國立交通大學
National Chiao Tung University

出國報告（出國類別：學研訪問）

「飛秒脈衝雷射之生物學應用」所需細胞移轉技術之交流訪問

Discussion and survey of cell manipulation techniques for our new project on biological application of femtosecond laser

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摘要

我們正計畫將飛秒脈衝雷射應用於生物學實驗，目的為開發活細胞元件以研究細胞學與藥物安全的檢測法。我們已熟悉飛秒脈衝雷射的特性，不過還需要知道更多雷射在生物實驗上引發的現象。本次赴日訪問旨在從現有的細胞元件相關研究中獲得經驗與靈感。本人訪問了東北福祉大學（ＴＦＵ，仙臺市）與奈良先端科學技術大學院大學（ＮＡＩＳＴ，生駒市），接待我的教授們熱心地展示新成果；１）ＴＦＵ的坪川宏（H. Tsubokawa）教授展示了神經與星狀細胞受刺激後的體積變化，他表示腦細胞的膨脹是一種神經與星狀細胞活動時的生理反應，此現象對於開發藥物測試方法是有幫助的。２）ＮＡＩＳＴ的細川陽一郎（Y. Hosokawa）教授利用單一飛秒脈衝雷射光分離並搬移培養中的細胞。目標細胞與雷射光焦點之間的距離會影響搬移作用；當此距離約小於２５微米時，細胞被推離焦點。而當距離大於２５微米時，細胞則被拉向焦點。所以２５微米是搬移作用的關鍵參數。

We have a plan to start new experiment relating to femtosecond laser application on biology. Our goal is development of living cell based devices for study of cytology and cell based assay system for safety test of drugs and chemicals. Although we well know the characteristics of femtosecond laser well, we need further information on laser and biology. The purpose of present visit to Japan is to obtain hints on cell based devices on point of view of femtosecond laser application. To this end, I have visited to Tohoku Fukushi University (TFU, Sendai) and Nara Institute of Science and Technology (NAIST, Ikoma). The host professors kindly presented their new informations. 1) Volume of neuron and astrocyte are changed by stimulation, which was shown by Professor H. Tsubokawa of TFU. He said the swelling of brain cells is one of the physiological responses associated with neuronal and astrocytic activation. This phenomenon will be useful to the development of cell based drug tests. 2) Single pulse of femtosecond laser can detach culturing cells and transfer them individually. The transportation of cells depends on the distance of a target cell and laser focal point. The cell is pulled from laser foal point if the focal point is near than 25 μm to the cell, while the cell is pulled in if the focal point is farther. The switching distance of pull-in and -out is 25 μm (Professor Y. Hosokawa of NAIST.)

目次

本文

一、目的

近來微陣列元件在基因體學與蛋白質體學研究上變得日益重要。除了 DNA 陣列、蛋白質陣列，使用活細胞作為微實驗室晶片上的功能性元件也成為熱門的選擇。我們團隊正投入細胞晶片這個領域，對於現有的雷射技術已十分了解，但仍需要更進一步瞭解雷射光對生物細胞的影響。我拜訪了在日本的兩位教授，並討論適合本計畫的細胞種類與方法。其中一位是服務於ＴＦＵ的神經生理學家坪川宏（Hiroshi Tsubokawa）教授，他同時指導ＴＦＵ與東北大學的學生。另一位是服務於ＮＡＩＳＴ的雷射生物物理學家細川陽一郎（Yoichroh Hosokawa）教授，在飛秒脈衝雷射光應用於細胞的研究上（特別是植物細胞），他是受到高度期許的年輕科學家。

[背景 Back ground]

近來日益受關注的微陣列元件，如 DNA 陣列、蛋白質陣列與細胞陣列，是基因體學及蛋白質體學等生物領域中重要的角色之一。從這個觀點來看，活細胞作為微實驗室晶片上的功能性元件也成為熱門的選擇。要製作一個以活細胞為基礎的微元件，細胞貼附於基材的時－空間控制與細胞網絡的引發是一個重要的課題。細胞貼附需要藉助細胞外間質蛋白（ECM），所以若在培養細胞的過程中，將 ECM 於特定時刻、特定圖樣固定至基材上，此舉可能幫助我們研究許多細胞生長過程；諸如細胞分裂、分化、遷移與凋亡。然而我們必須即時更改基材上的的 ECM 圖樣以研究以上過程。所以我們的目的是開發以活細胞建立的微元件，提供給細胞學研究與以細胞為主的化學藥物檢測系統。我們對於現有的雷射技術已十分了解，但仍需要更進一步瞭解雷射光對生物細胞的影響。本人感興趣的是以心臟與大腦組織建構於固體基材（如玻璃、石英及透明電極）上的元件，因為它們可能成為檢測藥物與化學物質的好方法。在這個元件上很容易監視藥物與化學物質的影響，對於發現新藥與毒性檢測將會有所幫助。

Recent growing interest in microarray devices such as DNA array, protein array, and cell array are one of the important roles in related biological fields such as genome and

proteome. Along the same line, use of living cells as functional elements is highly desirable for miniaturized laboratory-on-a-chip devices. To fabricate the living cell based microdevices, one of the key issues is the spatiotemporal control of cell adhesion to substrate and induction of cell-cell networks. Cell adhesion is mediated by extra cellular matrix proteins. Hence, if the extra cellular matrix proteins on a substrate are immobilized and patterned at the specific position and time during cell culture, novel opportunities may be provided for studying many cellular processes such as cell division, differentiation, migration, and cell death. However, the patterns on a substrate surface must be dynamically modified to study of such cellular processes. Our goal is development of living cell based devices for study of cytology and cell based assay system for safety test of drugs and chemicals. We well know the femtosecond laser; we need further information on laser and biology. I am interested in the devices that are constructed with part of brain and heart on a solid substrate (e.g. Glass, quartz, and transparent electrode), because they may be attractive for survey of drugs and chemicals. If it is easy to monitor the action of the drugs and chemicals, it will help the researchers on drug discovery and toxicity test of chemical.

[出差目的 Aims of the current duty-trip]

我們其中一個步驟是達成可逆的表面修飾技術，隨意控制基材表面功能性蛋白質的貼附與釋放，讓活細胞可以被置放於基材表面上。細胞的發生、分化、遷移與凋亡皆能被可逆的表面修飾技術控制。近來我們開發了以飛秒脈衝雷射將活細胞置放於玻璃基材上的新技術。此基材上塗布有疏蛋白質的兩性高分子（阻擋蛋白質附著於玻璃基材表面）可被高強度的飛秒脈衝雷射光剝離。目前我們正將此技術用於細胞培養過程中，製作玻璃基材上疏細胞的微圖案，以培養不同細胞（例如以正常細胞與癌細胞，研究癌細胞的轉移；以神經細胞與星狀細胞，模擬功能性的腦模型等）。此次出差有以下三目的：

One of our next steps is form reversible surface surface modification technique that is controllable to attach and detach functional proteins on a substrate surface on demand, which may enables living cell arrangement on a substrate surface. The cell development (発生), differentiation（分化）, migration（移動）, and death（死） will be control on the reversible surface modification technique. Recently, we developed new technique to arrange living cells on a glass substrate by femtosecond laser. The substrate covered with proteinphobic polymer having zwitterion (that inhibits protein adsorption on the glass surface) is easily ablated under physiological buffered solution (neutral pH, 25-37°C, ambient pressure) by intense femtosecond pulsed laser. We are now applying this technique to pattern cytophobic maicropatterns on the glass substrate during cell culture to form to form different cells (i.e. normal cell and cancer cells for study of cancerous cell

metastasis, neuron and astrocyte as a functional brain model, etc). There are three purpose of the current duty-trip.

1) 討論可逆性表面修飾技術的可能性。用新的高分子以塗布基材表面是選擇之一。已有微圖案的表面會被此種能夠在生理緩衝條件下覆蓋微圖案的新的高分子修飾。其中一位有名的是東京大學的高井（Madoka Takai）教授。

   Discussing possibility of the reversible surface modification method. Candidate is the new polymer to cover the substrate surface. One patterned surface will modified with the new polymer that can cover the one micropatterned area under physiological buffered condition. One of the most famous person is Professor Madoka Takai (高井まどか) in the University of Tokyo.

2) 以中樞神經、星狀細胞與微血管細胞建構的功能性腦細胞迴路之討論。坪川宏（Hiroshi Tsubokawa）教授享譽日本。他在日本仙臺的東北福祉大學（私立大學）與東北大學（國立大學）研究大腦功能。此次關於大腦功能的會議於東北福祉大學舉行，所以本人不僅與坪川 Tsubokawa 教授，還有小川誠司(Seiji Ogawa）教授（於美國貝爾實驗室發明功能性核磁共振影像，並被諾貝爾獎提名）討論。

   Discussing a development of functional brain cell-circuit that is constructed with central neuron, astrocyte, and vascular cells. Professor Hiroshi Tsubokawa（坪川宏）is well known in Japan. He is researching brain functions at Tohoku Fukushi University (Private University) and Tohoku University (National University) located in Sendai, Japan. The meeting about the brain function will be held in Tohoku Fukushi University, thus I attend the meeting and discuss with not only Professor Tsubokawa but also Professor Seiji Ogawa (小川誠司, He developed functional MRI at Bell-lab. in USA, and nominated to Nobel Prize.).

3) 了解飛秒脈衝雷射的非線性現象。本人與雷射生物物理學家 Yoichroh Hosokawa 會談。在飛秒脈衝雷射光應用於細胞的研究上（特別是植物細胞），他是受到高度期許的年輕科學家。我們之間的交流合作也在議題之中。

   Understanding non-linear phenomenon of femtosecond laser. I discuses with Professor Yoichiroh Hosokawa, who is a hope of yang generation in the interaction of femtosecond laser pulse and living cells (especially the plant cells) of Japan. Collaboration with him is also discussed during the discussion.

[預期成效 The result that is expected]

1) 討論出如何設計新的高分子以阻止細胞附著於基材表面（疏蛋白與疏細胞高

分子)。我們將決定採用何種高分子，且高井（Takai）教授可能在本人下次造訪該實驗室時，協助我們準備此種材料。

Design of new polymers to prevent cell adhesion on the substrate surface (proteinphobic and cytophobic polymer) will be cleared on the discussion. Candidate of polymer will be decided on the discussion and Professor Takai may prepare it for us at the next visit to Professor Takai’ s laboratory.

2) 坪川 Tsubokawa 教授能夠展示神經與星狀細胞在微圖案上的功能。

Functions of neuron and astrocyte on the microfabricated pattern may be shown by Professor Tsubokawa.

3) 鏊清飛秒脈衝雷射與其引發的機械力的關係。Hosokawa 教授表示他已發現雷射照射位置與物質位移的關係，此實驗結果目前尚未公開。

The relation of femtosecond laser and its mechanical force is cleared. Professor Hosokawa says he has an important experimental data about geographical condition where the laser irradiates at under water condition to move materials on the substrate, which is confidential.

二、過程

三月十五日 March 15 (Friday): Travel to Sendai

於晨間 6:30 啟程，搭乘 JAL 0802 班機前往日本，約於 18:00 抵達仙臺。原定晚間與 H. Tsubokawa 教授有一個討論，但因故取消會面。Tsubokawa 教授提供一篇相關的學術文章（請見附錄），並且透過電子郵件及行動電話來進行討論。

6:30-18:00: I departed at Toayuan international airport at10:00 and arrived at 14:00 at Narita international airport in Japan (JAL 0802). I moved from airport to Sendai by using high speed railway and arrived at around 18:00. Although I have appointment to discuss with Professor H. Tsubokawa, he called me at evening and told me that his father had died and he must prepare his funeral, thus he canceled the discussion.
He offered to me sending a related paper and discussing by e-mail and phone. I received an article named "GABAergic input contributes to activity-dependent change in cell volume in the hippocampal CA1 region" and the related papers **(附錄)**.
I stayed at hotel in Sendai today.

三月十六日 March 16 (Saturday): TFU, Sendai

於東北福祉大學參加有關腦科學的學術會議「Advanced individual preventive care system for a mental disorder」，與亀井（Kamei）教授（MRI 顯微影像專家）和小川誠司（Seiji Ogawa）教授（於貝爾實驗室發明 fMRI 上 BOLD 方法）討論活細胞核磁共振顯微術的可行性。亀井（Kamei）教授正在製作低磁場下顯微影像的原型機。

13:00 to 18:00: I attended a meeting named "Advanced individual preventive care system for a mental disorder" to search the brain science. I discussed with Professor Kamei（亀井裕孟; specialist on MRI micro-imaging) and Seiji Ogawa (小川誠二, He invented Blood Oxygenation Level Dependent (BOLD) method on fMRI at Bell Laboratories.). We discussed about ability of imaging of living cells by MRI micro-imaging. Professor Kamei is now making a prototype for micro-imaging under low magnetic field for feasibility test.

三月十八日。March 18 (Monday): NAIST, Ikoma

本人於招待所準備投稿至期刊 Lab on a Chip 的文章手稿。由於部分實驗是在 NAIST 進行，所以本人檢視了實驗設備與樣品的名稱。本人也選擇了欲轉移至交通大學的儀器設備。

10:00-20:00: I am preparing manuscript for Lab on a Chip. Since some experiments were done in NAIST, I checked the name of equipments and materials (objective, culture medium, conditions of femtosecond laser, SEM, XPS, etc). I have select instruments that I want to transfer from NAIST to NCTU
I stayed at resident room in NAIST.

三月十九 March 19 (Thursday): NAIST, Ikoma, and Funeral at Kawasaki (at night)

準備科學文章手稿。本人挑選、打包並寄送了許多文件至交通大學。本人選擇了欲轉移至交通大學的儀器設備，以在交大進行相關實驗。本人也準備了設備移轉手續。

9:30-14:00: Preparation of manuscript for Lab on a Chip. I selected, packed, and sent many documents to send NCTU. I select instruments (one cell PCR, incubator, etc) in NAIST. I have a plan to transport them to NCTU for our experiments in NCTU. I have checked the documents for for export them from JAPAN.
14:00-18:00: Movement from NAIST to Kawasaki
I attended a funeral for Professor Tubokawa's father.
I have stayed at my parents' house.

三月二十 March 20 (Wednesday): Holiday in Japan.

日本國定假日。

Today is holiday in the Vernal Equinox Day in Japan.
Translocation to Osaka.

三月二十一 March 21 (Thursday): NAIST, Ikoma

於ＮＩＡＳＴ與 Hosokawa 教授討論飛秒脈衝雷射的應用與設備移轉手續。

9:30-19:00: Discussion with Professor Hosokawa for the femtosecond laser application. I also discussed with Professor about transportation of my equipments (one cell PCR, incubator, etc) in NAIST.

三月二十二 March 22 (Friday): AIST, Ikoma

於ＮＡＩＳＴ與 Hosokawa 教授及其學生討論飛秒脈衝雷射的應用，包含應用於植物細胞上，以及脈衝雷射光引起的衝力如何可以轉移細胞。

9:30-19:00: Discussion with Professor Hosokawa and students for the femtosecond laser application, which included application on plant cells. Generation of impulse force by femtosecond laser, and its application for cell transfer was discussed.

19:00-24:00: Moving to Hotel near Haneda international airport.

三月二十三 March 23 (Saturday): travel to Hsinchu

搭乘 JAL 0802 班機，返回臺灣新竹。15:30-23:30: I departed at Haneda Tokyo international airport at17:55 and arrived at 14:00 at Narita international airport in Japan (JAL 0802).

三、心得及建議

1) 神經與星狀細胞體積變化的討論 Volume-change of neuron and astrocyte: Discussion with Professor Tsubokawa of TFU by telephone.

Tsubokawa 教授表示包含神經與星狀細胞的腦組織體積變化與神經活動有關。根據大腦切片觀察的實驗，他推測被培養的腦細胞也會在數分鐘之內改變體積，同時他也對培養中的腦細胞在藥物刺激下的體積變化感興趣。所以現在我們正在準備平面的細胞培養，藉由飛秒脈衝雷射光加工技術製作細胞晶片，使細胞呈陣列式生長，結合三維雷利散射顯微技術觀測單細胞體積的變化。以上方法將提供重要的科學證據，所以 Tsubokawa 教授已經樂意地借給本實驗室二氧化碳培養箱與桌上式潔淨操作臺，讓我們順利進行這個計畫。

He said the volume of brain tissue including neurons and astrocytes changes in relation to neuronal activity, which is one of the physiological responses associated with neuronal activation. The volume change and synaptic response is correlated. Although he has done experiments using brain slice, the cultured cells may change their volume within a few min. In his experiments, because his sample was brain slice (hippocampal from mus musculus), the neuron-astrocyte networks were ablated. Therefore he said that he is interested in the networks formed on the substrate on monitoring volume change (swelling) responding to drug simulation. Now we are preparing two dimensional (2D) culture, although three dedicational(3D) coculture is more suitable condition. There are some difficulties on conventional 3D coculture of neuron and astrocyte. Identification of neuron and astrocyte is very difficult without immunological staining that induce unknown effect on that cells. If we make patterns of neuron according to the design and covered with astrocyte layer, the neuron and astrocyte will be distinguished by their spatial locations. This can be achieved by our femtosecond laser-induced surface modification as cell array. The detection of volume change on each cells will be able to be monitored with 3D Rayleigh scattering imaging that is now developing in our laboratory. The water flow through aquapoline on the cell membrane may be possible to detect by our another method using correlation spectroscopy for micro area. Thus the information from Professor Tsubokawa is important for our study. Additionally, he kindly lent me CO_2 incubator and desk-top clean bench, that are already transferred from TFU to our laboratory in NCTU for our experiment. We want to collaborate together for monitoring the volume change on neuron-astrocyte circuit on a substrate, which was formed by femtosecond laser-based surface modification method.

2) NAIST 在近期內將飛秒脈衝雷射應用於細胞生物學研究上的進展 Recent progress in femtosecond laser application on cytology in NAIST: Discussion with Professor Hosokawa of NAIST.

*i) 利用飛秒脈衝雷射引發的脈衝力搬移細胞：*Hosokawa 教授發現細胞在脈衝力的作用下，有時候靠近雷射光焦點、有時候遠離焦點。他們探討了細胞至雷射光焦點與推拉作用的關係，並獲得了十分有趣的結果；當此距離約小於２５微米時，細胞被推離焦點。而當距離大於２５微米時，細胞則被拉向焦點。Hosokawa 教授表示此一現象可以被運動學理論解釋（由於學術論文正在準備中，細節尚無法對外公開）。

*ii) 利用飛秒脈衝雷射進行植物細胞的基因轉移：*由於植物細胞滲透壓的緣故，外來基因要進入植物細胞內是有困難的。Hosokawa 教授展示了這個研究的原型設備，不過它與本實驗室的計畫是無關的。

*iii) GIST-NAIST-NCTU 聯合研討會：*Hosokawa 教授建議我與學生一同參與這個研討會。其將於 11 月於 NAIST 舉行。

*iv) 儀器設備移轉：*由於運費十分昂貴（高達七十萬臺幣），且本實驗室已經沒有多餘的空間，我們決定只轉移小型的實驗設備，還有數種實驗用的動物細胞。

i) Cell manipulation by femtosecond laser induced impulsive force: Recently, our group has reported different direct patterning method using femtosecond laser matching biological window (700-900 nm) because of minimal damage of proteins and cells. In our method, the laser is focused in aqueous solution located in intermediate layer between laser targeted and post-patterned substrate, therefore their arrangement is fully carried out in aqueous solution. Functional proteins caged in insoluble articles and cells are released into liquid by cavitation bubbles accompanied with mechanical force generated by femtosecond laser, which were transferred to substrate, resultantly their pattern. This particle patterning was firstly developed by Professor Hosokawa. As I want to know the further results in his laboratory, I visited to him.

If the laser is focused in culture medium, the cells sometimes moves from laser focal point to further position and sometimes moved to the focal point. The reason is not cleared. His group has done a series of experiment by changing the distance of cell and focal point (Fig. 1.).

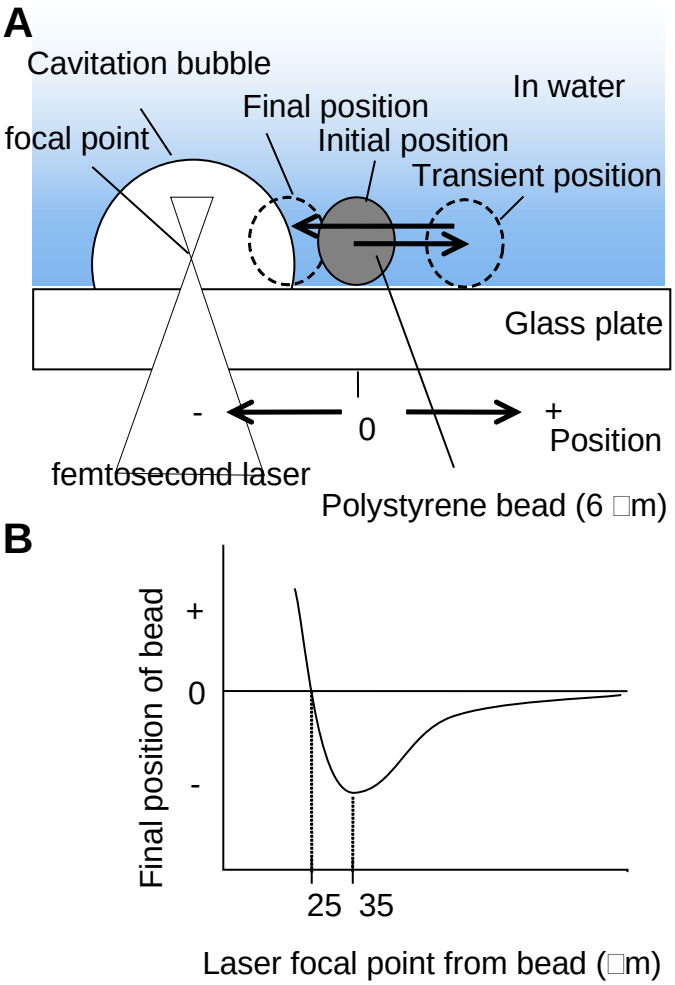

Fig.1. Micromanipulation of individual particles in water by femtosecond laser induced cavitation bubble. A) Schematics of movement of bead after the laser induced. B) Illustration of the result. Final position of the bead means the shifted position of the bead. If the bead is pushed from the focal point the final position shows plus, and if the bead is pulled toward focal point it shows minus.

If femtosecond laser pulse is focused in water, the shockwave and cavitation bubble is generated at its focal point. The cavitation bubble is quickly collapsed. If the bead (model of cell) was sit near the laser focal point, the bead is affected with force of generation and collapse of the bubble (A). The experimental data was as many as 1000 points. The result was very interesting. If the laser was focused near the cells less than 25 um, the cells were pulled from focal point. If the laser was focused farther than 25 um, the cells were pulled-in the focal point (B). Thus the force field of the cavitation bubble is local as several tens micrometers and the direction of force can be switched. He said this phenomenon is able to be explained theoretically by kinematics. (High confidentiality because of preparation of paper)

This information is very useful for our project, because the force of cavitation bubble

is as strong as detach a cell adhered on culture substrate.

ii) Gene transportation to plant cells by femtosecond laser: pressure of cytosol is higher than around in case of plant cells (osmotic pressure). It causes difficulty of gene transportation of plant cells. Although femtosecond laser easily make a hole to plant cell, this osmotic pressure inhibit the genetic transformation. His group is developing gene transfer method by laser-induced engraving the plant cells under high pressure. He shows a prototyping of the equipment. This is interesting but is not related in our study.

iii) GIST-NAIST-NCTU: Professor Hosokawa asked me to come to NAIST together with students of NCTU at GIST-NAIST-NCTU conference. The conference will be held in NAIST, November.

iv) Equipment and cells I used in NAIST: I have many equipment in NAIST. Although I have a right to be fair in transfer them from NAIST to NCTU, the transfer fee is expensive as high as 2,000,000 JPY (about 700,000 NT$). Additionally there are no space in our laboratory in NCTU. We decided we do not transfer the heavy and big equipment excepting small one that I can carry by aircraft. Equipment (big) are vacuum-chamber for oxygen sensitive chemical reaction (stainless steel, one piece), baking chamber (200°C, one piece), spinner for resist-coating on substrata (2 piece), program air-incubator for bacteria culture (2 pieces), and mechanical shaker (2 pieces). Small ones are single cell PCR (1 piece), electrophoresis set, UV illuminator, diode laser (473 nm, 1 piece), and bottle roller (1 deices). I have stored many cells at NAIST, such as normal human astrocyte, human neuronal pigment cell, normal human keratinocyte, PC12, C2C12, NIH3T3, HeLa, CHO, P19CL6,ST2. I can transfer them from NAIST to NCTU for our study. I have checked they are safely stored in liquid nitrogen or -80°C.

By the way, Prof. Tsubokawa of TFU kindly rent me a CO_2 incubator and a desktop clean bench, which were already transferred from TFU (Sendai) to NCTU.

四、附錄

以下為 Tsubokawa 教授提供之有參考價值的學術文章；

Open domain papers from Prof. Tsubokawa:

1) Sachiko Takagi, Kunihiko Obata, Hiroshi Tsubokawa, GABAergic input contributes to activity-dependent change in cell volume in the hippocampal CA1 region, Neurosci. Res. 44, 315-324 (2002)

摘要：腦細胞的增大為一種與神經活動有關的生理反應。作者分析了小鼠海馬體切片中細胞體積變化與突觸反應的交互作用。在 CA1區細胞增大的同時，近紅外光穿透率的增加與場興奮性後突觸電位也被記錄下來。實驗結果顯示流入 GABA-A 受器的氯離子對於突觸活動引起的海馬體 CA1區增大是有所貢獻的。

Abstract

Swelling of brain cells is one of the physiological responses associated with neuronal activation. To investigate underlying mechanisms, we analyzed interactions between changes in cell volume and synaptic responses in the hippocampal slices from rodents. Swelling within the CA1 area was detected as increases in transmittance of near-infrared light (IR), and field excitatory postsynaptic potentials (fEPSPs) were recorded simultaneously. High frequency stimulation (HFS) of afferent fibers induced a transient increase in IR transmittance in both somatic and dendritic regions, which was temporally associated with fEPSPs. Stimulus-induced increases in transmittance were strongly reduced in the presence of dl-2-amino-5-phosphonovaleric acid and 6-cyano-7-nitroquinoxaline-2,3-dione, indicating involvement of glutamate receptors.

Application of a GABA-A receptor antagonist, bicuculline, increased the amplitude and time course of the fEPSPs but rather decreased HFS-induced optical signals. When the extracellular Cl^- was reduced to 10.5 mM, HFS induced a decrease in transmittance, which was also blocked by bicuculline. In hippocampal slices obtained from mice deficient in the 65 kDa isoform of glutamic acid decarboxylase, HFS-induced signals were significantly smaller than in the wild-type mice, although fEPSP profiles did not differ. These results suggest that Cl^- influx through GABA-A receptors contributes to synaptically evoked swelling in the hippocampal CA1 region.

Keywords

Intrinsic optical signal; Cell swelling; High frequency stimulation; Hippocampal slice; Glutamatergic input; GABAergic input

2) R.D.Andrew, M.E. Lobinowich, E.P. Osehobe, Evidence against volume regulation by cortical brain cells during acute osmotic stress, Experimental Neurology, 143, 300-312 (1997)

摘要：神經與神經膠質細胞體面臨滲透壓力時，會於數分鐘內以被動的體積變化作為反應。本研究從海馬體組織切片來尋找支持以上單細胞現象的依據；在其 CA1區域中作者做了穿透影像、記錄了被動場電位還有組織電阻值以關聯體積的變化。作者發現在腦切片中的皮質細胞對滲透壓力並不顯示以往的體積變化，而且在施加滲透壓力的過程中，有體積變化的部分位於樹突而非細胞體。

Abstract

The cell bodies of neurons and glia examined in culture respond to severe osmotic stress (100 to 200 mOsm) by passive volume change that is followed within several minutes by volume regulation, even in the face of maintained osmotic change. However, in clinical situations, the brain does not experience such precipitous and severe changes in brain hydration. In this study we examined if there is evidence from the hippocampal slice preparation supporting the type of volume regulation observed in cultured brain cells. Within the CA1 region we imaged changes in light transmittance (LT), recorded the evoked field potential, and monitored tissue resistance (all measures of cell volume change) during the first hour of osmotic stress to search for evidence of volume regulation. During superfusion of hypo-osmotic aCSF (−40 mOsm), LT increased 24 to 28% in the dendritic regions of CA1 neurons. The LT reached a plateau which was maintained throughout a 45-min application interval, more than enough time to reveal a regulatory volume decrease. Upon return to control saline, LT immediately returned to baseline and settled there. Hypo-osmolality reversibly increased the relative tissue resistance (R_{REL}) measured across the CA1 region with a time course identical to the increase in LT. Conversely, hyperosmotic aCSF (mannitol, +40 mOsm)*decreased*both$R_{REL}$by 8% and LT by 15.5% with no indication of a regulatory volume increase. The CA1 cell body layer showed only slight hypo-osmotic swelling whereas exposure to the glutamate agonist quinolinic acid caused pronounced swelling in this region. Even when osmolality was decreased by 120 mOsm for 20 min, dendritic regions responded passively with no regulatory volume decrease. However, when aCSF $Cl^-$was substituted, the CA1 dendritic regions displayed immediate swelling followed by a dramatic volume reduction under nonosmotic conditions, indicating that such behavior can be evoked by extreme aCSF dilution. We conclude that in the brain slice preparation, the cortical cells do not exhibit classic volume regulation in response to sudden physiological changes in osmolality. Moreover it is the dendritic region, not the cell body region, that displays dynamic volume change during osmotic challenge.

3) C.E. Morris, J.A. Wang, V.S. Markn, "The invagination of excess surfce area by shrinking neurons, Biophys. J. 85, 223-235 (2003)

摘要：本研究利用共軛焦顯微術觀察培養神經元細胞在液壓改變下型態的改變，以討論當環境中的滲透壓改變時神經元細胞的調節機制。作者發現在滲透壓改變時，水流出神經元細胞表面的孔道。由此實驗他們推定神經元細胞內在滲透壓力和細胞孔道具有調節細胞形狀、形貌及功能之作用。

Abstract

Over most of their surface, neurons are surrounded by a narrow extracellular gap across which they make adhesive cell-cell contacts. Thus constrained, how do they regulate their geometry when osmotically perturbed? Specifically, are there any interesting consequences of local osmosis in such conditions? Using confocal imaging of shrinking neurons in culture, we observe water exiting into the cell-substratum gap. This water efflux generates a hydrostatic pressure that, at discrete (low adhesion) sites, causes the neuron's excess plasma membrane to invaginate, thus compensating for shrinkage with a pseudo-intracellular volume. To identify the minimal requirements of the process, a compartment/flux model was constructed. It comprises, essentially, a large liposome adhering in a labyrinthine fashion to a substratum. The model predicts that invaginations form at the cell-substratum interface under the influence of local osmosis, provided that adhesion across the gap is neither too tight nor too loose. Local osmosis in the central nervous system, in contrast to epithelia, is usually considered a mishap, not a physiological opportunity. We postulate, however, that local osmotic forces acting in conjunction with confined extracellular spaces could be harnessed in service of surface area, shape, and volume regulation when intense neural activity alters a neuron's osmotic balance.