Fabrication of Biological Neuronal Networks for the Study of Physiological Information Processing

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Biological neuronal networks have been proposed as intelligent models for studying physiological information processing. This article describes the fabrication of networks of cells in culture, where stimulation and response can be measured at various locations in a controlled manner. Our initial work used geometrical aids to grow neurons from the central nervous system to spatially regulate their synaptic connections in culture. However, in this culture system, we could not identify pre- and postsynaptic neurons, and we found it difficult to determine that the connections were anatomically wired. On the basis of these initial experiments, we are now exploring the formation of physiologically relevant synapses using morphologically identifiable neurons dissociated from the goldfish retinotectum projection. (Keywords: Central nervous system, Culture, Geometrical regulation, Neuron, Neuronal network, Synapse.)

INTRODUCTION

The UltraScale Computing Program of the Defense Advanced Research Projects Agency (DARPA) seeks to examine and develop technologies capable of achieving performance levels necessary for next-generation problem solving in defense systems. Defense computing needs continue to expand; emerging requirements are orders of magnitude beyond current technology. Higher speeds, increased storage density, lower cost, reduced power, and improved versatility are a few of the necessary specifications for computational systems in the next century. The objective of the work described in this article is to explore the foundation for computation in the physiological brain (biological intelligence). Its goal is to provide critical information in support of advances in computing architectures for developing the next generation of neural network processors.

The study of biological intelligence is one of the most powerful research areas in developing models for future computer architectures. Research is being conducted in a variety of fields, including computer science, neuroscience, molecular biology, and engineering.[1] Numerous new techniques and tools from these fields have contributed much intriguing scientific information. For example, computational models of neural networks have been useful in testing existing ideas of brain functions. Imaging techniques for tracing the functional flow of neuronal signals in animals have

provided new insights into the thinking mechanisms of the human brain. However, more research is needed in predicting how neuronal circuits function *in vivo* to pinpoint the minimal neuronal configuration. This work will increase our understanding of the principles of biological information processing.

An alternative way of investigating neuronal cell signaling is to work with dissociated neurons in culture. Working with cultured neurons offers simplicity of procedure, clarity of analysis, and experimental manipulation at the expense of losing some of the circuit's biological properties. Culture techniques have enabled researchers to isolate some neurons from central nervous system (CNS) tissue and to study their development in an environment where the growth conditions are experimentally controlled. However, the critical principles of neuronal circuit operation will not be found in how single neurons process signals; rather, these principles will be revealed by how groups of neurons interact. Thus, it is necessary to work on synaptically interconnected neurons when proposing a new way of studying biological communication.

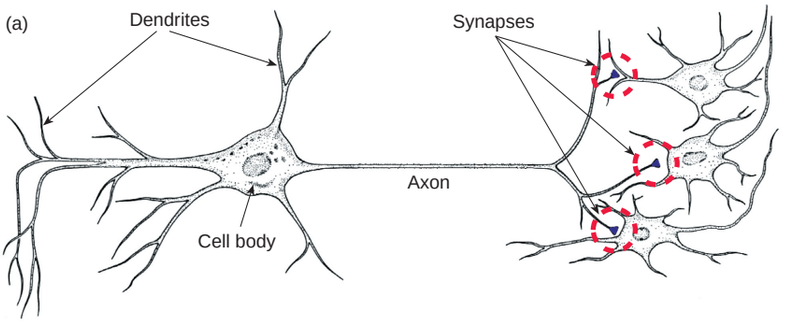
In this study, we first show the feasibility of forming a geometrically ordered arrangement of CNS neurons using organic thin films in culture. We then explore cultures of morphologically identifiable neurons (dissociated from the retinotectum projection of adult goldfish) to form physiologically relevant synapses. We are using neurons from the goldfish retinotectum projection to map the synaptic connections between neurons *in vitro* by stimulating one neuron and recording responses from one or more neurons near the stimulated neuron. The results of this project will help to answer several important questions for future computer designers:

- What are the commonalities between physiological computers and silicon-based machines?
- What is learning, and how are memories stored?
- What are the organizational rules that enable entire memories to be retrieved by minimal cues?
- What are the firing patterns of groups of neurons participating in collective computation?

MORPHOLOGY OF CNS NEURONS

During embryonic development, a CNS neuron initially develops a symmetrical appearance with several short neurites. The morphological characteristics of these neurites are not distinguishable. Then, at some point during embryonic development, a neuron rapidly elongates one of its short neurites, which begins to acquire axonal characteristics. As development continues, the rest of the short neurites develop dendritic characteristics and the neuron becomes morphologically polarized (Fig. 1a). The axon is then projected to its target neurons through synapses, and highly specialized, three-dimensional neuronal networks are formed (Fig. 1a). Generally, multiple-input signals are received at dendrites and then integrated at the cell body. The integrated information is transmitted by the axon as action potentials and transferred to the other neurons through the synapses. The efficacy of each synapse is strengthened or weakened during the development so that specific neuronal connections are formed.

Recent advances in cell culture techniques have enabled the growth of CNS neurons *in vitro*. Under an



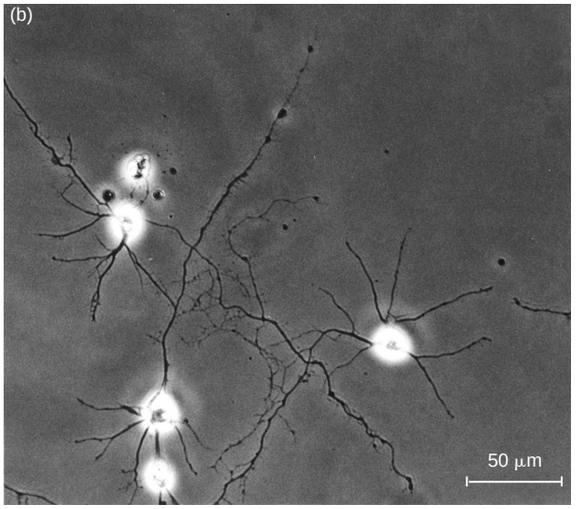


Figure 1. Morphology of central nervous system (CNS) neurons. (a) A schematic representation of neurons and their connections. An axon contacts other neurons (dendrites and cell bodies) through synapses. Typically, a neuron forms thousands of synapses with other neurons *in vivo*. (b) Hippocampal neurons grown in culture. With a standard culture technique, hippocampal neurons (CNS neurons) develop a morphology with a cell body (15 to 20 μm in diameter) and several neuritic processes. One of the processes extends beyond the others. Normally, the longest process differentiates into an axon, and other processes into dendrites. The neurons form random connections to each other.

appropriate culture condition, it has been shown that embryonic CNS neurons can develop a mature morphology featuring characteristics of their counterparts *in vivo* (Fig. 1b). Conventional techniques of electrophysiology have been applied to study fundamentals of the functional development of CNS neurons and their connections *in vitro*. However, unpredictable randomness in neuronal connections, which arises from the culture preparation of dissociated neurons, often hinders investigators from a repeatable functional analysis of neurons and their networks. Hence, the use of morphologically regulated networks of neurons is favorable for physiological study, because points of synaptic contacts are limited between individual neurons and the network architecture. The networks of these geometrically regulated neurons can serve as models to answer physiologically important questions.

FABRICATION AND EVALUATION OF NEURONS ON CHEMICALLY PATTERNED SUBSTRATES

We have patterned substrates with biomaterials to culture neurons and geometrically control their morphological development *in vitro*. The substrates were fabricated using chemical modification or a micropatterning technique. Glass cover slips were covalently modified with either octadecyltrichlorosilane (OTS), trimethylchlorosilane (TMS), or aminopropyldimethylethoxysilane (APDMS). Details of the film formation were described previously.[2] To make the APDMS surfaces biocompatible, the glass substrates functionalized with APDMS were further modified with laminin peptide using a heterobifunctional crosslinker, N-(γ-maleimidobutyryloxy)sulfosuccinimide ester (sulfo-GMBS).[3] The buildup of monolayer films is schematically presented in Fig. 2a. Patterning was done using either a 193-nm laser or a microcontact printing technique.

Patterning with a 193-nm laser involves taking a glass substrate modified with either OTS film or TMS film and modifying the pattern with deep ultraviolet lithography, as described elsewhere.[5,6] The use of the patterned substrate for the further peptide reaction allowed the coupling of laminin peptide to the surface amino groups.[3,7] Figure 2b shows a schematic view of the patterned surfaces.

Patterning by microcontact printing involves the use of an elastomeric microstamp fabricated from Sylgard (Corning) according to a previously described method.[8] A master plate for the stamp consisted of a pattern of either parallel lines or crossed lines (Kyodo Printing). A drop of phosphate buffer solution containing 10 μM laminin peptide was applied onto the stamp and spread using a cotton tip. The stamp was then placed in contact with a glass substrate that was

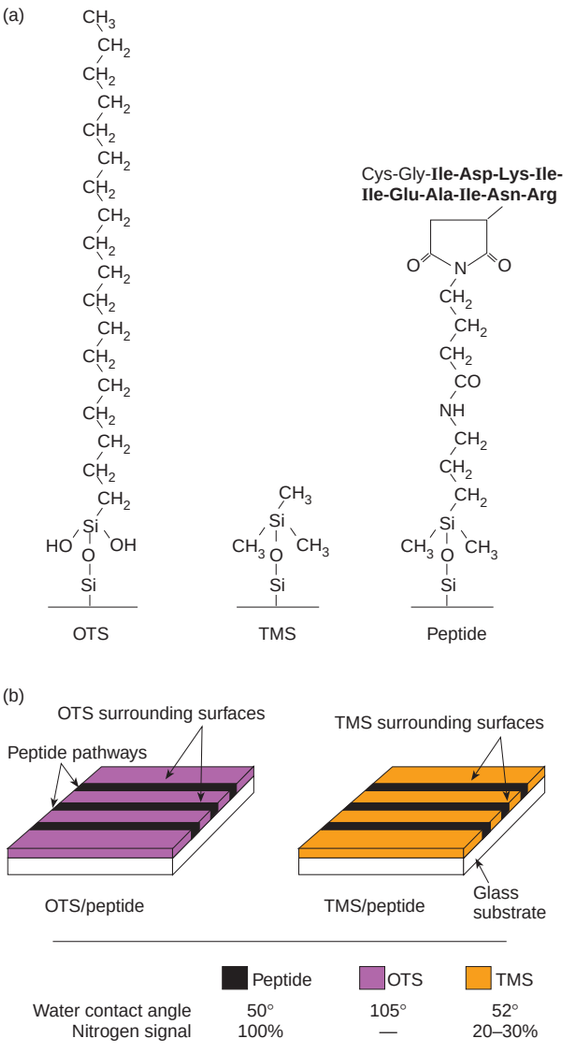


Figure 2. Schematics showing chemical modification of glass surfaces. (a) Surfaces of glass substrates chemically modified with organosilane compounds and laminin peptide. The synthetic peptide was first modified with Gly-Cys at the C-terminal part and then covalently bound to a glass surface. (Adapted from Ref. 3 by permission.) (b) Parallel peptide stripes (peptide pathways) formed on octadecyltrichlorosilane (OTS) and trimethylchlorosilane (TMS) substrates. A film of either OTS or TMS was first formed by chemisorption and then lithographyically patterned. The patterned surfaces were chemically modified with the synthetic peptide. A water contact angle, an indicator of surface hydrophobicity, was measured using a sessile drop technique. A nitrogen signal attributed to the number of amines and peptide bonding was detected using X-ray photoelectron spectroscopy and expressed as a percentage of nitrogen amount detected from peptide surface. (The dash indicates a negligible nitrogen signal.) (Adapted from Ref. 5 by permission.)

functionalized with amine and sulfo-GMBS. After 1 h of contact, the substrate was thoroughly rinsed with deionized water. The microstamped surface was visualized with an immunofluorescent technique using a rabbit antibody for laminin peptide, anti-PA22-2. The

technique allowed us to transfer micrometer-scale features onto the glass surfaces (Fig. 3).

HIPPOCAMPAL NEURONS ON PATTERNED SUBSTRATES

Our initial studies involved patterned substrates with hippocampal neurons. The hippocampus, named for its structural resemblance to the sea horse (in Greek, *hippo* means horse, and *kampos* means sea monster), is one of the best characterized CNS tissues. The hippocampus is organized by a relatively homogeneous population of pyramidal neurons. We used hippocampal tissues dissected from embryonic rats because there are ample data on the morphological and functional development of individual and slices of rat hippocampal neurons.

We cultured the tissues according to previously established techniques.[3,9,10] Briefly, hippocampal tissues were obtained from embryonic rats and dissociated into single neurons.[9] The cells were placed on the modified surfaces at a low density (5000 cells/cm^2) in a serum-free medium.[10] Cell attachment was allowed to occur for 1 h, after which unattached materials were removed by thoroughly rinsing the substrates and exchanging the culture medium. Neuronal growth was observed using an Olympus microscope equipped with phase contrast and fluorescent optics. The growth of neurons was monitored in real time using an Olympus microscope equipped with a Sony time-lapse recording system.

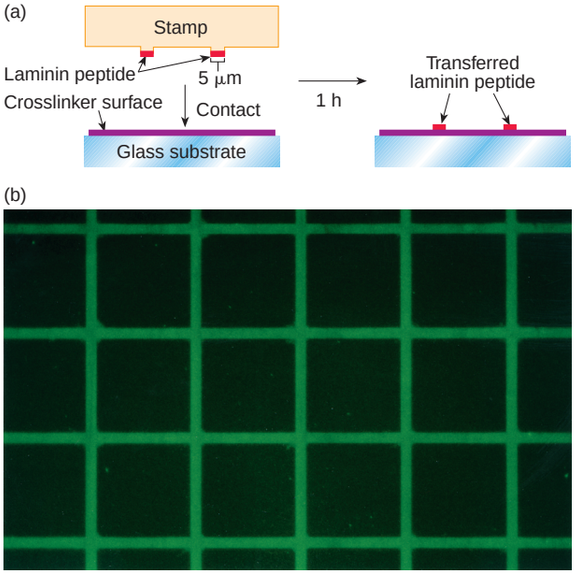


Figure 3. Chemical patterning of glass surfaces using microcontact printing. (a) Procedure for microcontact printing of laminin peptide. (b) An immunofluorescent image showing a pattern (from microcontact printing) of crossed lines formed with laminin peptide.

We first demonstrated the feasibility of controlling the geometry of individual neurons using substrates containing patterned parallel lines with a variety of line widths.[4] The parallel lines were modified with laminin synthetic peptides to promote cell attachment and neuritic outgrowth. Each line was surrounded by hydrophobic and passive surfaces of OTS films (60 μm in width, marked with asterisks in Fig. 4). Using these patterned substrates in culturing embryonic hippocampal neurons made it obvious that the width of the peptide lines affected the neuronal morphology. The neurons grown on peptide lines that were wider than twice the cell body diameter extended multiple neurites, which grew in a confined area without directionality (Fig. 4a). However, if the line width was approximately the cell body diameter, the neurons adopted a bipolar morphology and extended single long neurites, which are putatively axonal processes, along the peptide line. Patterned substrates without peptide modification (controls) did not promote the outgrowth of neuritic processes. These results suggest that the geometrical and biochemical properties of the patterned substrates play a role in controlling the geometry of single neurons. The peptide line width that promoted the longest extension of neuritic processes was regarded as optimal (Fig. 4b) for our later experiments.

To investigate whether neuronal outgrowth was regulated as a result of the growing neurite's preference for the laminin peptide or if it resulted simply from the repellent effect of the hydrophobic OTS surfaces, we conducted culture experiments using another type of patterned substrate. The substrates were fabricated so that, although the peptide lines had the optimal geometry described previously, the surrounding surfaces

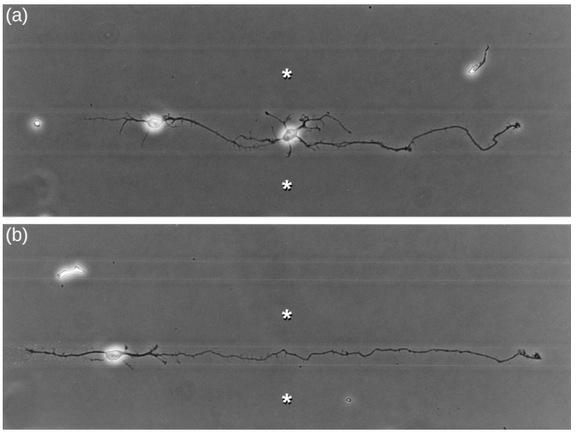


Figure 4. Geometrical control of hippocampal neurons in culture. Hippocampal neurons were grown on patterned substrates containing (a) a 25-μm-wide pathway and (b) a 13-μm pathway, surrounded by 60-μm-wide OTS films. (Adapted from Ref. 4 by permission.)

were formed by TMS films that had a hydrophobic/hydrophilic property equivalent to that of the peptide surfaces. The neurons grown on these substrates initially exhibited a bipolar morphology similar to that shown in Fig. 4b. The time-lapse recording technique revealed that the growth cones advanced using an exploratory movement between the peptide pathway and its surrounding TMS surfaces. The results indicate that the growth of neuritic processes was guided not by the hydrophobicity of the surrounding surfaces but rather by the peptide patterns (Fig. 5).

With a prolonged culture period (>72 h), however, we found that the neurons partially shed their bipolar phenotypicity and extended neurites away from the peptide pathway onto the surrounding TMS surfaces (Fig. 5b). These neurites tended to extend from the cell bodies and/or neurites at a right angle with respect to the alignment of the initial neurite outgrowth (Fig. 5b).[4] A similar tendency was seen when neurons were grown on patterned laminin substrates, which are recognized as substrates with more biological properties.[11] The tendency of neurite elongation at right angles is a defined natural property of hippocampal axons *in vivo*.[12] An expression of an axonal marker, 200-kDa neurofilament (NF) protein, in neuritic processes indicates the development of axonal differentiation (Fig. 5b). The observed directional neurite outgrowth on the peptide pathways was a unique property of the axon-growing neurons as opposed to migratory neurons.[4]

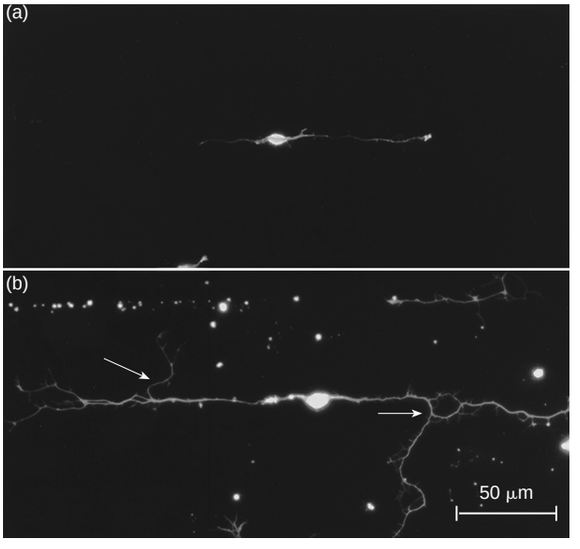


Figure 5. Immunofluorescent images of patterned hippocampal neurons on patterned TMS/peptide substrates. The neuronal morphology on parallel patterned substrates was revealed with neurofilament antibody. (a) The neurons attached on the peptide surface initially extended neuritic processes along the peptide pathway and showed a bipolar morphology. (b) The neuritic processes were then extended onto the TMS surface area and branched out. Arrows indicate neurites extending off the patterned parallel line.

The morphological observation of patterned hippocampal neurons led us to design an optimal substrate to form spatially uniform synapses. We utilized the tendency of hippocampal neurons to extend neurites at right angles by fabricating crossed-line pathways instead of parallel ones on the TMS film surfaces (Fig. 6a). With the use of such an aid, we found that the neuronal attachment occurred on the cross sections, whereas the outgrowth of neurites occurred along the pathways. The neurons readily adapted to the cross-shaped surface geometry and remained confined to those patterns. Neuronal contacts were observed to occur after 48 h in our culture condition. The majority of patterned neurons expressed a presynaptic marker, Synaptophysin, in their cell bodies and axonal processes and tips (Fig. 6b). The expression of synaptic vesicles is an indicator for synapse formation. These patterned neurons were found to be electrophysiologically functional and synaptically interactive (Figs. 6c and 6d) (Ref. 13; also Matsuzawa and others, unpublished data, 1999).

NEURONS FROM GOLDFISH RETINOTECTUM PROJECTION

As described in the previous section, hippocampal neurons were geometrically regulated, and neuronal connections could be formed between individual neurons. However, the synaptic pairs that formed on the substrates were random. Ordered synaptic pairs cannot be formed from randomly dissociated hippocampal neurons because of a lack of identification of pre- and postsynaptic cells. Thus, we have begun exploring the formation of biologically relevant synapses using morphologically identifiable neurons dissociated from the goldfish retinotectum projection. This system would allow us to better control the processing of information in the two-dimensional device.

In the goldfish retinotectum projection, retinal ganglion neurons located in the inner layer of the retina extend axons and form synaptic contacts with neurons in the tectum (Fig. 7). The projection occurs with a defined order. Unlike neurons from the mammalian nervous system, fish optic axons can be regenerated in culture even if the neurons are dissected from adult fish. It has been reported that acutely dissected retinal ganglion neurons from adult fish possess matured physiological characteristics with specific kinds of ion channels and distinct action potentials.[14] Given these anatomical and functional properties of the goldfish retinotectum projection, we feel that the formation of synaptic contacts between neurons from the retina and tectum is advantageous for studying physiological properties of networks.

To morphologically identify a presynaptic neuron (retinal ganglion cell) in culture, the optic nerve of a goldfish (10–12 cm in length) was crushed *in situ* before

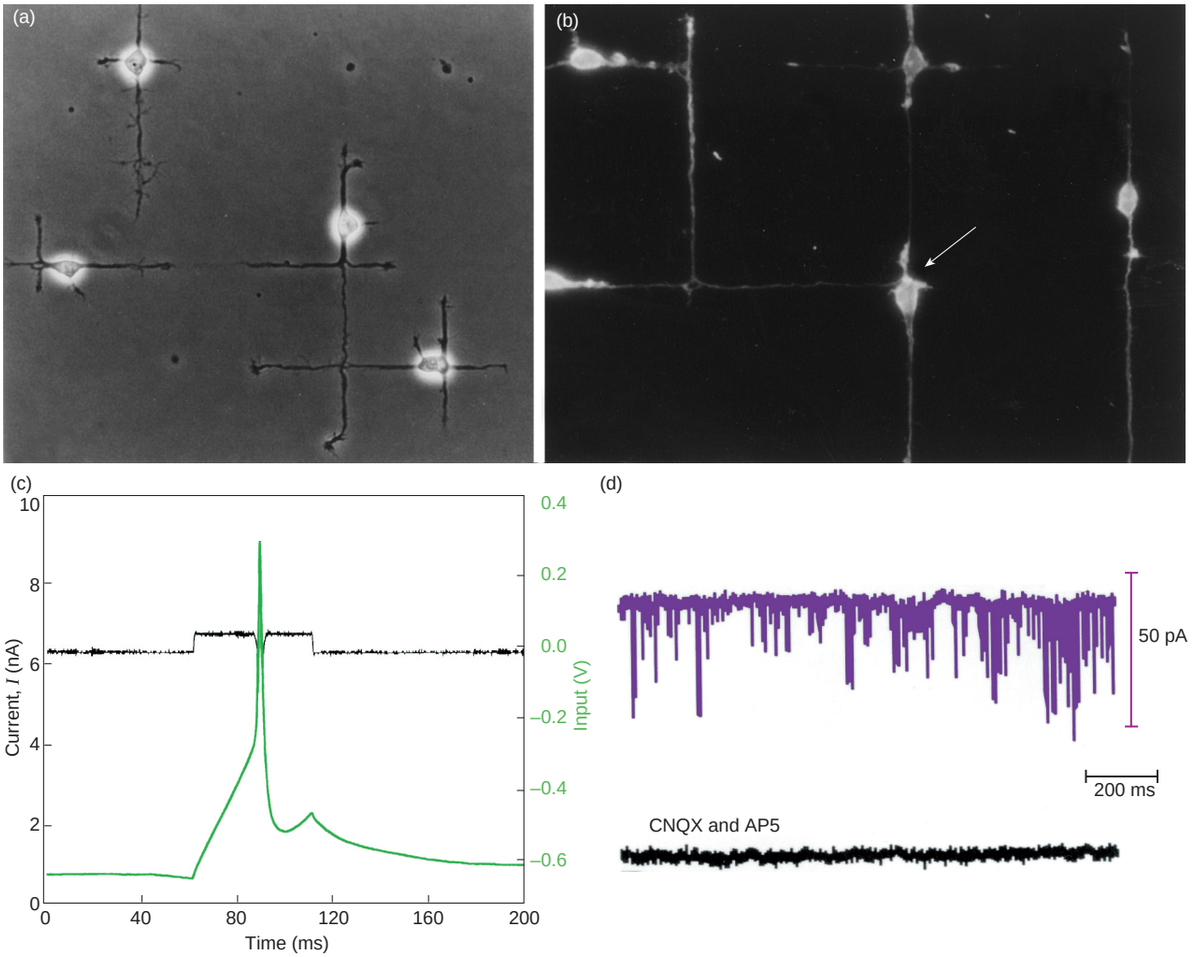


Figure 6. Geometrical regulation of synaptic connections in culture. (a) The use of a crossed pattern (TMS/peptide) regulated the neuronal morphology. A representative view of patterned hippocampal neurons is shown (spacing = 50 μm). (b) Fluorescent image showing the distribution of Synaptophysin in a patterned hippocampal culture (spacing = 100 μm). The arrow indicates strongly stained nerve terminals (one from the top neuron, the other from the left neuron). (c) A typical profile of action potential exhibited by a presumably presynaptic neuron. (d) Current traces obtained from the presumably postsynaptic neuron shown when the membrane potential was held at –90 mV. Random shots of spontaneous inward current were observed in the control bath solution. The shots of spontaneous inward current disappeared after adding 10 μM 6-cyano-7-nitroquinoxaline-2,3-dione and 100 μM DL-2-amino-5-phosphonopentanoic acid to the bath solution (labeled CNQX and AP5, respectively).

dissection.[15] (The nerve crush causes the reproduction of messenger RNA, making the neucleoli larger.) Then the nerve-crushed eye was dark-adapted for 30 min. The retina was removed from the nerve-crushed eye, placed in a sterile plastic dish (60 mm) filled with Ishida's solution,[14] and washed by exchanging the basic solution. The solution was then removed with a Pasteur pipette, and the dish was wiped dry with sterilized filter papers. The retina was spread flat on the bottom of the dish with the nerve fiber layer upward.

Next, the surface of the flattened retina was covered gently with a sterilized Millipore filter. Then the entire retina, including the Millipore filter, was picked up with tweezers and turned over so that the outer unclear layer faced upward. The exposed layer was covered with a second Millipore filter. The retina attached to the filter papers was cut into a few millimeter squares. The filter paper covering the outer layer of retina was gently removed, and the remaining retinal tissue was separated from the other filter paper using a tissue scraper.

The purified retinal tissue was incubated for 20 min in 5 mL of Ishida's basic solution, which contained 5 mg of protease (Sigma). The proteolysis was stopped by rinsing the tissue for 5 min in the basic solution containing 0.01% (weight:volume) bovine serum albumin. After the treatment, the tissue was dissociated into single cells by trituration. The suspended cells were placed on modified substrates in a modified Leibowitz medium.[15] Tectal tissues were removed from the goldfish brain and dissociated into single cells using a process similar to the retinal dissociation procedure just described.

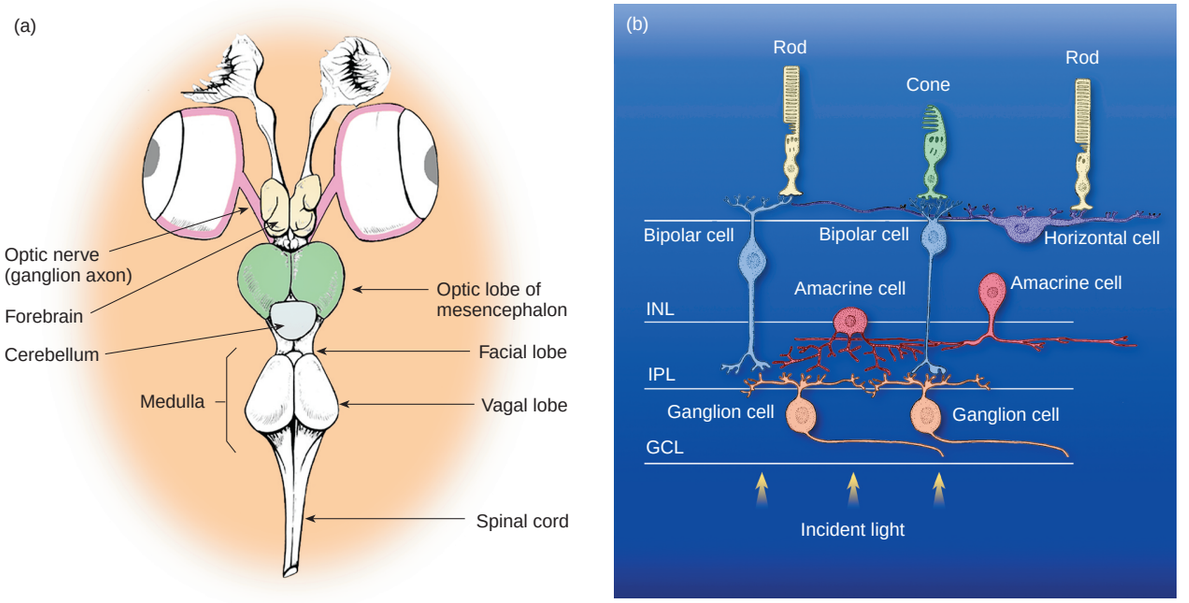


Figure 7. Nerve projection in the visual system of the adult goldfish and neuronal organization of the retina. (a) The retinotectum projection. The optic nerve arising from the retinal ganglion layer projected onto tectum in the brain. (b) Neuronal architecture in the retina. The ganglion layer is located in the innermost side of the retinal tissue (INL = inner nuclear layer, IPL = inner plexiform layer, GCL = ganglion cell layer).

Dissociated retinal ganglion cells and tectal cells were grown on substrates modified with laminin peptide. To allow the differential interference contrast (DIC) image observation, the modified glass substrate was assembled to serve as the bottom of the culture dish. In contrast with the hippocampal cultures, which provide purified neurons without other tissue components such as glial cells and connective cells, cultures of retinal ganglion cells contained degenerated cell bodies and cellular debris. With the DIC imaging technique, swollen nucleoli resulting from the nerve crush identified live ganglion neurons.

Morphological characteristics of retinal ganglion and tectal neurons were examined by immunostaining the neurons using a mouse monoclonal antibody for α-tubulin antibody. The neurons were visualized using an Olympus inverted microscope equipped with Nomarski DIC and fluorescence optics. Within a few days in culture, some cells regenerated the neuritic processes (Fig. 8a), while others went to degeneration

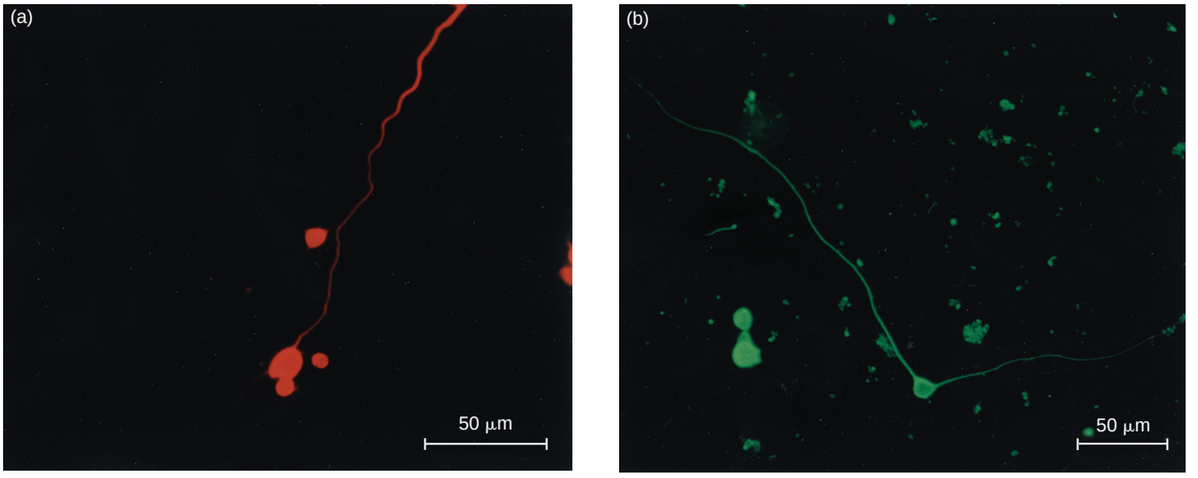


Figure 8. Retinal ganglion neuron and tectal neuron in culture. The neurons were immunostained with an antibody for α-tubulin protein. (a) The retinal neuron was indirectly visualized using a secondary antibody conjugated with Texas red. (b) The tectal neuron was indirectly visualized using a secondary antibody conjugated with fluorescein.

(the quantitative analysis is under way). Neurons grown on glass surfaces without chemical modification failed to regenerate their processes. The majority of live tectal neurons extended bipolar processes (Fig. 8b). Retinal and tectal neurons are morphologically distinctive in culture dishes. The two neuron types can be differentiated from each other in co-culture.

CONCLUSIONS

We have successfully demonstrated the formation of physiological synapses between a pair of geometrically regulated hippocampal neurons; we found that the formed synapses were made from randomly picked neurons from the hippocampus region of the brain. Such a neuron pair will be useful for some studies of synaptic functions, e.g., studies of the effects of pharmaceutical drugs on synaptic transmission. However, these neurons exhibit *de novo* characteristics of synaptic transmission that differ from their counterpart *in vivo*. The physiological analysis of neuronal communication cannot be done using such a preparation.

Hence, we explored experiments that use morphologically identifiable presynaptic and postsynaptic neurons, isolated from the goldfish retinotectum projection, to form physiological synapses. Although this work is still preliminary, we have successfully established culture conditions for both presynaptic neurons (retinal ganglion neurons) and postsynaptic neurons (tectal neurons). The next step is to form networks between these two different types of neurons in culture and to study their physiological behavior in two-dimensional circuits.

FUTURE WORK—OPTICAL RECORDING OF PHYSIOLOGICAL FUNCTION

The intracellular concentration of calcium ions is an important indicator for cellular physiology. We are currently investigating the resting calcium concentration of cultured retinal ganglion neurons using a calcium-indicative dye, fura-2, and fluorescent microscopy (Fig. 9). By attaching an automated monitoring system to the current setup, we will be able to monitor changes in intracellular calcium concentration in real time. The optical analysis of calcium changes in interconnected neurons will directly reveal acting and nonacting sites within the network. The optical analysis of calcium will yield a realistic model of how living neurons process and store information.

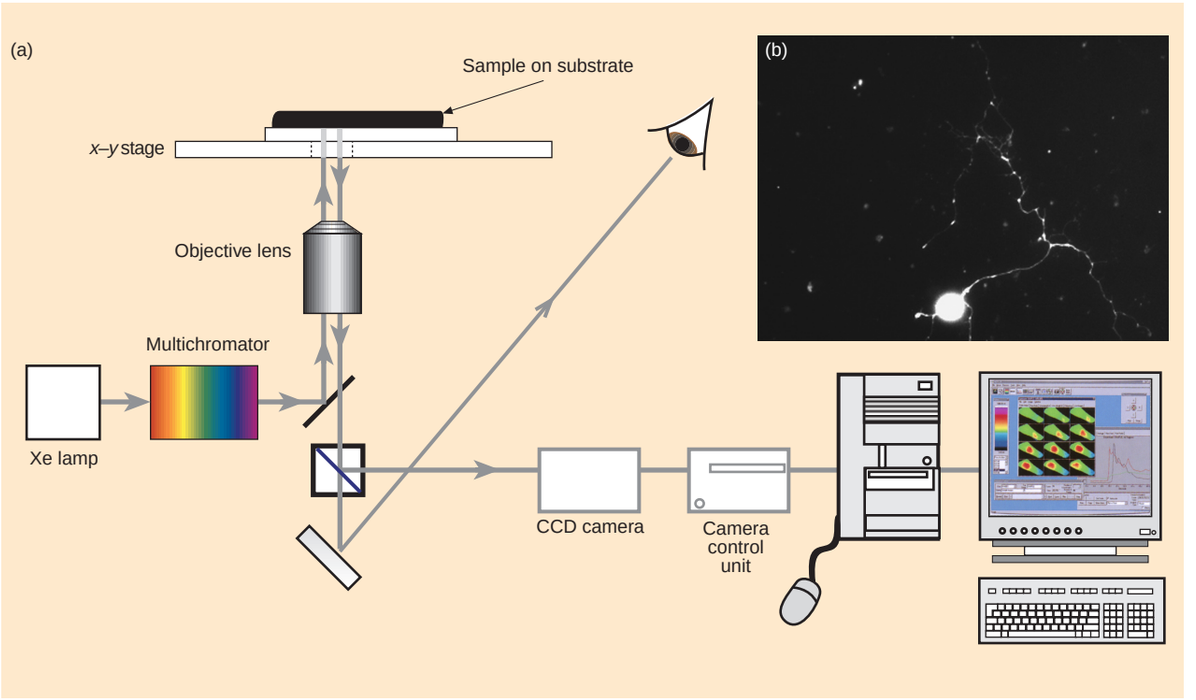


Figure 9. Optical recording of calcium flows using fluorescent microscopy and calcium-indicative dye. (a) Recording setup. A culture dish containing fura-2–stained neurons is placed on a microscope stage. The neurons are illuminated by dual wavelengths (340 and 380 nm), and fluorescence responses are recorded using a cooled charge-coupled device (CCD) camera. A ratio in fluorescence intensity (I_{340}:I_{380}) indicates calcium concentration within the neurons. (b) Fluorescent image shows a retinal ganglion neuron illuminated at 340 nm.

REFERENCES

[1]Mainen, Z. F., and Sejnowski, T. J., "Influence of Dendritic Structure on Firing Pattern in Model Neocortical Neurons," *Nature* **382**, 363–366 (1996).

[2]Matsuzawa, M., Tokumitsu, S., Knoll, W., and Sasabe, H., "Effects of Organosilane Monolayer Films on the Geometrical Guidance of CNS Neurons," *Langmuir* **14**, 5133–5138 (1998).

[3]Matsuzawa, M., Liesi, P., and Knoll, W., "Chemically Modifying Glass Surfaces to Study Substratum-Guided Neurite Outgrowth in Culture," *J. Neurosci. Meth.* **69**, 189–196 (1996).

[4]Matsuzawa, M., Tokumitsu, S., Knoll, W., and Liesi, P. L., "A Molecular Gradient Along the Axon Pathway Is Not Required for Axon Guidance," *J. Neurosci. Res.* **53**, 114–124 (1998).

[5]Matsuzawa, M., Potember, R. S., Stenger, D. A., and Krauthamer, V., "Containment and Growth of Neuroblastoma Cells on Chemically Patterned Substrates," *J. Neurosci. Meth.* **50**, 253–260 (1993).

[6]Matsuzawa, M., Umemura, K., Beyer, D., Sugioka, K., and Knoll, W., "Micropatterning of Neurons Using Organic Substrates in Culture," *Thin Solid Films* **305**, 74–79 (1997).

[7]Matsuzawa, M., Weight, F. F., Potember, R. S., and Liesi, P., "Directional Neurite Outgrowth and Axonal Differentiation of Embryonic Hippocampal Neurons Are Promoted by a Neurite Outgrowth Domain of the B2-Chain of Laminin," *Int. J. Dev. Neurosci.* **14**(3), 283–395 (1996).

[8]Singhvi, R., Kumar, A., Lopez, G. P., Stephanopoulos, G. N., Wang, et al., "Engineering Cell Shape and Function," *Science* **264**, 696–698 (1994).

[9]Banker, G., and Goslin, K., *Culturing Nerve Cells*, The MIT Press, Cambridge, MA (1991).

[10]Brewer, G. J., Torricelli, J. R., Evege, E. K., and Price, P. J., "Optimized Survival of Hippocampal Neurons in B27-Supplemented Neurobasal, a New Serum-Free Medium Combination," *J. Neurosci. Res.* **35**, 567–576 (1993).

[11]Matsuzawa, M., Kobayashi, K., Sugioka, K., and Knoll, W., "A Biocompatible Interface for the Geometrical Guidance of Central Neurons In Vitro," *J. Colloid Interface Sci.* **202**, 213–221 (1998).

[12]Ramon y Cajal, S., *Histology of the Nervous System for Man and Vertebrates*, The Oxford University Press, New York (1995).

[13]Offenhäusser, A., Sprössler, C., Matsuzawa, M., and Knoll, W., "Electrophysiological Development of Embryonic Hippocampal Neurons from the Rat Grown on Synthetic Thin Films," *Neurosci. Lett.* **223**, 9–12 (1997).

[14]Ishida, A. T., and Cohen, B. N., "GABA-Activated Whole-Cell Currents in Isolated Retinal Ganglion Cells," *J. Neurophysiol.* **60**(2), 381–396 (1988).

[15]Ishida, A. T., and Cheng, M-H., "Cold Inhibits Neurite Outgrowth from Single Retinal Ganglion Cells Isolated from Adult Goldfish," *Exp. Eye Res.* **52**, 175–191 (1991).

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