addition, the activated astrocyte will boost the neighboring inactivated astrocytes. Therefore, in a global view, the astrocyte signal will expand and attenuate while the information is averaged and enhanced in the

Figure 4.6: Protoplasmic astrocyte network in a certain layer

surrounding area (Fig. 4.6). There may exist other mechanisms concerning phase-locking and state-binding under certain frequency.

The function of interlaminar astrocyte in the network is still not clear. However, experiments suggest that interlaminar astrocyte has relation to the higher cognitive process and human intelligence. In the experiment, the author supposes that the GJC between interlaminar astrocyte and L2 protoplasmic astrocyte varies according to the type of the stimulus while only under certain circumstances is the GJC permeability large enough to transfer a calcium wave [71]. As a consequence, interlaminar astrocytes are only sensitive to a specific kind of stimulus. Normally, the neuronal input required to activate the interlaminar astrocyte is larger than the protoplasmic astrocyte since the long processes of the former extend to L3/L4 neuropile and hence demands reinforced driving power.

4.4 Simulation Definition and Results

The thickness of each cortical layer is listed in Table 4.1 with the data from [143]. According to the astrocyte:neuron ratio, the number of protoplasmic astrocyte is derived.

As the neuron body is about $10\ \mu m$ and the core width of a minicolumn is about $10\text{-}15\ \mu m$, the neuron body is limited in the core of the minicolumn. Let the neurons distribute evenly within the layer. Each L4 neuron could connect to all the neurons in 9 nearest neighboring minicolumns in L2/3. Each L2/3 neuron covers all the neurons in 9 nearest neighboring minicolumns in L5. Each neuron connects to all the neurons in the current minicolumn and 6 neighboring minicolumns in the local layer. Although the longest process of astrocyte can reach $100\text{-}200\ \mu m$ [144], the actual domain of an astrocyte is only about $80\ \mu m$ from Fig. 4B (See Fig. B.1) in [122] since its processes are tortuous and discrete. The width of a minicolumn is $50\ \mu m$ and the distance between the centers of two adjacent minicolumns is $130\ \mu m$. Therefore, it is assumed that an astrocyte does not extend to other minicolumns since the main volume is less than the sum of radius and spacing. Suppose that astrocyte is vertically aligned and the body is randomly distributed in a $10 \times 10 \times 10\ nm^3$ cube. Likewise, a protoplasmic astrocyte could contact 8 neighbors including 6 astrocytes of the same vertical position in the neighboring minicolumns and two vertically adjacent astrocytes in the same minicolumn. Thereafter, each neuron is allocated to an astrocyte by the location and the domain of each astrocyte is about a $80 \times 80 \times 80\ nm^3$ cube. Besides, each interlaminar astrocyte could contact all the L2/3 protoplasmic astrocytes in 3 nearest minicolumns via the long processes. Each minicolumn has only one interlaminar astrocyte. The author modulates the communication within a column and there are in total 91 minicolumns in a column which are patterned as hexagon cell (Fig. 4.2). 9 neighbors are selected from 6 minicolumns of the first layer and 3 neighbors of the second layers.

The voltage of electric pulses from intralaminar neighboring and interlaminar neighboring neurons are represented as V_{intra} and V_{inter} , respectively. Assume V_{intra} follows uniform distribution $U(0.4, 2.0)$ while V_{inter} has a distribution of $U(0.2, 4.4)$ [92]. The resting state of neurons has a voltage of $-70\ mV$ while the threshold for generating AP

Table 4.1: Parameter set

Layer	total neuron	pyramidal neuron or spiny	astrocytes
1	5	N/A	1
2/3	68	51	22
4	33	24	6
5	32	24	10

is 55 -mV. Eqn. 4.1 provides the condition of AP generation in an inactivated neuron.

$$AP = \begin{cases} Yes & \text{if } -70 + \sum V_{intra} + \sum V_{inter} > -55 \\ No & \text{Otherwise} \end{cases} \quad (4.1)$$

The calcium wave amplitude of astrocytes will attenuate after one time interval and it is assumed that the attenuation rate of Ca^{2+} amplitude is 0.1.

4.4.1 Local Communication within L4

The communication within L4 when thalamic inputs are imposed on L4 spiny neurons is analyzed. When about 25% input minicolumns are involved and in each minicolumn, an average of 50% of the total 24 neurons are selected to receive the inputs, not all the neurons within the columns are activated. Fig. 4.7 shows the inputs and outputs of the above trial. In total, 266 neurons generate APs in response to the thalamic inputs which are represented as star-dots in red. 867 local L4 neurons (in blue) also successfully elicit APs upon receiving APs from the 266 initially activated neurons. The remaining 1051 (in green) neurons fail to elicit AP which either receive no signals from the neighboring neurons or do not gather enough electric pulses to exceed the threshold.

The calcium waves in L4 protoplasmic astrocytes will not be initiated in one trial of thalamic inputs. Therefore repeated thalamic inputs are required to strengthen the outputs of the astrocytes. After 8 trials with the same neuronal inputs, the response of L4 protoplasmic astrocytes are plotted in Fig. 4.8. The black dots represent the activated neurons while the filled red circle denotes the activated astrocytes (221 out of 546). The

Figure 4.7: Neuron input and output from 25% activated minicolumns and 50% neurons within a minicolumn in L4

red circles are the 79 astrocytes which receive neuronal inputs but the accumulated Ca^{2+} fluxes are not enough to generate a stable wave. The remaining 246 astrocytes fail to receive any neuronal inputs and are depicted as blue circles. The activated astrocytes are clustered around the activated neurons.

Overall, minicolumns will strengthen the sparse input and produce a synchronization effect within a column.

4.4.2 From Thalamic Inputs to L2/3 Responses via L4

The L4 neuronal inputs would activate AP in L2/3 and successively activate the astrocytes in this layer. The impact of the sparseness in the activation of minicolumns and neurons in a single minicolumn is observed in Fig. 4.9 and Fig. 4.10 respectively.

Figure 4.8: Neuron input and astrocyte output from 25% activated minicolumns and 50% neurons within a minicolumn in L4

Under current configurations, if the ratio of activated neurons in a single minicolumn is fixed, the increasing number of minicolumns leads to an increasing number of activated L2/3 neurons. Besides, the speed of increase slows down when the ratio of minicolumns is larger than 60%. L2/3 neurons are all covered when 90% of the minicolumns are involved. The compensation of neighboring neurons could increase and enhance the coverage of information signals. Therefore, the "explosion-like" information transmission is sensitive to smaller stimulation.

When the number of selected minicolumns is fixed, the increasing rate of neurons in

Figure 4.9: Neuron input from different ratio of minicolumns and 10% neurons in each minicolumn in L4 and output in L2/3

a single column has a similar increasing pattern as in Fig. 4.9. All of L2/3 neurons in the corresponding column are activated when the sending neurons ratio reaches 100%. The result shows that the connection of neurons in the column is strong and a localized and dense input is capable of expanding to a larger dense area.

4.4.3 From Thalamic Inputs to Output of L5

Thereafter, the author tests the situation in a complete communication process where thalamic inputs are eventually transmitted to L5 neurons for execution. To analyze the transmission trend, a very sparse input is chosen rather than a saturated input in which the signal would definitely traverse the expected column. In this trial, 22 L4 neurons receive thalamic inputs. 1647 out of 4641 (35.48%) L2/3 neurons are activated while 1608 out of 2184 (73.63%) L5 neurons (Fig. 4.11) are successively activated. After 8 repetitive trials, 286 out of 2002 (14.3%) L2/3 astrocytes and 257 out of 910 (28.24%) L5 astrocytes

Figure 4.10: Neuron input from 12.5% minicolumns and different ratio of neurons in each minicolumn in L4 and astrocyte output in L2/3

generate calcium waves (Fig. 4.12). The amplification of input signal is 70-fold in L5. Hence, identifiable signals elicited by collaboration of neuron ensemble would conduce to the decision of execution. The map of activated astrocytes after 8 trials follows similar pattern of neurons and the storage of states is enhanced by expanding the responding area. Thus if successive execution is required, the stored states could be recalled.

4.4.4 Interlaminar Astrocyte Activated from Thalamic Inputs

Other than the perception-execution process, the interlaminar astrocytes could also receive signal from L2/3 protoplasmic astrocytes. As the calcium wave is more difficult to be initiated in an interlaminar astrocyte, it is assumed that the interlaminar require 3-fold astrocytic input compared to protoplasmic astrocytes. When repetitive 8 identical neuronal input of 50% minicolumns with 10% neurons in each minicolumn is imposed on L4 neurons, 836 (46.75%) L2/3 protoplasmic astrocytes and 34 out of 91 (37.36%)

Figure 4.11: Neuron input in L4 and outputs in L2/3 and L5

interlaminar astrocytes are activated (Fig. 4.13). The activated interlaminar astrocytes ratio is slightly less than L2/3 astrocytes which indicates that the perceptual states are also stored and processed in L1. The result is under the assumption that the input signal is desired while the GJC between L2/3 and L1 is open.

The above modeling is based on the basic biological facts while the numerical definitions are concluded from experiments which could be adapted if further research experiments yield new values for the parameters. Overall, the information flow is represented and the working mechanism within a column is studied. The function of astrocyte as a buffering pool for information and a confidential memory unit can thus be envisaged.

Figure 4.12: Neuron input in L4 and astrocyte output in L2/3 and L5

4.5 Conclusion

In this chapter, a conceptual communication model of neurons and astrocytes in the cerebral cortex has been developed. The chain of neuronal responses from the thalamic inputs to the generation of action in Layer 5 is simulated. In addition, the memory function of astrocytes over repetitive neuronal inputs are proposed and validated. The proposed model serves as a handy simulation model for biological researchers to gain insight into the communication between the astrocytes and neurons. It also facilitates the validation and extrapolation of their biological experiments. The model is designed such that it can be readily expanded and adapted with further results from future biological research.

Figure 4.13: Activated protoplasmic astrocyte in L2/3 and all interlaminar astrocyte in L1 from 50% minicolumns with 10% neurons in each minicolumn in L4

Chapter 5

Two-layer protocol of cluster based calcium signaling

In this chapter, a two-layer protocol stack is established which draws its inspiration from the standard network. The physical layer is concerned with the coding schemes, communication entities and channel while the network layer includes the routing protocols. The layered stack encapsulates the complicated biological process for analyzing the message transmission conveniently by abstracting the lower layer. Furthermore, a ring topology is introduced for more practical implementation of the protocol stack while the error probability is analyzed by considering the leakage to the uncontrolled clusters to make the results more complete and accurate. Relatively long distance communication is accomplished while the success rate is largely elevated. Given the many source-destination pairs in a typical biological trial, the author is able to model the communication path of a single source-destination pair. The comparison of the proposed model against biological experiments shows that the integration of nanotechnology directs the calcium waves and achieves considerable superiority in terms of communication distance and successful transmission rate.

The rest of this chapter is organized as follows. The two-layer stack framework is presented in Section 5.1. Section 5.2 describes the entities, coding and channel characterizations of the physical layer. The topology, communication protocols and routing

Figure 5.1: Two-layer stack

protocols in the network layer are explained in Section 5.3. The simulation parameters and definitions, the numerical results and analysis are discussed in Section VI. Possible medical applications of the network model are provided in Section 5.4. Section 5.6 concludes this chapter.

5.1 Two-layer Stack Overview

As presented in Fig. 5.1, the author proposes a network stack which comprises two layers: physical layer and network layer. The physical layer describes the actual transmission process in the cellular network. Characterizations of calcium signaling as well as a combination of nano devices introduced in Chapter 2 are utilized to fabricate a functional information channel. Human intervention at the essential nodes (i.e. central node and gateway nodes) realizes the simple routing according to the routing tables which enhances the message direction control and alleviate the bottleneck effect. Channel properties serve as a foundation for the upper layer construction. In addition, the separate encoding and decoding in distant cells are incorporated into a complete process. The network layer defines message types, topology, protocols and simple addressing schedule. The three-step communication procedure, in particular, the simple implementation of the routing protocols, are the most significant contents in our design.

Figure 5.2: Communication process in physical layer

5.2 Physical Layer

Calcium signaling emerges among patterned cells in various tissues. Thus the transmission process is conceived based on the existing biological infrastructure (Fig. 5.2). Message is transformed to calcium waves at the sender and restored at the receiver. Propagating from the sender to the receiver, the modulated waves travel across the cellular network. Physical-layer entities, coding mechanism and channel properties are illustrated as follows.

5.2.1 Entities

5.2.1.1 Sender

The sender is composed of a cell and an external controller (Fig. 5.3). Firstly, message is converted to the associated stimulation by the external controller. After that the calcium wave is initiated and propagates to the surrounding cells. The controller is the most essential component in the network stack. It consists of nano devices with different functions (Fig. 5.4). The processor has simple computing capabilities which involves stimulation, detection and memory modules. Memory unit stores the routing table and gateway table (discussed in Section 5.3.3.1) which could be read by the processor. The CNT and chemical storage make up the stimulation module which takes the responsibility of initiating calcium wave by releasing the preloaded chemical. The detection module is a biosensor which also contains CNTs. It is sensitive to the concentration of certain molecules. The detection module in Fig. 5.4 is only in the receiver (Fig. 5.3).

Figure 5.3: Constitution of physical layer entities

Figure 5.4: Controller in sender and receiver (biosensor is only applicable to receiver)

5.2.1.2 Communication Channel

The channel is made up of arranged cells whose permeabilities are non-identical. These cells connect the sender to the receiver.

5.2.1.3 Receiver

The receiver cell (Fig. 5.3) is also accompanied by an external controller (the nano sensor included). The biosensor takes charge of monitoring the cellular activity and informing the external controller.

5.2.2 Coding

The author adopts the communication model [16] in which both ATP and IP_3 pathways are incorporated to generate the calcium waves. ATP release from the stimulated cell declines in the travelling process together with the IP_3 diffusion and transformation. The participation ratio of the pathway differs according to the cell types. For instance, different areas of brain regions (cortex versus hippocampus and corpus callosum) are mediated by different pathways [22]. In addition, the pathway varies between different cell types, e.g. the astrocyte-astrocyte Ca^{2+} waves are relayed by GJCs while the astrocyte-Muller cells mainly rely on the ATP diffusion.

5.2.2.1 Encoding

Calcium waves are triggered by various external agonists, electrical stimulation and mechanical stimulations. In reverse, Ca^{2+} spikes take charge of controlling versatile cellular activities by influencing the formation and conversion of proteins, lipids and other compounds. For example, arterial smooth muscle cells produce calcium waves upon local phenylephrine stimulation [71].

The initiation of calcium wave is a complicated procedure (Fig. 5.5). The low $[Ca^{2+}]_i$ ¹ ($\sim 100\text{nM}$) is maintained by the collaboration of plasma membrane Ca^{2+} ATPase (PMCA) and sarcoendoplasmic reticular Ca^{2+} ATPase (SERCA) [16,23]. SERCA pumps Ca^{2+} into ER while PMCA pumps out Ca^{2+} to the extracellular environment. When the sender cell is exposed to the appropriate agonist or stimulation, the G protein-coupled receptors (GPCR) activate the phospholipids $C\beta$ ($PLC\beta$) while tyrosine-kinase receptors (TKR) activate $PLC\gamma$. The PLCs transform phosphatidylinositol 4,5-bisphosphate (PIP2) into IP_3 . Besides, ATP release which is triggered by the gating of the PMCCs, produces IP_3 after binding to the purinergic receptors (i.e $P2Y_2$) on the cell membrane. Then, IP_3 binds to the IP_3R on the surface of ER and opens Ca^{2+} channels. Consequently, abundant Ca^{2+} pours into the cytosol. The $[Ca^{2+}]_i$ elevation is accompanied with a trail of feedbacks. IP_3R activation is boosted and the Ca^{2+} -dependent PLC activation conversely further enhances the discharge of internal Ca^{2+} store. Correspondingly, the low $[Ca^{2+}]_i$ limits the Ca^{2+} release. As the positive and negative feedbacks take turns to dominate the trend of $[Ca^{2+}]_i$, cytosolic Ca^{2+} will reach the acme and abate to the basal level thus leading to the repetitive Ca^{2+} oscillations.

In the proposed design, the nano processor in the controller sends instructions to the stimulation module whereby agonists from the chemical storage are freed to the corresponding cell. After the stimulation, extracellular ATP is released from the stimulated cell which arouses IP_3 generation along the transmission route. Meanwhile, the cytosolic IP_3 at the initial cell diffuses through GJC along with the metabolic transition [16].

The method, duration and the amount of the stimulation [63] affect the quantity and speed of IP_3 generation rate, determining the IP_3R activation, which is translated from the message to the instructions towards CNT. Hence, the property of Ca^{2+} efflux from the internal store is influenced. Consequently, information could be encoded in the

¹ $[Ca^{2+}]_i$ denotes the concentration of cytosolic Ca^{2+} .

Figure 5.5: Initiation of calcium waves(sender cell)

calcium wave in terms of amplitude or frequency modulation [117]. For example, the calcium wave amplitude in astrocytes increases at higher whisker stimulation frequency and reduces after reaching the apex [145]. In [146], the frequency of calcium oscillations is under the control of the electric stimulation frequency in the astrocytes.

5.2.2.2 Decoding

The receiver cell has a sensitive component to distinguish the frequency or amplitude of the calcium wave. These components transform the encoded information into responses such as exocytosis, mitochondrial redox state and differential gene transcription via molecular machines including CaMKII and PKC [147].

For instance, the nuclear factor kappa B activation, protein phosphorylation, enzyme generation and other cellular activities are regulated by the Ca^{2+} wave frequency. In [148], for example, the generation of calmodulin kinase II autonomy has a positive

Figure 5.6: The schematic diagram of calcium wave generation in adjacent cells

correlation to the frequency of Ca^{2+} waves. On the other hand, Ca^{2+} wave amplitude can be decoded by calmodulin and protein kinase C.

The nano sensor could detect the activity by measuring the associated concentration variation of certain molecules or compounds and the message is eventually judged and reconstructed by the processor.

5.2.3 Channel Property

Generally, there are two kinds of techniques to govern Ca^{2+} wave direction, i.e. changing GJC permeabilities and extracellular ATP concentration.

The sender transmits calcium waves via the passive diffusion of IP_3 and ATP (Fig. 5.6). In effect, the presence of cytosolic IP_3 in the forwarding cells evokes local calcium waves. The mechanism of calcium spike occurrence resembles that in the sender cell except that the IP_3 originates from the adjacent cells. A forwarding cell delivers IP_3 to its neighboring cells due to the $[IP_3]_i$ ² gradient. The ATP generated at the stimulation point propagates in the extracellular space whose velocity and range approach that in

² $[IP_3]_i$ denotes the concentration of cytosolic IP_3 .

the cell-free area [22]. The ATP arrives at the wavefront and induces IP_3 production as in the sender cell. The occurrence of calcium wave is restrained by $[IP_3]_i$ with an upper limit and a lower limit. Calcium wave passes through the forwarding network following successive IP_3 generation.

Apart from the $[IP_3]_i$ in the surrounding cells, calcium wave generation is also influenced by ATP attenuation speed [67] and GJC permeability. The disparity of GJC permeabilities conduces to different efficiencies of IP_3 propagation, i.e. the calcium wave tends to spread to the cell with a higher GJC permeability. Furthermore, the permeability under a certain level would even prevent the calcium wave. The external factors which are capable of changing the GJC permeabilities are presented in Chapter 2. Likewise, the P2R blocking and the full hydrolysis of ATP before binding to the P2R would halt the calcium wave in certain areas [22].

5.3 Network Model

Considering the communication characteristics of calcium signaling, the author designs a cluster-based network topology in a cellular structure. Nodes have diverse functions according to their geographic locations within a cluster. Adjacent clusters are connected directly by gateway nodes situated at the cluster edges. Intra-cluster and inter-cluster data transmission modes are ensured by employing gateway cells at the edge of a cluster. Communication and routing protocols are designed to improve the success rates and propagation range.

5.3.1 Network Topology

The network topology is depicted in Fig. 5.7. A central node, gateway nodes and forwarding nodes comprise a cluster. Cluster sizes and the quantities of gateway nodes may be non-identical for each cluster. The constitution of the different nodes are listed as follows.

Figure 5.7: Network topology of cluster-based network

5.3.1.1 Central Node

The central node is the essential component in the network. The detailed design of a central node is elaborated in Fig. 5.8. The central node contains two physical layer entities: a sender and a receiver. An external controller is deployed to monitor the message to be sent and received. The message transmission begins with the transformation of a message to a stimulus by the controller. Then the sender cell generates a certain amount of IP_3 to induce the calcium wave correspondingly. On the other hand, when the calcium wave arrives at the central node, the receiver cell detects the frequency or amplitude and reacts with cellular activity. Such behavior is sensed and converted into a simple signal by the nano sensor. The controller eventually decodes the signal passed by the sensor.

Figure 5.8: Constitution of a central node

5.3.1.2 Forwarding Cell

The forwarding cells correspond to the communication channel in the lower layer. Message is routed by forwarding cells patterned with physical contact. These cells join the central node and the gateway nodes. Each message is transmitted across a number of cells until the $[IP_3]_i$ drops below the lower bound to induce the calcium wave.

5.3.1.3 Gateway Node

The gateway node also possesses these two entities but augmented with an additional capability of governing the cluster edge. This function is realized by inserting another stimulation module into the external controller which stores chemicals to affect the GJC permeability after the processor sends the instructions. A gateway node could judge the type of message and transmit the message to the external controller through the cooperation of the receiver cell and the nano sensor. In our design, each gateway nodes include three gateway cells to reduce bottleneck effect. Hence, the gateway node would receive the message if any one of the gateway cells encounters the calcium wavefront.

The external controller releases a signal to change the GJC permeability of all the

gateway cells. In this aspect, the gateway node serves as a switch to govern the passage to the other clusters. Gateway nodes in the same cluster exhibit different permeability alteration in response to the identical frequency or amplitude of calcium oscillations. Therefore, the statuses of gateway nodes at the edge of a cluster are regulated. Therefore, only pre-defined gateway nodes could navigate the message. The external controller could also intensify the original calcium wave.

5.3.2 Communication Protocols

5.3.2.1 Layered Stack

As explained in Section 5.1, the network structure consists of two layers. The layered stack of two adjacent clusters is illustrated in Fig. 5.9.

The address and data messages are encoded and decoded in the physical layer at the central node and gateway nodes. The gateway nodes merely receive and forward the calcium wave when all the gateways are open or after the gating status is set. Thereafter, the central node calculates the next cluster based on the routing protocols in the network layer.

Another message defined as gateway message is sent from the central node of each cluster to their gateway nodes. In this case, the gateway node will send it to its network layer and its controller will decide whether to change the gating status.

5.3.2.2 Three-step Communication

To enable relative long distance communication, message transmission should be targeted and shortest path transmission is necessary. In this case, address broadcasting and gateway regulation are augmented before the data message transmission (Fig. 5.10). Firstly, the source cluster disseminates the address of the destination to the rest of the clusters. The addresses are represented by messages using AM/FM. For example, if the network scale is limited, each address could be identified by a certain frequency.

Figure 5.9: Layered stacks in adjacent clusters

When the network scale increases, the address should be converted to a set of frequency combinations (several continuous short messages). Secondly, these clusters generate the gateway messages from their routing tables. Finally, data message is initiated from the original cluster and propagates through the shortest routing path to the destination cluster. The author employs the time-out mechanism to detect the failure. In addition, a reset message is produced before every communication in all clusters to remove the interference.

5.3.3 Routing Protocols

The routing tables are computed and stored at the central node in each cluster before the communication begins. The central node reads the table and maps the destination address to the next cluster address. Thereafter, the gateway message is generated according to the gateway table saved at the gateway node which comes in the form of <gateway message, next cluster> pairs. The table size is the number of gateway nodes in the current cluster. By this means, the gateway towards the anticipated cluster is open and the clusters on the shortest path constitute a controlled passage.

Figure 5.10: Three-step communication

5.3.3.1 Routing Tables

The routing table is a $N \times 1$ table where N is the total number of clusters. Routing tables of nodes 1 and 4 (Fig. 5.7) are presented in Table 5.1. Each item is a $\langle \text{destination, next cluster} \rangle$ pair. The right columns are the contents of the tables while the left ones are the destinations, order-listed for analysis. It is assumed that the distance between adjacent clusters is 1. In each row, the right column denotes the next cluster which is on

Table 5.1: Routing tables for the central nodes in two clusters

(a) Cluster 1		(b) Cluster 4	
1	1	1	2
2	2	2	2
3	3	3	3
4	2	4	4
5	2	5	5
6	3	6	3

Table 5.2: Routing tables

(a) cluster i		(b) cluster k	
1	i_1	1	k_1
2	i_2	2	k_2
3	i_3	3	k_3
·	·	·	·
·	·	·	·
j	k	j	m
·	·	·	·
·	·	·	·
N	i_N	N	k_N

the pre-calculated shortest path towards the destination. By tracing through the routing tables, the passage could be established. For example, search for the shortest path from clusters i to j through the following (routing tables are shown in Table 5.2):

- Find the j^{th} row in table i . Obtain $Next_i[j] = k$.
- Find the j^{th} row in table k . Obtain $Next_k[j] = m$.
- By this analogy, eventually it can be found in table s that $Next_s[j] = j$.

It is obvious that the shortest path acquired in the algorithm is the sequence $(i, k, m...j)$. Actually the $N \times N$ path table defined in the Floyd algorithm [149] could be divided into N columns and each column is exactly the routing table of a cluster in our algorithm. A brief demonstration of the algorithm is shown in the following paragraphs for completeness.

Firstly, prove that the clusters in this sequence are in the shortest path from i to j .

- Start from cluster k in the sequence. It is apparent that k is the next cluster in the shortest path in accordance with the definition. Denote the shortest distance from clusters a to b as $dist_{ab}$. Thus obtain $dist_{ij} = dist_{ik} + dist_{kj}$.

- If $k = j$, then the algorithm is successfully proven. Otherwise, search table k and get $dist_{kj} = dist_{km} + dist_{mj}$.
- If m is not in the shortest path, then there must exist cluster m' which is in the path. Hence, find the next cluster of k in the path and get $dist_{ij} = dist_{ik} + dist_{kj} \geq dist_{ik} + dist_{km'} + dist_{m'j}$. Combining the analysis above, obtain $dist_{ik} + dist_{km} + dist_{mj} \geq dist_{ik} + dist_{km'} + dist_{m'j}$. It could be derived that the path from k to j via m' is shorter than that via m which is against the assumption. Therefore, it can be concluded that m is in the shortest path from i to j .
- Similarly, the clusters in the sequence are all in the shortest path.

Conversely, there is a need to prove that the sequence is not a subset of the solution but exactly the shortest path. Assume that there exists cluster k_s which is in the shortest path but not in the computed sequence and k_s is between clusters k_{s-1} and k_{s+1} . Hence, in the shortest path ($k_1 = i, k_n = j$):

$$dist_{ij} = \sum_{m=1}^{s-2} dist_{k_m k_{m+1}} + dist_{k_{s-1} k_s} + dist_{k_s k_{s+1}} + \sum_{m=s+1}^{n-1} dist_{k_m k_{m+1}} \quad (5.1)$$

In our sequence:

$$dist'_{ij} = \sum_{m=1}^{s-2} dist_{k_m k_{m+1}} + dist_{k_{s-1} k_{s+1}} + \sum_{m=s+1}^{n-1} dist_{k_m k_{m+1}} \quad (5.2)$$

As $dist_{ij}$ is the shortest, we get

$$dist_{k_{s-1} k_{s+1}} \geq dist_{k_{s-1} k_s} + dist_{k_s k_{s+1}} \quad (5.3)$$

When calculating $dist_{k_{s-1}j}$ in the algorithm, get the next cluster $s+1$. If equation (5.3) is correct, then the next cluster of $dist_{k_{s-1}j}$ should be s . As in the sequence, clusters $s-1$ and $s+1$ are directly connected; this conclusion is contradictory to the previous calculation. It can therefore be inferred that the algorithm gives a complete solution of the shortest path.

There are many efficient algorithms to calculate the shortest path among cluster pairs, e.g Bellman-Ford, Dijkstra, etc [149]. The routing table could be modified to other shortest path algorithm under different circumstance.

5.3.3.2 Clusters Outside the Routing Path

In the algorithm, the author manipulates the routing table by defining rules to guarantee that there is only one shortest path from the source to the destination. Albeit the clusters outside the shortest path have open gateway nodes, they could not distribute the data message to their next clusters. This is because these clusters will not get the data message from their antecedent clusters.

Such conclusion is obvious from the definition of the shortest path. Assume that there is a cluster C_k outside the shortest path which receives the data message. Then it must have a train of precursors denoted as $(C_0, C_1 \dots C_{k-2}, C_{k-1})$ which have transmitted the data message successively. C_0 is the source cluster. There must be two adjacent clusters within the sequence $(C_0, C_1 \dots C_{k-1}, C_k)$: the previous one is in the shortest path and the latter one is outside the path. We denote the previous cluster as C_m . Therefore, C_m has at least two next clusters: C_{m+1} and the one in the shortest path. This is contradictory to the premise that one cluster only possesses a single open gateway.

The above conclusion is beneficial for the simulation as the author could neglect the failure in the rest of the clusters.

5.3.3.3 Gateway Tables

Items in the gateway table exist in the form of <next cluster, gateway message> pair while the table size is the number of gateway nodes. Since the address of the next cluster is acquired upon receiving the address message at the central node, the nano processor will read the gateway table and initiate a gateway message to be broadcast within the current cluster.

5.4 Results Analysis

The simulation is operated on a set of patterned clusters with 37 nodes in each cluster. Each node has 6 neighboring nodes. There are 8 link cells between any two of the clusters. The connection of a cluster with two neighbors is shown in Fig. 5.11. The number of gateway nodes could be more than two. In addition, link cells are supplied to provide a low-loss route for calcium waves. Only a few nodes in cluster 2 and link cells connecting clusters 1 to 3 are depicted in the figure for illustration purposes instead of all the cells as their structures are similar. The red node (node 1) denotes the central node in cluster 1 while the purple ones (nodes 1001-1010, nodes 1012-1014) are the link cells. The green nodes denote gateway cells in each cluster and a gateway node contains three gateway cells, e.g. nodes 20, 21 and 37 belong to the gateway towards cluster 2 while nodes 22, 23 and 24 correspond to the gateway towards cluster 3. The rest are forwarding nodes.

A ring network topology is envisaged while its performance is evaluated when the uncontrolled nodes are excluded and included.

5.4.1 Simulation Description

The actual transmission procedure of calcium signaling is complex with IP_3 metabolism and diffusion through the cellular network. Thereafter, the interaction between IP_3 and Ca^{2+} located in different areas (cytosol, internal store and extracellular environment)

Figure 5.11: Schematic presentation of a simplified three-cluster network in the simulation

conduces to the asynchronous calcium waves in the network. $[IP_3]_i$ and $[Ca^{2+}]_i$ are modeled in kinetic equations in [68]. To simulate the communication process on the proposed network, the author simplifies the cumulative transmission to a discrete event list. The proposed model only needs the property of whether a cell in a certain region can generate calcium waves. In this case, a simplification method which conforms to the statistical results of biological experiments is reasonable. The probability that calcium wave could be generated in a cell is uniquely determined by the concentration of IP_3 in the models introduced. Hence, the essential variable is $[IP_3]_i$.

The simulation configuration is presented in the following equations. W denotes $[IP_3]_i$ and t denotes the arriving time of calcium wavefront. These equations describe the relationships of time and $[IP_3]_i$ between the stimulated cell and its neighboring cells.

The parameters such as propagation speed [71] [150], calcium wave travelling range [151] and IP_3 thresholds are drawn from some biological experiments since the performances differ under different situations. In cultured rat astrocytes [150], the propagation speed of calcium waves is about 15-20 $\mu m/s$ which is approximately constant. Let the IP_3 propagation time (Eqn. 5.4) from a cell to its adjacent cell obey the normal distribution $N(3.0, 2.5)$. The degradation speed of $[IP_3]_i$ has a linear correlation with the propagation delay (Eqn. 5.5). $[IP_3]_i$ in the stimulated cells (the maximal IP_3 value in the system) and the lower bound of $[IP_3]_i$ are denoted as IP_{3max} and IP_{3min} , respectively. Set $IP_{3max} = 3.0\mu M$ and $IP_{3min} = 0.8\mu M$.

$$\Delta t = t_{next} - t_{local} = 3.0 \quad (5.4)$$

$$\Delta W = W_{next} - W_{local} = 0.104 \times \Delta t \quad (5.5)$$

5.4.2 Intra-cluster Communication

A couple of experiments are conducted to examine the correctness of the network model. The nodes in each cluster are classified into rows depending on their distances from the central node [150]. For example, in cluster 1 (Fig. 5.11), node 1 is in row 0, nodes 2 to 7 are in row 1, and so forth. A bigger isolated cluster with 8 cellular rows for the configuration decision is employed. The cluster has 169 nodes which is expanded from cluster 1. A scenario where message 1 is initiated from node 1 is tested. In the cells with $[IP_3]_i$ lower than IP_{3min} , calcium waves fail to be generated. The average percentage (shown in Fig. 5.12) of responding cells with induced calcium waves in all the eight rows (row 0 to row 7 in Fig. 5.11) is close to the results of Fig. 4A³ in [151]. $[IP_3]_i$ decreases in the process of spreading and self-degradation. The near-central cells tend to show a

³Fig. 4A is shown in the Fig. C.1

Figure 5.12: Average success rate comparison of designed model (extended cluster for intra-cluster transmission) and biological experiment

higher rate to induce calcium waves while cells in the more distant cellular row has a lower average rate (19.6% in row 7). If the cluster is bigger, the rate would diminish further. The gateway nodes are supposed to receive message 1 to set the gating status. However, cells in row 3 have a probability of missing the gateway messages due to the failure opportunity. This would definitely impact the successive data transmission.

5.4.3 Inter-cluster Communication

To test the propagation power of calcium signaling, experiments on inter-cluster communication between two clusters with/without message enhancement at the gateway node in the original and receiving clusters are conducted. To investigate the performance of the designed network, define the cellular rows from the center of cluster 1. The nodes in clusters 2 and 3 are also classified into rows in accordance with their distances from node 1 (Fig. 5.13). For example, nodes 1001, 1004 and 1006 are in row 4 (Fig. 5.11). In this

Figure 5.13: Definition of cellular rows in inter-cluster signaling

section, the devised network model which partitions the cells into clusters and the outer environment is compared to the natural calcium signaling within the same area, i.e the entire coverage shown in Fig. 5.13.

When the augment of $[IP_3]_i$ is applied at the gateway in the original cluster, the success rates are elevated consequently in the objective cluster. The $[IP_3]_i$ is gradually replenished and the gateway node works as a new source to transmit the calcium wave. Hence, the calcium wave can be transmitted to row 10 which acquires a wider range than the calcium signaling stimulated by focal ionomycin. It is inferred that the possibility of successful transmission in the gateway node has a great impact on the existence of calcium waves in the succeeding cluster.

Then further enhancement at the gateway node of the target cluster is examined. Success rate map of nodes within the clusters and the link are presented in Fig. 5.14. $[IP_3]_i$ reduces in steps from rows 0 to 3 in cluster 1 and nodes in the same row have similar

Figure 5.14: Average success rate map for inter-cluster message transmission of the designed model

$[IP_3]_i$. The fluctuation among a certain row is due to the variance of time distribution. The success ratios at the three gateway cells 37, 20, and 21 are 0.895, 0.844 and 0.896, respectively. However, the link cells possess even higher success rates, e.g. 0.992 for node 1001. The average rate of each cellular row is plotted in Fig. 5.15 which gives a clear view of the reversion at row 4. The reason for the reversion is the lessened bottleneck effect at the gateway node, i.e. the link cells would fail to generate calcium waves only if all the gateway cells fail at the same time.

The enhanced inter-cluster communication shows a superior performance compared to the biological experiments (Fig. 5.15). A significant difference between natural calcium signaling and the proposed calcium wave transmission method is that the latter is more effective. By utilizing clusters, the message does not flood to the outer environment or the cluster which is not expecting the message. Avoiding the wastage of transmission is important because the IP_3 diffusion process is unlike the communication in a typical computer network where messages can be completely replicated in the neighboring cells. The total IP_3 amount is quite fixed although a very small amount of IP_3 could

Figure 5.15: Average success rate comparison for inter-cluster message transmission of designed model and biological experiment

be synthesized in each cell. Thus unnecessary cell participation in the communication compromises the calcium wave coverage owing to the IP_3 consumption as the calcium wave traverses the cells.

The IP_3 decay among the link cells is very small since the propagation is almost in one direction. Besides, the width of the link path is not too broad thus reducing unnecessary calcium leakage. The secondary enhancement at the target gateway node ensures the sufficiency of IP_3 which could diffuse to the target cluster. If the link path has to be broadened or lengthened, there may exist further IP_3 loss accordingly.

5.4.4 Propagation Range

After measuring the basic properties of cluster-based topology, the two-layer protocol is examined. Firstly, the calcium wave coverage is tested by comparing the simulation with biological experiments. In [152], astrocytic Ca^{2+} wave in organotypic slices includes 20-60 cells per wave while the calcium wave travels to at least 35 cells in the Embryonic Ventricular Zone [153]. To check the success rate distribution in each cellular row rather

Figure 5.16: 7×7 -lattice cluster topology and the shortest path

than a general range, the author employs the results in [150]. Moreover, the wave range results could act as a reference to assess the performance.

The clusters are placed in a 7×7 -lattice topology and each cluster has four gateway nodes (Fig. 5.16). Similarly, the distances between adjacent clusters are considered to be 1. Denote the absolute horizontal distance from cluster 1 to cluster i as d_{h_i} while the absolute vertical distance is d_{v_i} . To ensure the uniqueness of the shortest path, the following rules are applied when two paths between clusters i and j have equivalent length:

- If $|d_{h_i} - d_{h_j}| < |d_{v_i} - d_{v_j}|$, then the routing path is through the vertical adjacent cluster. Otherwise, the next cluster is the horizontal one.

To investigate the success rates of unicast in different cellular rows, cluster 25 is set as the original cluster and data message is transmitted to a single cluster each time. The cellular row is defined as the distance from the central node in cluster 25 to the central node in the target cluster. The average success rates for clusters with equivalent distances

Figure 5.17: Average success rate comparison for the lattice-topology and biological experiment

along with the biological results are depicted in Fig. 5.17. The designed network has obvious superior performance compared to the biological experiments whose percentage of responding cells drops to 25% in row 7. The simulation shows a gradual degradation as the distance increases. The average success rate in most distant clusters (clusters 1, 7, 43 and 49) is 38.5% while the wave range is more than 220 cells in a single trial which is much larger than the range in [152].

This experiment proves that the long-distance unicast could be achieved through the three-step protocol. The disturbance in the cells not expecting the message is reduced via controlled transmission. Moreover, the success rates in distant rows are enhanced by exploiting enhancement at the gateway nodes.

5.4.5 Communication in ring network

In order to enable the communication among the edge of the system, a network with crucial clusters and several forwarding clusters is required to be established. In this scenario, the source cluster is requested to transmit the message to the internal clusters

Figure 5.18: Ring network and the shortest path

which will then channel the message to the destination. A simple ring network is shown in Fig. 5.18. Clusters outside the dotted circle in purple (13 to 24) are the end-clusters and clusters in blue (1 to 12) are the forwarding clusters. The rest (25 to 37) which has the same shape as clusters 1 to 24, are not actual clusters. They are composed of patterned cells without control. Calcium waves in these uncontrolled clusters are not considered here. In the controlled clusters, gateway table length is 2 or 3 which equals to the number of controlled neighbors, e.g. the effective neighbors of cluster 7 are clusters 1, 13 and 24. Thus the table size is 3, which means there is no gateway node towards "clusters" 26, 31 and 32. The routing table length equals to the number of end-clusters since the internal clusters are not supposed to be a destination, e.g. routing table of cluster 1 has only 12 items. Likewise, there are two rules to ensure the uniqueness of the shortest path:

Rule 1: It is forbidden to pass other end-clusters.

Rule 2: If two paths have equivalent distances, the clockwise one is chosen.

Figure 5.19: Success rates in ring network

Rule 1 has supreme priority if a conflict is encountered. For example, from clusters 13 to 23, choose (13, 7, 1, 6, 12, 23) though it is longer than (13, 7, 24, 23) according to rule 1.

The success rates from cluster 13 to other clusters are shown in Fig. 5.19. The horizontal axis indicates the number of clusters on the way towards the destination. The bars represent the simulation records while the line denotes the average success rate at the corresponding distance. There are more than one bar of the last three distances corresponding to the results of all clusters having the same distance, e.g. clusters 16 and 17 are at the same distance (i.e. 7 clusters away) from cluster 23. As shown, when the distance is short, there are few available source-destination pairs. The same applies when the source-destination pairs are at the two farthest points of the ring network (distance is 7). The success rate exhibits a decreasing trend as the distance increases which ranges from 31.57% to 93.3%.

5.4.6 Leakage of Ring Network

To obtain more accurate evaluation of the performance in the ring network, the author takes the uncontrolled clusters (25 to 37) into account to check the variation of success rate and the probability that messages are mistakenly received by unexpected end-clusters (referred to as the fault rate). Since the cluster edge of uncontrolled clusters are not guarded, these clusters are open to messages from all the neighbors. Therefore, the edge of arranged clusters whose adjacent cluster is an uncontrolled one, could receive the message delivered.

Fig. 5.20 presents the success rates where the leakage to the uncontrolled clusters is considered (plotted as the green line with triangles). In comparison to the previous results (blue line with squares), the overall success rate is substantially raised. The reason for the elevation is that the uncontrolled clusters supply an extra message route apart from the unique routing path. For instance, if the message is to be sent from clusters 13 to 14, the data message could also transmit along clusters 13-26-14 since the edge towards "cluster" 26 is always open, which boosts the success probability.

Furthermore, the fault rates to the other end-clusters are all 0. The zero fault rate to the rest of the crucial clusters is due to the following two reasons: a) The forwarding clusters have configured the gating status and the gate towards the destination address is open. If a forwarding cluster receives the message, it will pass the message towards the destination. In this way, the destination cluster could be viewed as a sink which gathers the messages in the network. A general trend of message flow to the expected cluster suppresses the divergent fluxes to other end-clusters. b) The degradation of calcium waves restricts the wave range. In the uncontrolled clusters without enhancement at the gateway, the received calcium wave possesses a weaker intensity which can hardly traverse the cluster.

Figure 5.20: Success rate comparison in ring network (normal, with leakage, 3 cells in central node and integrated)

5.4.7 Improvement of Ring Network

When the log file in the failed trials are traced, it is discovered that the bottleneck effect at the central node restricts the message receiving and influences the subsequent steps in the transmission. Therefore, the author increases the number of receiving cells around the central node (blue cells, labelled from 1 to 12, in the magnified cluster image of Fig. 5.11). The central node could identify the message if any of these cells receives the calcium waves. The performance (red line with diamonds in Fig. 5.20) is superior to the normal ring network. Similarly, there is no faulty message delivery to the rest of the end-clusters.

Finally, Sections 5.4.5 and 5.4.6 are integrated and considerable improvement in overall success rate (purple line with circles in Fig. 5.20) is achieved. Likewise, faulty message transmission is absent. The success rates range from 99.44% to 86.2%. The results indicate that the ring network is centrosymmetric and balanced while the transmissions towards distant destinations have similar and fairly good performances.

The ring network has been proven to be relatively accurate and effective for message

transmission especially when the uncontrolled clusters are considered and the central node is enhanced. It is feasible to be realized since human intervention is involved only at the central node and three responsible gateway nodes in each cluster. The ring network could be extended by adding more clusters.

The above simulations are implemented using a cluster-based architecture while realizing the long-distance communication. The role of unicast is significant since data is required to be accurately targeted with the least disturbance to the other cells in biological applications. By applying appropriate routing protocol, the shortest path is ensured. The data message could be guided to the destination with the least attenuation. A ring network is introduced which provides feasible topology for message transmission. Besides, the cluster sizes could be increased to 5 cellular rows with acceptable success rate of broadcast in a single cluster. This could further reduce the extent of human intervention.

5.5 Applications

5.5.1 Brain Function Repair

When a part of the brain is damaged and the connection to the surrounding area is absent, especially in the cerebral cortex, the function of astrocyte would be inhibited. Astrocyte plays a significant role in sustaining the homeostasis, supplying the energy and gathering information from the neurons. As Ca^{2+} is one of the messengers used by astrocytes, the proposed network stack could be implemented by implanting nano-controllers into the unconnected area and rebuilding the communication. Fig. 5.21 shows a schematic view of a cluster-based network and the surrounding tissues or cells outside of the clusters. Using Fig. 5.21 as an example, should one or more astrocytes fail in function, nano-implants can be substituted to allow communication within and outside the clusters to continue as per normal.

Figure 5.21: Rebuild communication to surrounding environment

5.5.2 Disease Detection

In many situations, diseases such as brain dysfunction cannot be diagnosed accurately. Through triggering cellular activity in the area which demands detection, the designed system may accelerate the therapy by initiating a message from a relatively safe site. In addition, the network could be expanded for more functions by loading other nano devices, e.g. imaging device.

5.6 Conclusion

In this chapter, a two-layer network protocol is designed based on calcium signaling. The communication characterizations and properties are discussed in the physical layer. The feasibility of dynamic control via human factor intervention is demonstrated. Coding scheme and entities are defined. The network layer arrange cells into clusters with cen-

tral node and gateway node to control intra-cluster and inter-cluster communications. Detailed framework including network entities, topology and protocols are presented for directed transmission. Simulation results and comparisons to the biological experiments prove the correctness of the proposed model. Long-distance unicast is realized while a ring network is envisaged for balanced and reliable unicast among end-clusters.

Chapter 6

Conclusion and Future Work

In the chapter, the author summarizes the thesis by examining the benefits of the schemes and sketching out directions for future work.

6.1 Conclusion

6.1.1 Conceptual Network Model from Sensory Neurons to Astrocytes of the Human Nervous System

The human nervous system is an intricate information processing center which regulates sensing and motion, as well as high level activities such as thinking and learning. To support neurological disease treatment and inspire the development of artificial intelligence, various experiments and models have been proposed. Specific sections of the entire system are studied such as the neuronal connection, tripartite synapse, etc. However, there lacks a complete model from the input signal to the output cognitive episodes. The vast amount of variables and difficulties in conducting in vivo experiments largely restrict the progress of building an accurate model.

The new functions of astrocytes, i.e. communication with neurons and peer astrocytes, as well as their diversity and increasing complexity in human, imply their significance in the nervous system. Astrocyte network increases the interaction in cerebral cortex, contributing to signal integration and further processing.

In Chapter 3, the author develops a conceptual network model based on the cellular characterizations of the nervous system. The ascending signal pathway, from the external stimulation upon sensory neurons, to the calcium waves in astrocytes, generates conscious episode in the human brain eventually. The author applies the astrocyte-centric hypothesis of which information processing is ultimately fulfilled by astrocytes. The information is supposed to be stored in the astrocytic network while a repetitive stimulus would evoke the memory. Conscious episode formation from primitive cognitive patterns is envisaged.

According to the anatomic structures and characterizations, the conceptual network model is divided into four layers along the signal pathway. Layer 1 specifies the stimulus-response of the sensory neuron and electric pulse signal transmission towards the neuron in the spinal cord. The intermediate neuron in layer 2 directly projects to interconnected layer 3 astrocytes. Layer 4 is presented by the state combination of individual astrocyte. Simulation results are validated against the available biological experiments and mathematical models. A test case of the integrated network is provided to present the entire signal transmission process. As the production of conscious episode in the human nervous system is still under intense research, the model serves as a useful tool to facilitate, complement and verify current and future study in human cognition.

6.1.2 Modeling of Communication Between Neurons and Astrocytes in Laminar Cerebral Cortex

Moreover, the highest processing center in human nervous system - cerebral cortex is studied. Based on the existing knowledge of laminar and columnar structures, which is divided by the ensemble of neurons, astrocytes are found to conform to the neuronal boundary. Neuron-astrocyte communication increases the information propagation speed in astrocytic network which compensates the slow calcium wave velocity. In contrast, the slow attenuation of calcium wave in astrocytes acts as a memory site. Thalamic inputs

towards the cerebral cortex is received in layer 4 first. Thereafter, layer 2/3 will acquire the signal and transmit to layer 5 eventually.

In Chapter 4, a nano-bio conceptual network model is built which integrates the function of neurons and astrocytes in the cerebral cortex. The chain of neuronal responses from the thalamic inputs to the generation of action in Layer 5 is simulated. Besides, the memory function of astrocytes over repetitive neuronal inputs are proposed and validated. The proposed model serves as a handy simulation model for biological researchers to gain insight into the communication between the astrocytes and neurons. It also facilitate the validation and extrapolation of their biological experiments. The model is designed such that it can be readily expanded and adjusted with further results from future biological research.

6.1.3 Two-layer Protocol of Cluster based Calcium Signaling

To design a bio-compatible nanonetwork, calcium signaling stands out due to its ubiquity in living creature. Besides, the coding scheme could be realized since encoding and decoding exist in vivo. Cells utilize calcium waves to carry intermediate messages, with environment stimulus and chemical detector to encode and decode the message, respectively. Nano devices development facilitates intensive control of calcium signaling which makes it possible for conveying signals in a more efficient way.

In Chapter 5, the author introduces a two-layer stack based on calcium signaling. The physical entities, coding scheme and channel properties are explained. A reasonable explanation of calcium wave origination and propagation is provided. Three-step communication protocols and routing methods in the network layer are introduced. Long-distance unicast is realized while a ring network is envisaged for balanced and reliable unicast among end-clusters. The simulation results is compared to biological experiments to demonstrate the validity and superiority of the proposed network model. The proposed network model is realisable biologically as researchers have shown the feasibility

of human intervention via nano-controllers. The incorporation of these nano-controllers allows researchers to utilize the characterizations of intercellular waves to realize more efficient controlled communication. Furthermore, the model can provide insights and facilitate biological researchers in carrying out more experiments on calcium signalling.

6.2 Future Research Directions

6.2.1 Modeling of Human Nervous System

The descending signal pathway, which starts from the astrocytic network and terminates at the motor neurons, could be incorporated into the current network model. Therefore a complete perception-action step would be simulated and behavioural experiments could be adopted to adjust the parameters.

Practical use of the model requires incorporation of different features of the various sensing systems. In addition, different sensory systems would converge in the cerebral cortex to generate effective perception information. For example, recognition of a type of food requires cooperation of visual, gustation and olfactory systems.

Moreover, the generation of global Ca^{2+} wave in layer 3 and the affection of past prototypical cognitive patterns in layer 4 as well as the relationship of conscious episode and the memory formation could be investigated.

6.2.2 Modeling of Communication Between Neurons and Astrocytes in Laminar Cerebral Cortex

With the discovery of the interlaminar astrocyte, especially the chemical power consumed in its bulbous end, its involvement in the cerebral cortex is to be studied.

Besides, more experiments testing the relationship of inherent frequency and neuronal activity could be conducted. Other coding methods such as temporal coding and phase information could be examined.

6.2.3 Two-layer Protocol of Cluster based Calcium Signaling

The network stack utilizes the time-out scheme to ensure successful message arrival. Thus a failure detection and re-transmission mechanism could be added to the current model to improve the reliability.

The gateway node placement strategy in the cellular structure is another issue. The optimal positions to handle the maximum number of cells with the least overlap could be calculated. Utilization rate and coverage could be improved by shrinking the cluster edge and changing the cluster sizes.

A better topology which will shorten the propagation time can be developed. In addition, multicast could be proposed by configuring some router clusters in the network to distribute the data message in multiple directions.

Furthermore, the compatibility of nano sensors and external controller is to be researched in the practical environment.

Appendix A

Conceptual Network Model from Sensory Neurons to Astrocytes of the Human Nervous System

A.1 Related Published Figures

The signal divergence and convergence published in Fig. 8-3A [115] is illustrated in Fig. A.1. The experiment results published in Fig. 6F and Fig. 6G [115] are duplicated in Fig. A.2.

A.2 Related Formulas

A.2.1 Hill Equation

Hill equation was originally introduced by A.V. Hill in 1910 [154] to describe the relationship of two variables (shown in Eqn. A.1). y_{max} , c and n are the parameters while x is the dependent variable.

$$y = \frac{y_{max}x^\alpha}{c^\alpha + x^\alpha} \quad (A.1)$$

A.2.2 Major Equation of Li-Rinzel Model Applied [1]

Intracellular Ca^{2+} is calculated in Eqn. A.2. J_{chan} represents the amount of Ca^{2+} affected by IP_3 and Ca^{2+} concentrations. J_{leak} is the leaked Ca^{2+} from ER while J_{pump} denotes

Figure A.1: Diagram of the excitatory synaptic connections among 3 receptors and the interneurons at the next two higher levels. The inset over each axon shows its relative rate of discharge during stimulation.

Figure A.2: Area of the astrocyte Ca^{2+} (A) and iGluSnFR (B) signals as a function of EFS stimuli and in relation to the territory of an s.l. astrocyte

Ca^{2+} pumped from the cytosol into the ER.

$$\frac{dCa^{2+}}{dt} = J_{chan}(Ca^{2+}, h, IP_3) + J_{leak}(Ca^{2+}) - J_{pump}(Ca^{2+}) \quad (A.2)$$

Appendix B

Modeling of Communication Between Neurons and Astrocytes in Laminar Cerebral Cortex

B.1 Related Published Figures

The experiment result published in [122] is shown below.

Figure B.1: Typical human protoplasmic astrocyte in the same scale. Scale bar, 20 μm)

Appendix C

Two-layer Protocol of Cluster based Calcium Signaling

C.1 Related Published Figures

The experiment result published in [151] is shown below.

Figure C.1: Analysis of intercellular calcium signaling generated in confluent rat astrocytes by focal application of ionomycin ($50 \mu M$)

Publications

Journals

- **Yiqun Yang**, Chai Kiat Yeo, “An Enhanced Cluster-based Network Model for Calcium Signaling”, Nanotechnology, IEEE Transactions on , vol.12, no.5, pp.788,795, Sept. 2013.
- **Yiqun Yang**, Chai Kiat Yeo, “Conceptual Network Model of Nervous System in Human Body”, Biomedical Engineering, IEEE Transactions on, vol.62, no. 7, pp.1843,1852, Jul. 2015.
- **Yiqun Yang**, Chai Kiat Yeo, “Modelling of Communication Between Neurons and Astrocytes in Laminar Cerebral Cortex”, submitted to Elsevier Neurocomputing Journal.

Conferences

- **Yiqun Yang**, Chai Kiat Yeo, “Molecular communication model through gap junction channel with selective permeabilities”, Networks (ICON), 2012 18th IEEE International Conference on, pp.256,261, 12-14 Dec. 2012.
- **Yiqun Yang**, Chai Kiat Yeo, “Design and Analysis of a Cluster-based Signaling Network Model”, Consumer Communications and Networking Conference (CCNC), 2013 IEEE, pp.574,579, 11-14 Jan. 2013 (best paper candidate and awarded Honorable Mention for Innovation).
- **Yiqun Yang**, Wenjie Zhang, Chai Kiat Yeo, “Modelling Calcium Signaling with a Two-Layer Cluster-based Communication Network System”, Accepted by BWCCA 2015.

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