計畫編號：BM08-00
計畫名稱：精神分裂症的致病病理研究：基因、神經生物學與認知科學取向
計畫主持人：梁庚晨/ 胡海國/ 符文美
計畫摘要(中)：

精神分裂症致病病理研究(SOPAS)是依據基因、神經生物與認知功能障礙之三種基礎精神病理所設計，是台灣大學神經生物與認知科學中心，整合基礎與臨床，跨理學院、醫學院、生命科學院、公衛學院、工學院之跨院際、跨學門的研究計畫，它含有一個核心主計劃與6 個子計畫，研究期限為三年。由主要的三個研究領域負責人共同主持。由臨床病人相關之精神科教授負責研究行政之執行。此SOPAS 的6 個子計畫含：(1) 精神分裂症候選致病基因的臨床與神經生物學研究，(2) 精神分裂症患者工作記憶的功能與結構連絡障礙研究，(3) 精神分裂症的神經心理功能輪廓像(profile) 與基礎認知機轉研究，(4) Orexin(下視丘胜肽鏈激發物)於精神分裂症致病病理研究，(5) 性賀爾蒙與心理—社會壓力在精神分裂症致病性基因DISC1 與NRG1 基因缺損鼠的交互作用研究；(6) 精神分裂症致病性基因 DISC1 與 NRG1 基因在神經細胞的發展與退化作用之研究。

精神分裂症是一個病情嚴重且具明顯烙印之精神疾病，並具高遺傳致病性(h2=0.70)。本 SOPAS 團隊，由過去系列性的研究，已發現精神分裂症有不同亞型、有潛在神經心理功能障礙，並發現多個候選致病性基因，與 20 個致病性 SNP 多態型。

此拔尖 SOPAS 計畫立意完成下列二項研究工作，以探究精神分裂症的致病病理，期能據以發展全新的有效治療與預防方法，以解除精神分裂症病人及其家屬的人生折磨：(1) 功能性基因群與神經心理/神經生物學功能障礙與臨床亞型病理相互間的相關性；(2) 以精神分裂症致病性基因中 DISC1 與 NRG1 基因有缺損的老鼠，在有壓力與無壓力之下，探討由 DISC1 與 NRG1 基因所導致的神經生物學與認知功能障礙。

計畫摘要(英)：

This Excellency Program Project (EPP) entitled “Study on Pathogenesis of Schizophrenia: genetic, neurobiological and cognitive approaches” (SOPAS) is sponsored by the Neurobiology and Cognitive

Science Center, National Taiwan University. The interdisciplinary collaborative work of basic neurobiology, cognitive science and clinical psychopathology schizophrenia is the main emphasis of this Research Center. The PIs are, thus, of three responsible professors from three main research domains. The research administration is executed by the Professor from Psychiatry as the study sample of patients is from clinically settings. And subsequently SOPAS is designed based on the psychopathological dimensions of genetic, neurobiological and cognitive disturbances of schizophrenia (SCH) and comprises one Core Major project and 6 subprojects in a 3-year research period. This EPP-SOPAS comprises 6 subprojects of (1) A Clinical and Neurobiological Study on Candidate Vulnerability Genes of SCH; (2) Functional and Structural Disconnectivity of Working Memory in Patients with SCH; (3) Psychological profiles of SCH and the underlying cognitive mechanisms; (4) Role(s) of Orexins, the Hypothalamic Peptide Agonists of a Novel Orphan GPCR, in the Pathogenesis of SCH; (5) Interaction between psychological or social stress and gonad hormones in pathogenesis of SCH: A study on DISC1 and NRG1 mice; (6) Role of SCH-related genes of NRG1 and DISC1 in neuron development and neuronal degeneration. This EPP-SOPAS team includes complimentary research expertise from related neuroscience Departments or Institutes including Psychology, Pharmacology, Anatomy, Life Science, Electric Engineer, Neuroimaging, Nuclear Medicine, Neurology and Psychiatry.

SCH is a devastating and stigmatized psychiatric disorder with high genetic loading (h2 = 0.70). This SOPAS team of National Taiwan University Hospital (NTUH) has found underlined neurocognitive dysfunctions and a three-subtype model of SCH. Besides, we have also found 28 candidate vulnerability genes (CVGs) of SCH, and potentially 30 risk SNP polymorphisms (RPMs) of SCH for further functional study.

This EPP-SOPAS intends to accomplish the following two major tasks to understand the pathogenesis of schizophrenia for developing novel treatment and prevention methods for the relief of the grave sufferings of SCH patients and the family.

The first, we want to find the correlation among the functional

genetic cluster (oligogeneitc etiological factor or gene x gene interaction epistasis effect), the neurocognitive / neurobiological impairments and clinical subtypes of SCH. Besides, we want to validate these CVGs and/or gene clusters of SCH using human postmortem brain tissues and human cell lines or lymphocytes. The second, we want to search for the neurobiological and cognitive evidences caused by the vulnerability genes of SCH by using DISC1 and NRG1 genetic defective mouse model with or without stress effects. This EPP-SOPAS also want to investigate the single and double genetic effects on the neurobiological and cognitive variables.

These 6 subprojects are designed using the same clinical and animal samples for mutual hypotheses testing and for economic purpose. This EPP-SOPAS team has sound infrastructure for smooth coordination and operation.

計畫編號：BM08-01
計畫名稱：精神分裂症致病性基因的臨床與神經生物學研究
計畫主持人：胡海國
計畫摘要(中)：

精神分裂症是一慢性重大的精神疾病，有明顯的神經生物的異常，屬多基因遺傳模式的複雜疾病。過去的研究已發現許多可能的候選基因，但候選基因彼此之間的交互作用，基因、神經生物異常及臨床症狀亞型之間的關聯性目前仍不清楚，是此第一個子計畫的研究主題。

台大醫學院精神醫學基因體研究群(GENOP)研究團隊過去在台灣的精神分裂症家族中已找到 28 個精神分裂症的候選基因，並以直接定序的方法尋找其中 15 個與神經傳遞、神經發展功能相關之基因中的危險基因多型性，將可找到 30 個危險性基因多型性。本研究團隊已確認本土精神分裂症病患有神經心理功能、神經生理功能缺損及腦影像的異常，並根據治療後臨床症狀的表現將此病分為三個臨床亞型，並發現與神經心理功能缺損有相關，也發現個別基因(DISC1，CACNG2，ANXA，PPP3CB)與神經心理功能缺損有相關。

本計畫的目標為(1)以統計及生物資訊的方法，澄清候選基因彼此間的交互作用及候選基因間形成的基因生化路徑。(2)於神經細胞株以 siRNA 減低特定候選基因的表現，以 microarray 研究受該候選

基因影響的基因以澄清基因彼此的關聯性；研究精神分裂症患者死後大腦組織的各候選基因的表現量及其之間的關聯性；以細胞生物學的實驗方法，證實目標一中形成的基因交互作用的假設及闡明其分子生物層次的交互作用細節。(3)收集完整的神經生理功能及腦影像檢查的資料，定出 23 個危險性基因多型性及文獻報告四個基因(COMT, GRM3, G72, BDNF)的 7 個功能性的基因變異的基因型(總共 30 個危險性基因多態型)，了解臨床症狀亞型，神經生物異常，及根據目標(1)及(2)形成的基因交互作用及生化路徑的候選基因群之間的關係。

計畫摘要(英)：

Schizophrenia (SCH) is a chronic debilitating mental disorder with neurobiological abnormalities, and it is a genetic complex disorder. Many candidate vulnerability genes (CVGs) of SCH have been found. However, the interaction between these CVGs, the correlations between these CVGs and neurobiological abnormalties and clinical subtypes of clinical samples remain unclear. This functional study on CVGs of SCH is the main task of this Subproject No.1. Our research team, Group of Genomic Study on Psychiatric Disorders (GENOP), have found 28 potential CVGs of SCH on Taiwanese samples. We have being undergoing direct sequencing on 15 most promising CVGs. We expect to find a total of 30 risk polymorphisms for further functional study.

We have confirmed the neuropsychological and neurophysiological impairments and brain image abnormalities in Taiwanese schizophrenia. We also found that there are potential 3 clinical subtypes of SCH, which is related to different neuropsychological impairments. We also found the association between individual CVG and specific neuropsychological impairments, including DISC1, CACNG2, ANXA7, and PPP3CB.

The specific aims are (1) To clarify the gene-gene interactions and genetic pathways using statistical and bioinformatic methods; (2) To clarify the interactions between specific CVGs and other genes by performing gene expression microarray after knocking down specific CVGs by siRNA, to clarify the correlations of gene expression in the postmorten brain tissue between these CVGs by realtime PCR method, and to confirm the hypothesis of gene-gene interactions proposed by

specific aim 1 and to clarify the detail molecular interaction mechanisms by molecule biology methods; (3) To clarify the correlations between clinical subtypes, neurobiological abnormalities, and the gene clusters proposed by specific aim 1 and 2, after obtaining 23 risk SNP polymorphisms (RPMs) from our sequencing study and 7 RPMs reviewed in literature (a total of 30 RPMs for this study).

The novelties of this project are (1) to validate the CVGs by clarifying and confirming the interactions and genetic pathways of CVGs to form gene clusters, which is novel in delineating the neurobiological mechanism of the oligogenetic etiological model of SCH. (2) to clarify the correspondence between genotypes and neurobiological phenotypes of SCH by analysis of a comprehensive data of clinical subtypes, neurobiological abnormalities and CVGs clusters. This will substantiate a future novel approach in clinical application.

計畫編號：BM08-02

計畫名稱：以功能性磁振造影與擴散頻譜造影研究精神分裂症患者工作記憶之功能性與結構性聯結異常

計畫主持人：曾文毅

計畫摘要(中)：

先前的研究已經顯示精神分裂症是一個同時受到異源性基因以及環境影響的疾病，其異源性使得意圖用單一理論去闡釋致病機轉變得極端困難，近來，胡教授團隊針對 139 位精神分裂症病人進行了為期五年之追蹤，根據病人的臨床症狀，社交能力和神經心理功能，分離出三種亞型，分別是持續的妄想症/幻覺，明顯的遲鈍，以及緩解型三種，根據三種亞型不同的臨床表現，三個假設性

的神經組織病變也被提及：(1) 持續妄想症/幻覺可能源於神經退化性疾病，(2) 明顯遲鈍可能源於神經發育性疾病，(3) 緩解型則為多巴胺精神錯亂。另一方面，我們也了解精神分裂症是神經系統之間的整合發生問題，亦即神經的聯結異常。基於這個假說，本子計畫致力於應用神經聯結造影的先進技術，如功能性磁振造影(fMRI)及擴散頻譜性磁振造影(DSI)，來釐清三種亞型病人在大腦功能以及構造上聯結異常的情況。這假說和近年來精神分裂症的功能性模型是一致的，即皮質-小腦-視丘-皮質-路徑的聯結異常，最後導致認知辨距不良。此外，語言工作記憶被認為是精神分裂症神經心理缺陷的核心，

而語言工作記憶的大小腦神經聯結網絡是廣為人知的，因此，本子計畫將會著重於語言工作記憶的功能及構造聯結。方法：我們將蒐集總共 60 位右利者之精神分裂症病人，每種亞型 20 位。另外 45 位性別，年齡，以及教育程度相仿的右利自願者。病人會在子計畫一中被診斷及分類，在三年期間，我們將陸續研究此三種亞型，每個病人及受試者都會在 3T 磁振造影儀中接受 fMRI 和 DSI 的掃描。fMRI 的資料用以定量語言工作記憶的皮質功能，DSI 的資料則用以評估工作記憶迴路上神經聯結節點處白質的完整性。然後再結合 fMRI 和 DSI，並使用結構方程式模型來計算每個節點的有效聯結。預期結果：相較於正常受試者，我們期望看到三種亞型在工作記憶網絡的功能、構造、及有效聯結均發生不同程度之改變。特別是，緩解型病人將會表現幾乎完整的大小腦聯結，而明顯遲鈍型病人將會在大小腦聯結的結構上出現明顯損傷，而持續性妄想/幻覺的病人不論是在功能、構造、或是有效聯結方面，將會表現出全面性的錯亂。我們會逐年分析三種臨床亞型的功能、構造以及有效聯結的資料，並和正常人相比。在第三年末，三種亞型的資料將互相比較，以顯示出每種亞型，各有其特異之聯結異常。結論：藉由本研究，我們能夠了解精神分裂症中易損基因群對神經組織之傷害，並明確找出此傷害乃是構成工作記憶網絡受損之主因。

計畫摘要(英)：

Previous studies have shown that schizophrenia is a disease with heterogeneous genetic and environmental attributes. The heterogeneity of schizophrenia makes it extremely difficult to elucidate the mechanism of this disease with a single theory. Recently, three clinical subtypes of schizophrenia have been distinguished by Hwu et al. according to disparate clinical profiles, social functioning and neuropsychological functions in a 5-year follow-up study on a cohort of 139 patients. These are the persistent delusion/hallucination, marked blunting, and remitted subtype. Corresponding to different clinical manifestations of three subtypes, three hypothetical neurobiological disorders were proposed: (1) neurodegenerative disorder for the persistent delusion/hallucination, (2)neurodevelopmental disorder for the marked blunting, and (3) dopamine psychosis for the remitted. It has been realized that schizophrenia is a result of abnormal functional integration of neural

systems, i.e., dysconnectivity. We further hypothesize that neurobiological disorders of the three clinical subtypes manifest three discernable patterns of functional and structural dysconnectivity. Based on this hypothesis, this subproject aims to combine advanced techniques for imaging connectivity, functional MRI (fMRI) and diffusion spectrum imaging (DSI), to characterize functional and structural dysconnectivity in these three subtypes of patients. These hypotheses are consistent with current functional models of schizophrenia where dysconnectivity has been proposed in the cortico-cerebellar-thalamo-cortico circuit (CCTCC), resulting in the phenomenon of cognitive dysmetria. In addition, working memory (VWM) has been identified as a core neuropsychological deficit in schizophrenia, and we do have a 16 well-established cerebro-cerebellar neural circuitry in VWM. Therefore, in this subproject we will focus on functional and structural connectivity of VWM. Methods: A total of 60 right-handed patients, 20 in each subtype, and 45 gender-, age-, and education-matched right-handed volunteers will be recruited and examined. The patients will be diagnosed and categorized by subproject #1, the three subtypes will be studied consecutively across the three years. Functional MRI and DSI will be performed on the subjects with a 3T MRI scanner. We will measure cortical function of the VWM based on fMRI data, assess white matter integrity of the fibers connecting nodal points of the VWM circuit using DSI and apply structure equation modeling to compute the effective connectivity between the nodal points. Expected

Results: We expect to see different patterns of altered functional, structural and effective connectivity of VWM in all three clinical subtypes compared to normal controls. Specifically, the remitted subtype will present virtually intact structural and cerebrocerebellar connectivity, the marked blunt subtype will show predominant impairment of structural and cerebrocerebellar connectivity, and the persistent delusion/hallucination subtype will manifest global derangement of functional, structural and effective connectivity. For each year, results of functional, structural and effective connectivity of a clinical subtype will be analyzed and their alterations with respect to normal control will be

characterized. At the end of the third year, the results of three subtypes will be compared to show the significant characteristics of dysconnectivity among three groups. Conclusion: this study could help understand the effects of candidate vulnerable genes on neurobiological systems and provide the structural and functional underpinnings of working memory impairment in schizophrenia.

計畫編號：BM08-03
計畫名稱：精神分裂症心理特徵與認知機制：臨床常模與發展的研究
計畫主持人：鄭昭明/ 花茂棪
計畫摘要(中)：

本子計畫旨在探討透過臨床症狀所分離出來的三種不同的精神分裂症病患的心智功能狀態。過去有一個理論認為精神分裂症患者由於基因異常，導致其無法如正常人一般形成左右腦的功能分化，因此造成某些與皮質相關功能由於左右腦半球分工的不平衡而產生各式的症狀。有鑑於此本子計畫將以四組不同的研究探討與左右腦半球分化共細緻為密切的語言與自我功能三個類型的精神分裂症患者是否有所差異。第一組研究將以神經心理測驗對於三個類型的病人作全面性的心智能力篩檢以及自我/社會功能的測試。第二組研究將專注於三個類型的精神分裂症患者語言知覺的研究，以期瞭解病人其語音知覺變化並探討其腦部的變化與經歷聽覺幻覺的關係。第三組研究將探討三種不同類型的病人對於語意瞭解的處理情形，並探討其語意瞭解的腦部活化及聯結狀態與正常人的差異。第四組研究將探討三個不同類型的精神分裂症患者在自我相關注意力、概念以及工作記憶上的表現情形，並與正常人加以比較，以瞭解自我功能在精神分裂症患者的運作。同時，以上所有的認知與社會行為資料都將以 Multinomial processing tree (MPT)統計/認知模型加以模擬，以期瞭解此一統計認知模型的相關參數在不同類型的病人與正常人是否具有不同，並企圖尋求模型參數所代表的實質意義。這些研究將有助於吾人深入的了解精神分裂症症狀表面底下的認知機制，並進一步推斷其疾病成因與發展治療方法。

計畫摘要(英)：

Schizophrenia has been viewed to be caused by failure of achieve hemispheric asymmetry in cerebral hemisphere caused by aberrant genes

(Crow, 2007). This abnormality yields functional imbalance of the two cerebral hemispheres and leads to indecision. It predicts that symptoms of schizophrenia should be related to deficits in language and other mental functions, such as self, attention control, and emotion/social functions, in which the left and right hemispheres are activated differentially in normal humans. Clinical evidence collected by Taiwan’ s Schizophrenia Research Group suggests that patients with schizophrenia in Taiwan may be categorized into three different subtypes. This subproject aims to test whether cognitive and social deficits may be differentially associated with subtypes of schizophrenia by comparing functions in language and self between patients with schizophrenia and normal controls. For the language abnormalities, this project will focus on speech perception and semantic processing. As for study of self, this project will focus on comparing attention, concept and working memory related to self. Four studies will be included in this subproject.

First, various language, cognitive and social functions will be assessed by neuropsychological tests and correlated with the various clinical symptom profiles of schizophrenia to detect clustering of specific psychological deficits under different subtypes. Second, whether patients with different 16 subtypes of schizophrenia show deficits in categorical speech perception and a distort speech organization will be examined, and the brain activation pattern for organizing speech sounds is examined in an fMRI study using the SVM data analysis. A longitudinal design will be adopted to examine whether speech perception, narrative ability and general cognitive ability are the predictors for later schizophrenia in adolescents at high-risk. A logistic regression and growth curve model will be used to assess the power and developmental pattern of predictors. Third, anomalies in the brain mechanism underlying semantic processing of language will be inferred from functional magnetic resonance imaging (fMRI) and the inherent effective connectivity delineated by dynamic causal modeling. This will be accomplished by comparing the patients with schizophrenia with normal subjects as they are carrying out a character association task in which the relatedness in meaning of two Chinese characters has to be judged. Finally, a model of deficits in theory

of mind (ToM) modularity and dissociation ability is proposed to account for the symptoms of schizophrenia. The Stroop task, modified implicit association task and n-back task will be applied to test the model on self related attention, self concept and self-related working memory. Multinomial processing tree models will be used to analyze various lines of experimental data gathered by this subproject and to test whether the three subtypes of schizophrenia could be delineated by evaluating different parameters in the models.

It is expected that the cohesive effort through from studies proposed in this subproject will further our understanding on psychological profile associated with and neuro-cognitive mechanisms underlying different subtypes of schizophrenia based on biogenetic analysis.

計畫編號：BM08-04
計畫名稱：Orexins,一個新的孤兒 G 蛋白偶合受器的下視丘生肽受質,在精神分裂症的致病機轉所扮演的角色-基礎與臨床研究
計畫主持人：邱麗珠
計畫摘要(中)：

Orexin A 和 B (又稱 hypocretin 1 和 2), 源自 preprohypocretin (HCRT), 是新的孤兒 G 蛋白偶聯受體 OX1R (HCRTR1)和 OX2R (HCRTR2) 的內生受質。Orexin neurons 主要分佈在 lateralhypothalamus 並投射至許多腦區, 包括掌管認知功能的視丘及前額葉(PFC)。MRI 顯示首發精神分裂症(schizophrenia)患者在 orexin fibers 密佈的丘腦-PFC 途徑可能有缺損。臨床發現可活化 orexin neurons 的提神藥物 modafinil 能改善 schizophrenia 的注意力及認知功能, 且非典型精神病藥物能活化 orexin neurons 並增加 PFC dopamine 濃度。因此,我們假設 orexin 系統功能低落可能是 schizophrenia 病人注意力缺損的原因之一, 而非典型藥物之有別於典型者能改善負性症狀,可能是激活 orexin 系統所致。為驗證假設,我們將以 subproject-1 募集的受試者, 配合 subproject-3 的認知功能測驗,與 subproject-2 的 fMRI 與 DSI 掃描，檢驗注意力缺損的 schizophrenia 病人的丘腦皮質前束是否缺損(目標一), 以 RIA 測得的血中 orexin A 是否較低, 並定其 HCRT, HCRTR1 和 HCRTR2 基因的 SNP (目標二)，分析臨床特徵,影像資料與 orexin A 濃度及基因 SNP 的關聯性。動物實驗將在 d-amphetamine 與 phencyclinde 動物

模式及 DISC1 基因轉殖小鼠，分別以 PET 掃描[18F]Fallypride 或 [11C]-(+)-PHNO 顯像與 microdialysis/HPLC 的技術，測量大鼠 PFC 的 dopamine，評估 orexins 的影響；並擬在衝動前抑制，潛在抑制，五項選擇連續反應時間等三種動物注意力行為模式，比較非典型藥物(clozapine)與典型藥物(haloperidol)的差別影響,觀察 orexin 在其中所扮演的角色(目標三)。此計畫結合基礎和臨床研究，期能揭開 orexin 在 schizophrenia 的角色，進而有助治療之發展。

計畫摘要(英)：

Orexin A and B, also known as hypocretin 1 and 2, derived from preprohypocretin (HCRT) are the endogenous agonists of the newly deorphanized orphan G-protein coupled receptors, OX1R (HCRTR1) and OX2R (HCRTR2). Orexin-containing neurons are localized mainly in the lateral hypothalamus and project widely to many brain regions, including the thalamus and prefrontal cortex (PFC), two crucial areas involved in cognitive function. An MRI study suggests schizophrenic patients with first episodes have a deficit in the thalamocortical system, where orexin fibers form synapses densely. A wake promoting agent, modafinil which could activate orexin neurons in animal studies, was unexpectedly found to improve attention and cognitive function in schizophrenia patients. The atypical, but not typical, antipsychotic agents, were found to activate orexin neurons and increase the dopamine levels in the PFC. We, therefore, hypothesize that orexin system hypofunction might contribute to attention deficit and cognition impairment in schizophrenia and atypical antipsychotics might exert their clinical effect, different from that of typical ones, through activating orexin system. To validate these hypotheses, we propose to examine if those schizophrenic patients with attention deficit have thalamocortical tract defects (specific aim 1) and lower orexin levels or SNP in HCRT, HCRTR1and HCRTR2 genes (specific aim 2), and to elucidate the role of orexins in the differential effect of atypical antipsychotic (clozapine), as compared with the typical one (haloperidol), on dopamine release in the PFC and the behavioral tasks resembling attention performance in animal models (specific aim 3). Clinical studies will be conducted in subjects recruited in subproject 1, with the neurocognitive function tests in subproject 3 and fMRI and DSI

imaging in subproject 2. Plasma orexin A levels measured with RIA and SNP in HCRT, HCRTR1and HCRTR2 genes revealed by genotyping will be correlated with the clinical features and imaging data of these patients. The dopamine levels in the PFC of rats will be measured by microdialysis with HPLC and the binding potential displacement of [18F]Fallypride or [11C]-(+)-PHNO, the selective and potent PET ligand of D2 dopamine receptors. We will use three animal models resembling schizophrenia based on the dopamine hypothesis (d-amphetamine-treated rats) and the glutamate hypothesis (phencyclinde-treated rats and mice), and the genomic study, the transgenic mice with a schizophrenia vulnerable risky gene, DISC1. Three behavioral tasks, prepulse inhibition, latent inhibition and 5-choice serial reaction time task, will be used to evaluate the attention performance of rats or mice. The effects of orexin A and B in those behavioral tasks and dopamine release in these three models will be examined. SB 33-4867, an OX1 selective antagonist and [Ala11,D-Leu15]-Orexin B, an OX2R selective agonist (no OX2 selective antagonist available now), respectively, will be used to verify if the effects are mediated by OX1R or OX2R. Schizophrenia is a devastating psychiatric illness with the pathogenesis remained unclear. From basic to clinical approach, this study hopeful can reveal the role of orexins in the pathogenesis of schizophrenia and might be of help in future development of novel therapeutic strategy.

計畫編號：BM08-05
計畫名稱:生殖賀爾蒙與心理或社會壓力間的交互作用於精神分裂症的致病病理研究：致病性 DISC1 與 NRG1 基因缺損小鼠為模式
計畫主持人：梁庚辰/賴文崧
計畫摘要(中)：

研究中發現基因遺傳在精神分裂症上扮演重要角色,但從同卵雙生子的研究中也發現雙生子的共病率從未超過百分之五十,這些證據顯示表觀遺傳扮演一定的調控角色。從精神分裂症通常好發於青春期可以推測青春期階段的心理與社會壓力可能促成精神分裂症之症狀的出現，特別好發於有基因缺損的群體中。為了進一步測試這個假說，在這個子計畫中使用了 DISC1 與 NRG1 基因缺損小鼠為模式，去探討壓力與賀爾蒙對於小鼠在與精神分裂症症狀相關之行為測驗

中的行為表現，包括了前脈衝抑制、痕跡恐懼制約與社會記憶等作業。這幾項作業可以作為測試精神分裂症的三個重要症狀，即注意力、認知能力以及社會能力不良的模式。在心理壓力的部分，將給予電擊與中性聲音或環境連結的制約學習後並用該中性刺激的重複呈現作為心理壓力源；在賀爾蒙調控的部分，將利用手術閹割及睪固酮注射的方式來調控小鼠體內雄性激素含量，將利用這些壓力與賀爾蒙的操

弄去研究是否會在基因缺損小鼠中有促發或舒緩精神分裂症相關症狀的效果。在這個子計畫中將利用 DISC1 與 NRG1 基因缺損小鼠為模式去探討四個主要的目標：(1)評量心理與社會壓力對於精神分裂症成因的影響；(2)評估賀爾蒙睪丸酮對於精神分裂症的促發所扮演的角色；(3)研究壓力及賀爾蒙兩種因素對於神經生理活動影響；(4)用電生理的方法去研究壓力及賀爾蒙對於神經突觸活動的影響。

計畫摘要(英)：

While evidence indicates a genetic basis for schizophrenia, the concordance rate in homozygotic twins inflicting schizophrenia never reaches 50%, suggesting epigenetic involvement in this disease. In view of appearance of major schizophrenic symptoms after the juvenile stage, it is conjectured that psychological or social stress encountered at the puberty may be a factor of precipitating the symptoms in a genetic prone population. To test this hypothesis, matured DISC1 or NRG1 mutant mice and the wild-type controls will be subjected to stress or hormonal manipulations and then tested on pre-pulse inhibition, trace fear conditioning and social memory tests, which are used to model the attention, cognitive and social or emotional impairments in schizophrenic patients. Psychological stress will be induced by pairing an otherwise neutral stimulus with a foot shock, while social stress will be induced by group housing of the subjects. It is expected that psychological or social stress will either precipitate or aggravate behavioral deficits in the mutant mice which are sexually matured. Further, sex hormones may play an enabling role in these deficits, thus gonadectomy before stress should be able to attenuate the effects of stress on the various behavioral indices. Findings from this project should contribute to our understanding of how

external factors in the environment or internal milieu in the body interact with genetic endowment to induce symptoms of schizophrenia. This subproject is expected to achieve four specific aims: (1) To assess the behavioral effects of psychological or social stress on pre-pulse inhibition, trace fear conditioning and social memory in DISC1 and NRG1 mice. (2) To evaluate the effects of gonad hormone depletion and supplement on pre-pulse inhibition, trace fear conditioning and social memory and involvement of these hormones in influences of stress in DISC1 and NRG1 mice. (3) To study the neurophysiological correlates of the stress or hormonal effects on pre-pulse inhibition, trace fear conditioning and social memory in behaving DISC1 and NRG1 mice. (4) To study the synaptic mechanisms underlying neurophysiological correlates of the stress and hormonal effects in brain slices or reduced preparation of DISC1 and NRG1 mice.

計畫編號：BM08-06
計畫名稱：探討與精神分裂症相關之基因 NRG1 及 DISC1 在神經發育及神經退化之角色
計畫主持人：符文美
計畫摘要(中)：

在本子計畫中我們主要是探討與精神分裂症相關的遺傳基因 Neuregulin-1 (NRG1)和 Disrupted-in-Schizophrenia-1 (DISC1)在神經發育及神經退化之角色，以期了解精神分裂症的病理機轉。

在本子計畫中我們將運用基因變異鼠的模式，致力於了解 NRG1 和 DISC1 基因變異鼠造成精神分裂的分子作用機轉。在家族遺傳所致的精神分裂症常見是由多個遺傳基因異常所造成的，因此我們已引進 NRG1 及 DISC1 基因變異鼠，並計畫將此二種小鼠交配得到二種基因均產生變異之小鼠，發展 NRG1 和 DISC1 同時變異的基因轉殖鼠，以便瞭解多重基因缺陷產生之影響，我們將評估這些老鼠的神經發育、神經可塑性及神經退化受到之影響，以研究精神分裂症的致病機轉。此外，我們要將小鼠放在有壓力的環境下觀察 NRG1 和 DISC1 變異對壓力感受性之影響。

我們在此三年計畫將探討下列事項：

1. 在 NRG1 及 DISC1 基因變異對新生鼠及成鼠腦部各區域包括大腦腦室、大腦皮質、海馬迴、ventral tegmental area, amygdala 以及

小腦等區域之神經或 glia 之巨觀變化。除了以組織染色外將用 MicroMRI 來掃瞄動物腦部。在有壓力之環境下觀察 NRG1 和 DISC1 變異對結構之影響。

2. 在 NRG1 及 DISC1 基因變異對新生鼠及成鼠腦部區域 frontal cortex, ventral tegmental area 的 dopaminergic neuron 之影響，除了做免疫染色外將用 MicroPet 以 F18-DOPA 為 probe 來掃瞄動物腦部。將小鼠關在束縛的小籠子並給予浸水下半身之壓力，觀察 NRG1 和 DISC1 變異對 dopaminergic neuron 之影響
3. 在 NRG1 及 DISC1 基因變異對新生鼠及成鼠腦部之大腦皮質、海馬迴、ventral tegmental area 及 amygdala 之 dendritic spines 的影響，在有束縛-浸水壓力之環境下觀察 NRG1 和 DISC1 變異對 dendritic spines 之影響。
4. 給予小鼠急性或慢性之情緒上的壓力，觀察在 NRG1 及 DISC1 基因變異對學習及記憶能力之影響，對電生理及 long-term potentiation 之影響也一併探討。
5. 觀察 NRG1 及 DISC1 基因變異對神經滋養因子 (包括 glial cell line-derived neurotrophic factor, brain-derived neurotrophic factor, EPO, VEGF) 及 glucose transporters 表現之影響。
6. 以斑馬魚為實驗動物模式，將神經特別以 GFP 螢光標定，再注入 NRG1 或 DISC1 之變異基因，觀察神經發育及神經退化受到之影響。在體外細胞培養的實驗中，利用已建立的神經細胞株 SH-SY5Y 、Neuro2A 和 PC-12，以細胞轉殖的方式將 NRG1 和 DISC1 的 shRNA 或 human mutant 送到細胞內，或直接以 NRG1 及 DISC1 基因變異胎鼠做 primary cultures，以探討在神經細胞抑制該等基因正常表現時，對神經軸的生長、神經細胞的分裂、細胞存活、dendritic spine 的產生以及細胞移行的影響。在神經系統中，神經生長因子對於神經細胞生理功能的調節極為重要，包括 Brain-derived neurotrophic factor (BDNF)、Glial cell line-derived neurotrophic factor (GDNF) 或 platelet-derived growth factor (PDGF)…等，都會刺激神經細胞的分化、存活以及神經軸的生長。因此本計畫也將探討抑制 NRG1 和 DISC1 的功能對於這些神經滋養因子的表現有何影響。

DISC1 及 NRG1 在 glial 細胞也有表現。我們也將探討這些基因在 glial cells 之功能，使用 human glial cell A172 來探討 BDNF、

GDNF、EPO、VEGF 等神經滋養因子的表現受到 DISC1 及 NRG1 的調節作用。此外，精神分裂症病人所發現的功能性失調包括在病人的大腦皮質會發現減少葡萄糖的利用，我們將在神經及 glial cells 探討在 NRG1 和 DISC1 抑制或產生變異的情形下， 對於 glucose transporter GluT1 和 GluT4 的影響。

計畫摘要(英)：

In this subproject, we will concentrate on the exploration of the molecular mechanism regarding the pathogenesis of the schizophrenia-related genes NRG1 or DISC1 using both transgenic mice and cell culture system. The NRG1 or DISC1 human mutant transgenic mice have been imported. Since schizophrenia patients appear to have multiple genes' defect, we will backcross these two kinds of transgenic mice to produce NRG1 and DISC1 double mutant mice. We will then evaluate the neuron development and neuronal degeneration in these mice in order to further understand the molecular pathology of schizophrenia. The following experiments will be performed in neonatal and adult mice in order to study the effects of NRG1 and DISC1 mutant on synaptic plasticity and structural plasticity. (1) Macrosopic histological examination in lateral ventricle, frontal cortex, hippocampus, ventral tegmental area (VTA), amygdala and cerebellum. Both neuron and glia will be examined. (2) Effect on dopaminergic neurons in ventral tegmental area and frontal cortex. (3) Effect on dendritic spines in various brain regions. (4) Since schizophrenia patients can not tolerate the stress, we will examine the mutant of NRG1 and DISC1 on dendritic spine formation or learning and memory activity under exposure to water immersion-restraint stress. The effect of stress on long-term potentiation in hippocampus or amygdala will also be examined. In addition, the electrical properties of dopaminergic neurons in ventral tegmental area in response to drug treatment or psychological stress will also be explored. (5) Effect on the regulation of neurotrophic factor expression in both neuron and glia (including glial cell line-derived neurotrophic factor, brain-derived neurotrophic factor, EPO, VEGF). In addition, the mutation of NRG1 and DISC1 on the expression of glucose transporters will also be explored in the mutant mice. (6) We will also use zebra fish as an

animal model to examine the role of both NRG1 and DISC1 on neuron development and neuronal degeneration. The neuron will be specifically labeled with GFP in order to examine the neuronal morphology in whole body.

In cell culture systems, we will knockdown the genes of NRG1 or DISC1 or transfect with human mutants of NRG1 or DISC1 in SH-SY5Y, Neuro2A, PC12 or glial cells (human glial cell line A172) to explore the role of these genes in neuronal proliferation, survival, migration, neurite outgrowth and neurotrophic factor secretion. In addition, the primary cultures derived from NRG1 or DISC1mutant mice will also be used. Neurotrophic factors are very important factors for regulating various neuronal functions. We have got preliminary data to show that DISC1 knockdown or overexpression of DISC1 mutant inhibited GDNF or BDNF expression in various brain regions of mutant mice and cell cultures. Therefore, we will examine the role of DISC1 and NRG1 in the synthesis and release of neurotrophic factors in both neuron and astrocyte. Furthermore, schizophrenia patients also show reduced glucose utilization in the prefrontal cortex. We will also investigate the regulatory role of NRG1 or DISC1 in the expression of GluT1 and GluT4 in neurons or glial cells.