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

利用神經系統性疾病和果蠅動物模式研究學習與記憶之分子機制暨篩選有效之治療藥物

（精簡報告）

計畫類別：個別型計畫

計畫編號：NSC 97-2320-B-039 -021 -MY3

執行期間： 98 年 8 月 1 日至 99 年 7 月 31 日

執行單位：中國醫藥大學 中西醫結合研究所

計畫主持人：林維勇

共同主持人：蔡輔仁、郭煌宗、陳永祥

計畫參與人員：陳靖眉 （專任助理）
陳威成 （兼任助理）
陳瑋鑫 （兼任助理）
劉娟秀 （兼任助理）

處理方式：本計畫暫不公開查詢

中 華 民 國 99 年 5 月 31 日

目錄

中文摘要

關鍵字: 神經系統性疾病、學習與記憶

神經系統性疾病或神經系統性異常會影響到學習與記憶的能力，而到目前為止，並沒有有效的藥物和治療方式能完全治癒此疾病。解開人類學習與記憶的分子機制一直是科學家們想要解決的議題。本實驗於本期(本計畫第二年)將以建立好的學習與記憶有障礙之果蠅模型，使用氣味試驗評估果蠅的記憶效果。預期本研究之效益為篩選出改善果蠅學習記憶的出有效中藥與天然化合物。

Abstract
Keywords: Neurological disorders; learning and memory

Neuronal systemic diseases and abnormalities will affect the ability for learning and memory formation. Until now, there is no effective drug and effective therapeutic method yet to cure these diseases fully. To understand the molecular mechanisms is still the important topics which scientists want to solve. In the second year of this project, we will use the established *Drosophila* model of learning and memory defects to determine the performance index by odor test. It is expected that the benefit of this study is screening the effective Chinese herbal medicines and natural compounds for improving the learning and memory of *Drosophila*.

Introduction

Exploring the molecular mechanisms of learning and memory is a topic that scientists want to solve to understand the neurological disorders. Neurological disorders are imbalance of central nervous system caused by congenital, acquire acute or chronic conditions, it is predicted to be related to the degeneration or disorder of brain cells.

Finding out relevant genes could help us to understand the mechanisms of learning and memory. Recently, there is a trend that many famous pharmaceutical and research institutes use fly to rapid screen drugs and genes that can be treated to human disorders. As a result, we use methods of transgenic fly to set up a platform for drug and gene screening. We plan to transfer genes that caused human disease to the fruit fly and make a humanized fly. Therefore, we can use this animal model to rapid screen drugs and genes for the mechanism of human neurological disorders. In this second year's project, we use this model to find genes and drugs that influence memory and learning. This study has the following effects: localization of genes that caused neurological disorders, set up a rapid screening animal model for drugs treating neurological disorders, study the molecular mechanisms of learning and memory disturbance of neurological disorders, and find effective drugs or other bioactive materials that can treat neurological disorders, etc. This treating model for neurological disorder of learning and memory disturbance can therefore further be applied to other new medical fields and enforcement preventive medicine.

Objective

In this project of second year, we will use the potential gene for neurological systemic disorders based on the SNP analysis to find the molecular mechanisms of learning and memory. Meanwhile, we will seek out the therapeutic drugs or other active compounds for neurological disorders, learning and memory defects aggressively.

Literature review

The study of brain neuronal science is focused on the functions of different regions in the brain and seeking out the working mechanisms of several behaviors expression and mind activities in the brain. There are several neurological disorders which will affect learning and memory, i.e. Kanner's autism (Makita 1977), Parkinson’s disease, Alzheimer’s disease, frontotemporal dementia, dementia with Lewy bodies, etc.

The most neuronal degeneration disease which people suffered is Alzheimer’s disease. Its major pathological zone located in the neuronal cells of hippocampus zone and cortex of the brain. The expression of acetylcholine is decreased and the functions of learning and cognition are affected. Neurological disorders induced dementia including the damage of learning and memory. In the part of memory, people will loss the ability for new messages formation gradually and forget the previous learned knowledge; In the part of learning, people will suffered aphasia, apraxia, agnosia and obstacles of performing function (planning, organization and abstract thinking)

Recently, the knowledge of neuroscience is break through. In 2000, Arvid

Carlsson, Paul Greengard and Eric Kandel got the Nobel Prizes by discovering the dopamine is neurotransmitter, conduction formation by chemical change, the signal transmission between cells is mediated by synapse, respectively. This finding brings new insights for the treatment of neurological disorders.

According to recent research researches, using transgenic *Drosophila* as animal model for curing the human neuronal degeneration can speed up the research progress and can imitate human disorders and speed up the effective therapeutic methods in mice and human. (Lin, Sahakian et al. 2003; Greenspan and Dierick 2004; Marsh and Thompson 2004).

In 2006, the famous international journal, Nature, use "article" format to publish the paper "Distinct memory traces for two visual features in the Drosophila brain". This new finding says "although *Drosophila* is small, the brain is complex and the sight memory function need operated by the circuit formed in the specific neurons of brain" (Liu, Seiler et al. 2006) The comment in the same issue of Nature thinks that this work tells us clearly that *Drosophila* can be the good model for investigation of neuronal structures. Thus, we plan to use *Drosophila* to investigational neurological disorders mediated the pathogenesis of learning and memory and hope for screen the potential therapeutic drugs by this advantaged animal model.

Meterials and Methods

1. Using UAS-GAL4 system to express candidate genes

We used transgenic *Drosophila* who carries candidate genes behind the UAS gene. After crossing with *Drosophila* who carry GAL4 gene, the progenies will express genes that associated with learning and memory all the time.

The next step, we used special apparatus “T-maze” to estimate the response of the gene and to screen effective Chinese herbal medicine.

2. Olfactory learning and memory in *Drosophila*

In order to observe the efficiency after cross, we use the special apparatus “T-maze” to estimate the response of the gene. First, flies were introduced into the upper tube that contains copper grid, then, OCT was offered with shock and MCH was offered without shock. Second, the flies were moved into the center of the T-maze and OCT was offered on the end of the arm and MCH was offered on the other arm. According to the number of flies in two arms, we can estimate whether the candidate gene cause impairment in learning and memory or not.

Results and discussion

We find that flies expressing**α**-synuclein, with learning and memory defects fed with Chinese herbal medicine 23 will increase middle term memory, but this experimental is not finished yet. We need further to verify if Chinese herbal medicine 23 (CHM23) has the ability for increasing the middle term memory (fig 2). CHM23 also can improve the motor function of flies expressing α-synuclein (Fig. 2.). Besides, CHM23 can improve the survival of flies expressing α-synuclein (Fig. 3.).

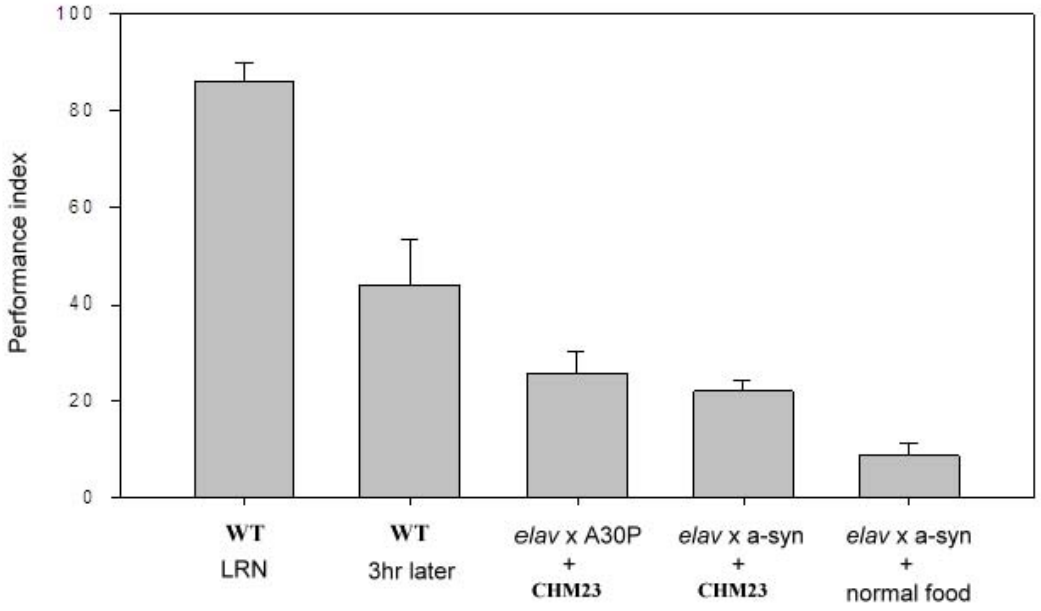

Fig. 1. CHM23 can partly rescue the 3hr memory defect of the flies expressing α-synuclein.

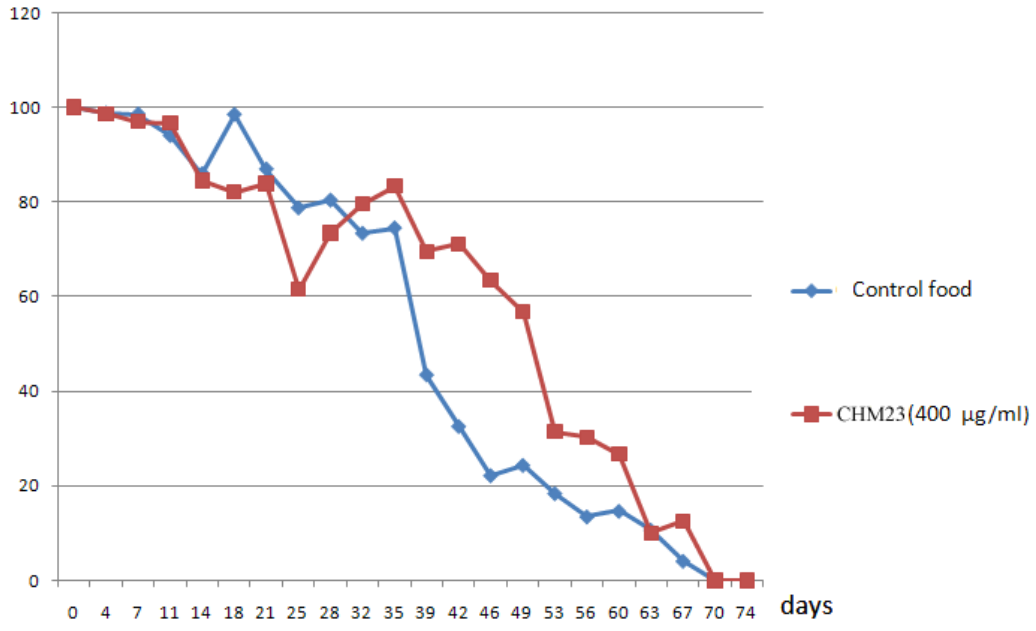

Fig. 2. CHM23 can improve the motor function of flies expressing α-synuclein.

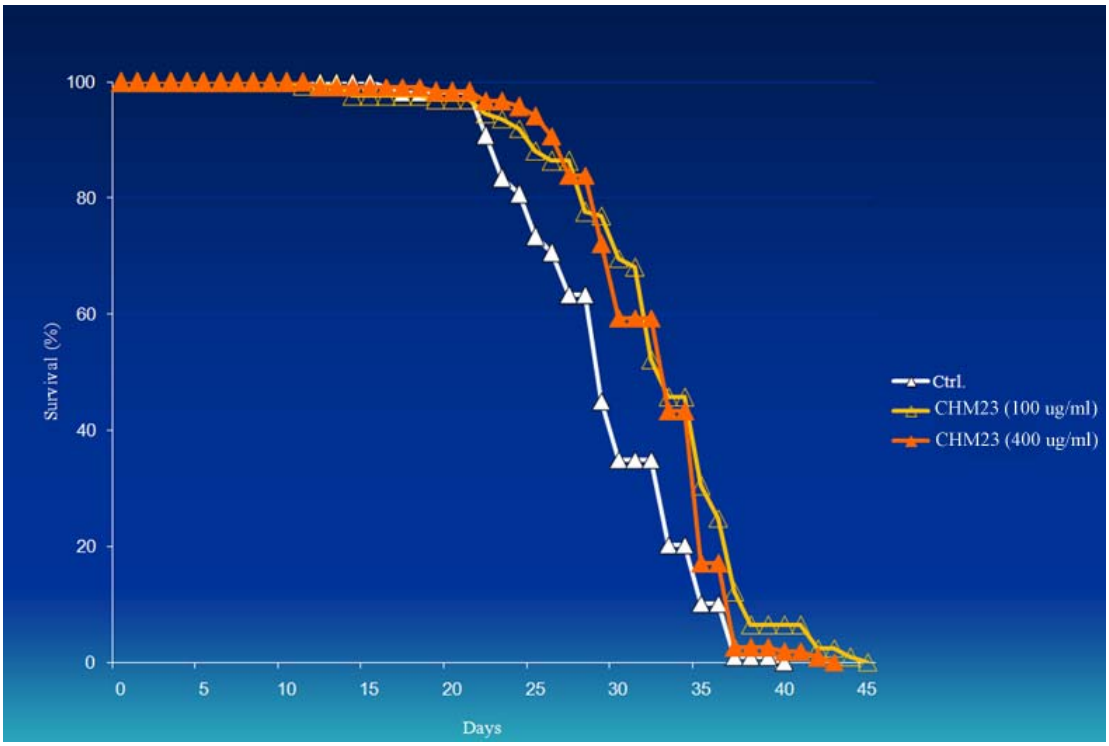

Fig. 3. CHM23 can improve the survival of flies expressing α-synuclein.

參考文獻

Baxter, M. G. (2001). "Effects of selective immunotoxic lesions on learning and memory." Methods Mol Biol **166**: 249-65.

Chiang, A. S., W. Y. Lin, et al. (2002). "Insect NMDA receptors mediate juvenile hormone biosynthesis." Proc Natl Acad Sci U S A **99**(1): 37-42.

Gaddum, J. (1965). "The neurological basis of learning." Perspect Biol Med **8**(4):436-74.

Greenspan, R. J. and H. A. Dierick (2004). "'Am not I a fly like thee?' From genes in fruit flies to behavior in humans." Hum Mol Genet **13 Spec No 2**: R267-73.

Grillner, S., L. Olson, et al. (2000). "[Arvid Carlsson, Paul Greengard and Eric Kandel are winners of the Nobel Prize in physiology and medicine in 2000. They are awarded for basic research on chemical signal transmission in the brain]."Lakartidningen **97**(42): 4685-92.

Hamilton, J. M., D. P. Salmon, et al. (2004). "A comparison of episodic memory deficits in neuropathologically-confirmed Dementia with Lewy bodies and Alzheimer's disease." J Int Neuropsychol Soc **10**(5): 689-97.

Heisenberg, M. (2003). "Mushroom body memoir: from maps to models." Nat Rev Neurosci **4**(4): 266-75.

Henatsch, H. D. and H. H. Langer (1985). "Basic neurophysiology of motor skills in sport: a review." Int J Sports Med **6**(1): 2-14.

Jaffard, R., T. Durkin, et al. (1989). "Experimental dissociation of memory systems in mice: behavioral and neurochemical aspects." Arch Gerontol Geriatr Suppl **1**: 55-70.

Kassel, D. B. (2004). "Applications of high-throughput ADME in drug discovery." Curr Opin Chem Biol **8**(3): 339-45.

Kishore, K. and M. Singh (2005). "Effect of bacosides, alcoholic extract of Bacopa monniera Linn. (brahmi), on experimental amnesia in mice." Indian J Exp Biol **43**(7): 640-5.12

Labutta, R. J., R. B. Miles, et al. (1994). "Motor program memory storage in Parkinson's disease patients tested with a delayed response task." Mov Disord **9**(2): 218-22.

Lin, J., D. C. Sahakian, et al. (2003). "The role of absorption, distribution, metabolism, excretion and toxicity in drug discovery." Curr Top Med Chem **3**(10): 1125-54.

Lin, W. Y., L. Wan, et al. (2006). "Vitamin D receptor gene polymorphisms are associated with risk of Hashimoto's thyroiditis in Chinese patients in Taiwan." J Clin Lab Anal **20**(3): 109-12.

Liu, G., H. Seiler, et al. (2006). "Distinct memory traces for two visual features in the Drosophila brain." Nature **439**(7076): 551-6.

Mai, J. K., S. Lensing-Hohn, et al. (1997). "Developmental organization of neurophysin neurons in the human brain." J Comp Neurol **385**(3): 477-89.

Makita, K. (1977). "[Kanner's syndrome]." Nippon Rinsho **35 Suppl 1**: 686-7.

Nesnidalova, R. and V. Fiala (1961). "[On the problem of Kanner's autism in children.]." Cesk Psychiatr **57**: 76-84.

Parng, C. (2005). "In vivo zebrafish assays for toxicity testing." Curr Opin Drug Discov Devel **8**(1): 100-6.

Quinn, W. G. (2006). "Neurobiology: memories of a fruitfly." Nature **439**(7076): 546-8.

Ray, B., T. S. Roy, et al. (2005). "Development of the human fetal cochlear nerve: a morphometric study." Hear Res **202**(1-2): 74-86.

Rippon, G. A., B. F. Boeve, et al. (2005). "Late-onset frontotemporal dementia associated with progressive supranuclear palsy/argyrophilic grain disease/Alzheimer's disease pathology." Neurocase **11**(3): 204-11.

Rubin, G. M. and A. C. Spradling (1982). "Genetic transformation of Drosophila with transposable element vectors." Science **218**(4570): 348-53.

Tully, T., R. Bourtchouladze, et al. (2003). "Targeting the CREB pathway for memory enhancers." Nat Rev Drug Discov **2**(4): 267-77.

Williams, J. A., J. Bauman, et al. (2005). "In vitro ADME phenotyping in drug discovery: current challenges and future solutions." Curr Opin Drug Discov Devel **8**(1): 78-88.

Xia, S., T. Miyashita, et al. (2005). "NMDA receptors mediate olfactory learning and memory in Drosophila." Curr Biol **15**(7): 603-15.

Yu, H. and A. Adedoyin (2003). "ADME-Tox in drug discovery: integration of experimental and computational technologies." Drug Discov Today **8**(18): 852-61.

Zhao, X., C. Li, et al. (2004). "An integrated view of copy number and allelic alterations in the cancer genome using single nucleotide polymorphism arrays." Cancer Res **64**(9): 3060-71.

Zhao, X., B. A. Weir, et al. (2005). "Homozygous deletions and chromosome amplifications in human lung carcinomas revealed by single nucleotide polymorphism array analysis." Cancer Res **65**(13): 5561-70.

計畫成果自評

To date, according to the results, we had estimated that we have corresponding progress. Our results own possibility to be published the findings in the future. Besides, we appreciate the support of the National Science Council, so that we could publish some results in 32nd European Conference on Visual Perception (ECVP), 9th Annual Meeting, Aug 24-28, 2009, University of Regensburg, Regensburg, German.