行政院國家科學委員會補助專題研究計畫 ☑成果報告 □期中進度報告

探討早年聽覺經驗對於中腦聽神經元突觸發育之影響

與其分子調控機轉

計畫類別：☑個別型計畫　□整合型計畫
計畫類別：☑ 個別型計畫　□ 整合型計畫
計畫編號：NSC　98-2320-B-009-001-MY2
執行期間：　2009 年 02 月 01 日至　2010 年 12　月 31 日

執行機構及系所：交通大學生物科技系
計畫主持人：曲在雯
共同主持人：

成果報告類型(依經費核定清單規定繳交)：□精簡報告 ☑完整報告

本計畫除繳交成果報告外，另須繳交以下出國心得報告：
□赴國外出差或研習心得報告
□赴大陸地區出差或研習心得報告
☑出席國際學術會議心得報告
□國際合作研究計畫國外研究報告

處理方式：除列管計畫及下列情形者外，得立即公開查詢
□涉及專利或其他智慧財產權，□一年□二年後可公開查詢

中　華　民　國　100　年　08 月 31 日

中文摘要

大腦從出生後仍具有可塑性。例如，聽覺皮層與中腦聽神經元之反應特性會因早年的聽覺環境而改變，而且其神經反應特性改變的結果會因受到環境聲音種類與開始接受此種聲音刺激的時間而有所不同。在文獻中已知以種因細胞活性改變而引起的神經可塑性，不僅會表現在反應特性上也會呈現在神經元的型態與神經迴路連結上。關於此種活性誘發的神經型態與迴路變化的假說有兩種，一種室認為活性會調控新突觸的形成，第二種則是認為活性會影響已形成的細胞突觸減少的速度或是其穩定性。在聽覺系統的研究中，不論是在動物實驗或是離體的單細胞或組織培養的實驗中均證實，若減少訊號傳遞而造成細胞活化機會減低的狀況下，其樹突的密度會降低，突觸的形態結構也會改變。至於，是否當增加聲音訊號輸入時，是否能引起神經元樹突密度，分布範圍，與突觸形態上有所改變則仍不清楚。因此本實驗的目的是要探討早年聽覺經驗除了可造成過去結果已知的中腦聽神經元反應特性改變外，是否亦會改變神聽元的型態結構，樹突密度與分部範圍，以及突觸的結構等。而此一神經形態上的改變是否只侷限在訊號輸入強度改變的區域(isofrequency laminar) 具有活性的專一性；或是此一形態改變的範圍亦會呈現在其他的區域 (laminae beyond the isofrequency laminar)。最後更要探討 MAP2 與 GAP-43 是否亦參與此一早年聽覺經驗所誘發之神經形態之調控機制中。

實驗組的幼鼠從出生後的第二週到第五週將接受連續四週，每週七天，每天十小時(22:00-8:00)的純音(4 kHz, 65 dB SPL)刺激。控制組的幼鼠飼養在相同的環境下但是無純音的刺激。幼鼠在聲音刺激結束後，將以單細胞外記錄法確定單一神經元的反應特性，在最佳反應頻率為 4 kHz 旁以 juxtacellularly iontophoresis 的方式注射 neurobiotin 於該單一聽神經元旁。在最後一個細胞注射的八小時後，將動物再度麻醉經全身灌流福馬林固定腦組織，再經脫水，切片及標準的染色步驟後，將在顯微鏡下檢查與評估神聽元形態變化情形。

實驗結果初步發現，再經過連續四週的聲音刺激後，中腦 4kHz 的聽神經元的突觸數目有明顯的增加，其樹突橫跨的範圍亦有增加的趨勢。電生理的實驗結果發現在經過幼年期的長期聲音刺激後，聽神經元的反應特性會變得較為複雜，且有較高比例的聽神經原僅對複雜聲反應。實驗結果初步證明，幼年期的長時間聲音刺激，不僅會造成聽神經元的反應特性改變，聽神經元的細胞型態亦會因為細胞活性的長期改變而在樹突的橫跨範圍與突觸數目上有所改變。

關鍵詞:

神經可塑性、聽覺中腦、樹突、突觸、長期純音刺激

Abstract:

The brain is till plastic after birth. Previous studies showed that characteristics of the central auditory neurons, particularly the cortical and collicular neurons can be altered after the neonatal sound exposure. Furthermore, the above function changes are highly dependent on the type of stimuli and the onset of the sound exposure. Activities driven neural changes are not showed in terms of the functions but also reflected on neural structures. In the auditory system, both the in vivo and in vitro experiments showed that the synaptic density and morphology are decreased and altered by reducing the input activities. Whether the increased activities, particularly during the neonatal stages, have similar effects on altering the neural structures is still undetermined. Furthermore, if the experience driven plastic changes on the neural structures are restrict on the local areas with the input specificity or globally revealed in the whole relays also remains unclearly. The aim of the study in the first year is to determine whether the neonatal sound exposure have effects on remodeling

the dendritic or axonal morphology of the midbrain.

The experimental animals were exposed to a mild sound with frequency and intensity at 4 kHz and 65 dB SPL from week-2 to week-5. Control animals were raised in the same environment without sound exposure. After the end of the sound exposure, animals were first characterized by using extracellular single unit recording technique following the end of the sound exposure. Then, neurons with best frequencies at 4 kHzwere were labeled by juxtacellularly injected with neurobiotin. Eight hours after final injection, the animals will be first anesthetized and perfused with the normal saline, followed by 4% papraformaldehye and standard staining procedures.

In comparison with the control group, neurons around 4 kHz iso-frequency laminae were with a larger dendritic field after neonatal sound exposure. In addition, these neurons were showed an increased complexity in dendritic morphology, specifically an increase in the density of dendritic spines following early sound exposure. The electro-physiological findings revealed that the response characteristics of 4-kHz were complex after long-term neonatal sound exposure. The results suggested that the increased activities, which are driven by neonatal sound exposure, not only changed neurons' responsiveness but also altered the neurons' morphology.

Keywords:
Neural plasticity, auditory midbrain, dendritic spine, sound exposure

Background:

The neural circuitries in the brain are not fixed after the birth. Though the experience dependent neural plasticity can be observed from early neonatal to adult, the plasticity is still most rapid and robust during the neonatal stage.The experience-driven neural plasticity has been studied in the auditory system especially at the level of the auditory cortical area. For example, the tonotopic maps of the auditory cortex is replaced by retinotopic maps in the congenital deaf human subjects or animals with neonatal ablation of the cochleae (Kujala and Näätänen, 2000; Kral, 2007). Experiments also demonstrate the size of the auditory map is highly regulated by input activities. For example, the size of the auditory maps is significantly reduced after reducing or eliminating the sound inputs by sound deprivation procedures whereas an expanded auditory maps were revealed in functional images of the musicians, which characterized by functional magnetic resonance imaging (fMRI) techniques (Pantev et al., 2003). Recently, a series of studies from Merzenich's group suggested that early tone exposure produced functional changes in the auditory cortex. Specifically, they found an accelerated emergence and expansion of isofrequency areas representing the priming tone, and a delayed development in the overall tonotopicity and the appearance of broader-than normal receptive fields (Zhang et al., 2001, 2002). Zhou et al. (2008) also reported the plastic changes in the auditory cortex also dependent on the temporal structure of the input signals. Specifically, the over-representation of the priming frequency in cortical map was revealed in rats exposed to a pure tone bursts whereas the distorted cortical map with the large-than-normal areas tuned to high frequencies and the smaller-than-normal areas tuned to low frequencies was observed in those rats exposed to a continuous pure tone. However, the above results still could not rule out the possibilities that changes of the cortical organization were partially reflected what already altered at the lower auditory relays. The neonatal sound exposure altered tuning characteristics of auditory neurons have been shown at the midbrain level. For instance, comparing with the naïve control rats,

the number of neurons with best frequency (BF) around the priming frequency was significantly increased after exposing to a continuous pure tone during the first month after birth (Poon and Chen, 1992).

In the auditory system, both the sensory deprivation by removing the cochlea and the increased activities by directly stimulating the input fibers or increased the sound stimulation in the environment showed that activities would modify the number of dendritic spines. For example, the cochlear removal in adult or young rats reduced the density of dendritic profiles of the medial superior olivary nucleus (MSO, Russell and Moore, 1999). The similar reduced activities induced changes on the dendrites also observed in the auditory cortex. The spine counts revealed that neonatally deafened rabbits had 38.7% fewer spines along their basal dendrites but no differences between experimental and control rabbits were found in terms of soma cross-sectional area, total number of basal dendrites, total number of dendritic branches and total basal dendritic length (McMullen and Glaser, 1988). On the other hand, the combined methods using the single-cell electroporation, live cell imaging, in vitro deafferentation, pharmacology, and electrophysiological stimulation showed that local alterations in synaptic input would affect dendritic branch structure in Nucleus Laminaris (NL). They suggested that balanced activation of inputs of NL dendrites is required for maintaining the relative amount of dendritic surface area allotted to each input (Sorensen and Rubel, 2006). However, the above results were either from experiments of sound deprivation or manipulating the input activities in acute preparations. It is still unclear that the over-activities can also alter the neuronal morphology especially on the dendritic field and the number of dendritic spines. Therefore, the first aim of this project is to determine whether the sound exposure not only alter the neural responses but also remodel the dendritic or axonal morphology of the midbrain.

Materials and Methods

Animals: The experimental animals were exposed to tone for 4 weeks from week-2 to -5 in the sound-treated chamber. The control animals were raised in the same sound-treated chamber but without the exposing tone.

Sound exposures:

All the experimental animals will be exposed to a 4 kHz continuous tone on a half-day schedule during the night (22:00 to 8:00 hr). The 4 kHz will be chosen since it is located in the low frequency region of the rat audiogram (Kelly and Mastron, 1977). In previous studies on rats, tones with such spectral property showed to be effective in producing a clustering effect on tuning characteristics (Poon and Chen, 1992). Sound was presented to animals through a free-field speaker (Pioneer SP77) placed at the ceiling of the sound-treated chamber. A moderate intensity level of 65 dB SPL was chosen and confirmed by the measurement with a calibration microphone (B&K 4149) placed at the position of animals. During the week following after the termination of sound exposure, rats were studied in terms of the extracellular single unit recording combined with juxtacellular neurobiotin injection.

Surgical procedures:

Rats were first injected with atropine (0.05 mg/kg SC) at 15 min before anesthetized with Urethane (Sigma, 0.5 g/kg, i.p.). Under anesthesia, rats were mounted onto a head holder. For the recording in the IC, the skull overlaying on one side of occipital lobe will be first surgically exposed and drilled opened. To access the midbrain, the dura will be resected. A small stainless steel post will cemented onto frontal skull for subsequent fixation to a special head holder. Rectal temperature was controlled at 38±0.5℃ by a thermal pad.

Electrophysiology

Free-field acoustic stimuli will be delivered to the animal inside a sound-treated chamber (1.5x1.5x2.5 m3) through a speaker (Pioneer SP-77) placed 70 cm in the horizontal plane, 30 degree in azimuth, contralateral to the IC being studied electro-physiologically. Unit responses were first detected using glass micropipettes of fine tips filled with a 2% neurobiotin in 1.0 M potassium chloride. The tip of the glass electrode was placed on the surface of the exposed occipital lobe and it was advanced by a remote controlled stepping micro-drive (Narishige). The neural signal will be first recorded with a pre-amplifier (A-M 1600), and then filtered (300Hz-3 kHz), amplified and monitored on-line using an oscilloscope and an audio system. Spike signals will be conditioned with a level discriminator into 0.5 msec wide rectangular pulses and their times of occurrence will be stored into a computer (HP Pavilion) for offline analysis using a computer interface (Tucker Davis Technology, system III, RZ5). Auditory units will be either identified by their spontaneous activities or time-locked responses to an acoustic click (0.1 msec pulse, 90 dB SPL) usually with latencies between 7-20 msec (details see Poon et al., 1992). After the identification and isolation of an auditory unit, frequency responses to a steady tone were determined audio-visually for their best frequencies (BF) and minimum threshold (MT, Chiu and Poon, 2000). Then, their tuning characteristics were further studied in terms of response area (RA, Chiu et al., 1998).

Injection of tracers

Neurobiotin: Once a unit with specific BF (4 kHz) is identified and characterized, an iontophoretic injection of neurobiotin was placed (2 μA positive pulsed current, 7 s on, 7 s off, for 2 min, Miller et al., 2005) at that location.

Histology

Once the electrophysiological recording and neurobiotin injections were completed, animals were given a lethal dose of urethane (Sigma) and perfused with 0.9% saline followed by 4% paraformaldehyde. Brains were removed from the scalp and rinsed in 30% sucrose in 0.1 M phosphate buffered saline solution (PBS) overnight, and the next day 40 μm sections will be cut on a freezing microtome and placed into 0.1 M PBS for histology. Tissue will be soaked in 0.3% Triton X-100 in 0.1 M PBS for 30 min followed by three PBS rinses. Tissue will be then soaked in an avidin/biotin solution (ABC, Vector Laboratories, Burlingame, CA, USA) for 1.5 h followed by one rinse in 0.1 M PBS and two rinses in 0.1 M phosphate buffer. To visualize the neurobiotin injections, sections will be reacted with a 0.05% 3,3 diaminobenzidine tetrahydrochloride solution (Sigma, with 0.003% hydrogen peroxide in 0.1 M phosphate buffer). Sections will be mounted on chrome-alum slides, air-dried, dehydrated in ethanol and xylene, and then coverslipped for subsequent microscopic analysis.

Data analysis

All sections will be analyzed using light microscopy. Digital photomicrographs will be taken using a CoolSnap digital camera mounted on a Nikon microscope (Nikon E400). Pictures will be digitally adjusted for color, brightness, or contrast at the time that the photograph was taken, but no further digital adjustments will be made to the photograph of the tissue. High magnification (100X) composite images were created by compiling photographic stacks using Image Pro Plus software (Media Cybernetics, Silver Spring, MD, USA). Locations of labeled cells and fibers were manually digitized (SummaSketch III, Xu et al., 1990) and reconstructed with software specially developed to visualize their 3D patterns of distribution.

Results and Discussions:

The neonatal sound exposure altered the BF-MT distribution by over-represented units tuned to the priming frequency (Fig. 1). In addition, the neurons' tuning selectivity and response properties were changed after 4 weeks neonatal sound exposure. Specifically, frequency tunings became broadened at regions around the priming frequency (mean Q10 dropped, $p<0.05$, Student's t-test; Fig. 2B). The effects of sound exposure further extended on the higher frequency regions ($p<0.05$; Student's t-test 2C). Furthermore, most units with BF around the priming frequency showed complex response area and responded selectively to frequency modulated (FM) tones with carrier frequency around the priming frequency.

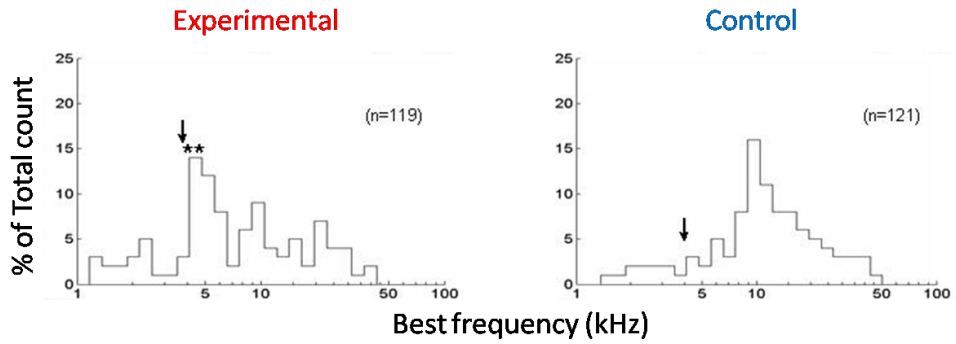

Figure 1: BF histograms of IC neurons that recorded from rats following exposure to a 4 kHz pure tone during the week-2 to -5 and their corresponding control groups. Note the prominent peak in the histogram around 4 kHz (arrows) was found in the experimental animals (**: $p< 0.01$, Chi-square test).

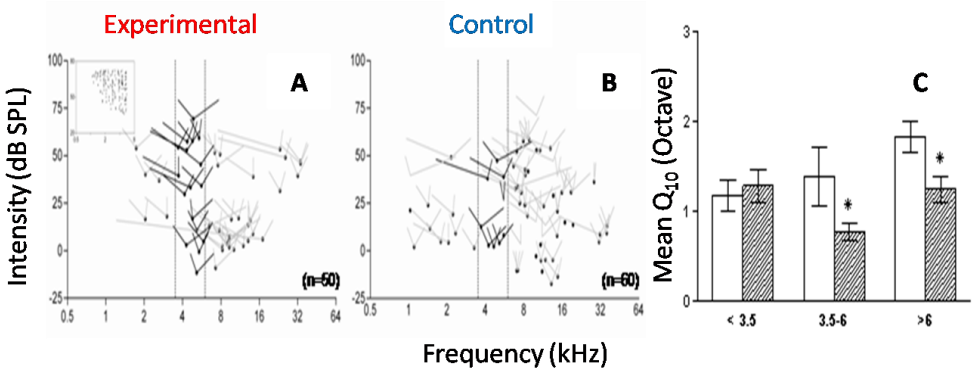

Figure 2: Effects of sound exposures on the frequency tuning of IC units. (A, B): graphic representation of the frequency tuning of individual units in the exposed and control groups: each 'V-shape' symbol is the approximation of the tip of tuning curve (details see Materials and methods: data analyses). Vertical dashed lines mark 3.5 and 6 kHz, or the gross frequency bounds of the exposing tone region; tuning curves of units within this region is highlighted in thick lines. (C): bandwidths of frequency tuning as expressed in Q10 for IC units in the control (open-bars) and exposed group (shaded-bars) according to different frequency regions. Note the broadening of frequency tuning in experimental groups. Insets: representative dot raster of a single unit with BF between 3.5 and 6 kHz.

The effects of sound exposure on cortical responses were also characterized by ECoG recording. The results showed that tone evoked gamma activities by around the priming tone were significantly higher than other frequencies (Figs 5 and 6). Furthermore, the gamma activities evoked by 9-kHz, 65 dB SPL tone pips significantly decreased (Fig 6). The results suggested that the neural activities around the priming frequency was enhanced while those activities higher the priming frequency was diminished. These results were consistent with those results showing the reorganized cortical maps and response characteristics by early

sound exposure (Zheng et al. 2001). In addition, such changes were similar to those found in the IC (Fig. 7).

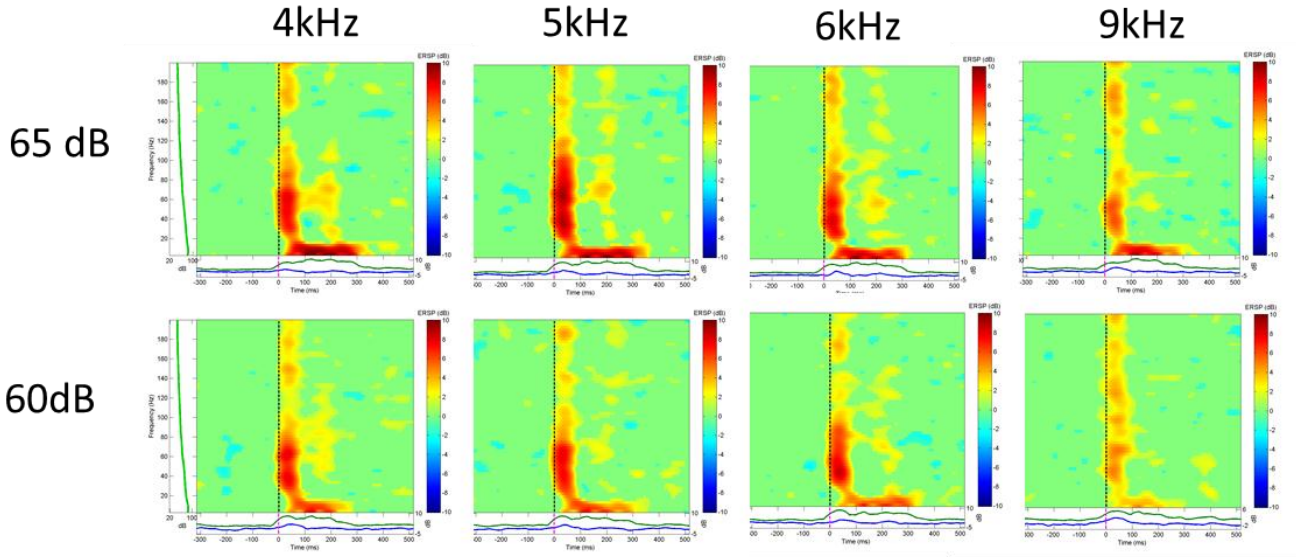

Figure 3: Sound-induced gamma responses showing Gamma band activity (up to 200 Hz) recorded at the auditory cortex of an anesthetized rat to 4, 5, 6 and 9 kHz tone burst (onset at vertical line) at intensity levels of 65 and 60 dB SPL with a single silver wire (ERSP: event related spectral perturbation, ITC: inter trial phase coherence and the corresponding AEP).

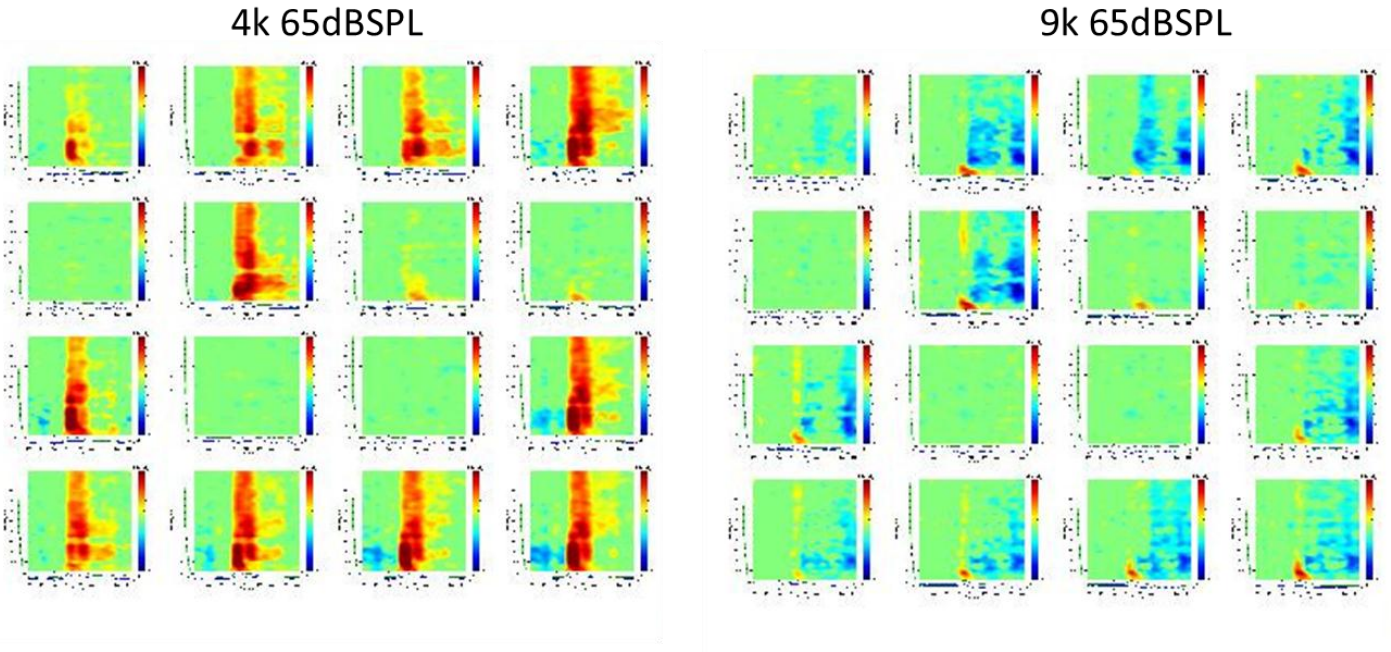

Figure 4: Sound-induced gamma responses showing Gamma band activity (up to 200 Hz) recorded at the auditory cortex of an anesthetized rat to 4 and 9 kHz tone burst (onset at vertical line) at intensity level of 65 dB SPL by a16-ch electrode array (ERSP: event related spectral perturbation, ITC: inter trial phase coherence and the corresponding AEP).

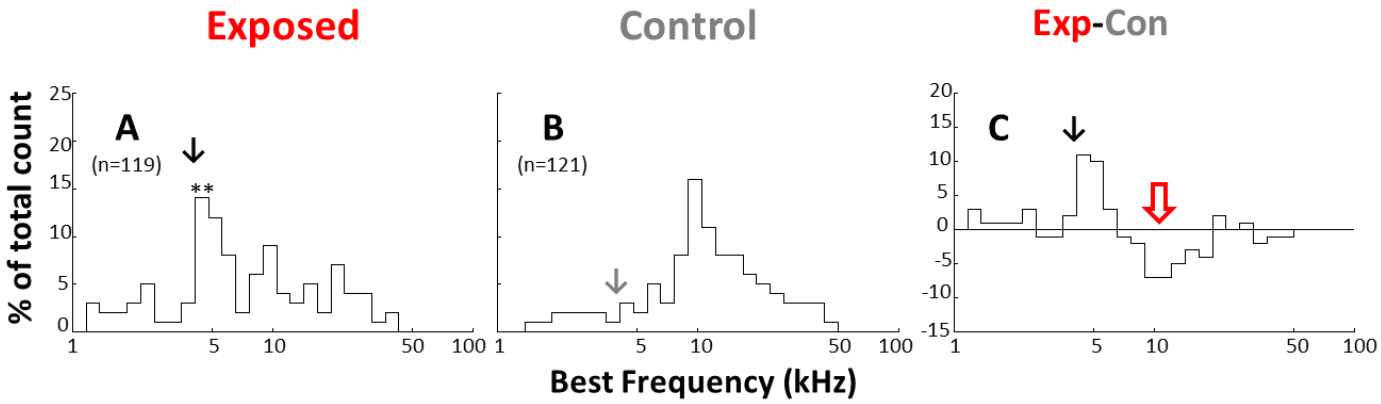

Figure 5: BF histograms of IC neurons that recorded from rats following exposure to a 4 kHz pure tone during the week-2 to -5 (A), its corresponding control group (B) and the difference histogram between the experimental and control groups. Note the prominent peak in the histogram around 4 kHz (black arrows) was found in the experimental animals (**: $p< 0.01$, Chi-square test) and the prominent valley around 9 kHz represented in the difference histogram.

Since the survival time might affect the results of staining, we first tested the effects of survival time on neurobiotin staring by sacrificing the animal at 30 min, 2 hrs, 4 hrs, 6hrs and 12 hrs after the neurobiotin injection. The results suggested that the best survival time is at 6 hrs after the neurobiotin injection. Therefore, the animals were sacrificed at 6 hrs after the neurobiotin injection.

The over-activities driven plastic changes also revealed on the neurons' morphology. Neurons were showed an increased complexity in dendritic morphology, specifically an increase in the density of dendritic spines following early sound exposure (Fig. 3, 4). Furthermore, Neurons around 4 kHz iso-frequency laminae were with a larger dendritic field after neonatal sound exposure (Fig. 5).

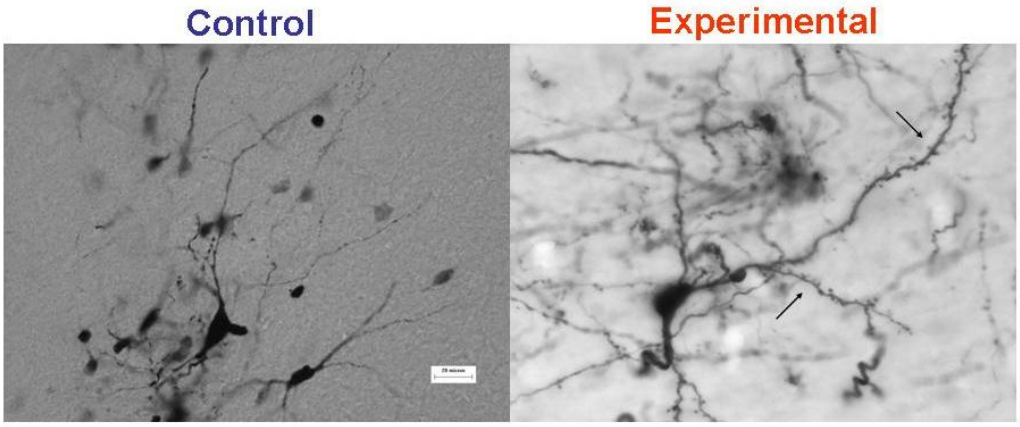

Figure 6: Increased dendritic spines shown in frozen section (40 μm) stained with neurobiotin.

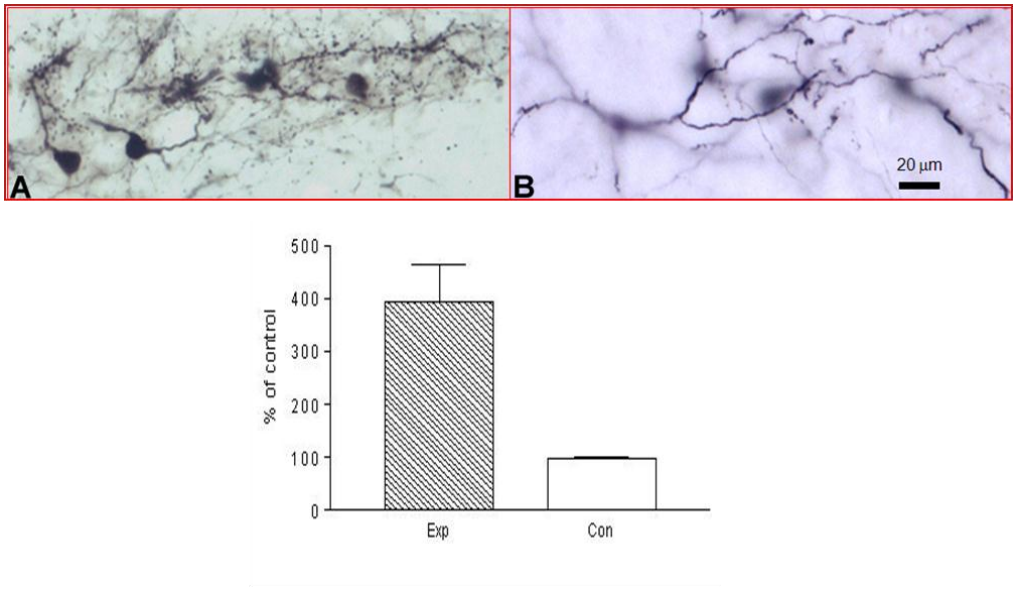

Figure 7: High power computer-enhanced digital photomicrographs showed labeling of boutons along the 4 kHz isofrequency laminar of the neurobiotin labeled region in the sound exposed (A) and control rats (B). The dendritic spines' density (number of spines per unit length) showed increment after week-2 sound exposure (C).

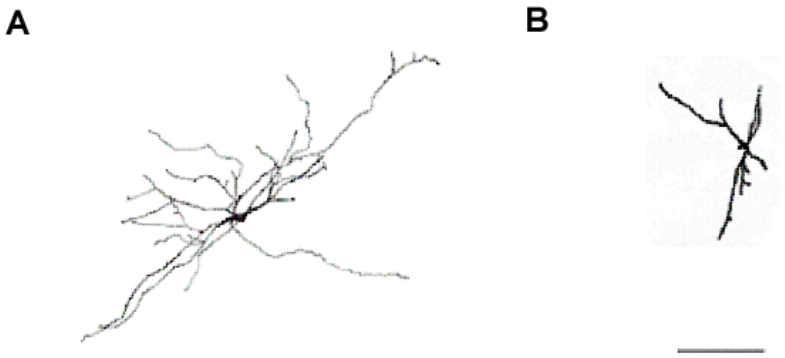

Figure 5: Neurobiotin-labeled cells at the 4 kHz laminae of experimental IC showing larger dendritic field (scale bar=125 µm).

The BF-MT distribution showed the number of neurons with BF at priming tone was increased following neonatal sound exposure is consistent with previous finding (Poon and Chen, 1992). Our results further revealed that effects of sound exposure on changing the tuning characteristics and complex sensitive. In addition, we found more 4kHz positive cells were identified from the external IC in comparison with the control animals. We speculate that the increased dendritic spines and dendritic field probably related to neonatal sound exposure increased the FM sensitivities of the midbrain neurons, which had demonstrated are with larger dendritic fields, more dendritic branching and more dendritic spines than the mixed FM or insensitive cells (Poon et al., 1992).

In this study, the MAP2 staining was not successful. The results of staining were not very convinced and this might relate to the choice of antibody or the procedures of the tissue preparation we used were not adequate for the MAP2 staining. We still need some time to fix all the problems and hope we can finished this

part of the experiment within one year.

References

1 Chiu TW, Liu YD and Poon PW. Transient frequency and intensity sensitivities of central auditory neurons determined with sweep tone. Chin J Physiol 41:133-8. 1998.

2 Chiu TW and Poon PW. Basic response determinants of single neurons to amplitude modulation in the auditory midbrain. Exp Brain Res 134: 237-45, 2000.

3 Kelly JB and Masterton B. Auditory sensitivity of the albino rat. J Comp Physiol Psychol 91: 930-6, 1977.

4 Kral A. Unimodal and cross-modal plasticity in the 'deaf' auditory cortex. Int J Audiol 46: 479-93, 2007.

5 Kujala T, Alho K and Näätänen R. Cross-modal reorganization of human cortical functions. Trends Neurosci 23: 115-20, 2000.

6 Miller KE, Casseday JH and Covey E. Relation between intrinsic connections and isofrequency contours in the inferior colliculus of the big brown bat, Eptesicus fuscus. Neuroscience 136: 895-905, 2005.

7 McMullen NT and Glaser EM. Auditory cortical responses to neonatal deafening: pyramidal neuron spine loss without changes in growth or orientation. Exp Brain Res 72: 195-200, 1988.

8 Pantev C, Dinnesen A, Ross B, Wollbrink A and Knief A. Dynamics of auditory plasticity after cochlear implantation: a longitudinal study. Cereb Cortex 16: 31-6, 2006.

9 Poon PW and Chen X. Postnatal exposure to tones alters the tuning characteristics of inferior collicular neurons in the rat. Brain Res 585: 391-4, 1992.

10 Poon PW, Chen X and Cheung YM. Differences in FM response correlate with morphology of neurons in the rat inferior colliculus. Exp Brain Res 91: 94-104, 1992.

11 Russell FA and Moore DR. Effects of unilateral cochlear removal on dendrites in the gerbil medial superior olivary nucleus. Eur J Neurosci. 11: 1379-90, 1999.

12 Sorensen SA and Rubel EW. The level and integrity of synaptic input regulates dendrite structure. J Neurosci 26:1539-50, 2006.

13 Xu JF, Poon PWF, Chen XY and Chung SN. Computer-based three dimensional reconstructions of nuclear subdivisions in the inferior colliculus. Comput Engin Applicat 6: 7-10, 1990.

14 Zhang LI, Bao S and Merzenich MM. Persistent and specific influences of early acoustic environments on primary auditory cortex. Nat Neurosci 4: 1123-30, 2001.

15 Zhou X, Nagarajan N, Mossop BJ and Merzenich MM. Influences of un-modulated acoustic inputs on functional maturation and critical-period plasticity of the primary auditory cortex. Neurosci 154: 390-6, 2008.

附件二

國科會補助專題研究計畫成果報告自評表

請就研究內容與原計畫相符程度、達成預期目標情況、研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性）、是否適合在學術期刊發表或申請專利、主要發現或其他有關價值等，作一綜合評估。

1. 請就研究內容與原計畫相符程度、達成預期目標情況作一綜合評估

☐ 達成目標

☑未達成目標（請說明，以 100 字為限）

☐ 實驗失敗

☐ 因故實驗中斷

☐ 其他原因

說明：此計畫內容分量應為三年的研究，以兩年的時間要執行完所有內容實屬困難，且因為新設實驗室，在儀器設備的建置與參與學生訓練上需花費較多時間。在探討相關機制的實驗目前遭遇到的一些問題，仍需要一段時間解決。

2. 研究成果在學術期刊發表或申請專利等情形：

論文：☐已發表 ☐未發表之文稿☑撰寫中 ☐無

專利：☐已獲得 ☐申請中 ☐無

技轉：☐已技轉 ☐洽談中 ☐無

其他：（以 100 字為限）

3. 請依學術成就、技術創新、社會影響等方面，評估研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性）（以 500 字為限）

此一研究成果證明出生後聽覺發育初期外在環境對於聽覺生經系統不論在聽覺中腦的功能與型態上皆有影響，此一結果顯示過去看到聽覺皮層的受到長期聲音刺激改變的結果可能部分只是反應聽覺皮層以下的神經系統變化，此外已可以推測過去在聽覺皮層除神經功能可受外在環境生改變外，其神經型態亦可能會受到改變，但其改變方式是否與中腦相同則須進一步的實驗證明，此外，早年下行聽覺神經迴路對於中腦的可塑性的影響與中腦變化對聽覺皮層的可塑性的影響程度也需要未來進一步實驗的探討。透過此一實驗可以發現幼年期的外在聲音刺激對於聽覺神經系統的影響十分重大，此一結果不僅可能發生在人類身上，人類幼年期的語言環境對於語言學習成果所扮演的角色也必須重視。此外此部分至少可以有兩篇相關論文發表，目前在在著手撰寫第一部分的實驗成果論文。

附件三

國科會補助計畫衍生研發成果推廣資料表

日期：___年__月__日

<table>
<tr><td>國科會補助計畫</td><td colspan="3">計畫名稱：
計畫主持人：
計畫編號：　　領域：</td></tr>
<tr><td rowspan="2">研發成果名稱</td><td colspan="3">（中文）</td></tr>
<tr><td colspan="3">（英文）</td></tr>
<tr><td>成果歸屬機構</td><td></td><td>發明人
（創作人）</td><td></td></tr>
<tr><td rowspan="2">技術說明</td><td colspan="3">（中文）

（200-500 字）</td></tr>
<tr><td colspan="3">（英文）</td></tr>
<tr><td>產業別</td><td colspan="3"></td></tr>
<tr><td>技術/產品應用範圍</td><td colspan="3"></td></tr>
<tr><td>技術移轉可行性及預期效益</td><td colspan="3"></td></tr>
</table>

註：**本項研發成果若尚未申請專利，請勿揭露可申請專利之主要內容。**

附件四

出席國際學術會議心得報告

計畫編號	98-2320-B-009-001-MY2
計畫名稱	探討早年聽覺經驗對於中腦聽神經元突觸發育之影響與其分子調控機轉
出國人員姓名 服務機關及職稱	曲在雯 國立交通大學 生物科技學系 助理教授
會議時間地點	2010 年 6 月 6 日 ~10 日 西班牙巴塞隆納
會議名稱	16th Annual Meeting of the Organization for Human Brain Mapping HBM2010
發表論文題目	Gamma band representation of amplitude and frequency modulation in Heschl's gyrus

一、參加會議經過

2006 年第一次參加此會議，並發表海報論文，與參與會議者的討論過程中得到相當多的建議,，對於後來的研究有相當大的幫助。最近，本人與合作團隊即將將研究結果整理與投稿，在定稿前，我們決定先將部份內容投到此次會議中發表，搜集其他相同領域的研究學者的意見，供做後面論文內容討論的參考。

二、與會心得

在此次會議中，發現 ICE 相關的研究愈來愈受到重視，對於本研究團隊是一項鼓舞。本研究團隊，在論文發表過程中也得到不少肯定。此外，也學到不少 fMRI 的相關研究資訊,包含實驗設計,資料分析等最新訊息,對於日後本團隊進一步設計結合 ICE 與 fMRI 的研究有很大的幫助。

三、建議

在此次會議中，發現台灣的參與者很少，發表口頭論文報告者僅有本人的研究團隊。反觀中國大陸，日本與韓國均有多篇論文發表，台灣的相關研究領域團隊需要更積極的將研究成果於口頭論文中發表，一方面可以得到更多的意見回饋此外也能提升台灣研究程在國際會議中的重要性。此外，應該鼓勵更多的研究生與年輕學者及臨床醫師參加此一國際會議，不僅能提升台灣在神經科學的研究深度，更能增加國際合作的可能性。

四、攜回資料

1. 大會手冊 (包括議程與論文摘要)。

(Poster number) 1072

Gamma band representation of amplitude and frequency modulation in Heschl's gyrus

Paul Wai-Fung Poon[1,2], Tzai-Wen Chiu[3], John F. Brugge[2], Hiroyuki Oya[2], Richard A. Reale[2], Hiroto Kawasaki[2], Kirill Nourski[2] , Haiming Chen[2] and Matthew A. Howard III[2]
[1]Dept of Physiology, National Cheng Kung University, Tainan, Taiwan; [2]Dept of Neurosurgery, University of Iowa, Iowa City, IA, USA; [3] Dept of Biological Science and Technology, National Chiao Tung University, Hsinchu, Taiwan

Introduction

Amplitude modulation (AM) and frequency modulation (FM) are important building blocks of speech signals. These sounds are processed by multiple auditory fields in the temporal lobe. Core auditory cortex in human is generally accepted as lying on the supratemporal plane occupying the posteromedial half of the Heschl's gyrus (HG). Surrounding the core is a belt of auditory cortex with a distinct functional organization. How AM and FM sounds are represented in these fields is not well understood.

Neural activity in the gamma band (70-180 Hz) may reflect neural processing of AM and FM signals. Based on intra-cortical recordings in neurosurgical patients, gamma band activity associated with repetitive transients and speech utterances [2,3,6] has been shown to be differentially represented between auditory core and the auditory belt on HG. To understand better the representation of temporally modulated sounds, we carried out a series of experiments on patients that had multi-contact electrodes chronically implanted in HG and perisylvian cortex as part of their surgical treatment plan. Protocols were approved by the University of Iowa Institutional Review Board.

Methods

Electrodes implanted in HG were clinical depth electrodes carrying 4 circumferential, 0.5 mm, macro-contacts separated by 10 mm, and modified with 14 additional micro-contacts (40 μm) arrayed along the electrode shaft. Following implantation, anatomical localization of recording sites was aided by high-resolution CT, structural MRI and intraoperative photography.

Sounds were AM or FM signals delivered through calibrated insert earphones which were integrated into custom-fitted ear molds. Sound intensity levels were comfortable to patients at about 50 dB suprathreshold. Patients listened passively in each 5-min session to 100 repetitions of the sounds.

AM sounds consisted of linear modulation ramps (40-150 Hz in 5 Hz steps, carrier: noise or a 2 kHz tone, see [1]). FM sounds were triangular FM ramps, ranging in frequency from 500 Hz to 2 kHz, with linear rising and falling phases. The ramps were of 3 different slopes (2.5, 4 and 10 Hz). A 500 Hz sine wave frequency modulated by a white noise low-pass filtered at 60 Hz created a 'random

FM' background, ranging from 250 Hz to 750 Hz, from which the linear FM ramps arose. Maintaining a constant random FM background avoided the onset transient that would have occurred against a background of silence. Click trains (5 clicks, 100 Hz) were used to help identify auditory fields on HG.

Data acquisition was performed in electrophysiological recording suites in the University of Iowa General Clinical Research Center. Subjects were awake and sitting comfortably during experiment. Electrocorticogram (ECoG) was acquired simultaneously from all electrodes at a sampling rate of 2034.5 Hz and with a bandpass of 1.6-1000 Hz using a TDT RX5 or RZ2 processor (Tucker-Davis Technologies, Alachua, FL, USA). Patients were studied over a period of two weeks.

Neural activity in the ECoG was analyzed in the time-frequency domain by computing, on a trial-by-trial basis, event-related the spectral perturbation (ERSP), which expresses changes in gamma activity relative to the prestimulus level (>95% confidence level) as a function of time. ERSPs were computed using programs adopted from an open source MATLAB tool box (EEGLAB version-7, http://sccn.ucsd.edu/eeglab/; [5]). ERSP is similar to event-related band power (ERBP) that shows changes in total power as a function of time [3,4,6].

Results

While there was inter-subject variability in the strength and locations of active cortical sites, the data shown for three subjects are consistent with those obtained in seven other subjects. AM sounds produced a strong gamma response centered in posteromedial HG, over the full range of modulation frequencies used. Gamma activity could be time locked to the modulation envelope, especially at low modulation frequency. AM-related gamma activity was relatively weak and of long latency in anterolateral HG, as compared with more posteromedial cortex. Click trains also produced strong gamma responses that overlapped those produced by AM. The distribution of cortical sites strongly responsive to AM and to click trains is consistent with earlier findings using click-trains of comparable repetition rates [2,3]. In contrast to AM and click responses, gamma-band responses to FM sounds were relatively weak in posteromedial HG, but were robust in anterolateral HG. The area of maximal response to FM did not overlap those responses on posteromedial HG to AM signals or to click trains. Comparing results from different FM ramps, it appeared that the durations of gamma-band responses were longer for slower FM sweeps.

Conclusions

Results suggest that amplitude modulated sounds are most strongly represented within core cortex on posteromedial HG. Frequency modulated signals, while capable of activating core cortex, appear more effective in activating what may be part of a belt system adjacent to the core.

Acknowledgements: supported by NIH Grants DC-04290, HD-03352, MH-070497 and MO1-RR-59 (General Clinical Research Centers Program), by the Hoover Fund and Carver Trust and in part by National Science Council, Taiwan

References

1. Artieda, J., Valencia, M., Alegre, M., Olaziregi, O., Urrestarazu, E. and Iriarte, J. (2004) 'Potentials evoked by chirp-modulated tones: a new technique to evaluate oscillatory activity in the auditory pathway'. *Clinical Neurophysiology,* vol.115, pp. 699-709.
2. Brugge, J.F., Volkov, I.O., Oya, H., Kawasaki, H., Reale, R.A., Steinschneider, M. and Howard, M.A. (2008). 'Functional localization of auditory cortical fields of human: click-train stimulation'. Hearing Research, vol. 238, pp. 12-24.
3. Brugge, J.F., Nourski, K.V., Oya, H., Reale, R.A., Kawasaki, H., Steinschneider M. and Howard, III M.A. (2009) 'Coding of repetitive transients by auditory cortex on Heschl's gyrus'. *Journal of Neurophysiololgy,* vol. 102, pp. 2358-2374.
4. Crone, N.E., Boatman, D., Gordon, B. and Hao, L. (2001) 'Induced electrocorticographic gamma activity during auditory perception'. *Clinical Neurophysiology,* vol. 112, pp. 565-582.
5. Delorme, A. and Makeig, S. (2004) 'EEGLAB: an open source toolbox for analysis of single-trial EEG dynamics including independent component analysis'. *Journal of Neuroscience Methods*, vol. 134, pp. 9-21.
6. Nourski, K.V., Reale, R.A., Oya, H., Kawasaki, H., Kovach, C.K., Chen, H., Howard III, M.A. and Brugge, J.F. (2009) 'Temporal envelope of time-compressed speech represented in the human auditory cortex'. *Journal of Neuroscience*, vol. 29, pp. 15564 -15574.

出席國際學術會議心得報告

計畫編號	98-2320-B-009-001-My2
計畫名稱	探討早年聽覺經驗對於中腦聽神經元突觸發育之影響與其分子調控機轉
出國人員姓名 服務機關及職稱	曲在雯 國立交通大學 生物科技學系 助理教授
會議時間地點	2010 年 11 月 13 日 ~17 日 美國聖地牙哥
會議名稱	2010 Society for Neuroscience 年會 2010 Society for Neuroscience Annual Meeting
發表論文題目	

三、參加會議經過

此次會議包含的主題相當的豐富，與會的人數也超過三萬多人，所以無法聽或看完所有的會議主題，只能選擇三到四個主要的項目。這次我參加 Experience-Dependent Synaptic Plasticity and Neurogenesis in the Degenerating and Injured Brain、Erasing Fear Memories with Extinction、Memory Enhancement Strategies for the Treatment of Cognitive Disorders 等主題的 symposium。在海報論文部分，則主要以 Cortical Reorganization and Plasticity、Auditory Processing: Neural Coding, Experiment, and Theory、Brain Machine Interface and the P300、Brain Machine Interface: Noninvasive Mechanisms、Auditory Processing: Circuits, Synapses, and Neurotransmitters、Novel Methods: Electrophysiology、Data Acquisition and Brain-Machine Interface: Practice and Theory 等。除此之外，在會議中亦參觀了 Exhibitor booth，了解目前研究相關儀器的進展，並與個人人所使用的儀器廠商討論一些儀器使用上的問題。合作的研究團隊亦在會議中設置了一個 non-profit exhibition booth，展示新研發的 miniature wireless ECoG system，在會場中獲得相當好評。此外，在最後一天的會議也購買了多本新書。

四、與會心得

本人已多年未參加基礎神經科學研究會議，此一會議為神經科學研究領域的最大會議，為希望能獲得相關最新相關研究的資訊，參加此會議聆聽相關的演講，研究海報介紹及與會的相關研究人員面對面討論是最好的辦法。在此次會議的最大收穫是在參觀海報論文的過程中，學習到一個可應用在大腦血管網研究的新方法，此方法能解決過去個人在研究上面臨到的一些瓶頸，且其方法不需要十分複雜得技術。在參加會議回來後，已開始積極準備測試此一方法在個人相關研究上的可行性。此外，合作團隊在會議中展示的新研發成果 miniature wireless ECoG system 獲得了相當不錯的反應，從來參觀的人的意見中，我們也看到了此一產品需進一步改進之處。在整個參加會議的過程中感受到跨領域整合研究的必要性，在會議多篇傑出的論文多出自於跨領域整合的研究團隊。在台灣近年也開始強調跨領域整合研究，但是如何有效率的整合研究所長與尋找一個適當的研究主題則是決定跨領研究團隊成敗的重要因素。很幸運的是個人目前所參與的交大-中國醫藥大學醫材中心跨領域合作的團隊目前已有不錯的開始，未來仍需更進一步的努力方能呈現最佳的成果。

五、建議

此一會議為神經科學領域的重要年會，若有機會應該多鼓勵與補助研究生參加，以增廣學生的學習視野，也能有機會讓學生能與不同國家的學生與研究人員有面對面討論的機會。

六、攜回資料

2. 大會手冊 (包括議程與論文摘要)。
3. CDs from Animal Welfare Information Center (Information Resources for Institutional Animal Care and Use Committees, Techniques and procedures, Information Resources on animals in Research, Environmental Enrichment Resources,AWIC Resources on the Literature Search for Alternatives)
4. Flyers for neuroscience products.
5. Flyer for “Ethical issue when writing a scientific paper”

附件五

國科會補助專題研究計畫項下赴國外(或大陸地區)出差或研習心得報告

日期：___年___月___日

計畫編號	NSC　　—　　—　　—　　—　　—		
計畫名稱			
出國人員 姓名		服務機構 及職稱	
出國時間	年　月　日至 年　月　日	出國地點	

一、國外(大陸)研究過程

二、研究成果

三、建議

四、其他

附件六

國科會補助專題研究計畫項下國際合作研究計畫國外研究報告

日期：___年___月___日

計畫編號	NSC　　—　　—　　—　　—　　—		
計畫名稱			
出國人員 姓名		服務機構 及職稱	
合作國家		合作機構	
出國時間	年　月　日至 年　月　日	出國地點	

一、國際合作研究過程

二、研究成果

三、建議

四、其他