Generic Modeling of Chemotactic Based Self-Wiring of Neural Networks

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Abstract

The proper functioning of the nervous system depends critically on the intricate network of synaptic connections that are generated during the system development. During the network formation, the growth cones migrate through the embryonic environment to their targets using chemical communication. A major obstacle in the elucidation of fundamental principles underlying this self-wiring is the complexity of the system being analyzed. Hence much effort is devoted to in-vitro experiments of simpler 2D model systems. In these experiments neurons are placed on Poly-L-Lysine (PLL) surfaces so it is easier to monitor their self-wiring. We developed a model to reproduce the salient features of the 2D systems, inspired by the study of bacterial colony’s growth and the aggregation of amoebae. We represent the neurons (each composed of cell’s soma, neurites and growth cones) by active elements that capture the generic features of the real neurons. The model also incorporates stationary units representing the cells’ soma and communicating walkers representing the growth cones. The stationary units send neurites one at a time, and respond to chemical signaling. The walkers migrate in response to chemotaxis substances emitted by the soma and communicate with each other and with the soma by means of chemotactic “feedback”. The interplay between the chemo-repulsive and chemo-attractive responses is determined by the dynamics of the walker’s internal energy which is controlled by the soma. These features enable the neurons to perform the complex task of self-wiring. We present numerical experiments of the model to demonstrate its ability to form fine structures in simple networks of few neurons. Our results raise two fundamental issues: 1. One needs to develop characterization methods (beyond number of connections per neuron) to distinguish the various possible networks. 2. What are the relations between the network organization and its computational properties and efficiency.
1 Introduction

The brain is probably the most challenging complex system that scientists can study [Abeles, 1991]. And indeed, much effort has been devoted to brain studies from the physiological level of ionic channels to the philosophical level where questions about intelligence and self-awareness are discussed [Penrose, 1996]. Yet, one of the fascinating aspects about the brain has almost been completely ignored until recently. We refer to the process during which a collection of individual neurons are transformed into a functioning network with unique capabilities—the brain. This emergence process cannot be totally determined by the stored genetic information. In a human brain, for instance, there are approximately $10^{11}$ neurons that form a network with more than $10^{15}$ synaptic connections. The human DNA is composed of about $10^9$ bases, so it lacks sufficient memory storage of the detailed structure of a brain. The alternative extreme explanation, of total randomness, could not be correct as well. After all, we know that while on the micro level (up to about $1\text{mm}$) the structure appears to be random, on the macro level (above $1\text{cm}$) the brain’s structure is quite deterministic. In addition, the brain structure varies from species to species, and, within a given species, some brain skills are inheritable (vs. learned). So clearly some elements of the brain structure must be dictated by stored genetic information.

The contemporary view is that the brain structure is essentially deterministic on a large scale but probabilistic on a small scale [Abeles, 1991]. As a consequence the network has no optimal structure. But we believe that neural networks can be constructed in an optimal way, and this optimal way is derived from the biological mechanisms that construct the network. In any approach to the construction of the network there must be a clear strategy by which the neurons find each other to establish synaptic connections.

At present we do not know which parts of the information about the brain structure are stored, and the general question about the role of randomness vs.
determinism during the emergent process is still open [Abeles, 1991].

Neuronal connections are formed when each neuron sends neurites that migrate through the embryonic environment [Tessier-Lavigne and Goodman, 1996]. At the leading tip of each neurite there is a region called growth cone, which is capable of “measuring” concentrations and gradients of chemical fields. Indeed, the growth cones navigate using sophisticated means of chemical signaling for communication and regulation, including repulsive and attractive chemotaxis.

At the beginning of the growth process the neurite has to migrate from its own cell’s soma. The neurite migrates to the area in which it is supposed to form a synaptic connection. In this area there are many neurons, each of which is a possible target cell for the neurite. When the neurite approaches one of the possible target cells, with which it will finally form a synaptic connection, it has to be attracted to that cell’s soma.

We describe here elements of a novel strategy for the emergent process. Since the proposed model is for simplified 2D systems, as such, it is far from being a full description of the brain’s adaptive self-wiring. Yet, if tested and shown to be correct, it will provide an important step towards understanding the emergence process in a real brain.

To clarify the problem at hand we show in figure ?? a self wiring process of neurons on a 2D silicone surface. Since we cannot distinguish between dendrites and axons, we refer to both as neurites from here on. The thin filaments are the neurites and during development they migrate over the surface to form synaptic connections.

Our model of self-wiring is inspired by communicating walkers model used in the study of complex patterning of bacterial colonies. We have developed [Segev and Ben-Jacob, 1998b, Segev and Ben-Jacob, 1998a] a simple model to reproduce the salient features of the 2D systems. We represent the neurons (each composed of cell’s soma, neurites and growth cones) by simple active elements
that capture the generic features of the real neurons. The model also incorporates stationary units representing the cells’ soma and communicating walkers representing the growth cones. The stationary units send neurites one at a time, and respond to chemical signaling. The walkers migrate in response to chemotaxis substances emitted by the soma and communicate with each other and with the soma by means of chemotactic “feedback”. The interplay between the chemorepulsive and chemo-attractive responses is determined by the dynamics of the walker’s internal energy which is controlled by the soma. These simple features enable the neurons to perform the complex task of self-wiring. In section 3 we elaborate on this model and its experimental predictions.

2 Biological and Experimental Background

In this section we present a brief summary of the existing biological knowledge relevant to the self-wiring of neural nets and their modeling.

2.1 Cell cultures

There are two major types of cell cultures used as a model (note that we use in this section a different meaning to the word “model”) for neuronal development: Primary cultures, which are prepared from cells taken directly from animal (usually rat embryo), and neuron-like cultures of cell lines. A cell line is usually derived from tumor cells that were cloned so as to obtain a genetically homogeneous population. Initially, the cell line has no particular biological function, instead, upon exposure to an external trigger (for example exposure to nerve growth factor (NGF) for PC12 cell line), it is pushed to develop neuronal-like processes. It develops morphological and functional neuronal phenotypes such as extending neurites, manufacturing neuro-transmitters and becoming electrically excitable. However, it is not clear how far these cells go in the route of neuronal differentiation (For example the PC12 cell line does not form real synapses). In
figure 1 we show cell culture of neuroblasticoma cells, which develop neuron-like processes when they are exposed to a serum free media.

2.2 Growth cones: structure and dynamics

The growth cone is capable of measuring concentration and concentration gradients of substances in the environment. It is composed of a central core which is an extension of the neurite itself, and is rich with microtubulets that provide the structural support. The core is rich with mitochondria, endoplasmic reticulum and vesicular structures. Surrounding the central core are regions known as lamellipodia, in which the contractile protein Actin is abundant. At the extremities of the lamellipodia there are very thin straight filaments known as filopodia. The filopodia are in constant motion, as they extend from the lamellipodia and retract back to it. The growth of the neurite occurs when filopodia extend from the lamellipodia and remains extended rather than retracts as the end of the lamellipodia advances towards the filopodia. The complexity and the dynamics of the growth cones hint that they might act as autonomous entities. Indeed, there are direct experimental observations of the activity of growth cones that support this view [Haydon et al., 1984]. In the experiment growth cones are cut off from their neurite. These isolated growth cones continue to extend and/or react to chemicals for a considerable long time after they were severed from their cells’ soma. This observation indicates that the measurements of chemotaxis gradients occur locally through receptors on the growth cones themselves, and do not require any signal from the cell’s soma.

The above observations are essential to the construction of our model. In particular, they lead us to represent each of the growth cones as an independent entity (walker) with its own internal energy as described in the next section.

Microscope observation [Braun, 1997] reveals that the movement of the growth cones appears to be a non-uniform random walk which has the highest probability to move forward (“inertia”) and high probability to move backward (“retrac-
tion”). The movement is not continuous, as there are time intervals during which the growth cones do not move. The growth rate is of the order of micrometer per minute [R.W. Gundersen, 1979].

2.3 Chemotaxis

Extensive studies in-vivo and in-vitro revealed that the movement of the growth cones can be affected by four types of guidance cues: attractive or repulsive cues that can be either local or non-local. The local cues are contact mediated by non-diffusive cell adhesion molecules (CAM) and extra cellular matrix (ECM) molecules. The non-local forces are mediated by emission of chemo-attractant and chemo-repellent substances which “pull” and “push” the growth cone from the soma or its neurites [Tessier-Lavigne and Goodman, 1996].

While the existence of attractive and repulsive agents has been established long ago, there recently has been an experiment which demonstrated the existence of a triggering agent. A gradient of brain-derived neuro-tropic factor (BDNF) was created with a micro-pipette near a growth cone [H. Song, 1997]. The specific kind of growth cone used in the experiment is usually attracted by the gradient of the BDNF. But the same gradient of BDNF induced a repulsive response of the growth cones when the growth cones were cultured in the presence of a competitive analogue of cAMP in the media. Apparently, the analogue of cAMP can trigger the growth cones to react differently to the same chemotaxis substance.

2.4 Chemical perception

Biological elements (growth cones, cells) sense the local concentration of a chemical via membrane receptors binding the chemicals molecules [Ben-Jacob, 1997]. They sense the concentration by measuring the fraction of occupied receptors, \( \frac{N_0}{N_f + N_0} \), where \( N_0 \) and \( N_f \) are the number of occupied and free receptors respectively. For a given concentration \( C \), \( N_0 \) is determined by two characteristic
times: the average time the receptor is occupied, $\tau_0$, and the average time the receptor is free, $\tau_f$. Since $\tau_f \propto 1/C$ with the proportion coefficient, $k$, determined by the receptor affinity to the chemical, we get:

$$\frac{N_0}{N_f + N_0} = \frac{\tau_o}{\tau_f + \tau_0} = \frac{C}{K + C}$$  \hspace{1cm} (1)

where $K = k/\tau_0$. Since growth cones measure changes in $N_0/(N_0 + N_f)$ and not in the concentration itself using eq (1) and assuming $\tau_0$ does not change in space we obtain:

$$\frac{\partial}{\partial x} \left( \frac{N_0}{N_0 + N_f} \right) = \frac{K}{(K + C)^2} \frac{\partial C}{\partial x}$$  \hspace{1cm} (2)

As we can see the growth cone measures the chemical gradient multiplied by a prefactor $K/(K + C)^2$. This law is known as the “Receptor’s Law”. Since at very high concentrations all the receptors are occupied the response is zero. At very low concentrations, due to internal and external noise, the response vanishes as well.

### 2.5 Constrains on the navigation distance

There are two related constrains which the distribution of a diffusible factor must satisfy to provide an effective guidance cue to a specific location [Goodhill, 1998]. First, as was explained in the previous section, the absolute concentration of the chemotaxis agent must not be too small or too large. Second, the gradient across the growth cone must be sufficiently large, since the growth cone measures the concentration differences over its width. These constrains are related because in both cases the problem is to overcome the statistical noise. Goodhill [Goodhill, 1998] investigated the limitation these constrains impose on the maximum guidance range of a diffusible factor, emitted by a cell away from the growth cone, by estimating the diffusion constant, the rate of production of a chemotaxis agent, the minimum and maximum of concentration and gradient detection. He came to the conclusion that maximum guidance distance may range up to 1mm.
2.6 Synaptic connections

An extending neurite does not make synaptic contacts with every cell that it encounters on its path, there has to be a signal which instructs the growth cone to slow its growth, contact one of the possible target cell, and form the synaptic connection. The nature of this signal is as yet unknown, but it is a reasonable assumption that it is related to some chemical interaction between the growth cone and the target cell. We did not include such interaction in our model, we simply assumed that the growth cone makes a synaptic connection when it first reaches a cell’s soma.

2.7 Spontaneous release of transmitter from growth cones

There is an experimental evidence for spontaneous release of neuro-transmitter acetylcholine from growth cones. The release of material from a growth cone may have a role in the interaction between the growth cone and its immediate environment. This observation led us to assume the existence of a chemical agent that the growth cones use to communicate with the target cells as we describe below.

3 The self-wiring model

Our model of self wiring is inspired by the communicating walkers model used in the study of complex patterning of bacterial colonies [Ben-Jacob et al., 1994, Ben-Jacob, 1995], and by the bions model used in the study of amoebae aggregation [D. Kessler, 1993]. How should one approach modeling of the growth of neural network? With present computational power it is natural to use computer models as a main tool in the study of complex systems. However, one must be careful not to be trapped in the “reminiscence syndrome”, described by J. Horgan [Horgan, 1993], as the tendency to devise a set of rules which will mimic some aspects of the observed phenomena and then, to quote J. Horgan “They
say: ‘Look, isn’t this reminiscent of a biological or physical phenomenon!’ They jump in right away as if it’s a decent model for the phenomenon, and usually of course it’s just got some accidental features that make it look like something.” Yet, the reminiscence modeling approach has some indirect value. True, doing so does not reveal directly the biological functions and behavior. However, it does reflect understanding of geometrical and temporal features of the patterns, which indirectly might help in revealing the underlying biological principles.

Another extreme is the “realistic modeling” approach, where one constructs an algorithm that includes in detail all the known biological facts about the system. Such an approach sets a trajectory of including more and more details vs. generalized features. The model keeps evolving to include so many details that it loses any predictive power.

Here we adopt another approach called “generic modeling” [D. Kessler, 1993, Ben-Jacob et al., 1994], where we seek to elicit, from the experimental observations and the biological knowledge, the generic features and basic principles needed to explain the biological behavior and to include these features in the model. We will demonstrate that such modeling, with close comparison to experimental observations, can be used as a research tool to reveal new understanding of the biological systems.

3.1 The model overview

In modeling the neurite navigation we were inspired by the bions model used in the study of amoebae aggregation [D. Kessler, 1993] and by the communicating walker model used in the study of bacterial colonies [Ben-Jacob et al., 1994] [Ben-Jacob, 1993] [Ben-Jacob, 1997]. In the model the growth cones are represented by walkers which perform off-lattice non-uniform random (biased) walk [Segev and Ben-Jacob, 1998]. The chemical dynamics (e.g. chemotactic agents, triggering field) are described by continuous reaction-diffusion equations solved on a tridiagonal lattice with a lattice constant $a_0 = 10\mu m$. Each of the soma
is represented by a stationary (not moving) unit occupying one lattice cell. The neurite are simply defined as the trajectory performed by the growth cone.

We assume that each of the soma cells continuously emits a repulsive agent whose concentration is denoted by $R$. In the model, $R$ satisfies the following reaction diffusion equation:

$$\frac{\partial R}{\partial t} = D_R \nabla^2 R + \Gamma_R \sum_{\text{soma}} \delta(\vec{r} - \vec{r}_j) - \lambda_R R$$  \hspace{1cm} (3)

Where $D_R$ is the diffusion coefficient, $\lambda_R$ is the spontaneous decomposition rate and $\Gamma_R$ is the emission rate by the soma cells.

When a neurite first sprouts it is mainly affected by the repulsive agent and moves away from its “mother” soma cell. It then continues to move on a trajectory which maximizes the distances from the surroundings soma cells (fig ??a).

When a neurite reaches a specific length determined by its soma cell via the internal energy (in a manners described below), it does two things: 1. It switches its chemotactic sensitivity from sensitivity to the repulsive agent to sensitivity to the attractive. 2. It emits a quantum of a triggering material (which satisfies an equation similar to eq. 3). Soma cells in the neighborhood respond by emitting a quantum of attractive agent if they sense an above threshold concentration of the triggering material. As a result, the growth cone moves towards the soma cell with the strongest attractive response (typically, the one closest to the growth cone, see figure ??b)

### 3.2 The growth cone’s movement in the absence of chemotaxis

At the absence of chemotaxis each walker performs off-lattice random walk of step size $d$ which is of the order $5a_0$, where $a_0 \sim 5\mu m$ is the lattice constant. The random walk is at an angle $\theta$ which is chosen out of 12 available directions $\Phi(n) = (n - 1)\pi/6$. The angle $\phi(n)$ is measured relative to the last direction of movement ($n = 0$ corresponds to a step in the last direction of movement). The
angle is chosen from a non-uniform probability distribution $P_0(n)$ shown in Fig 4. The highest probability is to continue to move in the same direction, the second pick in the probability distribution is to move backward. Thus the walker moves from its location $\vec{r}_i$ to a new location $\vec{r}_i'$ given by:

$$\vec{r}_i' = \vec{r}_i + d(\cos \theta, \sin \theta)$$ (4)

A typical trajectory of such a random walker is demonstrated in figure ??.

The hexagon represents the cell’s soma and the dot represents the walker. This random walker movement generates a neurite shape that is not in agreement with experiments [Dwir et al., 1996]. Thus we modify the motion rule as follows: the walker does not move every time step. After $\theta$ is selected, a counter for that chosen direction (given $n$) is increased by one. The walker performs a movement only after one of the counters reaches a specified threshold $N_C$. The movement is in the direction which corresponds to that counter. This process acts as a noise reduction mechanism. We show a typical trajectory of the walker in figure ?? The observed neurite shape is in better agreement with experiments [Dwir et al., 1996].

### 3.3 Growth cone movement in the presence of chemotaxis

In the presence of chemotactic materials, the probability distribution $P_0(n)$ (the relative probability of choosing from the available 12 directions) is modified. Since a growth cone is repelled (attracted) by the repulsive (attractive) agent, the probability is higher (lower) in the direction of low (high) directional derivative of the chemo-repellent agent’s concentration. In our model we assumed that the probability changes linearly in the chemo-repellent concentration gradient. A similar rule is used for the chemo-attractant concentration. The new probability of moving in the $n$-th direction is given by:

$$P(n) = P_0(n) + G_A \cdot S(A)\nabla_n A - G_R \cdot S(R)\nabla_n R$$ (5)
Where $A$ and $R$ are the concentrations of chemo-attractant and chemo-repellent, respectively. $\nabla_n$ is the directional derivative in the appropriate direction. The functions $S(A)$ and $S(R)$ are pre-factors which describe the receptor’s sensitivity as a function of the concentration (the “Receptor’s law” as described in section 2.4). $G_R$ and $G_A$ are functions of $\frac{dE}{dt}$. They determine the relative magnitude of response to chemo-attractant and the chemo-repellent. Since $\frac{dE}{dt}$ decreases with the neurites length (as we describe in section 3.5), and we assume that the growth cone is more sensitive to the chemo-repellent while it is close to its cell’s soma, $G_R$ is taken to be an increasing function of $\frac{dE}{dt}$ as described in figure ??a, to obtain the desired result. A similar rule is used for the chemo-attractive concentration. Since the walker is more sensitive to the chemo-attractant while it is far away from its soma we take $G_A$ to be a decreasing function of $\frac{dE}{dt}$ as described in figure ??b.

### 3.4 Chemicals concentration

We handle the corresponding continuous reaction-diffusion equations for the chemical concentrations by solving them on a triangular lattice. The equations for the chemo-repellent concentration $R$, chemo-attractant $A$ and triggering $T$ are given by:

$$\frac{\partial R}{\partial t} = D_R \nabla^2 R - \lambda_R R + \Gamma_R \sum_{soma} \delta(\vec{r} - \vec{r}_j)$$

(6)

$$\frac{\partial A}{\partial t} = D_A \nabla^2 A - \lambda_A A + \Gamma_{As} \sum_{soma} \delta(\vec{r} - \vec{r}_j) + \Gamma_{Agc} \sum_{emitting\ walkers} \delta(\vec{r} - \vec{r}_i)$$

(7)

$$\frac{\partial T}{\partial t} = D_T \nabla^2 T - \lambda_T T + \Gamma_T \sum_{emitting\ walkers} \delta(\vec{r} - \vec{r}_i)$$

(8)

where $D_R, D_A, D_T$ are the diffusion coefficient, $\lambda_R, \lambda_A$ and $\lambda_T$ are the rate of spontaneous decomposition of $R, A, T$ respectively, $\Gamma_R$ is the rate of emission of $R$ by the soma located at $\vec{R}_j$, $\Gamma_{As}$ and $\Gamma_{Agc}$ are the rate of emission of $A$ by the soma and the growth cone respectively and $\Gamma_T$ is the rate of emission of triggering agent by a growth cone located at $\vec{R}_i$. The summation in equation 3 is over all
the soma. The summation in equations 4 is over all the soma and walkers that are currently emitting chemo-attractant. We assume that $D_R$ and $D_A$ are of the same order. We further assume that $\lambda_R < \lambda_A$, so the chemo-repellent is long ranged with respect to the chemo-attractant.

### 3.5 The growth cone’s internal energy

The assignment of internal energy to describe the metabolic state of the growth cone is a crucial assumption in the model. The assumption was first motivated by the modeling of bacterial colonies [Ben-Jacob et al., 1994, Ben-Jacob, 1995] [Matsushita, 1998] in which such internal energy turned out to be a crucial feature in modeling systems composed of biological elements.

Since we assume that the walker changes its sensitivity to the chemo-repellent and the chemo-attractant as a function of its neurite length, the walker can migrate away from its cell’s soma at the beginning of the growth and attract to one of the target cell’s soma at the late stage of the growth. We propose that the dependence of the walker sensitivity on the neurite length enters via an energy function which depends on the neurite length. The walker changes its relative sensitivity to the chemotaxis as a function of the energy as we describe below.

This assumption is supported by the experimental observations that the growth cones are rich with mitochondria [Levitan and Kaczmarek, 1991]. We assume that the soma feed the growth cone with internal energy, which the growth cone utilizes for its metabolic processes. We further assume that the neurite consumes internal energy proportional to its length. Finally, it is natural that the growth cone spends internal energy at a constant rate for its metabolic process. Thus, the time evolution of the internal energy is given by:

$$\frac{dE_i}{dt} = \Gamma(N_j) - \Omega - \lambda L_i + K(A)A$$

(9)

Where $\Gamma(N_j)$ is the rate of internal energy supplied by the soma, it is a decreasing function of $N_j$, the number of neurites sent out by the soma. The growth
cone consumes internal energy at a rate $\Omega$, and its neurite consumes the internal energy at a rate $\lambda$ per unit length. The last term on the right hand side of eq. (4) describes the absorption of chemo-attractant by the growth cone. $A$ is the chemo-attractant concentration at the walker position and $K(A)$ is a absorption coefficient. We assume (as is usually the case [Tessier-Lavigne and Goodman, 1996]) that the chemo-attractant agent can be used by the growth cone as an energy source. $K(A)$ is already measured in units of energy.

3.6 Walker activity

The soma supplies energy at a higher rate than the walker’s consumption rate $\Omega < \Gamma(N_f)$ (equation 4). Hence initially (i.e. short neurite’s length $L_i$) the internal energy increases. At this stage of the growth the walker has to migrate away from its own soma, therefore we assume that the walker is very sensitive to the chemo-repellent and insensitive to the chemo-attractant when $dE/dt$ is positive.

In order to overcome the repulsive force and attract to one of the possible target cells at the late stage of the growth, we assume two assumptions: the walker reverts its response to the chemotaxis, and signals to the target cell about its presence when $dE/dt < 0$. Specifically we can write in the absence of chemo-attractant, for

$$L_i > L_c \quad \text{where } L_c \equiv (\Gamma - \Omega)/\lambda$$

(10)

the internal energy decreases. When $\frac{dE}{dt}$ first becomes negative, the walker emits a quantum of triggering material. The triggering substance diffuses through the media and is sensed by a possible target cell’s soma. The target cell’s soma, as we described above, react by emitting a quantum of attractive substance. The walker can find its way to the target cell’s soma using this attractive substance concentration gradient. We call this signaling mechanism of the walker to the target cell an “Attractive Chemotactic Feedback”. The walker response to the chemotaxis
changes during the growth process in a continuous manner as described in the next section.

After the walker emits the first quantum of triggering agent it waits \( \tau_T \) time units before the next quantum is emitted. If during \( \tau_T \) the growth cone senses sufficient concentration of chemo-attractant, or \( \frac{dE_i}{dt} \) becomes positive, it will not emit another quantum of the triggering agent. If \( \frac{dE_i}{dt} \) is negative for a sufficiently long time, so that \( E_i \) drops to zero, the neurite and its growth cone degenerate and are removed, as usually is the case for growth cones that fail to create an appropriate synaptic connection. When the walker reaches another cell or another cell’s neurite, it creates a synaptic connection and its metabolic processes are stopped.

4 Simulation of The Chemotactic Navigation

As was mentioned above the reaction-diffusion equations are solved on a tridiagonal lattice with a lattice constant \( a_0 \). Thus, the fact that the soma occupies one lattice cell is in agreement with the typical size of the neurons’ soma. The typical size of the simulated system is about \( 200 - 400 a_0 \) and the distance between cells’ soma is about \( 25a_0 \). A typical diffusion coefficient \( D \) of the chemicals is of the order of \( 10^{-6} - 10^{-7} cm^2/sec \). Time is measured in units of \( 1 sec \) and the dimensionless diffusion coefficients are of the order of 10. In the simulations, the walkers growth rate is about 0.01 in dimensionless units, which corresponds to \( \sim 1\mu/min \) in agreement with the measured advance rate of the growth cones.

In figure ?? we show the result of numerical simulation of self wiring of 50 cells system on a \( 200 \times 200 \) grid. We look for new experiments that will provide a crucial test to verify our model’s validity and its ability to form the delicate structure of connection.
4.1 Two-cell systems

To demonstrate the efficiency of the proposed chemotactic navigation strategy, we consider two-cell systems in various configurations. In figure ??, the synaptic connections are formed at about half-way between the cell’s soma. The wiring process is very efficient: five out of the six emitted walkers formed connections.

In figure ?? the cell on the right is a “normal” cell, while the one on the left is a “variance” which is incapable of emitting neurites. This choice is in order to make the wiring pattern more transparent. We see that even neurites which originally migrated away from the target cell change their path and migrate towards this cell, once the target cell is triggered to emit a chemo-attractant.

In figure ?? we consider a system as in figure ?? with a barrier in between, in the absence of chemotactic communication (figure ??a), the barrier prevents the formation of synaptic connections between the two cells. When included, the chemotactic communication enables the walkers to over come the barrier effect and the two cells are wired as is shown in figure ??b.

In figure ?? we show the effect of a saturated media with chemo-attractant on the growth process. The media in which the growth process takes place is enriched artificially with chemo-attractant agent. In our model we assume that the chemo-attractant feeds the walkers (the $K(A)A$ term in equation 3.6), therefore, in chemo-attractant rich media the internal energy time derivative remains at a high level for a long time. Since $dE/dt$ remains positive the growth cones do not emit the triggering agent, and the target cell does not emit chemo-attractant. As a result, the growth cones cannot find their path to the target cell. This computer simulation suggests an experiment to prove the validity of our assumption concerning the existence of internal energy. We predict that in an artificially enriched chemo-attractant media the growth cones will lose their ability to find their path.
4.2 The effect of cutting off the growth cone

According to our model when a growth cone is cut off from its soma, the growth cone will change its response to the chemotaxis. This observation give us an indirect way to test the existence of internal degree of freedom in the growth.

[H. Song, 1997, Haydon et al., 1984, R. Shirasaki, 1998].

4.3 Growth regulation via internal energy

In fig 12 we show a system of five cells: one normal, at the center, and four ”variance” cells at the corners. All the parameters in figures ??a and ??b are the same, but in figure ??b the cell at the center “feeds” the neurite at a higher rate. As a result, the growth cones trigger target cells when they are further away from their soma, and the soma is wired to all four neighbors and not only to two, as is the case in figure ??a.

The fact that the feeding rate has a dramatic effect on the efficiency of the wiring process brings to mind the idea that the soma can regulate the distance of connections by adjusting the feeding rate. In figure ?? we show the same system as in figure ?? but with additional 24 “variance” cells. The first two neurites are fed at a low rate. Hence they are connected to the nearest neighbors. The third neurite is fed at a higher rate. Thus it forms a connection further away.

4.4 Simulation of micro-pipette as a source of chemo-attractant

A direct way to prove that a Nerve Growth Factor (NGF) is a chemotaxis is to place a micro-pipette containing NGF near a growing axon in tissue culture, the axon turns and grows toward the NGF source [R.W. Gundersen, 1979]. This turning response to elevated concentrations of NGF represent the chemotactic guidance of the NGF. In our model, we assume that at the beginning of the growth process the growth cones are insensitive to the chemo-attractant, here we construct a similar experiment to show the validity of our assumption.
The model predicts that since the walker has low sensitivity to chemo-attractant at the beginning of the growth process, an artificial source of chemo-attractant will not affect the growth at early stages. We show a computer simulation of such a system in figure ???. We simulated one cell in the presence of an artificial source of chemo-attractant and the contours represent the concentration levels of the chemo-attractant. We can see that the walker’s growth is not affected from the presence of chemotaxis gradient.

4.5 The growth in the presence of 2-fold anisotropy

Recently, there have been experimental studies of the effect of imposed 2-fold anisotropy on the wiring process [Dwir et al., 1996]. In the experiments, Dwir et al cultured hundreds of neurons on a silicon wafer covered with Poly-L-Lysine stripes, along which the growth cones have higher probability to move. To mimic the imposed anisotropy we include in the model a comb of stripes $5a_0$ wide, on the grid. When a walker position is on a stripe it has a higher probability to move along the stripe. The effect of such imposed anisotropy in a two-cell system is shown in Figure ???. The anisotropy is both parallel, figure ??a, and perpendicular, figure ??b, to the line connecting the two cells. When the comb of stripes is parallel the walkers can find their path and form synaptic connections, but, when the comb of stripes is perpendicular the walkers cannot find their path and we can see that two out of 6 walkers lose their way.

We constructed a computer simulation of a large network and analyze whether the results agree with the experiments. In figures ?? we show the effect of 2-fold imposed anisotropy on the growth of a large network. The 2-fold anisotropy has varying distances between stripes: ??a. $5a_0$, ??b. $30a_0$, and ?? no stripes. In each figure there are 80 cells on a $200 \times 200$ grid which correspond to an average inter-cellular distance in a random cell distribution of $\sim 22a_0$. In comparison with this distance, the distance between stripes spans a range of spacings from smaller to larger than the inter-cellular distance. Small inter-line spacing ($5a_0$) leads to
strong alignment of the neurite with the stripes due to the high surface coverage (33% – 20%) with stripes. Large line spacing, which is beyond the average inter-cellular distance, makes the stripes too sparse to support neurite alignment and the produced patterns are similar to the one we show in figure ?? where there are no stripes at all. These observations are in contrast to the experimental results of Dwir et al [Dwir et al., 1996]. In their experiments small inter-line spacing reduced neurites alignment since neurites can form interconnections between cell across Poly-L-Lysine. In our model the walkers can migrate across the surface which is uncovered by PLL with no stripes. Therefore large line spacing reduces neurite alignment. We expect that in an experiment in which the surface is covered with Poly-L-Lysine and stripes are printed on it (i.e additional cover of PLL), we will observe similar patterns to our model’s simulations.

5 The connection distance histogram

We look for a quantitative way to compare our model results to experiments. We suggest to use the histogram of number of connections vs. lengths. In connections lengths we mean the straight line distance between two connected neurons and not the length of the neurite connecting them. We show the results in figure ??.

We can explain these results as follows: In our model there are two characteristic length scales. The inter-cellular distance $d_c$, and the critical length for the walkers’ sensitivity switch, $L_c$. When $L_c \sim d_c$ there is only one characteristic length scale, hence, most of the connections’ lengths are of the order of one length and there is only one maximum in the histogram. When $L_c > d_c$ there is another length scale that emerges as we show in figure ??b-d. There are two maxima in the connection histogram which correspond to the two length scales in the system.

In figure ??e we compare the case of chemotactic navigation with the non-chemotactic navigation. In the first case the histogram shows a concentrated peak
at the inter-cellular distance. The histogram of the second case is widely spread. We suggest to use these results to show the validity of our model in experiment.

6 Conclusions

We have presented a navigation strategy for micro-level network organization. This mechanism lead to the formation of neural networks with fine structures, which can be genetically dependent. Our results raise two fundamental issues: 1. One needs to develop characterization methods (beyond number of connections per neurons) to distinguish the various possible networks. 2. What is the relation between the network organization and its computational properties and efficiency.

To clarify what we mean by characterization method, we consider the following example in which the network is mapped onto a directed graph. Each neuron is represented by a vertex and each synaptic connection between neurons is represented by a directed edge between the two vertices representing the neurons. We can map the graph structure into an adjacency matrix where there is 1 in the i,j entry of the matrix if there is a directed edge between the i and j vertices and 0 otherwise. Then using an algebraic method, such as spectral theory of matrices, we can analyze the adjacency matrix of the graph which characterizes the network. In other words, just as astronomers study spectra to determine the make-up of distance stars, we try to deduce the principle properties and structure of the graph from its adjacency matrix spectrum [Chung, 1997].

The next step of the endeavor presented here is to include the effect of the network electrical activity on its self-wiring. Once some initial synaptic connections are formed, the network begins its electrical activity. It is natural to expect that from this point on the wiring dynamics depend on the electrical activity. An example to such a dependence could be that the neurons emit chemo-attractant while it bursts a train of spikes. In addition we assume that a growth cone is more sensitive to the chemo-attractant when its soma bursts. Using such naviga-
tion strategy we expect that neurons with correlated electrical activity will have higher probability to form a synaptic connection as we show in figure ??.

To conclude, we are still far from understanding the emergence of a functioning brain from a collection of neurons. Yet we believe our studies provide a useful first step towards this goal.

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Figure 1
Neuron’s culture grown on a Silicon wafer after 5 days (500× magnification, reflected light+Numarsky). The thin filaments are the neurites that connect the neurons.

Figure 2
Growth cones’ strategy for path finding. a. At the beginning of the growth the growth cone is mainly affected by the chemo-repellent. b. After the critical length determined by the growth cone’s internal energy, the growth cone emits a quantum of triggering agent and reverts its response to the chemotaxis.

Figure 3
The non-uniform probability distribution of the walker.

Figure 4
A simulation of a 50-cell system on a grid of size 100 × 100. The small hexagons represent the neurons’ soma, the filaments represent the neurites and the dots represent the walkers.

Figure 5
Random walker with the non-uniform probability in figure 4. The hexagon represents the neuron’s soma and the dot represents the walker.

Figure 6
Random walker with the “winner take all” rule.
Figure 7

a. The function $G_R$ as a function of $dE/dt$. b. The function $G_A$ as a function of $dE/dt$.

Figure 8

Two cells system: the hexagons represent the soma, the dots represent the walkers, and the filaments represent the neurites.

Figure 9

The effect of chemo-attractant on the efficiency of navigation. The cell on the right is a "normal" cell and the cell on the left is a "variance" cell that does not emit neurites. Note that even a walker that first migrates away from the target cell navigates towards this cell after it has been triggered. The contours correspond to different concentrations of the chemo-attractant.

Figure 10

The effect of turning off the chemotactic communication. a. When we turn off the chemotaxis communication the walkers are unable to reach their synaptic target. b. When we turn the communication on the walker can migrate around the barrier.

Figure 11

Simulations of five-cell systems. The central cell is "normal" and the four cells at the corners are "variance" cells. The contours represent the chemo-attractant concentration levels. a. Low rate of "internal" energy "feeding" so that $l_c$ is much smaller than the inter-cell distances. In this case the wiring is not efficient as the central cell is wired only to two of the four neighbors. b. Higher rate of "feeding"
so that \( l_c \) is approximately half the inter-cellular distance. In this case the wiring is more efficient and the central cell is wired to all its neighbor cells.

**Figure 12**

Simulations of a system composed of 29 cells. Only the cell at the center is “normal” and all other cells are “variance”. The central cell has four nearest neighbor (NN) cells and eight next nearest neighbor (NNN) cells. At the beginning of the growth \( l_c \) is about half the inter-cellular distance. Thus the central cell is wired only to its NN cells. After the central cell forms two connections the “feeding” rate doubles (doubling of \( l_c \)). The new neurites navigate to the NNN cells. It demonstrates the manner in which the cell’s soma can regulate self-wiring.

**Figure 13**

The effect of a saturated media with chemo-attractant on the growth process. The cell on the right is a “normal” cell and the cell on the left is a “variance”. Since the chemo-attractant feeds the walkers, \( dE/dt \) remains positive during the growth, and the walkers do not emit the triggering agent. As a result, the walkers cannot find their path to the target cell.

**Figure 14**

One cell and an artificial source of chemo-attractant: since we assume, in our model, that at the beginning of the growth process the growth cones are insensitive to the chemo-attractant, the walkers are not effected by the chemo-attractant field.

**Figure 15**

The effect of 2-fold imposed anisotropy: we impose a comb of stripes \( a_0 \) wide and \( 5a_0 \) wide between the stripes. The growth cones have higher probability to
move along the stripes. The strips are parallel to the line connecting the two cells for a, and perpendicular to that line for b. The resulting pattern agrees with experimental observations [Dwir et al., 1996].

**Figure 16**

A 80-cells system on a 200 × 200 grid with a flat surface (no stripes).

**Figure 17**

Systems of neuron growth on 2-fold anisotropy with varying distances between stripes: a. 5\(a_0\), b. 30\(a_0\). In each figure there are 80 cells on 200 × 200 grid which correspond to an average inter-cellular distance in a random cell distribution of ∼ 22\(a_0\).

**Figure 18**

The number of connections per distance histogram. a-d The characteristic length of the walkers’ response switch varies from 1.25\(a_0\) (low feeding) to 50\(a_0\) (high feeding). At lower feeding rates the dominant length scale is the inter-cellular distance which is ∼ 20\(a_0\). The connections histogram achieves its maximum at the inter-cellular distance. At high feeding rate, there are two distinct length scales: the inter-cellular distance and characteristic length of sensitivity switch. There are two picks at the connection histogram which correspond to each length scale. e. The growth cones perform random walker and establish connection with the first soma it encounter on its way (the widely spread histogram), the peaked histogram represent the case where \(l_c = 1.25a_0\).

**Figure 19**

Example of the effect of the correlation in the electrical activity. The central cell is normal, while the others are variances which are unable to emit neurites.
We choose to use variance in order to make the connection patterns more transparent to the eye. The central and the outer cells (up, down, left, right) fire simultaneously while the other cells fire at random. Indeed, as we expect the first connections are formed between the cells that fire simultaneously.
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