行政院國家科學委員會專題研究計畫 成果報告

睡眠剝奪對睡眠促進中樞細胞膜結構、離子型受器暨胞內離子分布表現之影響

研究成果報告(精簡版)

計 畫 類 別 ：個別型
計 畫 編 號 ：NSC 96-2320-B-040-015-
執 行 期 間 ：96 年 08 月 01 日至 97 年 07 月 31 日
執 行 單 位 ：中山醫學大學醫學系解剖學科

計 畫 主 持 人：張宏名
共 同 主 持 人：麥富德
計畫參與人員：大專生-兼任助理人員：徐卿晃
大專生-兼任助理人員：王一
大專生-兼任助理人員：李建興
大專生-兼任助理人員：蕭伯諺

報 告 附 件 ：出席國際會議研究心得報告及發表論文

處 理 方 式 ：本計畫可公開查詢

中 華 民 國 97 年 11 月 04 日

Applied Surface Science xxx (2008) xxx–xxx

Contents lists available at ScienceDirect

Applied Surface Science

journal homepage: www.elsevier.com/locate/apsusc

Up-regulation of Na^+ expression in the area postrema of total sleep deprived rats by TOF-SIMS analysis

Fu-Der Mai [a], Bo-Jung Chen [b], Yong-Chien Ling [b], Un-In Wu [c], Yi-Lun Huang [d], Hung-Ming Chang [d,*]

[a] Department of Biochemistry, School of Medicine, Taipei Medical University, Taipei 110, Taiwan
[b] Department of Chemistry, National Tsing Hua University, Hsinchu 300, Taiwan
[c] Department of Internal Medicine, National Taiwan University Hospital, Taipei 100, Taiwan
[d] Department of Anatomy, Faculty of Medicine, Chung Shan Medical University, Taichung 402, Taiwan

ARTICLE INFO

Article history:
Available online xxx

Keywords:
Sodium
Area postrema
Sleep deprivation
Cardiovascular function
TOF-SIMS

ABSTRACT

Area postrema (AP) is a circumventricular organ plays an important role in sodium homeostasis and cardiovascular regulation. Since sleep deficiency will cause cardiovascular dysfunction, the present study aims to determine whether sodium level would significantly alter in AP following total sleep deprivation (TSD). Sodium level was investigated in vivo by time-of-flight secondary ion mass spectrometry (TOF-SIMS). Clinical manifestation of cardiovascular function was demonstrated by mean arterial pressure (MAP) values. Results indicated that in normal rats, TOF-SIMS spectrum revealed a major peak of sodium ion counting as 5.61×10^5 at m/z 23. The sodium ions were homogeneous distributed in AP without specific localization. However, following TSD, the sodium intensity was relatively increased (6.73×10^5) and the signal for sodium image was strongly expressed throughout AP with definite spatial distribution. MAP of TSD rats is 138 ± 5 mmHg, which is significantly higher than that of normal ones (121 ± 3 mmHg). Regarding AP is an important area for sodium sensation and development of hypernatremic related sympatho-excitation; up-regulation of sodium expression following TSD suggests that high sodium level might over-activate AP, through complex neuronal networks involving in sympathetic regulation, which could lead to the formation of TSD relevant cardiovascular diseases.

1. Introduction

With the coming of industrialization, sleep deprivation is increasingly becoming a major public health issue and affects millions of people in many countries [1]. Chronic sleep deprivation would excite the sympathetic nervous system, which unavoidably leads to metabolic and cardiovascular disturbances [1,2]. Our previous studies have demonstrated that sleep deprivation would predispose the liver to oxidative injury and depress the metabolic state of afferent neurons involved in sympathetic inhibition [3,4]. Enhanced sympathetic activity has long been suggested to play an important role in initiation or development of sleep deprivation relevant metabolic diseases [5].

Area postrema (AP) is a circumventricular organ located in the dorsal surface of caudal brainstem. During the past few decades, AP has been implicated to play an important role in the control of sodium homeostasis and sympathetic functions [6,7]. Previous studies have indicated that AP is participated as a functioning part of complex neuronal pathway regulating sympathetic activities [8,9]. Electrophysiological reports also demonstrated that AP is endowed with numerous chemosensitive neurons that may serve as sodium enteroceptors and act as an essential site for cardiovascular regulation [10,11]. It has been indicated that increased plasma sodium levels would contribute to hypertension as a result of over-activation of AP sodium sensitive neurons [12,13]. Through complex neuronal networks interlinking AP with other brainstem sympathetic regions, the sodium-dependent activation of AP is hence considered to play a vital role in development of cardiovascular diseases [14].

However, although maintaining sodium homeostasis in AP may be crucial in regulation of sympathetic activity, it is still unclear whether sodium expression in AP would significantly alter under sleep deprivation, a stressful condition with sympathetic activation. Moreover, whether the probable change of AP sodium is positively correlated with clinical dysfunction is also remained to be explored. As an attempt to answer this question, and to provide sodium expression in a molecular imaging level, we decided to detect the in vivo sodium expression by the use of time-of-flight secondary ion mass spectrometry (TOF-SIMS). In addition, in order to examine the clinical dysfunction responding to sodium

* Corresponding author. Tel.: +886 4 24730022x11610; fax: +886 4 24739030.
E-mail address: anatomy@csmu.edu.tw (H.-M. Chang).

0169-4332/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.apsusc.2008.05.179

alteration, mean arterial pressure (MAP) measurement was further processed in the present study.

2. Experimental

2.1. Treatments and experimental animals

Adult male Wistar rats ($n = 36$, weighing 200–250 g) obtained from the Laboratory Animal Center of the National Taiwan University were used in this study. The experimental animals were divided equally into two groups. Rats in the first group were subjected to TSD for 5 days (TSD group), while those in the second group were housed in the TSD apparatus but were permitted to sleep (normal group). TSD was performed by the disc-on-water (DOW) method as described in our previous studies [3,4]. Sleep deprivation depends on the rat's aversion to water, since rats rarely entered the water spontaneously. As sleep deprivation begins, rats in the TSD group placing on the disc had to keep awake and walk against the direction of disc rotation to avoid being forced into the water. For normal group, rats were allowed to sleep wherein no disc movement was initiated. All experimental animals were exposed to an automatically regulated light:dark cycle of 12:12 at a constant temperature (25 ± 1 °C). Food and water were made available through grids placed on top of the chambers. In the care and handling of all experimental animals, the Guide for the Care and Use of Laboratory Animals (1985) as stated in the United States NIH guidelines (NIH publication no. 86-23) were followed.

2.2. Haemodynamic measurement

After 5 days of TSD, all rats were anesthetized by intraperitoneal injection of Inactin (100 mg/kg body wt) and placed on a heating pad. A polyethylene catheter (PE-10) was inserted into the right femoral artery. Haemodynamic variable of MAP was then measured using a pressure transducer (P23 ID; Gould, Glen Burnie, MD) connected to a polygraph and was recorded by a thermal recorder (7758 B System; Hewlett-Packard, Palo Alto, CA).

2.3. Perfusion and tissue preparation

For TOF-SIMS analytical study, rats were deeply anesthetized with Inactin and perfused transcardially with saline followed by 300 ml of 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4. The lower brainstem containing the AP were then removed and immersed in 30% sucrose buffer for cryoprotection at 4 °C. Serial 30-μm thick sections of the tissue were cut transversely with a cryostat on the following day and the collected sections were attached to silica wafers (1 cm × 1 cm).

2.4. TOF-SIMS analysis

TOF-SIMS analysis was carried out on a TOF-SIMS IV instrument (ION-TOF GmbH, Münster, Germany). Cryostated tissue sections (30 sections per animal with a total of 36 animals) were used for replicated analysis in this study. The Ga^+ primary ion beam (1 pA pulse current) operated at 25 kV was scanned over an area of 100 μm^2. Four random areas in AP were selected for scan in each section in which four spectra were separately acquired from each sample. Positive secondary ions flying through a reflectron mass spectrometer were detected with a microchannel plate assembly operating at 10 kV post-acceleration. Mass calibration of the ion spectrum was achieved by using a set of mass peaks (CxHy) like m/z 15 (CH_3^+), 41 ($C_3H_5^+$) and 55 ($C_4H_7^+$). The ion peaks at m/z 23 were used to identify and evaluate the molecular image of sodium expression. For statistical analysis, spectral intensity detected from each section were normalized to the ion intensity of CH_3^+ (serve as base line = 100%) and expressed as percentage above the base line. All the normalized spectra collected from each animal (four spectra per section with thirty sections per animal) were then averaged to get a representative data for that animal. The representative data acquired from animals belonging to the same experimental group were further averaged to gain a mean value for that corresponding group [4]. Comparisons between the mean values obtained from TSD and normal groups were subjected to Student's t-test. The statistical difference was considered significant if $P < 0.05$.

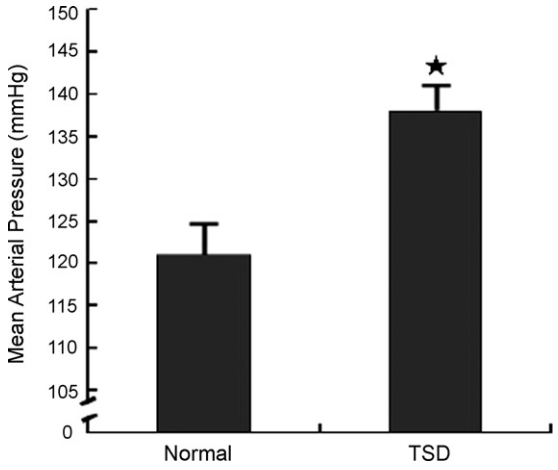

Fig. 1. Histogram showing the mean arterial pressure (MAP) in the normal and TSD rats. (★) $p < 0.05$, Student's t-test.

3. Results and discussion

3.1. Mean arterial pressure analysis

The changes of MAP of both experimental groups were summarized in Fig. 1. In normal rats, the MAP was estimated to

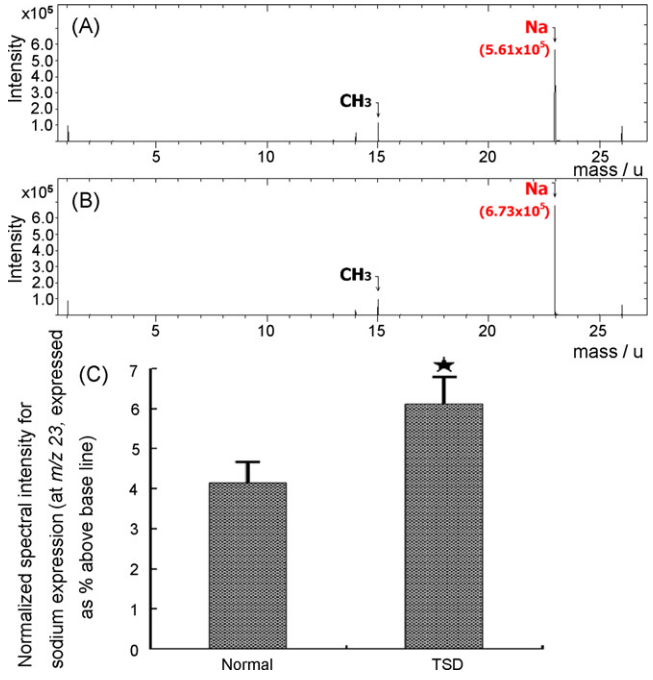

Fig. 2. TOF-SIMS positive ion spectrum (A and B) and histogram (C) showing the quantitative and replicated sodium ion intensity in the area postrema of normal (A) and TSD (B) rats.

Please cite this article in press as: F.-D. Mai et al., Up-regulation of Na^+ expression in the area postrema of total sleep deprived rats by TOF-SIMS analysis, Appl. Surf. Sci. (2008), doi:10.1016/j.apsusc.2008.05.179

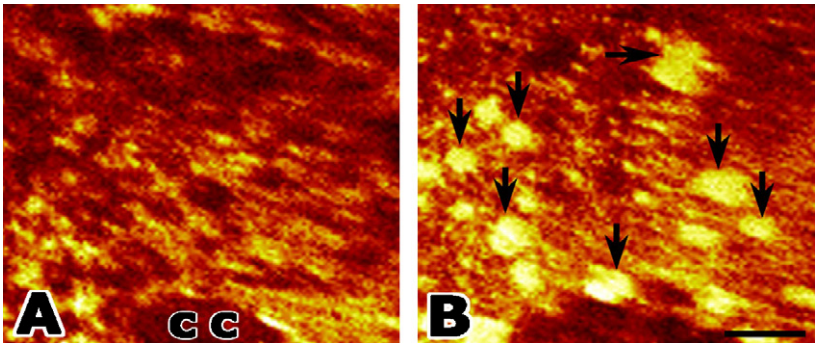

Fig. 3. TOF-SIMS positive ion image showing the sodium ion expression in the area postrema of normal (A) and TSD (B) rats. cc: central canal; scale bar = 25 μm.

be 121 ± 3 mmHg. However, in rats suffering from TSD, the MAP was significantly increased to nearly 138 ± 5 mmHg. These results suggested that TSD would exert a pressor effect on blood pressure, which might be elicited by potentiation of sympathetic activity.

3.2. TOF-SIMS mass spectra

Fig. 2 shows the TOF-SIMS positive ion mass spectra in the m/z of 23 that reflects the intensity distribution of sodium ion in the AP of both normal and TSD rats. The intensity for major peak of sodium in AP was counted to be 5.61×10^5 in normal rats (Fig. 2A). However, following 5 days of TSD, the spectrum for sodium ion intensity in AP was slightly increased to 6.73×10^5 (Fig. 2B). Mean values of the normalized spectra also showed a relatively higher expression in TSD group (6.118 ± 0.65%) as compared with that of normal ones (4.115 ± 0.5%) (Fig. 2C). It is worthy to note that the spectral intensities for normalization (i.e. CH_3^+ at m/z 15) were similar in both normal (1.35×10^5) and TSD (1.1×10^5) groups, suggested that the intensity change of sodium ion observed in current experimental paradigm is a distinct effect.

It has been reported that AP has a high number of sodium sensitive chemoreceptors that may involve in sodium homeostasis and cardiovascular regulation [10,11]. Previous report also demonstrated that increase sodium intake would contribute to the development of hypertension in which the pressor effect was proposed to be arisen from over-activation of sodium sensitive chemoreceptors within AP [12,13]. As one of the integrative sites for cardiovascular regulation, AP is shown to serve as a functional part of complex neuronal networks regulating sympathetic activity [8,9]. It is indicated that AP sends projections to numerous brainstem regions that have been identified as important areas for enhancing sympathetic activity (e.g. rostral ventrolateral medulla and parabrachial nucleus) [8,9]. Through the close interlinking between AP and these sympatho-excitatory areas, increased sodium levels in AP, as seen in our current TSD paradigm, would over-activate sodium responsive neurons and consequently lead to sympathetic associated cardiovascular dysfunctions [15,16].

3.3. TOF-SIMS molecular image

TOF-SIMS positive ion image has revealed that sodium ions were homogeneous distributed throughout the AP without any specific localization in normal rats (Fig. 3A). However, in TSD animals, the sodium ions were strongly expressed in AP with significant spatial distribution (Fig. 3B). Some sodium ion clusters with round profiles were found to be presented in distinct regions within AP, which might well mark the locations of over-activated sodium sensitive neurons within this area (Fig. 3B, arrows). It is suggested that elevated plasma sodium levels may play an important role in pathogenesis of the hypernatremic related hypertension [12,13]. Since AP is the major site for the control of sodium homeostasis [10], up-regulation of sodium expression in AP would evidently elicit cardiovascular changes by means of AP-mediated sympathetic activity. In the present study, we further observed a significantly higher MAP in TSD rats with apparent sodium increase in AP, which clearly demonstrate a possible mechanism of hypernatremic relevant cardiovascular diseases. However, the present study did not detect the potential changes of other brainstem nuclei or directly assess the sympathetic activity. As any cardiovascular change is closely correlated with complex neuronal activities, examining another neurochemical expression in other sympathetic regions should be performed in future study.

4. Conclusions

The present study is the first report employing TOF-SIMS analysis to provide molecular imaging evidence that sodium ions would relatively up-regulate in the AP of TSD rats. The clinical significance of enhanced sodium expression is functionally reflected by elevated MAP, a typical feature of sympathetic activation. To the extent of our knowledge, the present study has addressed for the first time that TOF-SIMS could be served as a useful tool for imaging the ion level and distribution in AP at the *in vivo* condition. Since TOF-SIMS has become one of the most sensitive analytical techniques used in current molecular cell biology, advanced use of TOF-SIMS in exploring other sympathetic regions following TSD will be greatly helpful in increasing our knowledge of TSD relevant cardiovascular diseases.

Acknowledgements

This study is supported in part by the research grants NSC 95-2320-B-040-012/NSC 96-2320-B-040-015 (to Dr. Chang) and NSC 96-2113-M-038-003-MY2 (to Dr. Mai) from the National Science Council, Taiwan.

References

[1] C. Lenfent, Metabolism 55 (2006) S50.
[2] B. Takase, T. Akima, K. Satomura, F. Ohsuzu, T. Mastui, M. Ishihara, A. Kurita, Biomed. Pharmacother. 58 (2004) S35.
[3] H.M. Chang, U.I. Wu, T.B. Lin, C.T. Lan, W.C. Chien, W.L. Huang, J.Y. Shieh, J. Anat. 209 (2006) 239.
[4] H.M. Chang, F.D. Mai, B.J. Chen, U.I. Wu, Y.L. Huang, C.T. Lan, Y.C. Ling, J. Anat. 212 (2008) 295.
[5] X. Zhong, H.J. Hilton, G.J. Gates, S. Jelic, Y. Stern, M.N. Bartels, R.E. Demeersman, R.C. Basner, J. Appl. Physiol. 98 (2005) 2024.

Please cite this article in press as: F.-D. Mai et al., Up-regulation of Na^+ expression in the area postrema of total sleep deprived rats by TOF-SIMS analysis, Appl. Surf. Sci. (2008), doi:10.1016/j.apsusc.2008.05.179

[6] P. Ylitalo, H. Karppanen, M.K. Paasonen, Nature 247 (2007) 58.
[7] T.M. Hyde, R.R. Miselis, Am. J. Physiol. 247 (1984) R173.
[8] D. van der Kooy, L.Y. Koda, J. Comp. Neurol. 219 (1983) 328.
[9] R.E. Shapiro, R.R. Miselis, J. Comp. Neurol. 234 (1985) 344.
[10] A. Adachi, M. Kobashi, N. Miyoshi, G. Tsukamoto, Brain Res. Bull. 26 (1991) 137.
[11] A.V. Ferguson, J.S. Bains, Front. Neuroendocrinol. 17 (1996) 440.
[12] V.L. Brooks, J.R. Haywood, A.K. Johnson, Clin. Exp. Pharmacol. Physiol. 32 (2005) 426.
[13] L.K. Dahl, Int. J. Epidemiol. 34 (2005) 967.
[14] D.B. Nahey, J.P. Collister, Am. J. Physiol. Heart Circ. Physiol. 292 (2007) H694.
[15] Y. Kawano, K.L. Barnes, C.M. Ferrario, J. Auton. Nerv. Syst. 35 (1991) 153.
[16] C.G. Wilson, A.C. Bonham, Am. J. Physiol. 266 (1994) H1075.

Please cite this article in press as: F.-D. Mai et al., Up-regulation of Na^+ expression in the area postrema of total sleep deprived rats by TOF-SIMS analysis, Appl. Surf. Sci. (2008), doi:10.1016/j.apsusc.2008.05.179

出席國際學術會議心得報告

計畫編號	NSC 96-2320-B-040-015
計畫名稱	睡眠剝奪對睡眠促進中樞細胞膜結構、離子型受器暨胞內離子分布表現之影響
出國人員姓名 服務機關及職稱	張宏名 中山醫學大學醫學系解剖學科　副教授
會議時間地點	2008/05/16 ~ 2008/05/20　俄羅斯　聖彼得堡
會議名稱	第十一屆國際壓力與行為神經科學會議
發表論文題目	睡眠剝奪鼠腦亟後區鈣離子升高表現之分子影像學研究

一、參加會議經過

承蒙行政院國家科學委員會暨校方於經費上之鼎力支持，職有幸獲准參加西元二００八年第十一屆國際壓力與行為神經科學會議。本屆會議於今年五月十六日至五月二十日於俄國文化之都聖彼得堡舉行。國際壓力與行為學會為一整合世界各國壓力與行為研究之學術組織，每年固定於俄羅斯聖彼得堡召開一次國際性學術會議，每次會議皆吸引相當多世界各地壓力與行為研究學者熱烈參與，與會者除了可在議程中，汲取壓力與行為研究發展的最新進展外，更可透過論文發表及展示，與世界各地之學者專家互相討論及分析，藉由彼此間的腦力激盪，不但可拓展自身的學術視野，更有助於學術交流的進行。職此次獲國科會暨校方各級長官的大力支持，代表醫學系解剖學科，公差假赴俄國參加本屆之國際壓力與行為神經科學會議，並在大會中發表學術論文，除增進自身之研究深度與廣度外，並積極與世界各國學者專家做學術交流，希望對提升台灣暨本校之國際學術形象與知名度有所助益。

職服務於大專院校期間，曾多次代表解剖學科出席國際上與神經科學研究相關之學術會議，包括近年內赴德國柏林參加第一屆世界睡眠醫學大會，並於會中發表學術論文，認識並結交不少國外學者。本次參加之神經科學學術會議由俄國主辦，議期為期五天，在俄國的文化中心聖彼得堡盛大舉行。此次會議共吸引了來自世界各地約六百位專家學者共襄盛舉，全球研究壓力與行為之神經科學家藉此機會齊聚一堂，除了可交換彼此的研究心得與經驗外，更可透過相互觀摩與討論的機會，達到拓展自身研究視野與增進良性學術交流的目的。

本屆會議探討之學術主題相當廣泛，大致上可分為十五個專區：

一、壓力 (stress)、恐懼 (fear)、焦慮 (anxiety) 與沮喪 (depression) 之行為分析與探討。本專區主要集中在探討患者之行為變異與成因，並據此設計一套客觀與正確之行為分析模式與架構，期能對患者之精神狀態做有效之評估與了解。

二、壓力之神經基因學 (neurogenetics of stress)。本專區主要探討誘發壓力的神經性因子，同時由分子生物學的角度審視基因在壓力形成過程中扮演的角色。

三、神經調節因子 (neuromediators and transporters)。本專區探討的主題亦集中在神經調節因子的生化功能，期能藉由操作神經調節因子於腦中的分布與濃度，達到改善患者壓力或減輕焦慮的目的。

四、中樞神經系統的神經性荷爾蒙 (neurosteroids in the CNS)。神經性荷爾蒙為神經系統運作的強效回饋劑，透過與細胞膜上特殊受器的接合，可大幅改變細胞的生理狀態與活化程度。有效控制中樞神經系統內神經性荷爾蒙的製造與多寡，將可大幅降低細胞異常活化或異常低落的現象。

五、壓力的神經化學與神經生理學 (neurochemistry and neurophysiology of stress)。本專區著重於神經化學分子 (如神經傳導物質、神經滋養物質) 與神經電生理狀態對壓力形成過程的影響，同時評估人為操作神經化學物質濃度對改善壓力病情是否有顯著成效。

六、神經心理藥理學 (neuropsychopharmacology) 與神經內分泌學 (neuroendocrinology)。本專區由心理藥理學與內分泌學的層面切入，探討神經用藥對壓力與外顯行為的潛在影響。

七、行為的實驗模式 (experimental models of behavior)。此專區是此次大會十數個專區中，除職本身之研究領域外，個人最感興趣的主題之一。專區旨在探討外顯行為的客觀研究模式，並比較各實驗模式的利弊得失，同時研討如何利用先進的電腦科技，正確並詳實的定量分析各外顯行為的組成部件。其中最令人印象深刻的是，來自世界各國的學者專家，紛紛都提出了別出心裁與獨具巧思的實驗創意，看到竟有那麼多實驗設計可以用來進行行為分析，好似經歷一場心靈與知識饗宴，收穫非常豐富。

八、壓力、記憶與學習 (stress, memory and learning)。此專區亦是相當熱門的討論區。記憶為學習之母，擁有良好的記憶方能成就有效的學習。目前學界已知適度的壓力有助記憶成形，並進而提升學習效率。然何謂適度的壓力，在適度的壓力下，其分子機制是否與過量壓

力有所不同，其所衍生的後續效應是否確是造成學習不佳的主因，諸如此類問題皆是本專區積極探討的課題。

九、心理生理學與神經心理學 (psychophysiology and neuropsychology)。本專區主要由心理生理學的觀念審視壓力與行為之因果關連，及其可能引發之精神後果。

十、心理神經免疫學 (psychoneuroimmunology)。本專區旨在探討壓力與行為潛在之神經免疫學成因。

十一、一般心理學 (general psychiatry)。本專區著眼於因壓力衍生之精神狀態評估與治療，同時深入剖析傳統心理分析和心理諮詢與現代神經藥理治療之成效差異。

十二、基因與環境之交互作用 (gene x environmental interactions)。目前學界已初步闡明某些精神疾病的發生與基因調節有密切關係。然生活環境的改變亦有可能導致基因發生突變。釐清環境與基因之交互作用，並藉此營造或避免某些優質或特殊環境，以提升生活品質，達到降低壓力與憂鬱的發生率，是此專區積極探討的主題。

十三、比較生物心理學 (translational research in biological psychiatry)。

十四、壓力的生物標記 (biomarkers of stress)。此專區亦是個人相當欣賞的討論主題之一。一般咸認壓力是種主觀的本體感覺，很難用客觀的方法加以精準的定量。患者主觀認知的壓力大小，常隨評估情境與施測環境而有很大的改變。在神經科學的研發上，目前已有一些相當穩定的生物標記 (如 c-fos 等)，可用來精準地定量細胞感受到的壓力大小。壓力生物標記的研發，無疑是對壓力研究學者提供一項分析的利器，藉由生物標記穩定且精準的標誌，可讓學者追蹤壓力影響的神經路徑，並進而研發和觀察精神用藥對抑低壓力的成效。

十五、其他腦或行為病變 (other brain or behavioral disorders)。值得一提的是，大會於議程進行中每天早上九點半至十一點，及每天下午兩點至三點半，各皆安排兩位世界上著名的壓力與行為研究學者做專題演講，講題為上述各學術討論專區主題之一，演講內容深入簡出，研究設計切入核心，聆聽大師精闢的演講，往往令人有收穫豐厚、不虛此行之感慨。於專題演講後，大會接續安排了半小時的休息時間，會議主辦單位提供了精緻的小點心與咖啡供各與會學者食用及品嚐，於此同時，與會學者亦可自由至壁報展示區瀏覽參展論文，並可駐足於有興趣的論文看板前，與論文作者做進一步的交流及討論。口頭論文的報告則安排於每日早上十一點十五至十二點，及每日下午三點四十五至六點等兩個時段舉行。由於口頭論文的

發表場地不只一處，同一時段通常有二至三個論文同時舉行，與會學者必須選擇最有興趣的一個主題前往聆聽，每一論文的報告時間為十五分鐘，接著由聽眾發表問題，最後再由論文作者逐一解答。職於參加大會口頭論文發表的過程中，不但親自見識到國外學者專家治學之嚴謹、研究思路之清晰、邏輯推理之正確，更重要的，是其發表問題之深入與回答問題之技巧，這些所得豐厚之見聞，是參加此一大型國際學術會議的重要收穫。此外，藉由瀏覽參展論文與聆聽口頭報告的機會中，職亦觀賞到了各國論文發表學者精美的海報版面與提綱契領的幻燈片製作，透過觀摩學習這些論文發表方面的實用技巧，對將來個人學術研發方面的成果展示上，亦是相當難能可貴的經驗。

二、與會心得

職於本屆大會中，用口頭報告的方式，發表了一篇學術論文。論文題目為：睡眠剝奪鼠腦亟後區鈣離子升高表現之分子影像學研究。腦亟後區 (area postrema) 位於下位腦幹 (lower brainstem)，是生物體偵測血液中鈉離子與鈣離子濃度高低的地方。腦亟後區缺乏血腦障蔽 (blood brain barrier)，血液中的鈣離子濃度高低，可直接藉由腦亟後區內特殊的感受細胞偵測，並將偵測結果轉換成化學訊號，透過綿密複雜的神經纏絡網路，將此一訊號散佈至腦幹其他核區，進而影響腦幹相關核區的活化程度，進一步促使生物體因應鈣離子濃度變化而改變部份生理功能 (如血壓上升、氧化壓力增加等)。睡眠剝奪已知將導致神經細胞氧化壓力上升，緣此，本研究之設計目的旨在探討腦亟後區鈣離子濃度上升是否即為神經細胞氧化壓力激增之潛在來源。我們與清華大學化學系合作，使用飛行式二次離子質譜儀 (Time-of-flight secondary ion mass spectrometry, TOF-SIMS) 直接觀察實驗鼠腦亟後區鈣離子之分子影像及分布。實驗同時結合攜鈣蛋白 (calmodulin) 免疫組織化學法，用專一性甚高之抗體直接標誌攜鈣蛋白於腦亟後區神經細胞內之活性表現。實驗結果發現，正常鼠腦亟後區僅有少量鈣離子隨機分布，其攜鈣蛋白之標誌情況亦不甚明顯。然經睡眠剝奪處理後，腦亟後區湧入大量鈣離子，且鈣離子分布之細胞內具非常強烈的攜鈣蛋白正向標誌反應。目前學界咸認為細胞內鈣離子濃度增高將導致興奮性毒性 (excitotoxicity)，進而改變細胞電生理狀態，嚴重者將導致細胞受損或死亡。我們據此實驗結果推測，睡眠剝奪鼠氧化壓力增高的成因之一即是腦亟後區鈣離子濃度急劇上升，誘使腦亟後區細胞活性發生改變，藉由腦亟後區與腦幹其他核區相互聯繫溝通的神經網路，腦亟後區的活性變化或受損將對腦幹相關核區產生間接影響，進

而致使生物體衍生血壓升高等病徵。由於目前學界尚不明瞭腦亟後區鈣離子分布表現與睡眠剝奪衍生病變之相互關連，本實驗首度提供鈣離子分布表現之分子影像學證據，直接且清晰地闡明鈣離子於睡眠剝奪病理生理演變過程中扮演的角色，除了提供睡眠研究學者一個降低臨床病變可能思考的方向之外，尚對細胞氧化壓力的成因，提出明確且嶄新的詮釋。我們的論文發表吸引了多數學者專家到場聆聽。期間亦有不少專家學者提出疑問或批評，職都一一詳細回答，對於建設性的討論與指教，我們更是銘記於心，並希望於返回校方服務後，做更進一步的研究或探討，將參與會議之所見所聞做詳實的整理與記錄，為壓力與行為之研究貢獻一己之力量。

職承蒙國科會及校方各級長官大力支持與鼎力協助，才得以於規定時間內完成各項出國手續，達成參加此一大型國際學術會議的計畫，職在此由衷的向各位曾經不遺餘力協助及支持職申請經費補助的各級長官與人員，表達深切誠摯的謝忱。職本身即為台灣少數睡眠醫學研究領域的學者之一，於本校服務期間，多次進行有關睡眠剝奪與細胞壓力變化衍生之相關議題，對睡眠剝奪所引致之神經系統損傷與抗氧化酵素可能具備之神經保護效果，皆有深刻的認知與了解。此次出席本屆之國際壓力與行為神經科學會議，即獲取了相當多寶貴的知識與實用的資訊，於大會進行期間，積極把握與各國學者專家做意見交流與討論的機會，如此不但可擴增自身之研究視野，並可將台灣之研究成果呈現於與會之世界學者專家眼前。然唯一美中不足的是，由於此次會議主辦單位宣傳不足，以致亞太地區參加學者不多，其中又以日本與韓國學者佔絕大多數，對於台灣科學研究於國際能見度的提升幫助極其有限，算是此行無法避免的一個小缺憾。

三、建議

參與國際醫學會議為醫學研究及醫療服務人員教育訓練的重要一環，在現今學術研究競爭激烈的環境中，身為基礎醫學研究領域的一份子，每個人皆應擁有強烈的使命感與宏偉的世界觀。當今政府正積極推展務實外交，要讓台灣的產業界及學術界與世界接軌，值此各方面皆蓬勃發展、且知識爆炸的時刻，職誠摯建議所有出席國際醫學會議的與會代表，皆能秉持為國家、為學校增光的原則，善加利用、把握此一難得的學習與外交機會，除了豐厚自己的學術識養外，尚能為學校與國家整體學術地位之提升，貢獻自身的一點心力！